Central Pattern Generator for Escape Swimming in the Notaspid Sea Slug *Pleurobranchaea californica*

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**Jing, Jian and Rhanor Gillette.** Central pattern generator for escape swimming in the notaspid sea slug *Pleurobranchaea californica*. J. Neurophysiol. 81: 654–667, 1999. Escape swimming in the notaspid opisthobranch *Pleurobranchaea* is an episode of alternating dorsal and ventral body flexions that overrides all other behaviors. We have explored the structure of the central pattern generator (CPG) in the cerebropleural ganglion as part of a study of neural network interactions underlying decision making in normal behavior. The CPG comprises at least eight bilaterally paired interneurons, each of which contributes and is phase-locked to the swim rhythm. Dorsal flexion is mediated by hemiganglion ensembles of four serotonin-immunoreactive neurons, the As1, As2, As3, and As4, and an electrically coupled pair, the A1 and A10 cells. When stimulated, A10 commands fictive swimming in the isolated CNS and actual swimming behavior in whole animals. As1–4 provide prolonged, neuromodulatory excitation enhancing dorsal flexion bursts and swim cycle number. Ventral flexion is mediated by the A3 cell and a ventral swim interneuron, lA3, the soma of which is yet unlocated. Initiation of a swim episode begins with persistent firing in A10, followed by recruitment of As1–4 and A1 into dorsal flexion. Recurrent excitation within the As1–4 ensemble and with A1/A10 may reinforce coactivity. Synchrony among swim interneuron partners and bilateral coordination is promoted by electrical coupling among the A1/A10 and As4 pairs, and among unilateral As2–4, and reciprocal chemical excitation between contralateral As1–4 groups. The switch from dorsal to ventral flexion coincides with delayed recruitment of A3, which is coupled electrically to A1, and with recurrent inhibition from A3/As to A1/A10. The alternating phase relation may be reinforced by reciprocal inhibition between As1–4 and lA3. *Pleurobranchaea*’s swim resembles that of the nudibranch *Tritonia*; we find that the CPGs are similar in many details, suggesting that the behavior and network are primitive characters derived from a common pleurobranchid ancestor.

**INTRODUCTION**

The escape swimming behavior of the mollusc *Pleurobranchaea californica* is a predator-avoidance mechanism that su-
percedes and suppresses all other behaviors (Davis and Mptisos 1971; Gillette et al. 1991). Previously, we identified a critical element, A1, of the central pattern generator for the escape swim and found that its activation caused suppression of feeding motor output, thereby elucidating a mechanism of behavioral switching (Jing and Gillette 1995a). Activation of A1 in both intact animals and isolated CNS drove profound inhibition in feeding command neurons, sites in the feeding network where feeding motor output is gated (Davis and Gillette 1978; Gillette et al. 1982). We also showed that this identified neuron resembled, in detail, a specific element of the previously described pattern-generating network underlying the similar escape swimming behavior of the nudibranch *Tritonia diomedea* (reviewed by Getting 1989b), and hypothesized the existence of a homologous network in *Pleurobranchaea*, despite gross differences between the animals in morphology, behavior, and ecological niche.

We have undertaken further study of the escape-swim pattern generator in *Pleurobranchaea* to elucidate its role in the repertory of the animal’s avoidance behaviors to provide a base for further investigation of mechanisms of decision making for avoidance versus feeding and to further probe its relationship to that of *Tritonia*. We have identified six more elements of the swimming pattern generator and have characterized partly a seventh through its postsynaptic effects. The roles of the neurons in the swim and their connectivity allow inference of the network mechanisms from which the motor pattern arises. We find detailed similarities with *Tritonia*’s swim network that argue strongly for conservation of ancestral neural circuitry. We also find differences in the existence of a novel command-like neuron, an extra putatively serotonergic cell, and more prominent electrical coupling in the network of *Pleurobranchaea*. These findings are interpreted in terms of their functional and evolutionary significance. Portions of these data have appeared in abstract form (Jing and Gillette 1995b; cf. Jing et al. 1997).

**METHODS**

Specimens (180) of *Pleurobranchaea californica* (3–600 g) were obtained from Sea-Life Supply (Sand City, CA) and Pacific BioMarine (Santa Monica, CA) and maintained in circulated artificial seawater at 14°C until use. All dissections were done under cold anesthesia at 4°C.

Whole animal preparations were made by accessing the cerebropleural ganglion through a 2-cm dorsal incision and pinning it to a wax platform (Jing and Gillette 1995a). Hooks retracting the incision partially supported and restrained the animal for stable intracellular recordings but left it capable of considerable movement, including vigorous swimming and feeding behavior. The preparation chamber was perfused constantly with fresh artificial seawater (14°C). Isolated CNS preparations included cerebropleural and pedal ganglia, occasionally with buccal ganglion attached, and were pinned to silicone elastomer (Sylgard) under saline (cooled to 13–14°C) of composition (in mM) 420 NaCl, 10 KCl, 25 MgCl₂, 25 MgSO₄, 10 CaCl₂, and 10 3-(N-morpholino)propanesulfonic acid (MOPS) buffer, adjusted to pH 7.5 with NaOH.

Intracellular and extracellular recordings were done with conventional KCl-filled glass micropipettes and polyethylene suction electrodes as previously described (Jing and Gillette 1995a). Data were recorded on chart recorder (Gould TA11; sampling rate: 250 kHz) and...
digitized on video tape for later measurements. Spike height measurements were taken when cells were spontaneously active at only low rates to avoid use-dependent attenuation.

To study fictive swimming, we selected animals that reliably swam in response to a mild electric shock applied to the dorsal mantle or tail; >90% of isolated CNS preparations showed fictive swim activity. When swimming episodes were induced repeatedly, trials were separated by ≥10 min to minimize habituation of swimming responses. Swimming in whole animal preparations and isolated CNS was usually elicited by shocks (monopolar, 2-ms duration, 3–15 V, 15 Hz, for 2–2.5 s) to the body wall nerve (BWN) of the cerebropleural ganglion, which innervates the dorsolateral mantle (Lee and Liegeois 1974); the effects of its stimulation are best analogous to noxious stimulation of the back and/or the tail. In those animals where shock was ineffective in eliciting a swim, withdrawal of the posterior part of the body occurred sometimes followed by a single ventral flexion. Postshock, whether or not a swim intervened, animals typically showed accelerated creeping locomotion accompanied by frequent turning. In tests of premotor neuron abilities to affect the swimming rhythm, experimental trials were sandwiched between two control trials. Experimental results were accepted only when both control trials were closely similar.

