Neuronal Elements That Mediate Escape Swimming and Suppress Feeding Behavior in the Predatory Sea Slug \textit{Pleurobranchaea}

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\textbf{SUMMARY AND CONCLUSIONS}

1. The white, bilaterally paired Al interneurons of the cerebropleural ganglion of \textit{Pleurobranchaea californica} fire rhythmic bursts of action potentials during escape swimming behavior. We studied the role of the Als in swimming behavior and pattern generation in whole animal and isolated CNS preparations.

2. The escape swim is a cyclic sequence of dorsal and ventral flexions of the body. During the swim, Al bursts precede and accompany the dorsal flexion phase of the cycle. Hyperpolarization of Al to prevent spike activity interrupts swimming behavior in the whole animal and fictive swimming in the isolated CNS. Stopped Al activity was not observed to cause swimming in whole animals, and was only occasionally sufficient to trigger fictive swimming activity in the isolated CNS.

3. In quiescent whole animal preparations, stimulation of a single Al normally causes a single dorsal flexion followed by body flexion to the side contralateral to the stimulated cell; characteristically, Al spike activity stimulates feedback inhibition coinciding with the end of dorsal flexion and the onset of contralateral flexion.

4. Al spike activity suppresses feeding behavior and causes proboscis retraction in whole animal preparations induced to feed. Al activity also suppresses fictive feeding driven by stimulation of the critical phasic paracerebral neurons (PC) of the motor network of feeding in the isolated CNS. Concomitantly, Al spikes cause potent inhibition of the PC interneurons.

5. The Als are specifically excited by noxious mechanical and chemical stimuli, but are not affected by feeding stimuli or the occurrence of feeding behavior.

6. We conclude that the Al neurons are elements of an escape swimming pattern generator, and that they are probably homologous to the similar C2 neurons of the nudibranch \textit{Tritonia diomedea}. One of their functions outside of generating the swim pattern may be the suppression of feeding behavior in response to noxious stimulation. These observations provide a neural mechanism for the original observations of the dominance of escape swimming behavior over feeding.

\textbf{INTRODUCTION}

Aversive behaviors are integral components of the behavioral repertoires of most animals. They let animals minimize accidental damage, they optimize foraging strategies, and they reduce risk of predation. In all phases of the life of a motile organism, aversive behaviors are integrated with those of foraging and reproduction. The interactions of aversive and feeding behaviors are basic to animal behavior, and it is thus important to elucidate how these interactions take place at the neural network level.

We have undertaken the study of the neural circuits underlying aversive behaviors and how they interact with the feeding circuitry in the predatory sea slug \textit{Pleurobranchaea californica}. The regulation of feeding behavior by neural mechanisms of learning and satiation is already partly understood at the network level (Davis and Gillette 1978; Kovac et al. 1986; London and Gillette 1986), and some interactions of feeding behavior with avoidance have already been documented in the suppression of local withdrawal responses by the active feeding motor network (Davis et al. 1974, 1977, 1980; Kovac and Davis 1977, 1980b). Aversive behavior often replaces feeding when animals learn to suppress feeding in a paired food-shock training paradigm, when they are satiated on food, or when noxious stimuli occur during feeding behavior. Such aversive behavior is drawn from a range of elements, including local withdrawal of affected body parts (e.g., oral veil, tentacles, rhinophores, gill, tail, and local areas of mantle and foot), aversive turns and crawling away from stimuli, and escape swimming (Gillette et al. 1991). The expression of escape swimming itself is a behavioral decision in which all other ongoing behaviors are suppressed (Davis and Mpitsos 1971).

Very little is known about the neural bases of aversive behaviors in \textit{Pleurobranchaea}, and until now no information has been available on the circuitry of escape swimming. We observed a bilateral pair of white neurons, named the Als, in the cerebropleural ganglion; these Al s could drive activity recorded in other neurons and in the axons of ganglion nerves. We tested the role of these neurons in escape swimming behavior because we recognized the possibility that both the neurons and the behavior were homologous to those previously described in the nudibranch \textit{Tritonia diomedea} (Getting 1977, Hume et al. 1982, Taghert and Willows 1978, Willows 1967; Willows et al. 1973). Escape swimming in \textit{Pleurobranchaea}, as for \textit{Tritonia}, is a response to noxious stimuli consisting of one or more cycles of an alternating sequence of dorsal and ventral flexions; this probably serves to lift the animal off the substrate to take advantage of prevailing currents to carry it from potential harm (Davis and Mpitsos 1971; Gillette et al. 1991). Completion of the swim sequence is followed by a minutes-long interval of substrate locomotion.

