Escape Swim Network Interneurons Have Diverse Roles in Behavioral Switching and Putative Arousal in *Pleurobranchaea*

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**Jing, Jian and Rhanor Gillette.** Escape swim network interneurons have diverse roles in behavioral switching and putative arousal in *Pleurobranchaea*. *J. Neurophysiol.* 83: 1346–1355, 2000. Escape swimming in the predatory sea slug *Pleurobranchaea* is a dominant behavior that overrides feeding, a behavioral switch caused by swim-induced inhibition of feeding command neurons. We have now found distinct roles for the different swim interneurons in acute suppression of feeding during the swim and in a longer-term stimulation of excitability in the feeding network. The identified pattern-generating swim neurons A1, A3, A10, and their follower interneuron A-ci1, suppress feeding motor output partly by excitation of the I1 feeding interneurons, which monosynaptically inhibit both the feeding command neurons, PCp, PSE, and other major interneurons, the I2s. This mechanism exerts broad inhibition of the feeding network suitable to an escape response; broader than feeding suppression in learned and satiation-induced food avoidance and acting through a different presynaptic pathway. Four intrinsic neuromodulatory neurons of the swim network, the serotonergic As1–4, add little to direct suppression of feeding. Rather, they monosynaptically excite the serotonergic metacerebral giant (MCG) neurons of the feeding network, themselves intrinsic neuromodulators of feeding, as well as a cluster of adjacent serotonergic feeding neurons, with both fast and slow EPSPs. They also provide mild neuromodulatory excitation of the PCp/PSE feeding command neurons, and I1 and I2 feeding interneurons, which is masked by inhibition during the swim. As1–4 also excite the serotonergic pedal ganglion G neurons for creeping locomotion. These observations further delineate the nature of the putative serotonergic arousal system of gastropods and suggest a central coordinating role to As1–4.

**INTRODUCTION**

Animals distribute their activities among foraging, predator avoidance, and reproduction by switching among behavioral suites, and within suites among their behavioral elements, and by context-dependent behavioral arousal (Tinbergen 1951). Switching and arousal have been studied in a variety of model systems where they are found to act with different time courses and by different mechanisms. Behavioral selection generally has been found to be mediated through motor network interactions (Arshavsky et al. 1994; Edwards 1991; Jing and Gillette 1995; Korn and Faber 1996; Kovac and Davis 1977; Krasne and Lee 1988; Norekian 1997; Shaw and Kristan 1997; Svoboda and Fetcho 1996). Arousal mechanisms tend to involve longer-term effects of neuromodulators in diverse sensory and motor networks that may augment general behavioral activity and reactivity to sensation (Byrne et al. 1991; Carew and Sahley 1986; Gillette and Davis 1977; Gillette et al. 1997; Katz et al. 1994; Kupfermann and Weiss 1981; McPherson and Blankenship 1991; Palovcik et al. 1982; Satterlie and Norekian 1996; Walters 1991; Weiss et al. 1978).

It might be expected that behavioral switching and arousal mechanisms would often act interdependently. We have examined the interdependence of switching and putative arousal mechanisms in the predatory opisthobranch mollusc *Pleurobranchaea californica*, a model animal system previously used for studying aspects of behavioral switching between feeding and avoidance, and for investigating the organization of the putative serotonergic arousal system. A notable example of behavioral switching in *Pleurobranchaea* happens during the escape swim, which is a vigorous and stereotypic avoidance behavior. The swim, often triggered naturally by the touch or bite of a cannibal conspecific, overrides all other ongoing behaviors, including feeding (Davis and Mpitsos 1971; Gillette et al. 1991). In a previous study, we showed that suppression of feeding behavior by swimming is caused partly by swim-induced synaptic inhibition of feeding command neurons whose activity is essential to feeding behavior (Jing and Gillette 1995). We also showed that activation of one critical element of the central pattern generator for the escape swim, the cell A1, caused similar inhibition of the feeding command cells. The swim central pattern generator (CPG) is now known to comprise a set of cells (A1, A3, A10, and I_{iv}s) that generate the swim motor pattern, and a set of serotonergic cells (As1–4) with apparent intrinsic modulatory arousal functions in the pattern generator (Jing and Gillette 1999), similar to the homologous CPG of the nudibranch *Tritonidae diomedeae* (Getting 1989).