Functional synaptic connections were examined in normal saline for postsynaptic potential (PSP) ability to follow presynaptic spikes one for one as a criterion of probable monosynapticity. Assays of probable mono- or polysynapticity also were conducted in high-divalent saline [which contained (in mM) 240 NaCl, 10 KCl, 125 MgCl₂, 25 MgSO₄, 30 CaCl₂, and 10 MOPS] to elevate spike thresholds and curtail polysynaptic activation (London and Gillette 1984).

Electrical coupling was assayed by passing hyperpolarizing current into one cell and measuring steady-state polarization in its partner. The steady-state coupling coefficient was taken as the ratio of the post- to presynaptic voltage change. No appreciable differences in coupling were observed in normal versus high-divalent saline (n = 8).

Nerve backfills and intracellular staining

Neurons with axons in specific nerves or connectives were backfilled via axons in the cut nerves with biocytin (Sigma). Neuron morphology was studied by intracellular injection of biocytin or neurobiotin (Vector, Burlingame, CA) from the recording electrode (Jing and Gillette 1995a). After an incubation of varying periods, tissues were fixed, and stain was developed and viewed in cleared whole mounts. Pressure injection of somata allowed staining of axon processes ≥2 cm from injection site after ganglia were incubated overnight at 8°C.

Immunocytochemistry and double labeling

Serotonin immunoreactivity was studied in whole mounts with the avidin-biotin peroxidase (ABC peroxidase) technique (Beltz and Burd 1989) as used previously (Sudlow et al. 1998). After fixation, immunoreaction with rabbit anti-serotonin (5-HT) primary antibody (Instar, Stillwater, MN) and stain development, tissues were cleared and viewed as whole mounts.

For double labeling with 5-HT antibody and neurobiotin, cells were injected with neurobiotin after identification and processed as above, except that the primary antibody (1:5,000 to 1:10,000 dilution, reacted at 4°C for 72 h) was visualized with rhodamine conjugated goat anti-rabbit secondary antibody (Cappel, Durham, NC) and neurobiotin was visualized by fluorescein-conjugated Avidin D (Vector) under confocal fluorescence microscopy. Images were stored as digitized image files (gray scale, 8 bit, 512 × 512 pixel) and processed with Adobe Photoshop software.

RESULTS

A cluster neurons: identification, cell morphology and serotonin immunoreactivity

The A cluster neurons lie in the dorsal cerebropleural ganglion in a group extending posteriorly from the base of the

FIG. 1. A cluster neuron somata. A: map of the A cluster neurons in the cerebropleural ganglion. Top: expanded view of the A cluster region. ●, swim interneurons; BWN, body wall nerve; sBWN, small body wall nerve; CBC, cerebrobuccal connective; aCPC, anterior cerebropedal connective; pCPC, posterior cerebropedal connective; CVC, cerebrovisceral connective; MN, mouth nerve; OVN, oral veil nerve; RN, rhinophore nerve; SCC, subcerebral commissure; TN, tentacle nerve. B: composite drawing of neuron somata with axons in left aCPC (a) and right pCPC (b) based on multiple camera lucida drawings from biocytin backfills. Soma positions on the contralateral sides were nearly symmetrical, especially for the A cluster. •••••, approximate boundary of the A cluster. Arrow heads show the connectives that were backfilled. ○, somata filled only infrequently.
rhinophore nerve up to the central commissure (Fig. 1A). This group comprises most cells of the dorsal ganglion sending descending axons to the pedal ganglia via the anterior cerebropedal connective (aCPC; Fig. 1B); most A cluster cells send their axons contralaterally. The aCPC corresponds to the cerebropedal connectives, and the posterior CPC (pCPC) corresponds to the pleuropedal connectives, of more primitive gastropods in which cerebral and pleural ganglia are not fused.

Identified neurons of the swim pattern generator are embedded in the A cluster. Most A cluster cells are either part of the swimming CPG or can modulate its output or receive inputs from it. Identified neurons are named alphanumerically, with ‘‘s’’ added to designate members of a 5-HT-immunoreactive population. For A cluster cells with no CPC axons, the term ‘‘rh’’ indicates a rhinophore nerve axon and ‘‘ci’’ designates interneurons with a commissural axon. In earlier preliminary reports (Jing and Gillette 1995b, 1996; Jing et al. 1997), a slightly different naming procedure was used, where As1–3 were called A3a–c, As4 was A8b, A3 was A3d, and A4 was A3e. Because As2 and As3 were indistinguishable (see also Table 1), the term As2/3 is used to refer to a single one of the pair, and As2–3 refers to them collectively. Only the swim interneurons with a commissural axon. In earlier preliminary preparations (Jing and Gillette 1995a), the second white soma, A2 (diam: 45–65 μm), is postero-medial from A1, separated by one to two orange somata, one of which is A7. Just postero-medial from A2 is a subcluster of seven cells with similar appearance: A-ci1, A-ci2, As1, As2, As3, A3, and A4. The relative positions of As1–3, A3 and A4 (Fig. 1A) vary slightly among preparations. These neurons are somewhat distinguishable by size: As1 and A3 tended to be larger, 60–90 μm diam, whereas others ranged 40–65 μm. In two preparations, all As1–3, A3, and A4 were identified and dye injected, thus confirming the existence of all.

Postero-medial to the As1–3 lie A10, As-rh, A8, and As4. The soma of A10 is medium-sized and translucent (65–85 μm diam) and sometimes separated from the As1–3 by another cell. The soma of A10 often is over lain partly by another cell and thus appears smaller than its actual size. As4 is postero- medial of A10 and is one of the largest somata (75–95 μm) of the A cluster. As-rh has a single axon in the ipsilateral rhinophore nerve. A8 has a single axon in contralateral aCPC (c-aCPC).

The morphologies of the swim interneurons are shown in Table 1.

