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DUAL MOTOR OUTPUT INTERNEURONS IN THE ABDOMINAL GANGLIA OF THE CRAYFISH *PROCAMBARUS CLARKII*: SYNAPTIC ACTIVATION OF MOTOR OUTPUTS IN BOTH THE SWIMMERET AND ABDOMINAL POSITIONING SYSTEMS BY SINGLE INTERNEURONS

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Summary

Many behavior patterns of the crayfish involve the positioning of the abdomen by the tonic motor system. Movements and positionings of the swimmerets are coordinated with these abdominal movements. Evidence from extracellular analyses suggested that single interneurons of the abdominal nerve cord could produce motor outputs in both the swimmeret and the abdominal positioning systems. Our intracellular investigation has revealed that many single cells can evoke outputs in both motor systems. Interneurons which produced fictive extension or flexion of the abdomen or inhibition of abdominal movement were also able to modulate a variety of swimmeret behavior including cyclic beating and excitation or inhibition of episodic outputs. Although interneurons were discovered that evoked each of the possible classes of dual-output combinations, those that evoked combinations frequently observed in the freely behaving animal were more common than those that evoked infrequently observed combinations.

Evidence also indicated that abdominal positioning inhibitors are present in greater numbers than previously suspected and that many are closely associated with the swimmeret circuitry. Interneurons with the ability to start and stop swimmeret cyclic outputs with current injections of opposite polarity are proposed to be higher-order cells, and some are shown to have the properties of trigger neurons. It is proposed that most dual-output cells are presynaptic to single-output cells and that groups of related dual-output cells may function together as command elements.

Introduction

Neural control of behavior patterns such as abdominal positioning and swimmeret movements has been examined extensively in crustaceans. These behavior patterns can be evoked by stimulation of abdominal cord interneurons. Wiersma and Ikeda (1964) first described 'command neurons' for swimmeret

Key words: crayfish, interneuron, swimmeret, abdominal position.

movements in crayfish, while Davis and Kennedy (1972*a*,*b*,*c*) examined similar interneurons in lobsters. Recent studies on control of the swimmeret system have been intracellular and have focused on the initiation, generation and coordination of behavior (Heitler, 1978, 1982, 1985; Heitler and Pearson, 1980; Paul and Mulloney, 1985*a*,*b*, 1986). Work on the control of abdominal positioning has been equally extensive. Extracellular studies have been conducted by Evoy and Kennedy (1967), Bowerman and Larimer (1974*a*,*b*) and Williams and Larimer (1980, 1981), while intracellular analyses in crayfish have been reported by Miall and Larimer (1982*a*,*b*), Larimer and Jellies (1983), Larimer and Moore (1984), Jellies and Larimer (1985, 1986), Moore and Larimer (1987, 1988) and in lobster by Jones and Page (1986*a*,*b*,*c*).

Observation of unrestrained animals has revealed that coupling can exist between abdominal positioning and swimmeret behavior. For example, cyclic beating of the swimmerets, consisting of alternating powerstroke and returnstroke phases, commonly accompanies abdominal extension. Extension can also occur without swimmeret beating, indicating that the two types of behavior can be uncoupled. During complete abdominal flexions, the swimmerets are held forward in the returnstroke position. Cattaert and Clarac (1983) obtained evidence by direct observations and EMG analysis that both abdominal positioning and walking are correlated with swimmeret beating in lobster. Kotak and Page (1986) have also reported that tactile stimulation of swimmerets produces abdominal extension behavior in lobster. The abdominal positioning system has also been shown to interact with the statocyst interneurons and uropod steering system (Takahata and Hisada, 1985). Other investigators have used various methods to show coupling of swimmeret positioning with giant-fiber-mediated abdominal flexions in the escape response (Wiersma, 1947; Cooke, 1985; Heitler and Darrig, 1986). Escape responses are not considered to be typical abdominal positioning behavior since they are subserved by separate sets of muscles and motoneurons (Kennedy and Takeda, 1965).

That activity in some interneurons might affect *both* abdominal positioning and swimmeret behavior was suggested by the extracellular analyses of Evoy and Kennedy (1967) and Williams and Larimer (1981). Since interneurons were known to exist which could initiate abdominal positioning or swimmeret beating separately, we used intracellular techniques to determine if any of these cells could affect both activities.

This paper reports the presence of many dual-output interneurons which influence both abdominal positioning and swimmeret movements, as well as some single-output cells which influence only one of the behavior patterns. Thus, the neural substratum in the abdominal cord appears to be able to support the coupled and uncoupled expression of the two types of behavior. We show that dual-output interneurons exist in all combinations; however, those evoking motor programs compatible with observed behavior are more prevalent than those which seem to initiate incompatible behavior patterns such as flexion with swimmeret beating. We also present evidence of a close association between abdominal positioning

inhibitors and the swimmeret circuitry. Finally, we comment on the probable arrangement of the command elements in this system.

Some of the material in this paper has been presented in abstract form (Murchison and Larimer, 1986).

Materials and methods

Crayfish (*Procambarus clarkii*) were obtained from Waubun Laboratories, Schriever, Louisiana. Animals of both sexes with rostrum to telson lengths between 7 and 12 cm were maintained in shallow tanks of dechlorinated water at 18 °C in LD 12 h: 12 h on a diet of commercial cat food. Gravid females or recently molted animals were not used.

Abdominal nerve cords were removed under cold anesthesia in a way that ensured at least 1 mm of each proximal ganglionic nerve remained undamaged. The cords were pinned, ventral side up, in Sylgard-lined plastic Petri dishes by anchoring extraneous ganglionic nerves to the Sylgard with fine-gauge insect pins. Cords were bathed in van Harreveld's solution (van Harreveld, 1936) at 15–18 °C.

Intracellular microelectrode techniques were used to survey the neuropil of abdominal ganglia for neurons able to evoke abdominal positioning motor outputs and/or swimmeret outputs. The third abdominal ganglion (A3) was sampled extensively, while the fourth (A4) and fifth (A5) ganglia were examined to a lesser extent. Fictive behavior was monitored by extracellular suction electrodes. At least one first nerve, containing the axons of the swimmeret motoneurons (MNs), one second nerve, containing the axons of the abdominal extensor MNs, and one superficial third nerve, containing the axons of the slow flexor MNs were monitored simultaneously. In some experiments an extracellular stimulating electrode was attached to the first nerves of A1 and was used to stimulate fictive swimmeret beating (Heitler, 1978).

Microelectrode impalements were made ventrally through the desheathed ganglion at various levels in the neuropil. Microelectrodes (impedence 80–360 MΩ) were prepared by filling the tips with a 3 % aqueous solution of Lucifer Yellow CH and the shanks with 1 mol l^{-1} lithium chloride (Stewart, 1978). Microelectrodes were used with a WPI M701 microprobe system with a bridge circuit extended to 500 MΩ, while extracellular electrodes were coupled to Tektronix 122 preamplifiers. Electrophysiological data were recorded as polaroid photographs of storage oscilloscope sweeps.

Impaled cells were initially injected with high levels of depolarizing current (10–15 nA) to elicit any motor outputs they might have. High current levels also acted as a test of impalement quality, since poor penetrations cannot be maintained at such levels. Lower levels of current were then used to determine output thresholds.

After current injection and data accumulation, impaled neurons were filled with Lucifer Yellow dye by passing 4–8 nA of hyperpolarizing current into the cell in 900 ms pulses at a rate of 0.4 Hz for between 1 and 90 min. Tissues from longer fills

(usually 30 min or more) were often allowed to sit for 2 or 3 h prior to fixation to permit dye diffusion and transport along the axons to reach adjacent ganglia. Tissues were fixed in 4% paraformaldehyde, rinsed with Sorensen's buffer, dehydrated with ethanol, cleared in methyl salicylate and examined in whole-mount under a fluorescence microscope. Cell morphologies were recorded using a Zeiss drawing tube.

Interpretation of data

Crayfish and other arthropods have both excitatory and inhibitory motoneurons (MNs). Spiking of a peripheral inhibitor (PI) motoneuron decreases excitation to target muscles. PIs to the abdominal positioning muscles can be identified in extracellular records by spike sizes and firing patterns (Kennedy and Takeda, 1965; Kennedy *et al.* 1966; Wine *et al.* 1974). This allows qualitative classification of abdominal positioning cells (as flexion, extension or inhibitor interneurons).