We have further investigated corollary outputs of the swim CPG in modulating other CNS circuitry and functions. We find that activation of all the known pattern-generating elements of the swim CPG and of a CPG follower interneuron, A-ci1, potently inhibits the feeding command neurons. Moreover, inhibition of the feeding network by swimming differs from that previously described for feeding suppression resulting from either food avoidance conditioning or satiating animals (Davis and Gillette 1978; Davis et al. 1983; London and Gillette 1986) because inhibition is more widely spread and is complete among the network elements, which may be appropriate to the importance of escape. In contrast, the serotonergic elements of the CPG do not inhibit, but provide long-lasting excitation of major feeding network elements via long-duration EPSPs, which are acutely masked during the swim by phasic inhibition. Moreover, the...
serotonergic elements monosynaptically excite the serotonergic neurons of both feeding and locomotor networks with both phasic and long-duration EPSPs and may thus generally activate much of the rest of the putative serotonergic arousal system of the animal’s CNS. Portions of these data have appeared in abstract form (Jing and Gillette 1998).

METHODS

Specimens of Pleurobranchaea californica (5–400 g) were obtained from Sea-Life Supply (Sand City, CA) and Pacific BioMarine (Santa Monica, CA) and were maintained in artificial seawater at 14°C until use. For isolated nervous system preparations, the CNS, including cerebropleural, pedal, and buccal ganglia, was dissected under cold anesthesia at 4°C and pinned to Sylgard in saline which was composed of (in mM) 420 NaCl, 10 KCl, 25 MgCl$_2$, 25 MgSO$_4$, 10 CaCl$_2$, and 10 MOPS buffer (pH 7.5) at 14°C.

Intracellular and extracellular recordings were done with KCl-filled glass micropipettes and polyethylene suction electrodes, respectively, as previously described (Jing and Gillette 1995). Data were recorded on chart recorder (Gould TA11; sampling rate, 250 kHz) and were videotaped for later examination and figure preparation. Functional synaptic connections were examined in normal saline for PSP ability to follow presynaptic spikes one-for-one as a criterion of probable monosynapticity and were examined in high-divalent saline where MgCl$_2$ was increased to 125 mM and CaCl$_2$ to 30 mM by replacing NaCl on an equimolar basis to elevate spike thresholds and curtail polysynaptic activation. Electrical coupling was assayed by passing hyperpolarizing current into one cell and measuring steady state polarization in its partner. Steady-state coupling coefficients were measured as ratios of post- to presynaptic voltage change.

In the isolated CNS, the swimming motor program was 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 are functionally equivalent to noxious shocks of the dorsal mantel in their ability to induce swims. The fictive feeding motor program was elicited by intracellular stimulation of feeding command neurons, the PC$_P$s and PSEs, and the resulting rhythmic feeding output was recorded in nerve root 3 of the buccal ganglion.

Cell identification

The neurons of the feeding and escape swimming networks studied here were all located in the cerebropleural ganglion and identified on the basis of position, color and known physiology. Figure 1 shows the relative positions of the neuron cell bodies. The feeding command neurons were identified by their spontaneous and prominent IPSPs, axon spike recorded in the ipsilateral CBC, and the ability to drive fictive feeding (Gillette et al. 1982). The PSEs were identified with similar axon spike recorded in the ipsilateral CBC, and the ability to drive fictive feeding motor program was elicited by intracellular stimulation of feeding command neurons, the PC$_P$s and PSEs, and the resulting rhythmic feeding output was recorded in nerve root 3 of the buccal ganglion.

FIG. 1. Line drawing of the dorsal surface of the cerebropleural and pedal ganglia indicating locations of identified neuronal somata of networks for feeding (inset: MCG, PC$_P$, PSE, PC$_T$, 2 I1s, and 3 I2s), escape swimming (inset: A1, A1–4, A10, A3, and A-ci1), and locomotion (G neuron cluster). Filled circles: serotonin immunoreactive somata. Whereas bilaterally symmetrical, somata are only shown unilaterally for convenience. Cell abbreviations: MCG, metacerebral giant neuron; PC$_P$, phasic paracerebral interneuron; PSE, polysynaptic excitor of the PC$_P$; PC$_T$, tonic paracerebral interneuron; I1, Interneuron 1; and I2, Interneuron 2. Nerve abbreviations, cerebropleural ganglion: 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, rhinophoral nerve; SCC, subcerebral commissure; and TN, tentacle nerve. Pedal ganglion: aLBWN, anterior lateral body wall nerve; pLBWN, posterior lateral body wall nerve; PC, pedal commissure; pPC, parapedal commissure; aPN, anterior pedal nerve; mPN, medial pedal nerve; and pPN, posterior pedal nerve.

most, if not all, of these neurons are 5-HT immunoreactive (Sudlow et al. 1998).