<table>
<thead>
<tr>
<th>Cell Name</th>
<th>Axon Path</th>
<th>Cell Size, μm; Color, Serotonin Immunoreactivity</th>
<th>Electrophysiology</th>
<th>Spontaneous Spike and PSPs Activity</th>
<th>Activity When No Swim Occurs Upon BWN Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>c-aCPC + PC</td>
<td>70–100, white</td>
<td>−48.6 ± 4.3</td>
<td>70.3 ± 4.9</td>
<td>3.2</td>
</tr>
<tr>
<td>As1</td>
<td>c-aCPC + PC</td>
<td>60–90, translucent, 5-HT positive</td>
<td>−49.7 ± 4.3</td>
<td>87.9 ± 5.3</td>
<td>2.6</td>
</tr>
<tr>
<td>As2 and As3</td>
<td>c-aCPC + PC</td>
<td>40–65, translucent, 5-HT positive</td>
<td>−48.1 ± 3.7</td>
<td>77.1 ± 6.3</td>
<td>2.4</td>
</tr>
<tr>
<td>A3</td>
<td>c-aCPC</td>
<td>60–90, translucent</td>
<td>−47.5 ± 3.8</td>
<td>89.6 ± 4.9</td>
<td>2.2</td>
</tr>
<tr>
<td>As4</td>
<td>c-aCPC + c-pCPC + peripheral nerves</td>
<td>75–95, translucent, 5-HT positive</td>
<td>−46.0 ± 3.9</td>
<td>82.8 ± 4.7</td>
<td>5.1</td>
</tr>
<tr>
<td>A10</td>
<td>i-pCPC + PC</td>
<td>65–85, translucent</td>
<td>−47.6 ± 3.4</td>
<td>85.3 ± 4.6</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. RP, resting potential; SH, spike height; SD, spike duration at half amplitude. PSP, postsynaptic potentials; c-aCPC, contralateral anterior cerebropedal connective; IPSP, inhibitory PSP; 5-HT, serotonin; EPSP, excitatory PSP; c-pCPC, contralateral posterior CPC; PC, pedal commissure. * Percent of preparations observed. † Slow bursting: in some preparations, As2/3 spontaneous activity followed a very slow, cyclic bursting form, with peak spike frequencies near 3 Hz, burst durations of 16–40 s, and cycle periods of 80–160 s. This slow bursting activity occurred frequently after body wall nerve (BWN) stimulation whether or not a swim episode was initiated.
Fig. 2 and summarized in Table 1. Of them, the neurons A1 (Jing and Gillette 1995a), As1–4, A3 each have at least one axon crossing the central commissure to exit in the c-aCPC. As4 also has an axon branch going to the contralateral pCPC and additional fine branches going to the periphery in the contralateral tentacle, rhinophore, and body wall nerves. The A10 axon exits in the ipsilateral pCPC (i-pCPC) and crosses from one pedal ganglion to the next in the pedal commissure.

A study of 5-HT immunoreactivity in the Pleurobranchaea CNS (Sudlow et al. 1998) located five immunoreactive neurons in the A cluster region. These cells were identified with intra-cellular electrodes, injected with neurobiotin, processed for 5-HT immunocytochemistry and found to be As1–4, and As-rh (Fig. 3). Other A cluster neurons tested in double-labeling experiments were immunonegative (A10, A-ci1, A-ci2, A3, A4, and A8).

Central pattern generator

Phasic activity of the pattern-generating elements. The activities of the various CPG neurons during fictive swims were related to the rhythmic firing recorded in the A1, aCPC, and/or the anterior lateral body wall nerve (aLBWN) of the pedal ganglion. Previously we showed that the A1 burst during the swim in the whole animal occurred just before and during the dorsal flexion phase (Jing and Gillette 1995a). We also established that escape swimming in the whole animal and fictive swimming in the isolated CNS are indistinguishable with respect to both A1 and other premotor activity recorded in the aCPC and that spike activity in the aLBWN is a useful monitor of the motor output of escape swimming (Jing and Gillette 1995a).

After nerve stimulation, a swim episode began with the induction of sustained activity in A10. The dorsal flexion phase of each swim cycle began with the onset of bursts in the putatively serotonergic As1–4 neurons, followed by recruitment of the burst of A1 (Fig. 4). During dorsal flexion, a decline in the initially high As1–4 activity was reversed as A1/A10 spike rate accelerated, causing two distinct peaks in As1–4 activity during this dorsal swim burst (Fig. 5).

During swims, burst activities of A1 and A10 were closely synchronized in both phase and duration (Figs. 4, 7B, and 8), an apparent consequence of the strong electrical coupling described later. However, it was notable that A10 activity differed from all other swim interneurons in that it continued spiking at a low rate (2–4 Hz) during the hyperpolarized interburst interval without complete spike inhibition. The spiking frequency during A10 bursts was typically higher than for A1 (peak and mean frequency ranges 40–60 and 15–20 Hz vs. 10–30 and 5–12 Hz, respectively).
The putatively serotonergic As1–4 were coactive in the same phase of dorsal flexion (cf. Fig. 9). The burst durations of the ensemble members were similar except that when the spike activity in bursts declined late in a swim episode, As1 and As4 bursts ceased somewhat before As2–3. Spike rates in the bursts of the two neurons As2 and As3 were virtually identical, whereas those for As1 and As4 were lower (peak and average frequency ranges 10–25 and 6–15 Hz vs. 5–12 and 3–8 Hz, respectively). Spike rates for As1–4 varied with cycle period, such that more intense spiking occurred in the earlier, shorter cycle periods (Fig. 5).

The transition from the dorsal to the ventral flexion phase was attended by bursting in A3. A3 activity began when A1 spike activity was highest and ended with or shortly after the A1 bursts (Figs. 4, 7B, and 11). The burst duration of A3 (0.7–1.6 s) was shorter than for the other identified CPG members. A3 burst intensity (6–17 Hz) tended to fade over successive swim cycles as the cycle period lengthened, especially as A1 burst intensity waned.

CONTRIBUTIONS OF THE PATTERN-GENERATING ELEMENTS TO INITIATION, PATTERNING, AND MAINTENANCE OF THE SWIM. A10. Among the swim interneurons, only A10 could consistently drive swim motor output (Figs. 6 and 7A). Stimulation of a single A10 at spike rates of 10–25 Hz drove cyclic activity appropriate to the swim rhythm in 14 of 19 isolated CNS preparations. In the four cases where A10 activity alone was unable to drive the cyclic activity of the swim, BWN stimulation did not initiate the fictive swim episode either. However,
in these four cases, when A10 activity was driven shortly after BWN stimulation, the swim rhythm could be activated successfully. In four of five whole animal preparations, driving a single A10 induced swimming behavior similar to that caused by BWN stimulation save that the ventral body flexion of the cycle was not as strong. The ability of A10 to drive patterned swimming output contrasted with the much weaker ability of A1, which was shown previously to be effectual in only a small fraction of isolated CNS preparations and never so in whole animal preparations (Jing and Gillette 1995a). Where we examined both A10 and A1 in six isolated CNS preparations, only A10 was able to drive coordinated swim activity.