In this report we present evidence that the Al neurons are necessary neural elements of the animal's swimming escape response, whose activity causes suppression of feeding behavior, and that they potentially contribute to other aspects of aversive behavior. Some of these data have been presented in abstract form (Jing and Gillette 1994; Jing et al. 1993).

\textbf{METHODS}

Specimens of the notaspid sea slug \textit{P. californica} (3–600 g) were obtained from Sea-Life Supply (Sand City, CA) and Pacific...
ment records were fitted to the intracellular record (Fig. 3). With a photodiode, brightness changes caused by swimming movements were plotted by commercial graphics programs. Behavioral records of isolated neurons were fed into a computer, and spike activity of the Al neuron was fed into an eight-channel digitizing unit (Neurodata). Data on video tape were played back to an A-D converter (Axon) as digital files and played through an eight-channel digitizing unit.

For isolated CNS preparations, the cerebropleural, pedal, and visceral connectives were performed under cold anesthesia (4°C). Whole animal preparations were made for intracellular recording as previously described (Gillette and Davis 1977; London and Gillette 1986) by exposing the cerebropleural ganglion through a 2-cm dorsal incision and pinning it to wax on a micromanipulator. Animals were supported and partially restrained for stable recordings, but were capable of a considerable degree of movement, including vigorous swimming and feeding behavior. The preparation chamber was constantly perfused with fresh artificial seawater (14°C). The outer ganglion connective sheath was removed in whole animal or isolated CNS preparations, intertrial intervals lasted at least 10 min. Swim episodes were typically induced by electric stimulation of a body wall nerve (BWN) of the cerebropleural ganglion with monopolar shocks 2 ms in duration, 3–15 V, 15 Hz, for 2–5 s. When burst durations of the Al neurons were measured during swim episodes, the first burst of a sequence was not included, because those bursts were usually markedly longer than subsequent bursts owing to a slow acceleration of initial spike activity.

The phasic paracerebral neurons (PCs) were identified by location; records of the cerebrobuccal axon pathway; the occurrence of spontaneous and prominent, phasic inhibitory postsynaptic potentials (IPSPs) (1–5 mV); and the ability to drive fictive feeding (Gillette et al. 1982). Putative swim motor neurons were located in the pedal ganglia via biocytin backfills of the anterior lateral body wall nerve (aLBWN), which innervates longitudinal musculature presumed to mediate swimming behavior. The neuron cluster so found comprises ~15 medium-sized somata (30–70 μM) lying in the dorsal anteromedial region (Jing et al. 1993; unpublished data). In physiological preparations, motor neurons were identified on the basis of soma position, recordings of orthodromic spikes in the aLBWN, and stimulation of antidromic spikes by shocking the same nerve.

Electrophysiological data were recorded on a chart recorder (Gould model 2800 or TA11) and a video tape recorder through an eight-channel digitizing unit (Neurodata). Data on video tape were played back to an A-D converter (Axon) as digital files and plotted by commercial graphics programs. Behavioral records of whole animal preparations were made with an 8-mm video tape recorder; in some cases, spike activity of the Al neuron was fed from the intracellular amplifier into the audio channel for a permanent record correlating behavior and Al activity, as shown in Fig. 5. Records correlating spike activity and movement during the swim were subsequently made from the video tape by recording, with a photodiode, brightness changes caused by swimming movements; using the audio channel spike record as a reference, movement records were fitted to the intracellular record (Fig. 3).

For isolated CNS preparations, the cerebropleural, pedal, and buccal ganglia were dissected with connectives intact and incubated in saline containing 0.2% Pronase E (Sigma) for 2 min to arrest spontaneous contractions of the muscular outer sheath. The CNS was then pinned to Sylgard under saline cooled to 13–14°C. Normal saline composition was (in mM) 420 NaCl, 10 KCl, 25 MgCl₂, 25 MgSO₄, 10 CaCl₂, and 10 3- (N-morpholino)propane-sulfonic acid (MOPS) buffer, pH adjusted to 7.5 with NaOH. Tests for probable mono- or polysynapticity were conducted in a high-divalent saline (composition, in mM: 240 NaCl, 10 KCl, 125 MgCl₂, 25 MgSO₄, 30 CaCl₂, and 10 MOPS) in order to elevate spike thresholds and curtail polysynaptic activation (London and Gillette 1984). The outer and inner sheaths covering the Al region were removed by careful dissection. Recordings were made as described above.