Cells were classified as evoking extension if depolarization caused spiking of excitatory MNs in the extension nerves and inhibited firing of MNs in flexion nerves, or did not affect their firing, and/or fired the PI to the slow flexors. Flexion cells were identified if depolarization caused firing of several flexion nerve excitor MNs, either inhibited or left unaffected the firing of extension MNs, and/or excited the PI to extensors. Interneurons with mixed abdominal positioning outputs had features of both flexion and extension cells, typically firing several MNs in both flexion and extension recordings. Cells categorized as abdominal positioning inhibitors (APIs) reduced the activity of excitatory MNs in flexion and/or extension recordings and/or fired the PI to flexors and/or extensors. Abdominal positioning outputs are considered reciprocal if antagonistic excitor MNs are inhibited or the PI to the antagonist muscles is excited coincident with the excitation of agonist MNs.

If swimmeret motoneurons (SWMNs) began spiking when an impaled cell was stimulated, the cell was categorized as producing SWMN excitation. If spiking of SWMNs was inhibited, the cell was placed in the inhibiting SWMNs category, and if swimmeret records were unaffected, the category was 'no swimmeret influences'. A few cells that showed excitation of one swimmeret nerve and inhibition of another or that reset the phase of the rhythm without noticeably exciting or inhibiting the MNs were classified as having 'other' swimmeret outputs.

Cells were considered to influence the cyclic swimmeret rhythm if current injection had any effect on the rhythm including: resetting of the cycle phase of the rhythm, changing the phase duration, or stopping/starting the rhythm. Cells were considered to start a swimmeret rhythm if, when injected with current following a prolonged bout (at least 10 s) of episodic or absent swimmeret output, a rhythmic output of several cycles was established. Likewise, cells were considered to stop a swimmeret rhythm when current injection during a well-established rhythm (continuing at a stable frequency for several cycles) abolished the rhythm for at least 4 s following the end of current injection. Data were only taken if the cells

showed qualitatively the same output during two or more trials and were not taken if the output varied between categories.

Because peripheral inhibitors and other motoneurons of swimmeret muscles are not identified, there is some uncertainty in interpretation of swimmeret outputs when that output consists of firing in a single SWMN. However, most outputs consisted of firing in numerous SWMNs, often in the characteristic bursts which could be identified from whole nerve records as subserving swimmeret powerstrokes or returnstrokes. Extracellular examination of SWMN records in virtually intact animals revealed that during cyclic swimmeret output, SWMN spikes of the powerstroke phase were of approximately twice the amplitude of the largest returnstroke phase spikes. Small-unit activity was seen to accompany episodic movement in the returnstroke direction, while large-unit activity was associated with episodic movement in the powerstroke direction.

Dual-output cells are those having motor outputs in both the abdominal positioning and swimmeret systems. It is not meant to imply that these are the only motor outputs produced by those cells. Indeed, it is likely that many of these cells are 'multiple-output' cells with outputs also occurring in motor systems that were not monitored in these experiments. Similarly, the term 'single-output cell' was applied to those cells having motor output in either the abdominal positioning or the swimmeret system but not in both. Again, these cells may have outputs in motor systems other than those monitored in this study.

Interpretation of swimmeret rhythms required data taken at slow oscilloscope sweep speeds (1 or 2 s division^{-1}) while unambiguous interpretation of abdominal positioning outputs required data to be taken at much faster sweep speeds (0.1 or 0.2 s division^{-1}). For the purposes of this paper, photographs from slow sweep speeds were deemed most informative. Although these data show clearly our points concerning swimmeret outputs, the nature of abdominal positioning output is often obscured. To limit the figures in the paper, most of the fast sweep speed data, showing definable abdominal positioning outputs, have been omitted. However, in all cases where abdominal positioning outputs are described, the interpretation of the nature of the output (extension, flexion or inhibition) was made from fast sweep speed data. Care was taken to ensure that a representative sample of background motor activity was visible on the photograph prior to current injection.

Most of the electrophysiological records shown here do not display balanced bridges because production of definitive motor outputs often required higher levels of current injection than could usually be balanced (7–15 nA). However, balanced bridge data were taken for virtually all cells at lower currents to determine the cells' abilities to spike.

Results

Coupled motor outputs

Examination of more than 380 cells yielded interpretable data in 363 examples

Table 1. *Summary of data from 363 intracellular microelectrode impalements separated into abdominal positioning categories and swimmeret output classes*

Extension cells	$N=76$	Flexion cells	$N=123$
Non-dual output	$N=16$	Non-dual output	$N=32$
Dual output	$N=60$	Dual output	$N=91$
Swimmeret excitation	$N=41$	Swimmeret excitation	$N=50$
Swimmeret inhibition	$N=13$	Small or single units	$N=33$
Other swimmeret outputs	$N=6$	Powerstrokes during current injection	$N=9$
Swimmeret rhythm influence	$N=29$	Powerstrokes after current injection	$N=8$
Mixed output cells	$N=24$	Swimmeret inhibition	$N=31$
Non-dual output	$N=5$	Other swimmeret outputs	$N=10$
Dual output	$N=19$	Swimmeret rhythm influence	$N=32$
Swimmeret excitation	$N=15$		
Swimmeret inhibition	$N=4$		
Swimmeret rhythm influence	$N=7$		
Abdominal positioning inhibiting cells (APIs)	$N=75$	Swimmeret cells	$N=65$
Non-dual output	$N=14$	Excitation	$N=46$
Dual output	$N=61$	Inhibition	$N=12$
Swimmeret excitation	$N=36$	Other outputs	$N=7$
Swimmeret inhibition	$N=17$	Swimmeret rhythm influence	$N=29$
Other swimmeret outputs	$N=8$		
Swimmeret rhythm influence	$N=29$		

Numerals indicate absolute numbers of cells encountered in each category.

Cells were included in the 'swimmeret rhythm influence' category based on their abilities to start, stop, reset or otherwise after a cyclic swimmeret output when injected with current.

Criteria for assigning a cell to one class or another are described in the 'interpretation of data' section.

and revealed that 231, or 64 %, of the cells had dual motor outputs. The remainder were classified as single-output cells and were evenly divided between those that produced only swimmeret outputs and those that produced only abdominal positioning outputs. Since these data were taken from cells impaled at random, the percentages should approximately reflect ratios of dual-output to single-output cells present in the third abdominal ganglion and probably also in the abdominal ganglia of other swimmeret-bearing segments. Each of the possible combinations of swimmeret and abdominal positioning outputs (see Table 1) is represented by a group of interneurons able to evoke that combination.

Coordination, similar to that in the intact animal, between the motor outputs of the swimmeret and abdominal positioning systems can occur in the isolated abdominal cord, as seen in Fig. 1. The observation that these coordinated outputs occur in completely isolated cords indicates that neither sensory feedback nor higher centers are necessary for them to take place. Instead, central interactions within the abdominal ganglia are sufficient to coordinate motor outputs from th

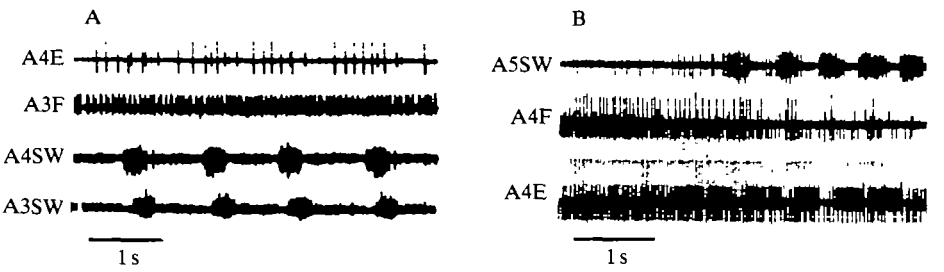

Fig. 1. Spontaneous coordination of swimmeret and abdominal positioning activity. (A) Coordination of extension motoneuron spiking with cyclic swimmeret output. Firing of extension motoneurons is seen to correlate with large bursts of spikes from swimmeret powerstroke motoneurons during fictive swimmeret beating. (B) Coordination of abdominal positioning outputs with spontaneously initiated swimmeret rhythm. The onset of cyclic swimmeret activity is correlated with a decrease in the rate of flexion motoneuron firing and the appearance of extension motor output, seen as a decrease in the rate of firing of the peripheral inhibitor to extensors and a 'bursty' organization of the extensor excitors. Abbreviations used in this and subsequent figures are as follows: A3–A5, third to fifth abdominal ganglia; F, flexion nerve recording (superficial third nerve); E, extension (second) nerve recording; SW, swimmeret (first) nerve recording; IN, intracellular recording; I, current monitor; MN, motoneuron; SF, slow flexion; SE, slow extension; PI, peripheral inhibitor.

two systems. This coordination could be mediated by the numerous dual-output cells present.