Intracellular staining

To view neuron morphology, cells were intracellularly injected with biocytin or neurobiotin (Vector, Burlingame, CA) from the recording electrode and processed as previously described (Jing and Gillette 1995) for whole-mount viewing. These procedures allowed staining of axon processes ±2 cm from injection site after ganglia were incubated overnight at 8°C.

RESULTS

The interneurons of the escape swimming network and an output interneuron

Known premotor neurons that either compose the central pattern generator for escape swimming or receive outputs from
it are found on the dorsal cerebral region of the cerebropleural ganglion, in a group called the A cluster (Fig. 1). The swim CPG is composed of at least 8 bilaterally paired neurons: the A1/A10 ensemble, the serotonergic ensemble of As1–4, A3, and the IVS which is as yet unlocated (Jing and Gillette 1999). We previously showed that the motor pattern of the swim was generated largely from the synaptic interactions of the dorsal flexion swim interneurons A1/A10 and As1–4 with the ventral flexion interneurons A3 and IVS. A major contribution of the As1–4 neurons, which are active during dorsal flexion, appears to be neuromodulatory excitation that drives multiple cycles of swimming in the pattern generator neurons. An important output of the CPG is a commissural interneuron of the A cluster, A-ci1 (soma diam 40–65 μm; Jing and Gillette 1999), whose single axon crosses to the contralateral cerebral lobe and ramifies in the neuropil of both sides (Fig. 2A). A-ci1 was rhythmically active during the dorsal flexion phase of escape swimming (Fig. 2B; n = 18). The neuron lacked effective inputs to the swim CPG; neither hyperpolarizing nor driving single A-ci1 neurons had detectable effects on the swim pattern (n = 4) and thus it is an output but not an element of the CPG. A-ci1 was excited by driven activity in the ipsilateral A1 and A10 (Fig. 2C; n = 8). A-ci1 was less reliably excited by As1–3 activity in normal saline (n = 6 of 12). All these connections were eliminated in high-divalent saline (n = 5), suggesting a polysynaptic pathway. The connection to A-ci1 from the contralateral As1–3 was somewhat stronger than the ipsilateral. Thus the strong bursting activity of A-ci1 during swims may largely result from the summed inputs of all the known oscillator neurons, and the cell might receive additional inputs from as yet unidentified elements.

The seven swim interneurons and A-ci1 act on the feeding network with diverse effects, as described in the following two sections.

Inhibition of feeding command neurons by A1/A10, A3, and A-ci1

Feeding behavior is inhibited during swim episodes in part by spike activity in the swim neuron A1, which was previously shown to activate a strong polysynaptic inhibitory pathway to the PCp feeding command neurons (Jing and Gillette 1995)
were also exerted on the PCp s and PSEs by A-ci1, the output activity is, by itself, significant. Powerful inhibitory effects the PCp s and PSEs (Kovac et al. 1983b; London and Gillette interneurons. The I1 interneurons, monosynaptic inhibitors of neurons was mediated monosynaptically by the identified I1
barrages coincide with both dorsal and ventral flexion (Jing and Gillette 1995), indicating a probable polysynaptic origin.

During the swim episode the feeding command cells receive biphasic inhibition during each swim cycle, such that IPSP barrages coincide with both dorsal and ventral flexion (Jing and Gillette 1995). Prolonged stimulation of A1 or A10 (>10 s) often induced cyclic barrages of IPSPs in the PCp s and PSEs (Fig. 3A; for A1, n = 8 of 18; for A10, n = 12 of 15), consistent with the abilities of those neurons to drive the swim motor program. The IPSP barrage in the ventral flexion phase may be driven partly by A3 (Fig. 4), whose swimming activity coincides with the transition from dorsal to ventral flexion (Jing and Gillette 1995, 1999).