In the initial phases of this study, the frequency of isolated CNS preparations showing fictive swimming was quite low. The frequency increased appreciably when we selected for animals that showed swimming in response to a mild electric shock applied to the dorsal mantle or tail (40-V bipolar stimulus, 10 ms in duration at 20 Hz, delivered via bipolar 20-gauge chlorided silver wire electrodes separated by 2 cm).

Where swimming and/or feeding episodes were repeatedly induced in whole animal or isolated CNS preparations, intertrial intervals lasted at least 10 min. Swim episodes were typically induced by electric stimulation of a body wall nerve (BWN) of the cerebropleural ganglion with monopolar shocks 2 ms in duration, 3–15 V, 15 Hz, for 2–5 s. When burst durations of the Al neurons were measured during swim episodes, the first burst of a sequence was not included, because those bursts were usually markedly longer than subsequent bursts owing to a slow acceleration of initial spike activity.
Backfills of nerves and connectives were performed by incubating the cut ends in 4% biocytin (Sigma) in 50 mM tris (hydroxymethyl)aminomethane (Tris, pH 7.4 buffered with HCl) for 18 h. Intracellular fills of A1 were performed with iontophoretic or pressure injection of 4% biocytin in a solution of 500 mM KCl and 50 mM Tris at pH 7.4 (Horikawa and Armstrong 1988) or of 5% Lucifer yellow CH in distilled water from recording electrodes. Ganglia were processed as previously described for viewing and photographing Lucifer yellow under fluorescence (London and Gillette 1984). Pressure-injected biocytin fills allowed filling of axon processes up to 2 cm when ganglia were incubated overnight at 8°C before fixation. For biocytin, the tissue was fixed with 3.7% formaldehyde in Pleurobranchaea saline overnight and washed in 500 mM NaCl and 20 mM sodium phosphate buffer (PBS, pH 7.5). After dehydration in ethanol, tissue was reacted with 0.5% H2O2 in absolute methanol, then rehydrated and washed in 150 mM NaCl and 20 mM PBS (ph 7.5). Tissue was then incubated in 5 ml PBS with one drop each of Vectastain A and B solutions (Vector) and 0.25% Triton X-100 for 19-24 h. Tissue was rinsed in PBS for 24-48 h, then rinsed in 50 mM Tris (pH 7.4) and incubated in 0.05% 3,3′-diaminobenzidine tetrahydrochloride (Sigma) in 50 mM Tris (pH 7.4) for 2 h, after which H2O2 was added to a concentration of 0.05% for the final reaction (4–15 min). Tissue was rinsed again in Tris buffer for 2 h, dehydrated, and cleared for viewing as a whole mount.

RESULTS

The A1 neurons are members of the “A cluster” population, which is a bilaterally paired dorsal cluster of 18 somata with axons that descend in the cerebropedalc connectives to the pedal ganglia. The A clusters lie posterolaterally to the origin of the rhinophore nerve on each side (Fig. 1). In each cluster, the A1 neuron soma is the larger and more anterolateral white soma of only two white somata in the cluster; it is separated from the other white soma and from the nerve by one to three of the other, mostly orange, somata of the ganglia. The A1 cell bodies vary in size with the animal, ranging from 50 to 110 μm in animals weighing from 10 to 600 g; they are slightly larger than most nearby neurons and easily identified. The anatomy of A1 is shown in Fig. 1. Intracellular biocytin (N = 8) and Lucifer yellow (N = 2) staining indicates that the A1 soma sends a single axon into the subjacent neuropil; this gives off fine neurites and turns medially to then cross the commissure and enter the contralateral anterior cerebropedal connective (aCPC); fewer fine neurites branch in the contralateral neuropil. On entering the contralateral pedal ganglion the axon gives off numerous neurites, then turns into the pedal commissure (PC) where it could be traced to near the ipsilateral pedal ganglion. Backfills of the aCPC with biocytin stained a prominent soma of appropriate size and contralateral location for A1. On stimulation of A1s with depolarizing current, orthodromic action potentials were recorded in the contralateral aCPC and also in the PC that couples the bilaterally paired pedal ganglia (Fig. 2A). Antidromic action potentials, following shocks delivered to the contralateral aCPC or to the PC at frequencies up to 15 Hz, were recorded from the A1 soma (Fig. 2B). Evidence for A1 axon branches in the other connective, commissurals, and pedal nerves was not found. Thus the A1 neurons send axons via the aCPC to the contralateral pedal ganglia, which then exit into the PC, apparently coursing to the ipsilateral pedal ganglia.