Cells evoking abdominal extension

Seventy-nine percent of extension cells (60/76) also had swimmeret outputs and were considered dual-output cells. The remaining 21% had only extension outputs. Most dual-output extension cells (41/60) excited swimmeret motoneurons (SWMNs), and many of these (16/41) were able to influence the swimmeret rhythm (see Table 1). Fig. 2 shows data from an extension cell which excited SWMNs.

Twenty-two percent (13/60) of dual-output extension cells inhibited firing of SWMNs and nine of these were able to influence the rhythm. These cells may operate during extension behavior when swimmeret beating would be inappropriate. Data from some cells of this class are displayed in Fig. 3.

Sixteen (21%) of the extension cells had no swimmeret outputs. They neither excited nor inhibited SWMNs nor had any observable effect on the swimmeret rhythm. Evidence from these extension cells indicates that mechanisms exist at an abdominal neuronal level for both the coupled and the uncoupled expression of abdominal positioning and swimmeret behavior.

Cells evoking abdominal flexion

Of 123 flexion cells encountered, 91 (74%) had swimmeret outputs, whereas 32 (26%) did not. Only 35% (32/91) of the dual-output flexion cells were able to

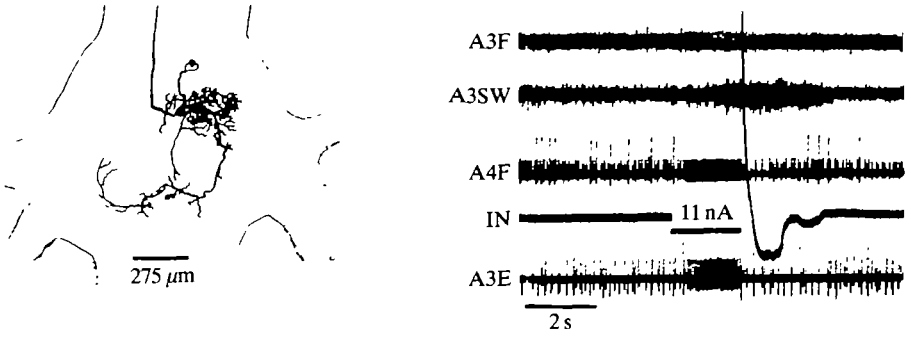

Fig. 2. A cell evoking abdominal extension and excitatory swimmeret output. This identified cell produces reciprocal extension by firing several extension motoneurons (SEMNs) and the peripheral inhibitor (PI) to the slow flexors while inhibiting other slow flexion motoneurons (SFMNs). In one preparation, depolarizing this cell caused firing of swimmeret motoneurons (shown), and in other preparations, with active swimmeret rhythms, depolarization reset the rhythm (not shown). This pattern of motor effects is common in cells of this category. Dual-output thresholds were achieved with less than 4 nA of current, and threshold firing rate was about 50 Hz. Apparent serial homologs occur in abdominal ganglia 3, 4 and 5. Another cell of this class (Miall and Larimer, 1982*b*; Fig. 10, no. 3) is now identified with apparent serial homologs in A3 and A4. Depolarization of that cell fires SWMNs, produces reciprocal extension and can reset a swimmeret rhythm (not shown). All cell morphologies are from A3, and the rostral direction is towards the top of the page.

influence a swimmeret rhythm. This was the lowest percentage of cells with such influence in any of the output categories (see Table 1).

Fifty-five percent (50/91) of dual-output flexion cells excited SWMNs. Since complete abdominal flexion behavior is accompanied by episodic positioning of the swimmerets in the returnstroke position, it is not surprising that 66% (33/50) of these flexion cells did not evoke the large unit first nerve burst typical of a swimmeret powerstroke. Instead, these cells excited either single units (probably peripheral inhibitors) or small-amplitude units (probably returnstroke MNs) as seen in Fig. 4.

Flexion cells (17/50) that paradoxically excite apparent powerstroke MNs fell into two subclasses which appear to influence the SWMNs by different mechanisms. One set of eight flexion cells produced swimmeret powerstrokes at various delays after depolarizing current injection. Four of these were also able to initiate an apparently normal rhythm of alternating powerstrokes and returnstrokes. Probable returnstroke MNs were usually excited during the stimulus. Fig. 5 shows data from some cells of this subclass. In both cases, strong reciprocal flexion was prolonged beyond the duration of the stimulus, probably indicating recruitment of other flexion interneurons. In contrast, another subset of dual-output flexion cells contained nine examples that were able to excite powerstroke MNs during depolarizing current injection. Six produced single powerstrokes during the

stimulus, and three were able to initiate a swimmeret rhythm. Data in Fig. 6 are from an interneuron of this type. Although some of these cells did not start a swimmeret rhythm when stimulated, each produced swimmeret outputs with a caudal to rostral metachrony that is present during a typical rhythm. This implies that the output is activated *via* the intersegmental coordination system. While it is clear that these two classes influence the swimmeret rhythm by different mechanisms, it is uncertain whether they act on different components of the central pattern generator (CPG), by using different transmitters or at different levels in the pattern-generating hierarchy.

Because the swimmerets seldom move during complete abdominal flexions, it is not surprising that, of the 91 dual-output flexion cells examined, 31 inhibited

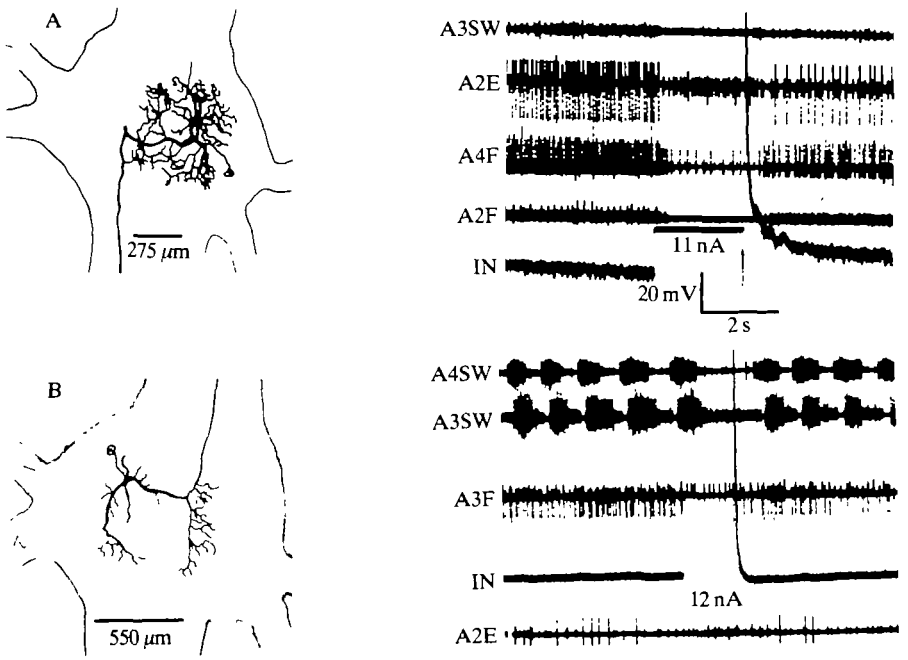

Fig. 3. Cells producing abdominal extension and SWMN inhibition. (A) Data from a tentatively identified A3 interneuron which produces reciprocal extension and inhibition of SWMN firing. Depolarization of this cell inhibits the firing of SFMNs in both immediately adjacent ganglia, inhibits the firing of the PI to extensors, fires other SEMNs, and inhibits episodic activity in the local swimmeret nerve. Dual outputs of this cell were inseparable at threshold current of 2 nA which elicited spiking at 40 Hz. (B) Data from a tentatively identified interneuron in A3 showing reciprocal extension and swimmeret rhythm influence. Depolarization of this cell inhibited excitatory SFMNs while exciting the PI to flexors and the SEMNs. Swimmeret returnstroke duration was lengthened during the stimulus and the cycle phase was apparently reset. This cell has an apparent serial homologue in A4. In B, determination of swimmeret inhibition was made from data (not shown) taken during a bout of episodic SWMN firing, as seen in Fig. 3A, and current was monitored on a different oscilloscope from that used to record motor records.