The strong electrical coupling between A1 and A10 made it difficult to separate the contributions of the individual cells to inhibition of the feeding command cells because depolarization or hyperpolarization of either cell would affect the other cell’s synaptic outputs too. However, spike activity in a single A10 comparable to its firing rate during swim episodes (~16 Hz) significantly inhibited driven activity in a PCp and suppressed feeding motor output driven by the feeding command neuron (Fig. 5; n = 4). Thus whatever the exact contributions, A10 activity is, by itself, significant. Powerful inhibitory effects were also exerted on the PCp s and PSEs by A-ci1, the output neuron of the swim CPG (Fig. 6A; n = 9).

Inhibition of the feeding command neurons by the swim neurons was mediated monosynaptically by the identified I1 interneurons. The I1 interneurons, monosynaptic inhibitors of the PCp s and PSEs (Kovac et al. 1983b; London and Gillette 1984), were weakly excited by A1/A10 (n = 3) and more strongly by A-ci1 (n = 3). Discharge of A1/A10 caused moderate depolarization in I1s that occasionally induced spik-

![Fig. 4. Inhibition of PCp and PSE by the swim interneuron A3 in normal saline. A3 stimulation induced similar IPSPs from common presynaptic inhibitors of the feeding command cells. Calibration bar: vertical, 40 mV for top record; horizontal, 3 s.](image)

(Fig. 1). We found here that cells A10, A3, and A-ci1 also inhibit the PCp s as well as nearby and similar feeding command neurons, the PSEs (Kovac et al. 1983a). The inhibition caused by A1, A10, and A-ci1 was somewhat stronger than that caused by A3 (Figs. 3–6). High-divalent saline abolished the IPSPs (cf. Jing and Gillette 1995), indicating a probable polysynaptic origin.

In contrast to the inhibition of the feeding command neurons caused by the pattern-generating swim neurons, the effects of discharging the serotonergic As1–4 neurons on the PCp and PSE were predominantly long-lasting depolarization that occasionally induced spiking (Fig. 7; n = 14). During the swim episode, these effects must be masked by the strong inhibition driven by the other pattern-generating swim neurons. As1–4, and in particular As2/3, also excited both the I2s (n = 5) and the I1s (Fig. 7; n = 3). The excitation of the I2s was largely caused by monosynaptic IPSPs from I1s, simultaneous with inhibition in the feeding command neurons (Fig. 3B); this observation provides functional significance to a negative feedback connection from the I1s to the I2s reported earlier but not understood (London and Gillette 1984) (see DISCUSSION).

**Long-lasting excitation of feeding neurons by serotonergic swim neurons**

In contrast to the inhibition of the feeding command neurons caused by the pattern-generating swim neurons, the effects of discharging the serotonergic As1–4 neurons on the PCp and PSE were predominantly long-lasting depolarization that occasionally induced spiking (Fig. 7; n = 14). During the swim episode, these effects must be masked by the strong inhibition driven by the other pattern-generating swim neurons. As1–4, and in particular As2/3, also excited both the I2s (n = 5) and the I1s (Fig. 7; n = 3). The excitation was slow in onset and long-lasting. The excitatory effects from As2/3 were variable among preparations, but usually caused spiking in the I2s. It is not known whether these connections are mono- or polysynaptic.

A particularly notable observation was the excitation of the MCG by the serotonergic swim interneurons. Direct stimula-

![Fig. 5. Activation of a single A10 suppresses fictive feeding in the isolated CNS. Driving the PCp induced rhythmic feeding motor output monitored in cerebrobuccal connectives (CBC) and buccal motor root 3 (R3). Driving A10 caused inhibition in the PCp and halted cyclic feeding bursts in R3. Intense spiking in PCp and the feeding motor program in R3 resumed on termination of A10 spike activity.](image)
tion of As2/3 (Fig. 8) or indirect stimulation by nerve shock (cf. Fig. 2B and Jing and Gillette 1999) induced spontaneous slow bursting activity of the cell at \( \approx 3 \) Hz, which lasted for minutes and coincided with induction of similar slow and spontaneous rhythmic activity in the MCGs (Fig. 8). Excitatory connections to the contralateral MCG were stronger than for the ipsilateral MCG, with EPSPs that followed As2/3 spikes one-for-one. In high-divalent saline both fast and slow excitatory connections from As1–4 to the contralateral MCG remained (Fig. 9). Typically, the EPSPs from As2/3 to the contralateral MCG (\( n = 5 \)) were the strongest of As1–4, with amplitudes ranging from 0.6 to 1.2 mV for the fast, unitary EPSPs and from 1.5 to 6 mV for the slow EPSPs with durations \( \leq 20 \) s and latencies to peak of 2–3 s. The fast components of the EPSPs from As1 and As4 to the MCG (\( n = 4 \)) could not usually be resolved (Fig. 9B), presumably because the EPSPs were too small and/or distant from the recording site; their slow EPSPs were also smaller, ranging from 0.7 to 1.5 mV. The As2/3 connections to the ipsilateral MCG in the normal saline were variable and decremented markedly with repeated tests; only a weak slow EPSP \( \sim 0.6 \) mV from one of the As2/3 pair remained in high-divalent saline (Fig. 9A; \( n = 3 \)). The weaker and more variable effects of the serotonergic swim neurons on their ipsilateral MCGs were probably mediated by the electrical and chemical coupling of contralateral As1–4 populations (Jing and Gillette 1999) and/or weak electrical coupling between the bilateral MCGs (coupling ratio, \( \sim 0.015 \)).