Role of A1 in escape swimming in the whole animal

During the swim cycle, the onset of cyclic A1 activity somewhat preceded (latency 400–1000 ms) the beginning of the dorsal flexion phase, and peak spike frequency generally occurred during the rapid phase of dorsal flexion (Fig. 3). In the whole animal, A1 was often quiescent on impalement, with a resting potential of −50 to −55 mV, and commonly exhibited spontaneous IPSP activity 1.5–3 mV in amplitude; otherwise A1 spiked spontaneously at 0.1–0.3 Hz. For reasons unknown, many Pleurobranchaea do not swim in response to noxious stimulation; however, swimming was elicited successfully 11 times in 5 of 20 whole animal preparations by electric stimulation of a BWN with an en passant suction electrode. Typically, whole animal preparations were relatively relaxed, exhibiting occasional very slow, low-amplitude torsions and flexions of the body. Whenever a swim occurred (N = 11 times in 5 animals) it was accompanied by cyclic bursting in A1. During a swim episode, the initial behavioral response to BWN stimulation was an extension of the body lasting for several seconds and followed by dorsal flexion. A1 spike activity preceded and accompanied dorsal flexions; A1 was generally hyperpolarized at the peak of dorsal flexion and during most of the
succeeding ventral flexion. For the robust episode of Fig. 3, and of one measured from another preparation, A1 instantaneous spike frequency during the bursts ranged from 4 to 20 Hz and from 3.5 to 17 Hz, respectively. Burst durations ranged from 1.4 to 2 s and from 1.3 to 2 s, and cycle periods ranged from 3 to 6 s and from 5.4 to 6 s, respectively.

In both the intact animal and in the isolated CNS, BWN stimulation was followed by increases in A1 spontaneous spike and IPSP activity that endured for several minutes (Fig. 4). This did not depend on the successful initiation of a cyclic swim episode (see also Fig. 9A). The records of Fig. 4 also show a prolonged and appreciable depolarization typically induced in the A1 by BWN stimulation; such depolarizations reached amplitudes of 5–10 mV and could outlast swim episodes by many seconds to minutes (other examples are shown in Figs. 6B, 7, A and B, and 13). However, in some other preparations the depolarization was less prolonged and returned to baseline within 10 s (Figs. 5 and 6A).

Figure 5 shows a record from one of three experiments in which we tested the necessity of A1 activity for swimming behavior in the whole animal. When induced swim episodes were separated in time by 10–30 min in an animal, the episodes were closely similar in timing and number of swim cycles. We compared control swim episodes (Fig. 5A) with experimental swims in which we hyperpolarized the A1 after the first or second cycle of the episode (Fig. 5B). In each experiment, during intervals of A1 hyperpolarization the swim cycle was interrupted, as the animal appeared to be maintained in the dorsal flexion posture. In each case, on release of hyperpolarization, A1 resumed intense cyclic activity and so did the swim episode. For the example shown in Fig. 5B, a second imposed period of hyperpolarization again stopped both A1 bursting and the swim, neither of which resumed when hyperpolarization was relaxed.

Role of A1 in fictive swimming in the isolated CNS

Fictive swimming could be evoked in the isolated CNSs of 26 preparations of 37 animals by electric stimulation of peripheral nerves or the cerebropedal connectives (Figs. 6 and 7). In two seemingly typical preparations we measured A1 instantaneous spike frequency during bursts ranging from 4 to 22 Hz and from 3 to 34 Hz, burst durations ranging from 1.7 to 2.5 s and from 1.3 to 1.5 s, and periods ranging from 3.5 to 6 s and from 4.2 to 7.5 s, respectively, which were comparable with those observed during swim episodes in the whole animal preparation (above).
The premotor activity of fictive swimming can be observed in the spike activity of descending axons in the aCPC, which include those of Al and other swim interneurons (Jing and Gillette 1995; J. Jing, L. C. Sudlow, and R. Gillette, unpublished data); aCPC activity was closely synchronous with Al soma spikes in both the swim of the whole animal and the fictive swim of the isolated CNS (Fig. 6). The presumed final motor output of swimming was recorded in the aLBWN of the pedal ganglion (Figs. 6 and 7), which was accessible to recording in the isolated CNS but not in the whole animal preparations. The aLBWN innervates longitudinal musculature presumed to mediate swimming behavior. The aLBWN bursts showed a small phase delay of several hundred milliseconds from the onset of Al activity (Figs. 6B and 7; also see Fig. 8).

We then tested the role of Al in fictive swimming in six preparations, in which we compared effects of hyperpolarizing Al during a swim episode with control episodes in the same preparations. In each case, hyperpolarization of a single Al during the swim cycle delayed the onset of the subsequent activity.
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burst cycle for the duration of hyperpolarization, judged by comparison with control fictive swims evoked in the same preparations (Fig. 7), and occasionally terminated the swim entirely. These observations suggest that activity in the Al neurons is necessary to the generation of the swim pattern.