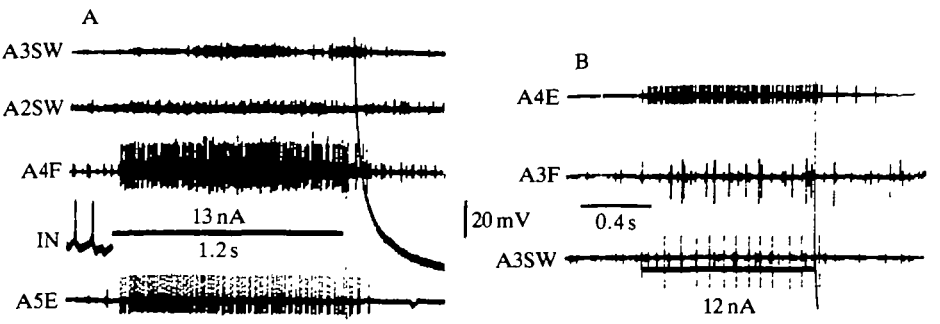

Fig. 4. Dual outputs of cells evoking abdominal flexion and exciting several small-amplitude SWMNs or a single SWMN. (A) Reciprocal flexion with firing of probable swimmeret returnstroke MNs. (B) Reciprocal flexion with firing of probable swimmeret peripheral inhibitor. In both cases, several SFMNs are recruited with the PI to extensors. The intracellular recording is not visible on the photograph in B.

SWMNs when depolarized. This was the largest class of dual-output cells to display swimmeret inhibition (see Table 1). Data from cells of this type are shown in Fig. 7. No cells of this class that were able to stop a swimmeret rhythm when depolarized were seen to originate in the ganglia of the swimmeret-bearing segments. This implies that cells with this property generally originate in A6 or in ganglia rostral to A1 and may represent high-order neurons for the inhibition of swimmeret outputs during abdominal flexions. Other cells of this class inhibited episodic swimmeret outputs without observable effect on cyclic outputs or, as in Fig. 7B, influenced the cyclic output without resetting the rhythm. This implies that some cells of this class may have their effects on the swimmerets through circuits that are separate from the pattern-generating apparatus or are postsynaptic to the CPG.

Flexion cells without swimmeret outputs made up the largest class of single-output abdominal positioning cells. The rarity of swimmeret movements accompanying abdominal flexion behavior makes this an expected result. The majority of interneurons in this class showed the bipolar morphology of the previously identified 'T' cell subclass. This cell type has been encountered frequently in intracellular studies of the abdominal positioning system. These T cells appear to be present only in the third abdominal ganglion. Typical examples of the morphology of this identified subclass have been published by Miall and Larimer (1982*b*, Fig. 5, type 1–3), Larimer and Jellies (1983; Fig. 5A,B), Larimer and Moore (1984; Figs 2B3 and 4E) and Jellies and Larimer (1985, Fig. 8A). Cells of this type produce strong flexion in several segments, and most have no detectable swimmeret outputs.

Cells inhibiting abdominal positioning

Most abdominal positioning inhibitors (APIs) influenced both flexion and extension outputs, although some inhibited only flexion, and a few were found to

inhibit only extension. Eighty-one percent (61/75) of the APIs had dual outputs. A high percentage of dual-output APIs is not unexpected, since observed behavior might lead one to anticipate that interneurons inhibiting flexion would excite swimmerets or that cells inhibiting extension might also inhibit swimmerets. The data presented here show that APIs are more prevalent in the abdominal nervous system than was previously believed and that they appear to play an important role in the coordination of swimmeret outputs with abdominal positioning.

Of the dual-output APIs, 59% (36/61) excited SWMNs, with 44% of these

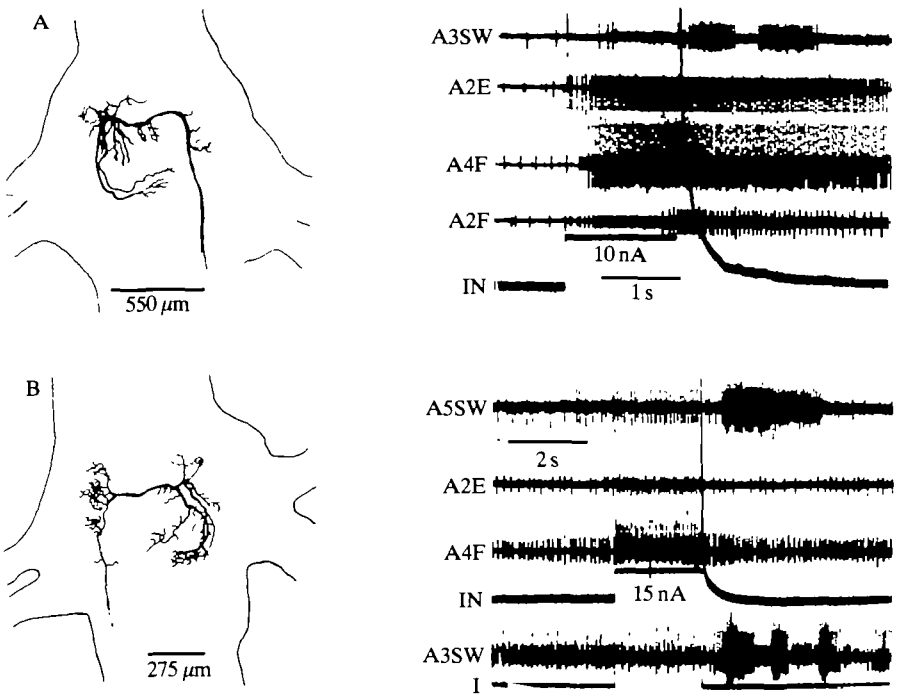

Fig. 5. Cells evoking abdominal flexion and swimmeret powerstroke activity after injection of depolarizing current. (A) Data from tentatively identified dye-coupled cells of almost duplicate morphology which produced a prolonged, patterned, reciprocal flexion output accompanied by excitation of small-amplitude swimmeret units during the stimulus and by excitation of swimmeret powerstroke units after the stimulus. Note that these cells have caudally directed axons, yet the output is first detected by activity in the peripheral inhibitor to extensors in the next rostral ganglion. Local flexion output follows, with caudal flexion occurring next and the onset of local swimmeret activity having the greatest delay. Because the rostral output must be mediated by at least one intercalated interneuron, it is likely that all the observed effects are polysynaptically produced. (B) Data from a tentatively identified cell from A3, with probable serial homologs known in A4. This cell was an unusual flexion cell in that threshold for swimmeret output was well below that for flexion. 3 nA depolarizing current caused this cell to spike at 85 Hz and provided threshold level excitation of SWMNs. 10 nA was required to evoke reciprocal flexion and 12 nA to elicit a swimmeret rhythm.

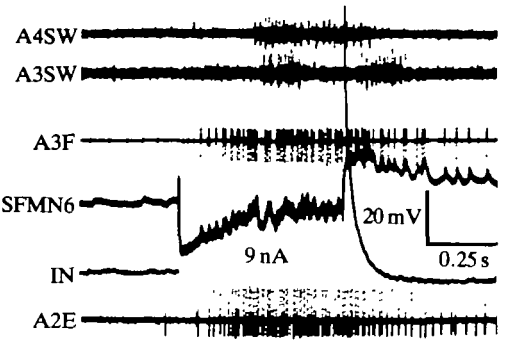

Fig. 6. A cell producing abdominal flexion and swimmeret powerstrokes during depolarizing current injection. This identified cell (see Larimer and Jellies, 1983; Fig. 5C,D) has probable serial homologs in A3, 4 and 5. Reciprocal flexion output accompanies simultaneous expression of swimmeret powerstroke output. This cell had threshold reciprocal flexion evoked by 1.5 nA depolarizing current which caused the cell to spike at 10–15 Hz. Twice the current was required to induce local swimmeret output and more was needed to elicit the output caudally. This cell was encountered during an experiment in which SFMN6 was also recorded intracellularly.

(16/36) able to influence a swimmeret rhythm. Eleven of these cells showed spontaneous correlation of cell spiking with cyclic swimmeret nerve activity, indicating a very intimate association of these cells with the CPG controlling the swimmerets. This was the only category of dual-output cells in which more than two or three examples of such correlation were observed. Also, thresholds for swimmeret outputs tended to be lower than those for inhibiting abdominal positioning. Thus, many of the cells of this class have properties which make them potentially critical elements in the coordination of swimmeret outputs with abdominal positioning. Examples of APIs that excited SWMNs are shown in Fig. 8. Dual-output APIs, such as those in Fig. 8A, B and D, which inhibit only flexion and/or the peripheral inhibitor to extensors, can be considered as extension permissive cells and may function in coupled extension and swimmeret beating behavior.