However, the fictive swim driven by A10 activity was incomplete compared with swim episodes induced by BWN stimulation in that the swim episode showed no signs of being self-sustained and halted immediately when depolarizing stimulation of A10 was halted. Moreover, A10 activity did not effectively recruit burst activity in As1–3 (Fig. 7). In five preparations, As1–3 burst activity driven by A10 in As1–3 was either quite weak (n = 3) or absent (n = 2). This is consistent with the observation that A1 and A10 are only weakly synaptically coupled to As1–4 (see Connectivity). Recruitment of As1–3 by BWN stimulation may be necessary to the normal swim episode, for when a fictive swim was induced by A10 depolarization after BWN stimulation (as described earlier), As1–3 bursts were obviously strengthened (n = 3).

Hyperpolarization of a single A10 interrupted the swim induced by nerve stimulation for the duration of current injection (n = 4, Fig. 8), pointing to an obligatory role in pattern generation. This also directly hyperpolarized A1, to which A10 is coupled electrically (see Electrical coupling between A1 and A10). Hyperpolarization of A1 was itself previously shown to effectively block expression of swim motor activity (Jing and Gillette 1995a). The strong electrical coupling between A1 and A10 made it difficult to differentiate the functions of the two cells, and we consider them both necessary to pattern generation.

As1–4. Through their synchronous activity during swims, shared 5-HT immunoreactivity and mutual excitatory connections (see Connectivity), the As1–4 cells appeared to act collectively in the pattern generator as a functional unit. Individually their effects were relatively weak: in only one of seven cases did hyperpolarization of a single As1–4 affect the fictive swim pattern. However, in three of three cases where we hyperpolarized two cells at a time, the ensuing burst cycle was delayed for 0.5–2.7 s, and the fictive swim episode was terminated early relative to pre- and postcontrol measures (Fig. 9), results with a random probability of <0.002 (Bernoulli distribution).

The effects of driving As1–4 on the fictive swim were tested in 14 preparations. In eight of these, driving a single As1–4 (As1, n = 3; As2/3, n = 3; As4, n = 2) prolonged the swim
episode by one to two extra cycles relative to both pre- and postcontrol measures (Fig. 10). For the 14 cases, the increased number of cycles on the second test was significant \((P < 0.002; \text{Fisher’s exact test})\). For the eight cases, the random probability of this exact result occurring was \(< 6 \times 10^{-8}\). In the six cases where the number of swim cycles was not increased, the mean spike frequency of the A1 bursts was enhanced on average \(87\%\) \((P < 0.02; \text{2-tailed t-test})\). By themselves, As1–4 were not effective in inducing a swim episode. The distinctly suppressive effects of hyperpolarizing two of the eight As1–4, and the stimulatory effects of depolarization of single cells, indicate that collective As1–4 activity significantly contributes to excitation of the swim CPG.

**A3.** Driving A3 tonically during the swim episode hyperpolarized A1 and suppressed the swim episode for the duration of A3 activity (Fig. 11; \(n = 7\) observations). In two cases, a complete swim cycle rebounded after release of A3 depolarization. Hyperpolarization of single A3 neurons during fictive swim episodes had no discernible effects \((n = 5)\). The inhibitory effects of A3 on the swimming pattern generator and the cyclic activity of the neuron during the swim are consistent with a role in terminating the dorsal flexion phase of the burst cycle, in particular the A1/A10 bursting, shared with another interneuron(s), I\(_{\text{VS}}\).

**I\(_{\text{VS}}\).** Activity in A1/A10 activates inhibitory feedback from this interneuron (Jing and Gillette 1995a), the soma of which remains unidentified. Inhibition from I\(_{\text{VS}}\) is distributed among all swim interneurons active during dorsal flexion (A1/A10, As1–4) and is presumed to make the major contribution to termination of dorsal flexion and to the duration of the ventral flexion phase. The onset of activity in the I\(_{\text{VS}}\) pathway as observed in the feedback inhibition of A1 is coincident with inhibition of the feeding network, and the cell potentially has widespread effects in the CNS.

**Connectivity**

In summary to this point, the cells A1, A10, As1–4, and A3 are part of the CPG for escape swimming. Of these, A1, As1–4, and A10 drive the dorsal flexion phase of the swim cycle. They compose two functional ensembles with distinct firing patterns: A1/A10 and As1–4. Activity in A3 leads up to ventral flexion, can contribute to the dorsal/ventral flexion phase transition, and in conjunction with I\(_{\text{VS}}\) may mediate inhibition to A1/A10. Inhibition to As1–4 is provided only by I\(_{\text{VS}}\). The synaptic coupling among the neurons described in the following sections is consistent with these roles.

**ELECTRICAL COUPLING BETWEEN A1 AND A10.** A most prominent feature of the connections among the A cluster neurons was abundant electrical coupling. Among the swim interneurons, appreciable coupling occurred between A1 and A10 and among As2, As3, and As4. Within a unilateral A cluster, the highest electrical coupling ratio was found for A1 and A10 (Table 2A, Fig. 12A). This coupling was asymmetric: the coupling coefficient for steady-state voltage change with current passage from A10 to A1 (0.41) was...
1.4 times that for A1–A10 (0.29). The strong A1/A10 coupling was expressed in frequent simultaneous spiking in the cells and in the subthreshold spike-like potentials in A1 synchronous with A10 spikes (Figs. 4, 7, and 8).

Electrical coupling between the bilateral homologues of A1 was also strong (Fig. 12B) with an average coupling ratio of 0.16. A1 appeared to be directly coupled to contralateral A10 because the coupling ratio between contralateral A1 and A10 cells was higher than between bilateral A1s (Table 2B, Fig. 12B). Reciprocal coupling between bilateral A10s must be effected entirely through the A1s instead of directly because the A10 axons do not cross the commissure (Table 2B). The strong electrical connections among the ipsilateral and contralateral A1/A10 ensembles can explain the observations that hyperpolarization of only one of these cells is sufficient to suppress swimming (Jing and Gillette 1995a) (Fig. 8).

RECURRENT EXCITATION WITHIN AS1–4: ELECTRICAL COUPLING AND LONG-LASTING COMPOUND EPSPS. Electrical coupling was found among ipsilateral As2, As3, and As4 (Fig. 13A, I and 2). The coupling between As2 and As3 was symmetric (Table 2A). The As2–3 pair also had mutually excitatory chemical connections; firing one cell induced a long-lasting excitatory PSP (EPSP) in the other, superimposed on the electrically mediated depolarization (Fig. 13A4).