Adjacent to the MCG lies a cluster of medium to large 5-HT immunoreactive somata (80–150 \( \mu \)m) (Sudlow et al. 1998). Some of these neurons are electrically coupled to the MCG, burst cyclically in the retraction phase of the feeding motor program, and send axons to the oral veil (Gillette and Davis 1977). The cells of this cluster that we sampled received chemical excitation from As2/3 (Fig. 10; \( n = 9 \) pairs in 3 preparations) like the MCG. These cells were also weakly electrically coupled to As2/3 of both ipsilateral and contralateral sides (coupling ratios 0.008–0.017). Other neurons adjacent to the MCG, but not electrically coupled to As2/3, were not excited by As2/3 (Fig. 10B).

The excitation of the MCGs and its neighbors by the As1–4 led us to examine relations with another major serotonergic neuron population, the G neuron cluster on the dorsal side of the pedal ganglion (Sudlow et al. 1998). The pedal G neurons are a cluster of 20–25 large serotonergic neurons; more than one-half of them were weakly electrically coupled, with coupling ratios ranging from 0.008 to 0.033 (\( n = 14 \) pairs in 4 preparations). No chemical connections among them were observed in high-divalent saline.

In high-divalent saline, As1–4 excited nearly all G neurons sampled in the contralateral pedal ganglion to varying degrees (\( n = 19 \) recordings in 4 preparations), with fast and slow EPSPs like those in the MCG (Fig. 11). The effectiveness of individual As1–4 differed from the pattern seen with the MCG. As4 had the largest excitatory effects on the G neurons, with fast unitary EPSPs ranging 0.4–1.2 mV and slow EPSPs 1.5–4.1 mV, durations of 18–24 s, and times-to-peak of 3–5 s. In only a single case did we find a neuron located in the G neuron area, on the medial edge of the ganglion adjacent to the pedal commissure, that was inhibited by As1–4.

Reciprocal connections to As1–4 from the MCG, its neighbors or the pedal G neurons were not found, other than the weak electrical coupling of As2/3 with the MCG neighbors.
Thus most connections between swim interneurons As1–4 and other identified serotonergic neurons in the feeding and locomotor networks of Pleurobranchaea originated from As1–4.

Effective connections from A1, A10, and A-ci1 to the MCGs were weakly inhibitory. Weak inhibition of the MCG by A1 in normal saline ($n = 3$, Fig. 12A) was eliminated in high-divalent saline, whereas a weak, slow IPSP from A-ci1 remained (Fig. 12B). In normal saline, A1 excited the G neurons in the contralateral pedal ganglion, possibly acting partly through its excitatory connections to As1–4 (Jing and Gillette 1999). In high-divalent saline, the connections from A1 to the pedal G neurons were still excitatory, but weak (compound EPSPs of 0.3–0.8 mV) and variable.

**DISCUSSION**

The motor network underlying the escape swim of Pleurobranchaea comprises two distinct sets of neurons with largely separate pattern generating and neuromodulatory functions. This study further distinguishes the two neuron sets on the basis of their corollary outputs during swim pattern generation. The set primarily responsible for generating the swim pattern itself, neurons A1, A10, A3, and their output cell A-ci1, are found to mediate the potent inhibition of feeding behavior during the swim. The serotonergic set of As1–4 neurons appears to have a neuromodulatory role outside of the swim network; they monosynaptically excite other serotonergic cells with both fast and slow EPSPs in both feeding and locomotor networks. The slow excitation of the feeding neurons is occluded by inhibition during the swim but is significant in the postswim period when As1–4 spontaneous discharge continues at a relatively high rate.