Seventeen APIs inhibited swimmeret outputs, and eight of these were able to influence a swimmeret rhythm. Cells of this type could be useful for terminating behavior which is subordinate to other commands or for setting a 'neutral' behavioral state prior to the initiation of a different behavior. Fig. 9 describes two identified cells of this class.

Some dual-output APIs appear similar to cells described by Paul and Mulloney (1985*a*,*b*, 1986) in *Pacifastacus leniusculus*. These investigators have proposed that the cells are important components of the swimmeret CPG and intersegmental coordinating systems. We have found that cells with similar properties and morphologies in *Procambarus* also have roles in coordinating abdominal positioning. Data from some of these cells are presented in Fig. 10.

The single-output class comprised 19% of all APIs observed. Two cells of this

type have been identified. One is a local interneuron with morphology published by Jellies and Larimer (1985, Figs 11B-1 and 11B-2). This cell inhibited local flexion output and had no effect on swimmeret outputs when depolarized or hyperpolarized. This cell is apparently non-spiking and has probable serial homologs in A3, A4 and A5.

Single-output swimmeret cells

Almost half (65) of the single-output cells were swimmeret interneurons or motoneurons. These cells evoked swimmeret outputs but had no abdominal positioning effects. Membrane potential oscillation or spiking in 18 of these cells occurred in phase with a swimmeret rhythm. Swimmeret interneurons with oscillating potentials could be components of: (a) the swimmeret rhythm central pattern generator; (b) the interganglionic coordinating system; (c) the bilateral coordinating system; (d) other premotor systems receiving fairly direct CPG input. Fourteen cells affected swimmeret outputs when either hyperpolarizing or depolarizing current was injected; the effect of one polarity of current was

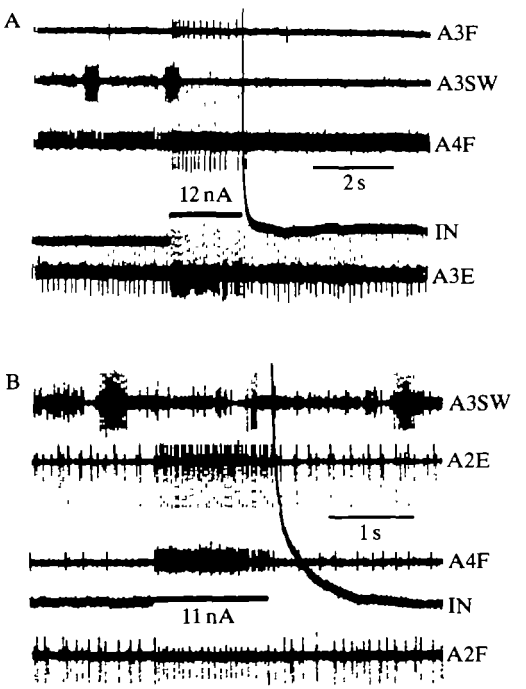

Fig. 7. Cells evoking abdominal flexion and swimmeret inhibition. (A) An example of output from unidentified axons. Depolarization caused strong reciprocal flexion and stopped an established swimmeret rhythm. (B) Data from an identified interneuron (Miall and Larimer, 1982*b*, Fig. 5, type 1–1) that produced strong reciprocal flexion and reduced the expression of powerstrokes in an existing rhythm during the stimulus without resetting the rhythm. Another identified interneuron (Miall and Larimer, 1982*b*, Fig. 5, type 2A–1) was found to produce reciprocal flexion and inhibition of episodic swimmeret output (not shown).

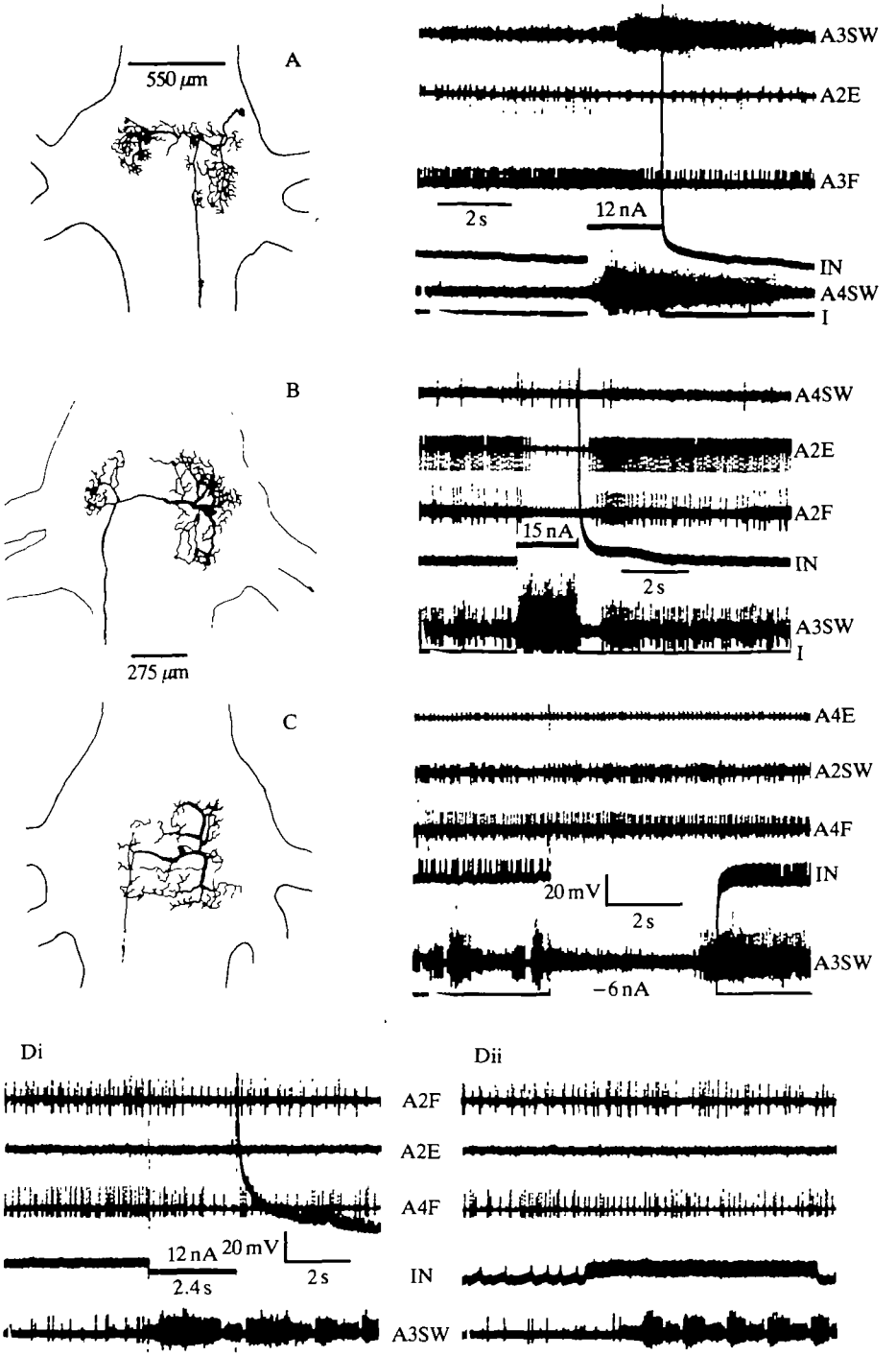
A
550 μm
A3SW
A2E
A3F
2 s
12 nA
IN
A4SW
I
B
275 μm
A4SW
A2E
A2F
15 nA
IN
2 s
A3SW
I
C
A4E
A2SW
A4F
IN
20 mV
2 s
A3SW
−6 nA
Di
Dii
A2F
A2E
A4F
12 nA
20 mV
2.4 s
2 s
IN
A3SW