Lacking electrical connections with other neurons in this ensemble, As1 made reciprocal, excitatory, and monosynaptic chemical connections with As2–4 demonstrable in high-divalent cation saline (Fig. 13A, 3–5), causing long-lasting, slow EPSPs. The amplitude of the compound EPSPs induced by a train of 10–20 spikes ranged from 1 to 4 mV (4–9 observations were made for each neuron pair). Timing of compound EPSPs in the different neurons was comparable with time to peak of 3–4 s and decay occurring during 10–20 s.

Contralateral connections among As1–4 were similar to their ipsilateral connections. Contralateral As2–4 were electrically coupled (Table 2B, Fig. 13B2); the coupling ratios were low relative to A1s and A10s, except for the contralateral As4s with a high coupling ratio of 0.38. As1 also was coupled weakly electrically to its contralateral counterpart (Fig. 13B1) but not to the contralateral As2–4. Generally coupling coefficients among this population ipsilaterally and contralaterally were smaller than those for A1/A10 populations.

Reciprocal excitatory connections mediating slow EPSPs were found between contralateral As1 neurons and between As1 and contralateral As2/3. The contralateral connections between As1 were strong enough to drive postsynaptic spiking even in high-divalent saline. Characteristics of timing and amplitude were similar to ipsilateral connections, reaching amplitudes of 1–3.5 mV and enduring to 16 s (Fig. 13B3, n = 7 pairs). The compound EPSPs from As1 to contralateral As2/3 (1.6–2.4 mV; Fig. 13B4, n = 4) and from As2/3 to As1 (2.2–5.5 mV; Fig. 13B5, n = 4) were similar.

RECURRENT EXCITATION AND INHIBITION BETWEEN A1/A10 AND AS1–4. A1 and A10 made mixed chemical synaptic connections with the ipsilateral As1–4 (Fig. 14A). In normal saline, a train of spikes in A1 evoked early excitation followed by
inhibition in As1–4 (n = 34 of 39; in 5 cases the connection was quite weak). The connection from As1–3 to A1 was excitatory; PSPs from As1 to A1 were observed more frequently (n = 10 of 14) than for those from As2/3 to A1 (n = 12 of 24), and the amplitude was typically larger. The connection from As4 to A1 is similar but weaker. Where connections were not observed, synaptic potentials may have been buried in the synaptic noise. High-divalent cation saline significantly suppressed the connection strength from A1 to As1–3 and from As1–3 to A1, suggesting that some components were polysynaptic. For the connections from A1 to As1–3, both the early excitatory and late inhibitory components were suppressed. Consistently, in high-divalent saline, connections from A1 to As1–3 remained as biphasic excitatory-inhibitory connections of attenuated amplitude (n = 9), whereas the connection from A1 to As4 was purely inhibition (n = 5, Fig. 14A3), of 0.7- 

### TABLE 2. Electrical coupling ratios (steady state) among swim interneurons

<table>
<thead>
<tr>
<th></th>
<th>A1</th>
<th>A10</th>
<th>A3</th>
<th>As2/3</th>
<th>As4</th>
<th>As1</th>
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<tr>
<td><strong>A. Coupling between ipsilateral neurons</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Post/Pre</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>—</td>
<td>0.41 ± 0.018 (26)</td>
<td>0.01 ± 0.001 (3)</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A10</td>
<td>0.288 ± 0.012 (23)</td>
<td>—</td>
<td>0.01 ± 0.002 (3)</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>0.0174 ± 0.003 (5)</td>
<td>0.021 ± 0.002 (3)</td>
<td>—</td>
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<td>—</td>
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<tr>
<td>As3/2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.088 ± 0.005 (41)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>As4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.086 ± 0.009 (10)</td>
<td>—</td>
</tr>
<tr>
<td><strong>B. Coupling between contralateral neurons</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contra-Ipsilateral</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>0.156 ± 0.014 (13)</td>
<td>0.166 ± 0.015 (6)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>A10</td>
<td>0.157 ± 0.01 (7)</td>
<td>0.109 ± 0.011 (2)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>As2/3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.60 ± 0.007 (12)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>As4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.376 ± 0.025 (4)</td>
<td>—</td>
</tr>
<tr>
<td>As1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.024 ± 0.004 (7)</td>
<td>—</td>
</tr>
<tr>
<td>A3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.120 ± 0.016 (7)</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE, number in parentheses indicates the number of measurements. *Electrical coupling between As2/3 and contralateral As4 was observed in three preparations, but accurate measurements of coupling were not obtained.

![FIG. 13. Ipsi- and bilateral connections in As1–4 groups. A: ipsilateral connections. Electrical coupling was observed between As2 and As3 but not with them and As1 (A1) and among As2, As3, and As4 (A2). A, 3–5: slow, monosynaptic and reciprocal excitation between As1 and As2–4. B: contralateral As1–3 connections. Electrical coupling was found between contralateral As1 (B1) and among the contralateral As2/3, but not with contralateral As1 (B2). B, 3–5: monosynaptic excitations between contralateral As1 (B3) and between As1 and contralateral As2/3 (B, 4 and 5). Calibration bar: vertical, 40 mV for all top traces and 4 mV for all bottom traces; horizontal, 4 s. All recordings were made in high-divalent saline.](image-url)
2-mV amplitude, 6-s duration, and time to peak of 2.4 s. Connections between A10 and As1–3 were similar to that between A1 and As1–3 but were somewhat weaker. Unlike A1, A10 still weakly excited As4 in high-divalent saline.

Recurrence inhibition from A3 to A1/A10. A3 received excitation from both A1/A10 and As1. A1/A10 and A3 were connected weakly electrically (Table 2A), a connection observable only in high-divalent cation saline where background synaptic noise was suppressed. A3 also was connected electrically to its contralateral homologue (Table 2B). In normal saline, excitatory connections from A1 and A10 to A3 (Fig. 14B1) were variable and dependent on presynaptic firing rate. At higher discharge rates, the initially small EPSP in A3 facilitated and reached spike threshold. In tests following closely on BWN stimulation, EPSPs showed apparent heterosynaptic facilitation. This connection disappeared in high-divalent saline, leaving behind only the weak electrical coupling (n = 9) and suggesting its polysynaptic origin. Also, a one-way monosynaptic excitatory connection from As1 to A3 was found, of amplitude 0.9–1.6 mV, average duration of 7.2 s, and time to peak of 1.2 s (n = 4; Fig. 14B2).