The activity of single Al neurons does not appear to be sufficient to trigger a full fictive swimming episode. In examination of 19 preparations that responded to BWN stimulation with a swim episode of three or more cycles, in only five cases did Al stimulation drive cyclic motor output like that of swimming (Fig. 8). Simultaneous stimulation of both Als might have increased the frequency of swimlike motor output, but was not tested. In no case did Al activity trigger cyclic motor activity that outlasted the stimulation of Al by current injection, as would be expected of a full fictive swim episode.

**Actions of Al in nonswimming animals**

In most of the whole animal preparations examined, swimming behavior could not be elicited (15 of 20). Moreover, frequently after an animal was induced to swim it was refractory to induction of another swim for several to some tens of minutes. In all such cases, Al reacted to BWN stimulation with excitation similar to that observed initially in swimming animals, but cyclic activity failed to appear (Fig. 9A).

In none of the whole animal preparations, even those that swam in response to BWN stimulation, did induced firing of single Al neurons activate swimming (N = 20), an unsurprising finding in view of the low frequency with which Al drove fictive swimming in the isolated CNS. Behaviorally, depolarizing a single Al to fire rapidly (12–23 Hz in the example of Fig. 9B) had a biphasic effect: the animal showed an initial straightening of the body resembling the dorsal flexion of the swim, then went to a laterally flexed position that was suggestive of the animal's body curvature during an active turn toward the side contralateral to the Al neuron being stimulated. The activity of the Al changed characteristically during stimulation (Fig. 9B): the initial high rate of discharge that induced the animal's dorsal flexion slowed markedly after several seconds (to 3–10 Hz in the figure) with the appearance of large IPSPs in the record; the consequent partial hyperpolarization was main-

**Suppression of feeding behavior by Al spike activity**

The suppression of feeding behavior by Al activity was a marked observation of this study. Whole animal prepara-

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**Fig. 8.** Rare fictive swim elicited by Al depolarization. An anterior lateral body wall muscle motor neuron (aLBWM MN; a putative swim motor neuron) of the contralateral pedal ganglion was recorded simultaneously.

**Fig. 9.** Al activity in nonswimming whole animal preparations. A: BWN stimulation (bar) typically caused a single burst, prolonged depolarization, and increased spike and PSP activity in the absence of the swim episode. B: tonic depolarization of Al induced inhibitory synaptic feedback that transiently suppressed Al activity and slowed its subsequent firing. Insets (boxes): expanded portions of the record to show PSP activity preceding the inhibition (1) and during initial feedback inhibition (2), and IPSPs during the subsequent depolarization (3). See text for further details. Action potentials are clipped in the insets.
FIG. 10. A1 activity inhibits feeding behavior in the whole animal preparation. Proboscis extension (Prob. ext.) and cyclic biting activity was induced by application of 100 mM trimethylglycine to the oral veil. A1 stimulation stopped biting and caused proboscis retraction (Prob. ret.). Cessation of A1 firing was succeeded by renewed proboscis extension and biting in the continued presence of the food stimulus. Line drawings depict the behavior of the preparation as seen from the ventral surface, indicating actions of the proboscis and jaws.

tions can often be stimulated to appetitive and consummatory feeding activity by application of strong feeding stimuli to the animal's oral veil, rhinophores, and mouth region (Gillette and Davis 1977; Gillette et al. 1978; London and Gillette 1984, 1986). In six experiments we stimulated relatively complete feeding behavior by the application of the appetitive chemical trimethylglycine (10–100 mM in seawater, pH 8.0) (Huang and Gillette 1985) while recording from an A1 neuron. Such activity consisted of orienting oral veil movements, proboscis extension, and cyclic biting. In these experiments, depolarization and firing of single A1s at 5–20 Hz caused strong suppression of feeding. Proboscis retraction was a rapid response occurring within 5 s and the animals' mouths appeared firmly closed (Fig. 10). The animals assumed the contralaterally flexed body position in response to A1 stimulation, as described in the previous section. Induced A1 firing frequencies used to suppress feeding were comparable with those occurring during swim pattern bursting. For instance, in the example of Fig. 10, the A1 instantaneous spike frequency within the initial 300 ms of depolarization ranged from 20 to 40 Hz, then dropped to 10–19 Hz for the next 1 s and was maintained at a level of 4–10 Hz for the remaining period.

In these experiments, feeding activity did not result in significant changes in spike or postsynaptic potential (PSP) activity in A1. The instability in the records of Fig. 10 arose largely from movement artifacts during the vigorous activities of feeding.