Fig. 8

Fig. 8. Abdominal positioning inhibitors providing swimmeret motoneuron excitation. Cells shown in A, B and C are tentatively identified. (A) Depolarization of this cell produced prolonged bursts in swimmeret powerstroke motoneurons, first in A4 and then in A3, while also inhibiting firing of the PI to A2 extensors and suppressing activity in the A3 flexion nerve during and after the stimulus. (B) Depolarization inhibited excitatory activity in the flexion nerve and the PI in the extension nerve while firing a single SWMN in A4 and powerstroke MNs in the local first nerve. Effects on rostral abdominal positioning must have occurred through the recruitment of ascending inhibitory elements. This cell sometimes spiked in phase with the local segment swimmeret powerstroke (not shown). Threshold swimmeret output was evoked by 2.5 nA depolarizing current, at which level the cell spiked at 35 Hz. Approximately 7 nA was required to achieve threshold for abdominal positioning. This cell is related to a class of single-output swimmeret cells which have similar morphology and swimmeret output. (C) Hyperpolarization of this cell was able to interrupt an existing swimmeret rhythm. Depolarization (not shown) elicited prolonged powerstroke activity in A2 and A3 and fired the flexor and extensor PIs. It also displayed a 33% increase in spiking rate during spontaneously initiated episodic swimmeret powerstroke activity (not shown). This cell has an apparent serial homolog in A4. (D) Abdominal positioning inhibitor able to start a swimmeret rhythm when depolarized. Data from a large-diameter (30 μm) axon in A3. (Di) Depolarization inhibited flexion in both adjacent ganglia and started a swimmeret rhythm in the local ganglion. (Dii) A spontaneous increase in the rate of firing of this cell from 2 to between 20 and 25 Hz correlates with the start of a swimmeret rhythm. Firing of this cell was almost certainly responsible for at least part of the initiation of the spontaneous rhythm. It was not necessary for the maintenance of the continuing rhythm, however, because hyperpolarization during the rhythm had no effect.

antagonistic to the effect of current of the opposite polarity. Many swimmeret cells neither oscillated nor influenced the rhythm but had only episodic outputs. This parallels the situation with many of the dual-output cells and may indicate that separate paths are available for mediating cyclic and episodic swimmeret outputs.

Most of the single-output swimmeret cells (71%) excited SWMNs when depolarized, and half of them were able to influence a rhythm. In contrast, cells having only inhibitory swimmeret effects were encountered just 12 times. It is possible that cells of this latter class are underrepresented since it is difficult to detect them in preparations with low rates of swimmeret activity.

Possible swimmeret trigger cells

Of all cells examined, eleven were found which could start and stop a swimmeret rhythm with current injections of opposite polarity. Three of these cells had only swimmeret outputs, while four also had extension outputs, three had flexion outputs and one was an API. Only one cell with the properties described above was found to originate in the non-terminal abdominal ganglia. This suggests that cells with these properties generally originate either in A6 or in ganglia rostral to the abdomen, possibly the brain. It has been shown by Larimer and Moore (1984) that similar abdominal positioning neurons can project the length of the nervous system. It is interesting to note that most of these cells showed the physiological

properties attributed to 'trigger' cells in leech. Such cells are able to initiate an enduring cyclic behavior in response to a brief depolarizing stimulus. Spiking in the cell triggers behavior, but continued spiking is not necessary for the completion of the behavior. Cells with this property are considered to be high-order command elements in leech (Brodfuehrer and Friesen, 1986). The possibility then exists that cells with these types of properties may be important high-level command elements for the swimmeret CPG. Data for two of these neurons are shown in Fig. 11. Processes from most of these axons were sparse and projected to diverse portions of the ganglion without much sign of substantial localization. Axonal diameters ranged from less than 10 μm to about 30 μm. They resembled putative swimmeret command fibers found by D. H. Paul and B. Mulloney (personal communication) morphologically (as described above) and physiologically in that some spiked in phase with the swimmeret rhythm and reset the rhythm when injected with current.

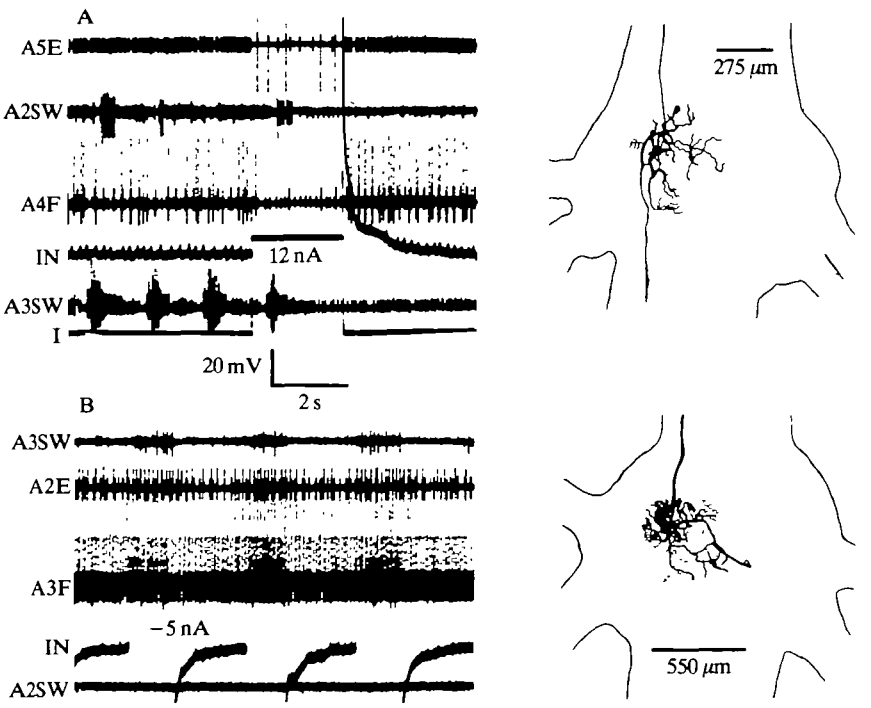

Fig. 9. APIs inhibiting swimmeret outputs. (A) Depolarization of this identified cell inhibited both flexion and extension activity, fired the PI to extensors and stopped a swimmeret rhythm. Serial homologs of this cell exist in A3, A4 and A5, and extension cells of similar morphology are also known. (B) Depolarizing this identified cell inhibited firing of flexion and extension MNs in local and more rostral ganglia and inhibited firing of SWMNs locally (not shown). Hyperpolarization of this cell is shown to release inhibition of MNs for flexion, extension and swimmerets. Spontaneous spiking of this cell was seen to correlate with cyclic swimmeret output; spikes were seen only in the interval between local powerstrokes (not shown). A morphology of this cell has been published by Miall and Larimer (1982*b*, Fig. 12–2).

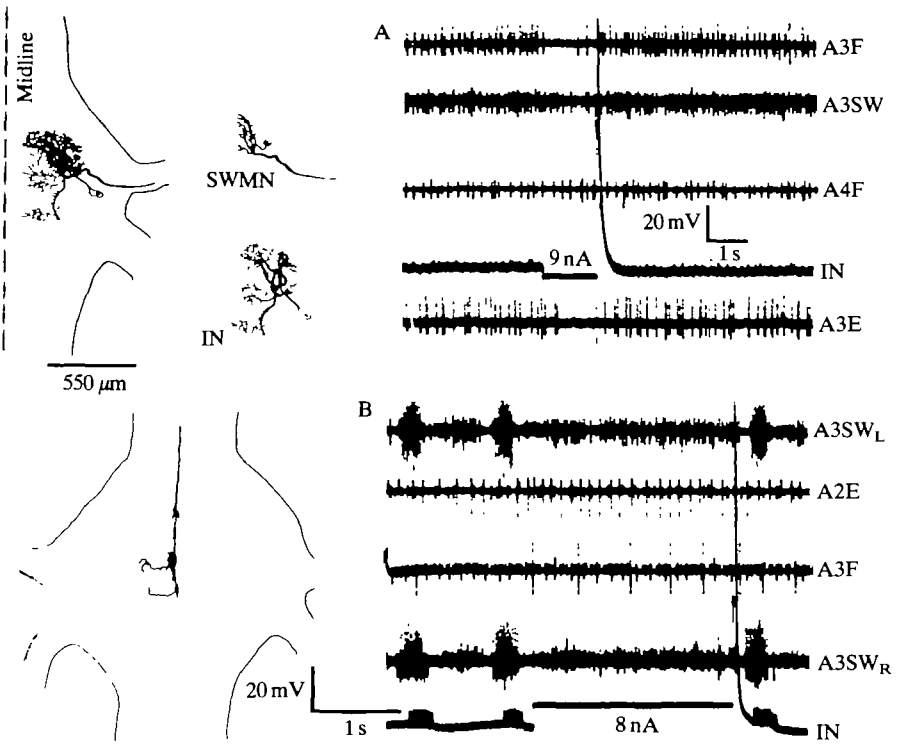

Fig. 10. Other dual-output APIs. (A) Morphology and output of an unidentified, unilateral local interneuron coupled to a SWMN and showing similarity to IN1A of Paul and Mulloney (1985*b*). Depolarization inhibits episodic activity of probable returnstroke SWMNs, as well as local activity of both flexion and extension MNs. All dendrites of this cell are hemiganglionic and the main branches are fairly large, as in IN1A. Both this cell and IN1A are coupled to a SWMN; the membrane potentials of both cells oscillate in phase with a swimmeret rhythm and depolarization of them inhibits swimmeret outputs. The main differences between the two cells are that IN1A is non-spiking, receives hyperpolarizing input from the swimmeret CPG and is coupled to an apparent returnstroke MN, while this cell spikes, receives depolarizing CPG input and is coupled to a probable powerstroke MN. (B) Morphology and output of an unidentified, unilateral termination. Depolarizing this cell activated both extension and flexion PIs, excited returnstroke SWMNs and influenced the cyclic output. This cell also spiked in phase with the rhythm. The primary known difference between this cell and unilateral terminations found by Paul and Mulloney (1985*a*) is that the arborizations of this cell do not appear to be restricted to the lateral neuropil.