A3 sent phasic and facilitating unitary IPSPs to A1 with amplitudes of 0.15–0.5 mV and duration of ~0.6 s (Fig. 14B3) and to A10 with smaller amplitudes that were resistant to high-divalent cation saline (n = 9), but not to As1–4. The summed IPSP from a driven burst of A3 spikes (10–20 Hz) to A1 had an amplitude of 3–5 mV, duration of 3–10 s, time to peak of 0.9 s; that from A3 to A10 was similar but smaller (not shown). This inhibitory connection may account for the suppressive effects of A3 on swim pattern generation when it is driven tonically (Fig. 11).

Reciprocal inhibition between As1–3 and I_{VS}. One source of inhibition from A1 to As1–3 could come from the I_{VS} neuron, which was excited by A1 activity to mediate strong feedback inhibition (Jing and Gillette 1995a). The onset of feedback inhibition in A1 coincided with the late inhibition in As1–3 (see Fig. 15C); suggesting that I_{VS} inhibited As1–3 as well. Moreover, As1–3 appeared to make effective reciprocal inhibitory connections with I_{VS}; the feedback inhibition in A1 from I_{VS} was suppressed when As1–3 were coactive with A1 (n = 3; Fig. 15).

FIG. 14. Synaptic connections among ipsilateral A1, As1–4, and A3. A: reciprocal excitation between A1 and As2/3 in normal saline (A, 1 and 2). A barrage of unitary inhibitory postsynaptic potentials (IPSPs) outlasted the induced depolarization in As2/3 (A1). In high-divalent cation saline (HD, A3), A1 was connected to As4 by a largely inhibitory connection but was connected to As2/3 in biphasic excitation-inhibition, attenuated from that recorded in normal saline (A1). B: A1 excitation of A3 (normal saline, B1) and 1-way excitation from As1 to A3 (high-divalent saline, B2). Monosynaptic and facilitating IPSPs from A3 to A1 remained in high-divalent cation saline (HD, B3), riding on the electrically coupled depolarization. Calibration bar: vertical, 40 mV for all top records, 8 mV for bottom records except for A3, B2, and B3 for which bottom records were 2, 4, and 0.2 mV, respectively; horizontal, 4 s. Spikes were clipped in bottom record of B1.

FIG. 15. As1 activity suppressed the feedback inhibition in A1 from I_{VS} (normal saline). A and C: activation of A1 by depolarization caused feedback inhibition in A1 from I_{VS}. Inhibition suppressed A1 activity in the later part of the depolarization. B: activation of As1 simultaneously with A1 suppressed the feedback inhibition, suggesting that As1 inhibits I_{VS}. Activation of As1 alone (D) had little effect on A1 spike activity. Bars under traces indicate duration of current injection in that neuron. Calibration bar: vertical, 40 mV for all top traces and 20 mV for all bottom traces; horizontal, 2 s. Spikes in As1 were clipped.
that proposed for Tritonia 664 J. JING AND R. GILLETTE

prominence of feedback inhibition in the pattern generating mechanisms (see 664)

 dorsale to ventrale flexion in Pleurobranchaea

1.4 I VS mediates the alternation of dorsal and ventral flexion with 1.4

83 A1/A10 are shown as one ensemble. I VS, the soma of which is unlocated, is 83

139 A10 neurons, the axons of which cross the pedal commissures (A1, As4), or ipsilateral (A10); bilateral activity in 139

reinforcement of their own activity and contribute to the prolonged depolarization of A1/A10 that endures throughout and after the swim episode. Recurrent excitation within the As1–4 ensemble and between it and the A1/A10 ensemble may thus both contribute to dorsal flexion and sustain multiple cycles of the swim episode. The maintained activity of A10, presumably distributing excitation further to CPG elements, must evoke the swim pattern as an emergent property of the CPG connectivity.

The bilateral CPG halves are likely to be largely coordinated during the swim by the electrical connections between the contralateral A1s and A10s, As4s, and As1–3. Premotor activity so synchronized descends to motoneurons of the pedal ganglia through axons running to contralateral connectives (A1, As4), or ipsilateral (A10); bilateral activity in pedal ganglia motoneurons may be further reinforced by the innervation of both pedal ganglia by all of the A1 and A10 neurons, the axons of which cross the pedal commissure.

Once triggered, the swim episode may be maintained in part by 5-HT released from the 5-HT-immunoreactive As1–4 ensemble. These neurons do not have a critical role in pattern generation as the coordinated CPG output can be driven by A10 activity without their recruitment; however, they appear to have a significant modulatory role. As1–4 activity demonstra-

Mechanisms of pattern generation of escape swimming

Seven neurons, about a third of the A cluster, take part in escape swimming pattern generation. Each fires cyclically in phase with the swim rhythm, and all seven are coupled by electrical and/or chemical connections. Moreover each as an individual or part of an ensemble can perturb the ongoing rhythm by its discharge or hyperpolarization. At least one other element, I VS, remains to be located. The CPG interneurons mediate either the dorsal flexion phase of the swim (A1/A10, and As1–4), or ventral flexion (A3 and I VS). The pattern of electrical and chemical connections is summarized in the diagram of Fig. 16A. The pattern of connectivity is simplified further in 16B, where the hypothetical CPG structure is shown in terms of functional ensembles. The synaptic mechanisms

Initiation and maintenance of the swim is likely to be dependent on activity in the A10 neuron, the tonic activity of which begins with the triggering stimulus (Fig. 4) and is both necessary and sufficient to the patterned motor output. Subsequently, the As1–4 ensemble is recruited into the first burst of dorsal flexion, succeeded by A1. The full source of the excitation that recruits the As1–4 ensemble is not yet clear; it cannot be from A10 activity, which does not effectively drive the likely serotonergic ensemble. Thus we presume that the As1–4 are themselves activated directly or indirectly by nociceptive afferents. The activation of the As1–4 group brings recurrent excitation in slow compound EPSPs, which may both reinforce their own activity and contribute to the prolonged depolarization of A1/A10 that endures throughout and after the swim episode. Recurrent excitation within the As1–4 ensemble and between it and the A1/A10 ensemble may thus both contribute to dorsal flexion and sustain multiple cycles of the swim episode. The maintained activity of A10, presumably distributing excitation further to CPG elements, must evoke the swim pattern as an emergent property of the CPG connectivity.