FIG. 9. As1–4 more strongly excited the contralateral than ipsilateral MCG. Recordings in high-divalent saline. A: connections from As2 and As3 in the same preparation to the contralateral MCG (c-MCG) had two components; a fast unitary EPSP following presynaptic spikes one-for-one and a slow EPSP lasting $>30$ s. One of the As2–3 pair also excited the ipsilateral MCG (i-MCG) but with lesser intensity (A1). B: A1 excitation of the contralateral MCG. A fast EPSP in the c-MCG was not distinguishable. Calibration bar: vertical, 40 mV for top record, 4 mV for bottom records; horizontal, 3 s.

FIG. 10. As2–3 excited both ipsilateral and contralateral neurons of the cluster adjacent to the MCG. All recordings were from the same preparation in high-divalent saline. A: As2 and As3 excited an ipsilateral MCG neighbor (i-Mn) with both fast unitary EPSPs and a slow depolarizing component lasting $>30$ s. B: As2 similarly excited a contralateral MCG neighbor (c-Mn1) that was weakly electrically coupled to As2 but did not excite an adjacent cell, c-Mn2, to which it was not electrically coupled. A third cell, c-Mn3, also excited by As2 and i-Mn were both electrically coupled to As2 (not shown). Bottom: positions of the cells recorded in the experiment (dorsal view). Calibration bar: vertical, 40 mV for top record, 4 mV for bottom records; horizontal, 3 s.

**Suppression of feeding motor output by the escape swimming CPG**

A repeated theme in mechanisms of behavioral selection in molluscs, annelids, arthropods, and fish is the ensurance of...
unitary behavioral expression through inhibition between the neural networks underlying conflicting behaviors (Edwards 1991; Jing and Gillette 1995; Korn and Faber 1996; Kovac and Davis 1977; Krasne and Lee 1988; Norekian 1997; Shaw and Kristan 1997; Svoboda and Fetcho 1996). Concordantly, we found that the dominance of escape swimming in the animal's behavioral repertory arises from inhibition of the feeding motor network by corollary outputs of the swim CPG. In the present study, two features of the neural inhibition of feeding by swimming have emerged. 1) Inhibition of the feeding CPG is specifically exerted by the pattern-generating subset of swim interneurons, and 2) the inhibition itself is more broadly distributed in the feeding network than previously realized. Earlier it was shown that expression of the escape swim was accompanied by cyclic inhibition in the feeding command neurons; barrages of IPSPs were phase-locked to the dorsal and ventral phases of the swim cycle (Jing and Gillette 1995). In that same study we found that discharge of the A1 neuron, normally active during dorsal flexion, caused polysynaptic inhibition of the feeding command cells. This study adds A10, A3, and A-ci1 to the cells that mediate inhibition of feeding by the swim. The fuller picture of the degree and scope of suppression of feeding by the swim is summarized in Fig. 13. Some intervening interneurons remain to be found.

A large part of the feeding inhibition is mediated by the A-ci1 interneuron, an output of the swim CPG. A-ci1 is activated polysynaptically by the A1/A10 ensemble to fire a burst of spikes during dorsal flexion. The axon paths of A-ci1 pass through neuropil areas where critical neurons of the feeding network (the I1s and I2s) ramify. Functionally, A-ci1 resembles the CD interneurons of the feeding network that suppress oral veil withdrawal by corollary discharge during feeding (Kovac and Davis 1977, 1980). Thus our observations agree with earlier speculation that in many instances behavioral choice is mediated by inhibitory interactions between competing motor systems, at the level of higher order interneurons (Kovac and Davis 1977, 1980).

**FIG. 12.** A1 and A-ci1 weakly inhibited the MCG. A: in normal saline A1 transiently inhibited spontaneous firing of the ipsilateral MCG. B: a weak, slow IPSP from A-ci1 to the ipsilateral MCG was present in high-divalent saline. Calibration bar, vertical, 40 mV except bottom record of B, 2 mV; horizontal, 6 s for A, 3 s for B.