Suppression of feeding output and inhibition of feeding interneurons by A1 activity and fictive swimming

In the isolated CNS, fictive feeding can be stimulated by driving the PCp interneurons. The PCp s are coordinating interneurons that link the cerebral and buccal feeding oscillators in a powerful positive feedback loop (Gillette et al. 1978, 1982; Kovac et al. 1983a,b) and are necessary to the initiation of consummatory feeding behavior (Gillette et al. 1978, 1982). They are also sites in the feeding motor network where the behavior is regulated by motivational and learning mechanisms (Davis and Gillette 1978; Davis et al. 1983; Kovac et al. 1985, 1986; London and Gillette 1986; Morielli et al. 1986). In each of seven experiments where PCp stimulation drove robust fictive feeding, stimulation of a single A1 neuron interrupted the feeding motor output, hyperpolarized the stimulated PCp and suppressed its spiking activity, and suppressed other cyclic neuron activity recorded in the cerebrobuccal connective (Fig. 11). A1 spike activity in these experiments was comparable with that which in the whole animal suppressed feeding behavior; for the example shown, instantaneous spike frequency of A1 in the first 300 ms was 20–33 Hz, for the following 760 ms it was 10–17 Hz, and it declined to 5–10 Hz.

A1 activity caused hyperpolarization of PCp s via IPSPs (Fig. 12A). These driven IPSPs began at several hundreds of milliseconds from the beginning of A1 stimulation and did not follow one-for-one with A1 action potentials. In high-divalent saline the A1-driven IPSPs in the PCp s were largely blocked (Fig. 12B). These data indicate that the pathway from A1 to PCp, however potent, is di- or polysynaptic.

Intracellular recordings made simultaneously from both A1 and PCp neurons during fictive swimming show that the inhibition of the feeding interneurons was biphasic during the swim cycle. Thus, the PCp s exhibited discrete intervals of synaptic inhibition phase-locked to A1 bursts during dorsal flexion and as well during the interval of ventral flexion (Fig. 13).

Sensory stimuli and A1 activity

Tactile stimuli were delivered manually to whole animal preparations as mild prods and rubs with a blunt glass probe. Such stimuli are normally mildly aversive; although stimuli were not quantified, stronger stimuli appeared to evoke larger excitatory postsynaptic potentials. Larger stimuli occasionally elicited a few action potentials (Fig. 14A).

Noxious stimuli, such as application of 0.5 M KCl, that induced quite strong aversive behavior in intact, unrestrained animals, also induced depolarizing PSPs in A1 (Fig. 14B). Excitatory postsynaptic potentials were associated with increase in A1 spiking activity and the occurrence of small,
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CBC
PCP
Al
40 mV
10s

FIG. 11. Stimulation of a single Al suppresses fictive feeding in the isolated CNS. Feeding motor output was driven by tonic stimulation of the phasic paracerebral neuron (PCp) and monitored by recordings of motor root 3 (R3) of the buccal ganglion and the cerebrobuccal connective (CBC). Al activity halted cyclic feeding bursts in the radular retraction neurons of R3 and in the protraction interneurons of the CBC. Al also hyperpolarized and halted spiking in the PCp, whose axon spike can be observed in the CBC. Records are continuous.

fast depolarizing potentials that we interpret as the attenuated spike potentials of at least five other electrically coupled neurons found in the cerebropleural ganglion (Jing and Gillette 1995; Jing et al. 1993; unpublished data).

DISCUSSION

Relatively little is known about the neuronal circuitry underlying Pleurobranchaea's aversive behaviors. Kovac and Davis (1977, 1980b) studied interactions of aversive withdrawal and feeding behaviors, identifying putative motor neurons for oral veil withdrawal and inferring the existence of a withdrawal command neuron. In other gastropods, neural mechanisms of various aversive behaviors have been studied, including gill withdrawal in Aplysia (Castellucci et al. 1970), defensive whole body withdrawal in Lymnaea (Ferguson and Benjamin 1991a,b), defensive withdrawal behavior in Helix (Balaban 1979), and defensive reactions of Planorbis (Arshavsky et al. 1994a-c). Escape swimming in Tritonia has been studied in some detail as a model system for central pattern generation (Getting 1981, 1983; Getting and Dekin 1985b; Getting et al. 1980; Lennard et al. 1980; Taghert and Willows 1978; reviewed by Getting 1989), and recently for its intrinsic neuromodulation by serotonin (Katz et al. 1994; McClellan et al. 1994). However, mechanisms of interaction at the network level between appetitive and aversive types of behavior are as yet insufficiently well documented. Escape swimming in Pleurobranchaea lies on the end of a spectrum of aversive behaviors induced by noxious stimulation, by contact with conspecifics, or by food stimuli in aversively conditioned animals. In a search for premotor neurons that could mediate aversive behavior, particularly transitions from feeding behavior to avoidance, the Al neuron presented itself prominently.