Cells evoking mixed abdominal positioning outputs

A small percentage (6%) of cells produced mixed abdominal positioning outputs. These may be associated with discrete abdominal geometries in which some segments are flexed and some extended. Episodic or cyclic swimmeret activity often accompanies this sort of positioning, as in swimmeret cleaning behavior (D. Murchison and J. L. Larimer, personal observation). Accordingly, 79% (19/24) of these cells had dual swimmeret outputs, with most of them (15/19;

79%) exciting SWMNs, and 40% (6/15) able to influence a rhythm. Less than one-third of the mixed-output cells originated in the non-terminal abdominal ganglia, suggesting that most cells of this class originate in more rostral ganglia or in A6. Mixed-output cells could represent command elements for certain discrete abdominal positioning movements. However, the finding of Murphy *et al.* (1989), concerning the mixed quality of most abdominal positioning outputs, indicates that more will have to be learned about these cells before their role in behavior can be ascertained.

Physiological observations

In some dual-output cells the two outputs were activated at different voltage thresholds. Although there were exceptions, dual-output extension cells did not usually have different output thresholds. In dual-output flexion cells, abdominal

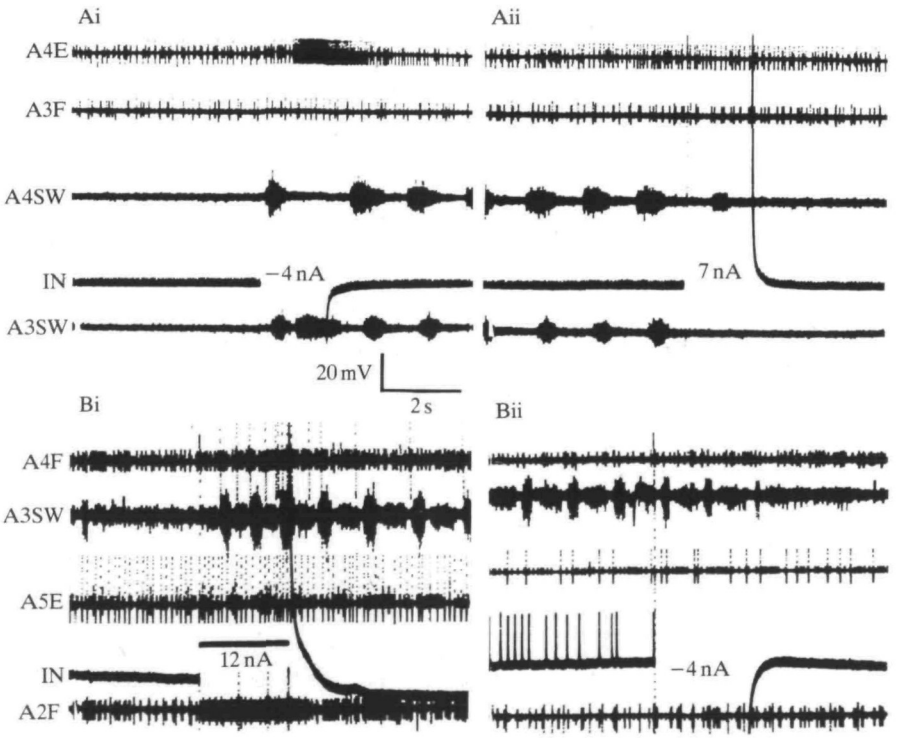

Fig. 11. Cells able to start and stop swimmeret rhythms with current injections of opposite polarity. (Ai) Hyperpolarization releases inhibition of SEMNs, fires the flexion PI and starts a swimmeret rhythm. (Aii) Depolarization of this cell stops a swimmeret rhythm and inhibits some abdominal positioning MNs. (B) An unidentified axon in A3 showing non-reciprocal flexion output and the onset of a swimmeret rhythm when depolarized (Bi) and the termination of a rhythm when hyperpolarized (Bii). (The gain differed in the records for B.) This cell was excited by 7 nA current to spike at 20 Hz and evoke threshold flexion. An additional 5 nA was required to evoke the start of a swimmeret rhythm.

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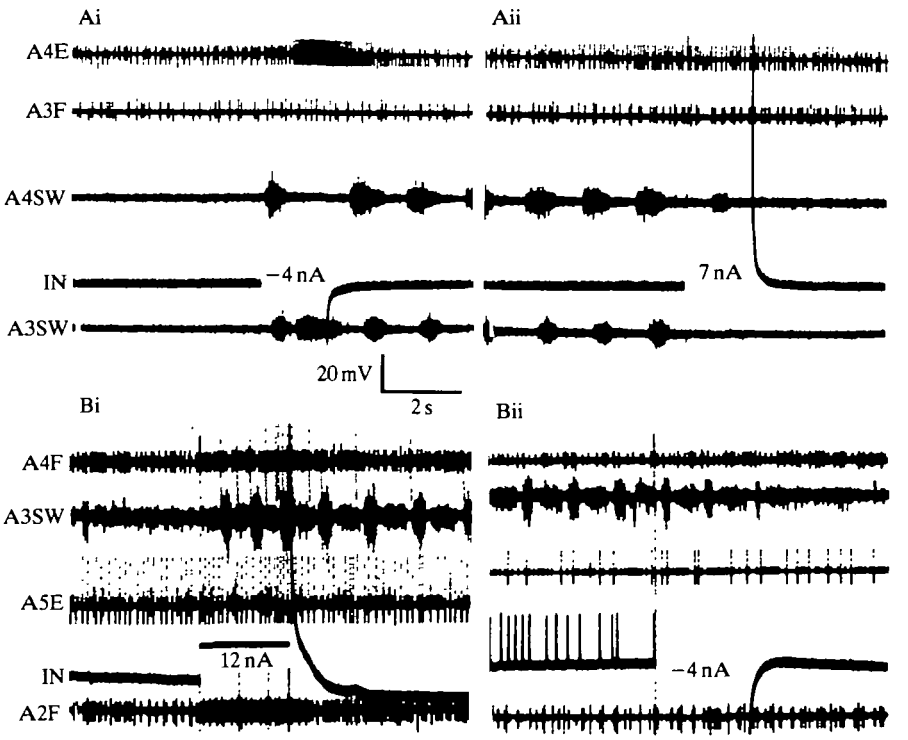

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positioning thresholds were generally below those for swimmeret outputs. Among the APIs, thresholds for swimmeret outputs tended to be lower than those for inhibiting abdominal positioning. Dual outputs of mixed cells could not usually be separated by threshold. While it is possible that in some cases different output thresholds were due to the position of the microelectrode in the dendrites and failure of low current levels to invade all dendritic output regions, in most instances the threshold output current invaded the spike-initiating zone sufficiently to produce a moderate rate of spiking. This implies that the different thresholds are due to differences in synaptic efficacy somewhere in the output circuitry. Such output threshold differences could allow some dual-output cells to function as single-output cells at low levels of activation.

The abdominal ganglia of crayfish contain numerous non-spiking cells (Heitler and Pearson, 1980; Heitler, 1982; Paul and Mulloney, 1985*a*,*b*; Takahata and Hisada, 1986). However, we failed to find dual-output cells that appeared to be non-spiking. Our evidence indicates that non-spiking interactions may occur between spiking cells in the system, since hyperpolarizing currents could evoke motor outputs from quiescent spiking cells (see Figs 9B, 11A). This suggests that continuous transmitter release may occur in such cells.