It is likely that the biphasic excitatory/inhibitory connection from A1 to As1–3, and the entirely inhibitory connection to As4, contribute to the termination of As1–4 spike activity in dorsal flexion slightly before A1. The consequent termination of As1–4 activity slightly earlier than for A1/A10 would subtract from the excitation of A1/A10 and thereby contribute to their own burst termination. However, a more potent factor is recurrent inhibition in terms of the negative feedback to A1/A10 from the A3/I VS cells. A3 enters activity late in the dorsal flexion phase, and its inhibitory effects on A1/A10 mark the transition between dorsal and ventral flexion. Two more factors bring the onset of inhibition from I VS, which mediates the ventral flexion phase and inhibits all the dorsal swim interneurons: I VS is excited potently by A1 and disinhibited by cessation of As1–4 activity. Possibly the duration of ventral flexion is set by the decay of activity in I VS. The re-onset of As1–4 activity, riding on their own slow EPSPs, and decline of activity of I VS because of waning excitatory input from A1 and inhibition from As1–4 then marks the beginning of the next cycle.

The bilateral CPG halves are likely to be largely coordinated during the swim by the electrical connections between the contralateral A1s and A10s, As4s, and As1–3. Premotor activity so synchronized descends to motoneurons of the pedal ganglia through axons running to contralateral connectives (A1, As4), or ipsilateral (A10); bilateral activity in pedal ganglia motoneurons may be further reinforced by the innervation of both pedal ganglia by all of the A1 and A10 neurons, the axons of which cross the pedal commissure.

Once triggered, the swim episode may be maintained in part by 5-HT released from the 5-HT-immunoreactive As1–4 ensemble. These neurons do not have a critical role in pattern generation as the coordinated CPG output can be driven by A10 activity without their recruitment; however, they appear to have a significant modulatory role. As1–4 activity demonstra-
inhibition between IVS and As1–4 may depend on physical cation of the activation of the CPG and the nature of reciprocal interest and significance for future study. In particular, clarifi-

an adenosine 3

and Gillette 1995) or variously potentiates (Huang et al. 1998)

branchaea

and the nudibranch

ergic neurons of

mechanisms in a variety of molluscan cells. In other seroto-

(2008), 5-HT depolarizes and activates bursting

episode (Fig. 9), similar to the action of the likely homologous
dorsal swim interneurons (DSI) in the swim CPG of Tritonia
(Lennard et al. 1980). 5-HT activates (Sudlow

-pleurobranchaea, 5-HT activates (Sudlow and Gillette 1995) or variously potentiates (Huang et al. 1998)
an adenosine 3',5'-cyclic monophosphate-gated Na⁺ current; a similar current is present in the swim CPG neurons (Jing et al. 1997) that could underlie their prolonged recurrent excitation and bursting.

This work has provided a partial characterization of the swim CPG in Pleurobranchaea and leaves several issues of interest and significance for future study. In particular, clarification of the activation of the CPG and the nature of reciprocal inhibition between IVS and As1–4 may depend on physical identification of the sensory inputs to the swim interneurons and of IVS.

Comparative neurobiology of the premotor networks for swimming and locomotion in opisthobranchs

The escape swimming behaviors of the notaspidean Pleuro

branchaea and the nudibranch Tritonia are similar in their patterning and episodic natures. Comparison of the CPG circuits of the animals, as summarized in Table 3, points to numerous possible homologies in neuron identities and connection patterns. Moreover, the likely pattern-generating mechanisms appear to be well conserved in both networks (cf. Getting 1989b).

We previously reported similarity of Pleurobranchaea’s A1 neuron to C2 of Tritonia (Jing and Gillette 1995a). We now show that the interneurons As1–3 resemble the Tritonia dorsal swim interneurons DSI-A-C, sharing morphological characters, functional roles (Getting et al. 1980; Lennard et al. 1980), and 5-HT immunoreactivity (Katz et al. 1994; McClellan et al. 1994) and show similarities down to conserved spike rates during bursts (Getting 1981). A fourth 5-HT-immunoreactive member of the network was identified in Pleurobranchaea as As4, the counterpart of which is described in Tritonia, is suggested by anti-5-HT staining (cf. Sudlow et al. 1998). We have reviewed evidence that the As1–4 may be highly conserved among opisthobranchs (Sudlow et al. 1998); it may be added to this that in Aplysia the serotonergic CB1 neuron, which heterosynaptically facilitates the gill-siphon withdrawal circuit (Mackey et al. 1989), resembles As4 in soma position and axon paths to ontogenetically corresponding regions (Wright et al. 1995).

Extrapolating from the extensive similarities, we expect that the differences in reported composition of the swim CPGs of Pleurobranchaea and Tritonia largely reflect incompleteness of description of both networks. Notably, quite different command-like elements, able to drive the motor program, have been found in both species: A10 of Pleurobranchaea and the “dorsal ramp interneuron” (DRI) of Tritonia (Frost and Katz 1996). These cells are functionally distinct and not apparently homologous; it is possible that their counterparts still will be found in the two species. If so, the contrasting functions of the two command-like neurons will be of interest to compare.