The present evidence suggests that Al is an integral part of a central-pattern-generating circuit for escape swimming. It fires rhythmically before and during the dorsal flexion phase of swimming. Nerve stimulation that can induce swimming also causes long-lasting increases in spontaneous PSPs and spike activity in Al, whether or not an actual swim occurs. Hyperpolarization of Al reversibly interrupted a swim; thus Al activity appears necessary to the pattern-generating mechanism of the swim. However, despite the apparently critical role of single Al neurons to the swim, stimulated Al activity was sufficient to initiate fictive swim motor output in less than half of the experiments, and in no instance was the cyclic activity self-sustaining as would be expected for a full swim episode. In the larger majority of cases in which Al firing did not drive cyclic motor output, it simply induced the feedback inhibition already described. The effects of stimulating both Als simultaneously was tested only once, with negative results, and although stimulating both Als is likely to be more efficacious than stimulating only one, it seems unlikely that Al neurons have a unique "command" role in triggering a full, self-sustaining swim episode. Rather, the Al neurons are likely to act in concert with other neurons both in initiation and maintenance of swimming behavior.

Al responded specifically with excitation to mechanical and noxious stimulation delivered anywhere on the animal's body, including the oral veil. Al did not respond to tastant stimuli delivered to the oral veil, even when such stimuli had definite appetitive effects in the whole animal preparations. The finding that activity in the swimming pattern genera-

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The present evidence suggests that Al is an integral part of a central-pattern-generating circuit for escape swimming. It fires rhythmically before and during the dorsal flexion phase of swimming. Nerve stimulation that can induce swimming also causes long-lasting increases in spontaneous PSPs and spike activity in Al, whether or not an actual swim occurs. Hyperpolarization of Al reversibly interrupted a swim; thus Al activity appears necessary to the pattern-generating mechanism of the swim. However, despite the apparently critical role of single Al neurons to the swim, stimulated Al activity was sufficient to initiate fictive swim motor output in less than half of the experiments, and in no instance was the cyclic activity self-sustaining as would be expected for a full swim episode. In the larger majority of cases in which Al firing did not drive cyclic motor output, it simply induced the feedback inhibition already described. The effects of stimulating both Als simultaneously was tested only once, with negative results, and although stimulating both Als is likely to be more efficacious than stimulating only one, it seems unlikely that Al neurons have a unique "command" role in triggering a full, self-sustaining swim episode. Rather, the Al neurons are likely to act in concert with other neurons both in initiation and maintenance of swimming behavior.

Al responded specifically with excitation to mechanical and noxious stimulation delivered anywhere on the animal's body, including the oral veil. Al did not respond to tastant stimuli delivered to the oral veil, even when such stimuli had definite appetitive effects in the whole animal preparations. The finding that activity in the swimming pattern genera-

tor accompanies inhibition of feeding network interneurons provides a neural correlate and a mechanism for the original behavioral observations by Davis and Mptsos (1971) that escape swimming suppresses feeding behavior in the intact animal. Inhibition is biphasic, occurring during both dorsal and ventral phases of the swim cycle. A1, active during dorsal flexion of the cycle, would contribute to the inhibition occurring during dorsal flexion. Stimulation of a single A1 suppressed ongoing feeding behavior when performed in the whole animal at frequencies normally occurring during swimming, and suppressed fictive feeding when similarly driven in the isolated CNS. Because both A1s are active during the swim, their effects are probably additive. Feeding behavior itself occupies a central position in the animal’s behavioral repertory; active feeding tends to suppress other behaviors such as reflexive righting in an upside-down animal, mating, and local withdrawal from tactile stimuli (ibid.). The apparent dominant position of escape swimming in the behavioral repertory is further paralleled at the network level by the observations that the occurrence of feeding activity in its appetitive and full consummatory forms was not even reflected in observable changes in spontaneous PSP activity in A1. The data indicate that the A1 neurons exert their effects on the motor network of feeding in part through potent polysynaptic inhibition of the major cerebral interneurons, the PC+ s. The PC+ s are critical to feeding behavior and are sites where feeding pattern generation is also inhibited in both satiated and food-avoidance-conditioned animals (Davis and Gillette 1978; Davis et al. 1983; London and Gillette 1986). Thus the A1 neurons are not only apparently important elements of the circuit of aversive swimming, but corollary output of their activity also contributes to adaptive suppression of behavior. These actions, and the response specificity of the A1s to noxious stimuli, suggest that one of their major functions is to suppress feeding activity during aversive behavior.