Morphological observations

Flexion and extension cells occur in several morphological types. However, only a single example of a local flexion or extension interneuron and no example of a distal axonal termination has been found in the ganglia of the swimmeret-bearing segments. Thus, most flexion and extension interneurons appear to be multisegmental; they may originate in any of the abdominal ganglia, but they apparently terminate only in A6 or in ganglia rostral to A1 (for a review, see Larimer and Pease, 1988). In contrast, the APIs and single-output swimmeret cells were encountered frequently in all morphological types, including local interneurons and mid-abdominal terminations. This suggests a fundamental difference in the organization of the circuitries between the extension/flexion system and the API and swimmeret systems and also that the apparent close association of the API and swimmeret systems is made possible by a similar circuitry.

The lateral neuropile (LN) is considered to be the site of interaction between SWMNs and interneurons (Paul and Mulloney 1985*a*). Leise *et al.* (1986) propose that the LN may also be a site of interaction between abdominal positioning systems and the swimmeret circuitry. Our findings tend to support this possibility as virtually all dual-output cells had dendrites overlapping the LN. However, strong dual outputs could also be obtained from cells with no apparent arborization in the LN (see Fig. 10B).

The tonic abdominal positioning MNs have extensive dendritic arborizations in several ganglionic domains (Leise *et al.* 1986, 1987). Most abdominal positioning interneurons also have extensive arborization domains. It is not apparent, though, that dual-output or single-output status corresponds to complexity of dendritic

arborization. Furthermore, no clear correlation of output categories and dendritic domains was evident.

In each output category, 60–80 % of the interneurons have processes extending across the mid-line in the ganglion of origin, while those of most (75 %) through axons were restricted to the ipsilateral hemiganglion. This suggests that most of the bilateral interactions occurring in a ganglion could be mediated by the cells originating there.

Discussion

We have shown that most cells involved in abdominal positioning also have outputs in the swimmeret system and that different classes of these cells could mediate a wide variety of interactions between the two motor systems. We have also shown that cells having properties consistent with important roles in the swimmeret system (i.e. ability to reset swimmeret rhythm, oscillating input from the swimmeret CPG) are also involved in coordinating abdominal positioning outputs. In this system, cells involved in an episodic motor behavior (abdominal positioning) can influence both episodic and cyclic outputs of another motor system (swimmerets), and a cyclic motor pattern (from the swimmeret CPG) can drive elements involved in an episodic motor program (especially the APIs). Thus, it seems that the premotor interneurons investigated here coordinate the activity of separate motor systems into unified behavioral outputs.

Most dual-output cells mediated motor output combinations of a type frequently seen in the freely behaving animal. For example, many extension cells were found to excite swimmeret outputs, while many flexion cells were found to inhibit them. However, several cells were found which produced the apparently incongruous combination of abdominal flexion accompanied by swimmeret beating. There are several ways to account for the occurrence of these latter cells, despite the fact that cyclic swimmeret output has not been reported during complete abdominal flexion in either the crayfish or the lobster. The simplest explanation is that flexion cells producing swimmeret powerstroke or cyclic activity do not underlie complete abdominal flexion behavior, but are instead involved in the production of a mixed, or partially flexed, abdominal position. This possibility is supported by the observation that evoked abdominal positioning frequently displays a mixed type of motor pattern which can be subserved by activity in groups of flexion and extension interneurons (Murphy *et al.* 1989). At least two activities have been observed to include swimmeret beating and a partially flexed abdomen in the intact animal (D. Murchison and J. L. Larimer, personal observation). Although certain activities are seldom observed, their occurrence could account for the presence of neurons with unusual or unexpected output combinations. Another possibility is that the higher thresholds for swimmeret outputs in most of these cells are not usually reached during typical behavior in the animal. At the low firing frequencies observed in similar cells in a

semi-intact preparation (Jellies, 1984; Jellies and Larimer, 1986; Murphy *et al.* 1989) only their flexion outputs would be seen. Inhibition could also mask part of the motor output to prevent incongruous behavior. These sorts of inhibition could be mediated by sensory input or by properties of the networks in which these cells are normally operative (as suggested by Jellies and Larimer, 1985).

It now seems apparent that there are many more abdominal positioning inhibitors present in the abdominal cord than was previously suspected. In addition to the important role implied for APIs in the abdominal positioning circuitry (Jellies and Larimer, 1985), it appears that the APIs also have an important role in the swimmeret circuitry. The APIs had the highest percentage of dual-output cells, the highest percentage of cells receiving cyclic input from the swimmeret CPG, and a very high percentage of cells able to influence the swimmeret rhythm. In addition, the morphological diversity of the APIs resembles that of the swimmeret cells more than that of the excitatory abdominal positioning cells. These properties of the APIs make it likely that some are involved in the coordination of outputs from the two motor systems. It is also probable that some of the APIs mediate the dual-output properties of the giant escape fibers, as postulated by Kuwada and Wine (1979).

An important consideration concerns the mechanism by which dual-output cells influence the swimmeret circuitry. Cells able to influence a swimmeret rhythm could do so by input to the CPG, by input to post-CPG 'gating' cells or by input to the SWMNs directly. Interneurons with 'trigger' properties (as in Fig. 11B) or that altered rhythm phase durations (as in Figs 3B, 8C) probably act on the CPG. Cells which influence the rhythm without resetting (as in Fig. 7B) or which have influences only during current injection (as in Fig. 8B) are likely to act postsynaptic to the CPG. Most cells able to influence a swimmeret rhythm also had effects on the SWMNs during episodic outputs. There were also many cells that influenced only episodic swimmeret outputs. This latter type almost certainly acts post-CPG, perhaps by pathways separate from those of the CPG.

Our observations allow some conclusions concerning the arrangement of the premotor interneurons of this system. Most single-output cells are probably either lower-order or in parallel with the dual-output cells. Single-output cells are unlikely to have strong excitatory connections to dual-output cells; if they did, complex circuit properties would be required to mask the output of the dual-output cells. For this reason also, it is likely that higher-order single-output cells would represent parallel circuits, separate from or inhibitory to dual-output circuits. Continuing with this reasoning leads to the prediction that most dual-output cells are probably presynaptic to single-output cells and mediate their dual outputs by acting on groups of single-output cells from both the swimmeret and abdominal positioning pools.

Other data have suggested that interneurons of the abdominal cord function as elements in a command group, each element responsible for only a portion of the behavioral output subserved by that group (Jellies and Larimer, 1985, 1986; Larimer *et al.* 1986; Moore and Larimer, 1987; Murphy *et al.* 1989). This

conclusion is reinforced by the findings presented here. Numerous cells were found, such as those shown in Figs 5A and 8B, which had strong outputs extending well beyond the stimulus duration or in opposite directions from the axonal projection. Also, hyperpolarization of active cells during spontaneous motor output caused only a partial deficit in the output. These output patterns are consistent with the command element hypothesis. In the intact animal, each of the output classes may represent one or more command groups with elements of each group acting together to produce a coordinated behavior.

Experiments involving intracellular recordings of synaptic interactions between pairs of these cells may help to establish which cells belong to the same command group. This type of experiment could also reveal any hierarchical or parallel relationships between single-output and dual-output cells and at what level in the swimmeret circuitry the dual-output cells act. The actual assignment of the type of behavior subserved by the command groups would require intracellular examination of cells in almost intact animals.

This paper attempts to document interneurons which synaptically evoke outputs in more than one motor system in crustaceans. The findings presented here illustrate the diversity and abundance of such cells in the crayfish abdominal nervous system. Until recently, evidence of cells with multiple motor output properties was restricted to giant fiber escape systems. In crayfish, the lateral, medial and segmental giants have been shown to activate not only the fast abdominal motoneurons involved in the escape response but also various motoneurons controlling movements of the legs, swimmerets, telson and uropods (Larimer *et al.* 1971; Cooke, 1985; Heitler and Darrig, 1986). The crayfish giants have also been shown to inhibit the excitatory slow abdominal motoneurons and excite the slow inhibitor (Kuwada and Wine, 1979; Kuwada *et al.* 1980). The dorsal giant of the cockroach has also been found to be able to drive either the running motor program or the flight motor program, depending on the sensory context of the animal (Ritzmann *et al.* 1980). That multiple motor output properties are not confined to giant interneurons is evident from the results of this investigation. Additional evidence of dual motor outputs by non-giant cells in crustaceans is provided by Dickinson *et al.* (1988) and Barthe *et al.* (1988). Dual motor output cells are also described in a mollusc by Kyriakides and McCrohan (1988). In leech, Kristan *et al.* (1988) report a cell active in gating two different behavior patterns. Thus, complex motor output properties of single cells onto multiple motor systems may be a common feature at certain levels of the nervous system in different animals.

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