### Table 3. Comparison of the escape swimming premotor networks of Pleurobranchaea and Tritonia

<table>
<thead>
<tr>
<th>CPG Composition</th>
<th>Pleurobranchaea</th>
<th>Tritonia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Network elements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediating dorsal flexion</td>
<td>A1</td>
<td>C2*</td>
</tr>
<tr>
<td></td>
<td>Serotonergic As1–3</td>
<td>Serotonergic DSI-A-C*</td>
</tr>
<tr>
<td></td>
<td>Serotonergic As4</td>
<td>Not reported (see DISCUSSION)</td>
</tr>
<tr>
<td>Mediating ventral flexion</td>
<td>IVS (inferred from network connectivity)</td>
<td>VSI-B*</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>Possibly VSI-A, but connectivity variance makes this less certain</td>
</tr>
<tr>
<td>Commandlike</td>
<td>A10: providing electrical excitation to A1</td>
<td>DRI: providing monosynaptic excitation to DSIs; not a likely homologue of A10</td>
</tr>
<tr>
<td><strong>Pattern of connectivity and proposed pattern-generating mechanisms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent excitation</td>
<td>Lasting compound EPSPs among ipsilateral As1–3 (As4)</td>
<td>Lasting compound EPSPs among ipsilateral DSIs*</td>
</tr>
<tr>
<td></td>
<td>Lasting compound EPSPs among contralateral As1–3 (As4)</td>
<td>Not present among contralateral DSIs</td>
</tr>
<tr>
<td></td>
<td>Electrical coupling between ipsilateral As2–3 (As4), and contralateral A1, and As1–3</td>
<td>Electrical coupling between ipsilateral DSI-B-C, and contralateral C2, and DSI-A-C*</td>
</tr>
<tr>
<td></td>
<td>Between A1 and As1–3 (As4)</td>
<td>Between C2 and DSI-A-C</td>
</tr>
<tr>
<td>Recurrent inhibition</td>
<td>From A1 to IVS</td>
<td>From C2 to VSI-B (appears weaker, because of virtual absence of feedback inhibition in C2)*</td>
</tr>
<tr>
<td>Reciprocal inhibition</td>
<td>From A1–10 to A3</td>
<td>Between DSI-A-C and IVS</td>
</tr>
<tr>
<td></td>
<td>Between As1–3 and IVS</td>
<td>—</td>
</tr>
<tr>
<td>Strength of electrical coupling</td>
<td>Left A1–right A1 (0.16)</td>
<td>Left C2-right C2 (0.02, weaker)</td>
</tr>
<tr>
<td></td>
<td>A1–A10</td>
<td>— (see DISCUSSION)</td>
</tr>
<tr>
<td></td>
<td>Among As2–3 (ipsilateral: 0.09, contralateral: 0.06, weaker)</td>
<td>Among DSI-B-C (ipsilateral: 0.19, contralateral: 0.14)</td>
</tr>
</tbody>
</table>

VSI, ventral swim interneuron. * Possible homology.
An apparent interspecific difference between the escape swim CPGs is in the strengths of the electrical connections (Table 3). This might extend to coupling of the C2 cell of *Tritonia* and a possible A10 homologue because there is no indication in the published C2 records or our occasional recordings in *Tritonia* C2 of the frequent attenuated spike potentials of the A10 homologue. The possible difference in electrical coupling could explain why hyperpolarization of only a single A1 completely suppresses the swim in *Pleurobranchaea* (Jing and Gillette 1995a) but hyperpolarization of both C2s is required to just phase shift the cycle in *Tritonia* (Getting et al. 1980; Taghert and Willows 1978).

Weaker coupling of As2–3 neurons is observed than for the DSI-B-C (Getting 1981), consistent with a less intense spiking during the swim. Also when we compared records in high-divalent saline, chemical connections between A1 and As1–3 appeared weaker than for C2 and the DSIs. As the spike activity of both As1–4/DSIs populations is correlated with cycle period and number of cycles in an episode, this observation is consistent with our impression that the swim cycle of *Pleurobranchaea* averages fewer cycles and shows a broader range of cycle periods (2.4 – 8.8 s).

The apparent conservation of the escape swim and its CPG circuitry in *Pleurobranchaea* and *Tritonia* has interesting implications for molluscan evolution. Anatomical and developmental evidence suggests that nudibranch snails evolved from pleurobranchomorph ancestors with the loss or translocation of the gill and changes in other characters. Reconstruction of a hypothetical ancestor of *Nudibranchia* and *Pleurobranchomorpha* could appear quite similar to a living pleurobranchid (Schmekel 1985). The action pattern of escape swimming behavior is found in multiple *Pleurobranchaea* species of the family *Pleurobranchaeinae*, which lack an internal shell and is not found in the only other pleurobranchomorph family, the *Pleurobranchinae* (cf. Gillette et al. 1991). Thus by extension our data suggest that members of the genus *Pleurobranchaea* most closely resemble the ancestor(s) of the nudibranch radiation from which the tritoniids conserve a primitive escape swimming behavior and CPG. Although we presently cannot exclude the possibility that the animals independently elaborated a similar swim network from homologous cells, such an hypothesis requires more assumptions and so is less likely.

Comparative studies of identifiable neurons and circuits help to understand the evolution of the nervous system (Arbas et al. 1991; Bulloch and Ridgway 1995; Gillette 1991; Katz and Tazaki 1992). In this light, it is of interest to speculate on the evolution of the escape swimming CPG of *Pleurobranchaea* and *Tritonia*. This CPG differs from other molluscan swimming CPGs in being located in the cerebropleural ganglion complex rather than in the paired pedal ganglia. Swimming pattern generation in other opisthobranchs so far investigated emerges from interactions among pedal interneurons mediated via pedal commissural axons, including animals that swim with symmetrical and simultaneous “clap and fling” movements of the parapodia such as *Clione limacina* and *Aplysia brasiliana*, and those that swim with lateral undulations of the body as *Melibe* (Arshavsky et al. 1985a–c; Lawrence 1997; McPherson and Blankenship 1991; Parsons and Pinsker 1988; Satterlie 1985; Thompson 1974). In *Pleurobranchaea* and *Tritonia*, the cerebropleural location of the oscillator CPG suggests that it is derived from premotor neurons that mediate the motor decisions for pedal locomotion, body withdrawal, and turning movements. On the basis of their relative location and axon paths, these neurons are possibly homologous to premotor neurons identified in *Clione* (Panchin et al. 1995; Satterlie and Norekian 1995) and *Aplysia* (Fredman and Jahan-Pawar 1983; Gamkrelidze et al. 1995) that initiate swimming or pedal creeping or modulate ongoing swimming movements.

The present description of the CPG for the escape swim in *Pleurobranchaea* provides a fuller circuitry context in which to probe the alternative expression of feeding and avoidance behaviors at the neural network level. Getting (1989a) suggested that neurons of the swim CPG function outside of the escape swim in reflex withdrawal. Other of our data suggest that the swim neurons are specifically sensitive tonoxious stimuli and contribute to performance of avoidance turns when the escape swim CPG is not active (Jing and Gillette 1995a, 1996; unpublished data). Future research may test the possibility that some CPG neurons and others of the A cluster compose a multifunctional network specifically devoted to mediating a range of avoidance and defensive behaviors.

We thank Drs. Leland Sudlow, Windsor Watson, and Jian M. Ding for technical advice and K. Huang and Y. Zheng for occasional assistance in data processing. Confocal microscopy was performed in the Beckman Institute Visualization Facility. This research was supported by National Institute of Neurological Disorders and Stroke Grant RO1NS-26838.

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Received 22 December 1997; accepted in final form 1 October 1998.

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