Inhibition between networks that mediate mutually exclusive behaviors is emerging as a common theme in neuroethological analyses of behavioral choice and switching. In Clione limacina, retraction of parapodial wings (local withdrawal) was found to take precedence over swimming (locomotion), a switch mediated by one-way inhibition from a retraction interneuron to the central pattern generator for swimming (Huang and Satterlie 1990). Inhibition between command systems for feeding and escape behavior in crayfish has also been demonstrated (Edwards 1991; Krasne and Lee 1988; Vu et al. 1993). In Pleurobranchaea, a transient inhibitory effect on feeding behavior caused by rough mechanical stimulation of the oral veil (Kovac and Davis 1980a) could potentially be mediated in part by A1 activation.

**Homology of A1 and C2 neurons**

The characteristics of the A1 neurons indicate that they are probably homologous to the C2 neurons of the nudibranch Tritonia (Getting 1977; Snow 1982; Taghert and Willows 1978), which have been well studied for their roles in generation of swimming behavior closely similar to that of Pleuro-
branchae (Audesirk and Audesirk 1985). This interpretation is shored up both by the animal’s phylectic closeness and a broad spectrum of similarities. Nudibranchs such as Tritonia probably evolved from the notaspid pleurobranchomorphs (Schmekel 1985; Thompson and Slinn 1959). Other likely neuronal homologies in Pleurobranchaea and Tritonia CNS have been readily identified in previous studies (Dickinson 1979, 1980). The neurons A1 and C2 themselves share common anatomic characters in soma position, distinctive whiteness of the soma, and axon pathway (Getting 1977, 1989; Getting et al. 1980; Longley and Longley 1987; Taghert and Willows 1978). They also share functional character as critical elements in pattern generation of a fixed action pattern, itself closely similar for the two animals. On these grounds it is likely that A1 and C2 have a common evolutionary origin. Longley and Longley (1987) have suggested that a homologous neuron is also present in nonswimming aeolid nudibranchs.

However similar, the A1 neurons do not appear to be exactly functionally identical to the C2s. In particular, in none of the published records for C2 can we find instances of the marked feedback inhibition A1 activates in nearly all the Pleurobranchaea preparations we have examined, nor have we observed it in recordings of C2 in four Tritonia preparations we have performed for comparison (unpublished data). This difference may reflect differences in the structure and/or connectivity of the swim pattern generator; our unpublished observations have documented a variety of neuron types coupled chemically and electrically to A1 that are unreported for Tritonia (cf. Jing and Gillette 1995).

Escape swimming and other aversive behaviors in Pleurobranchaea

The differences between A1 and C2 may in some way be related to differences between the two opisthobranchs in the structure of the swimming pattern generators, which may in turn reflect a difference in the animals’ propensities to exhibit swimming escape behavior. It is our experience that swimming is often more difficult to induce in Pleurobranchaea than in Tritonia. Whereas Tritonia is readily stimulated to swim by dropping a pinch of salt on its back or by the touch of a predatory starfish (Willows et al. 1973), these stimuli are rarely effective for Pleurobranchaea. Our impression is that the most effective stimulus for swimming in Pleurobranchaea, an enthusiastic cannibal in the aquarium and the wild (Cattaneo-Vietti et al. 1993; Davis and Mpitsos 1971), is the touch or the bite of a conspecific. Indeed, Pleurobranchaea has no known heterospecific predators, due at least in part to its defensive acid secretion (Gillette et al. 1991). Thus the differing sensitivities to sensory stimuli must reflect evolutionary pressures on the sensory paths to the pattern generator, on the structure and threshold of the pattern generator, or all of these.

Getting (Getting 1989; Getting and Dekin 1985a) suggested that the swim-generator circuitry of Tritonia could act in different states of coordination to serve different functions, either swimming or reflexive withdrawal. The contralateral flexion induced by stimulation of a single A1, in the absence of swimming, is suggestive of an aversive turn; a role for A1 in mediating aversive turns or reflexive withdrawal is presently an interesting speculation and awaits future tests.

In summary, we have identified neuronal elements in Pleurobranchaea that mediate escape swimming, that potentially act in other aversive behaviors, and that suppress feeding behavior. These findings provide a base for further documentation of the pattern-generating circuitry, its outputs, and its interactions with regulatory elements of the feeding network.

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