**FIG. 13.** Summary diagram of synaptic actions between the networks for escape swimming and feeding, and the locomotor neurons. Escape swimming network is composed of the neurons A10, A1, As1–4, A3, and IVS. Relevant identified neurons of the feeding network are the command neurons PCp and PSE, the I2 and I1 interneurons, and the MCG and adjacent serotonergic neighbors of the cerebropleural ganglion. The rest of the feeding network is represented as a generalized half-cell oscillator shown as populations of retraction (R) and protraction neurons (P). Suppression of feeding by escape swimming is largely caused by inhibitory inputs to PCp, PSE, and the I2s from the nonserotonergic swim neurons A1, A10, and A3, at least partly through the follower neuron A-ci1. "?" indicates other unidentified cells that may also contribute to the excitation of I1 interneurons. Serotonergic cells As1–4 and MCG and its neighbors are embedded as intrinsic neuromodulators in their respective swim and feeding motor networks. The locomotor G neurons are all serotonergic, as far as is known. As1–4 swim interneurons provide potent monosynaptic, neuromodulatory excitation to the G neurons and to the MCG and its neighbors and weaker excitation to the I2s and the I1s. Thus transient inhibition of the feeding network during the swim is succeeded by an underlying long-lasting, serotonergic excitation. Weak and variable excitation of PCp and PSE by As1–4 is not shown. Synaptic connections described in earlier reports (Gillette and Davis 1977; Gillette et al. 1982; Jing and Gillette 1999; Kovac et al. 1983a,b, 1986; London and Gillette 1984) are shaded. Switching vs. arousal.
Like suppression of feeding by the swim, learned and satiation-induced suppression of feeding behavior is also based on synaptic inhibition of the feeding command neurons, the PC's and PSEs, by the I1 population (cf. Davis and Gillette 1978; London and Gillette 1984, 1986). However, the inhibition from the swim differs significantly from that caused by food-avoidance training and satiation. In learned and satiation-induced suppression of feeding the I1s are monosynaptically driven by tonic activity in the I2 population, themselves hyper-excited by food stimuli. In contrast, during the swim the I2s are inhibited and the I1s must receive their excitation from a different source.

The differences in neural mechanisms of feeding inhibition between swimming and learning/satiation are reflected in behavioral function. The I2s are major interneurons that during feeding fire cyclically in the radular retraction phase of the protraction/retraction cycle and drive many other retraction neurons (London and Gillette 1984). In learning- or satiation-induced inhibition of feeding, the I2s fire tonically to virtually lock feeding network activity in the retraction phase of the feeding cycle (London and Gillette 1986). It is notable that in this mechanism for feeding suppression a great deal of excitation is still present in the oscillator, and patterned feeding activity can still be released suddenly if feeding stimuli are increased to high levels (Davis and Gillette 1978). In contrast, during swimming the inhibition of the elements of the feeding oscillator appears to be much broader than that occurring due to learning or satiation, because during the swim the I2s were inhibited. Thus during swimming both major retraction I2 interneurons and the major protraction paracerebral interneurons were effectively removed in a broad shutdown of the feeding motor network. The inhibition of the I2 neurons during the swim must arise partly from monosynaptic inhibitory inputs from the I1s (London and Gillette 1984), whose significance has been unappreciated until now.

The adaptive significance of the two mechanisms of feeding suppression may be that more complete inhibition of feeding should be desirable during the escape swim, but the feeding inhibition due to learning or partial satiation should be more subject to cost-benefit decisions based on feeding stimulus quality and incentive. Thus the decision to swim instead of feed is virtually irreversible; whereas in feeding suppression by learning or satiation, active feeding can be rapidly released once an elevated stimulus threshold is exceeded.

Coupling within the putative serotonergic arousal system: intrinsic neuromodulatory and internetwork coordinating roles for As1–4

In molluscs, 5-HT is considered a general arousal factor (Kupfermann and Weiss 1981; Sakharov 1990), a view supported by its neuromodulatory roles in diverse sensory and motor networks (Gillette and Davis 1977; Katz et al. 1994; Kupfermann and Weiss 1981; McPherson and Blankenship 1991; Satterlie and Norekian 1996; Walters 1991; Weiss et al. 1978), its involvement in both short and long-term sensitization of defensive avoidance reflexes (Byrne et al. 1991; Carew and Sahley 1986), and its augmentation of general behavioral activity following artificial elevation of the animal’s serotonin level (Gillette et al. 1997; Palovcik et al. 1982).

In Pleurobranchaea, exogenous 5-HT generally increases spontaneous activity and reactivity to sensory stimuli, notably lowers feeding thresholds in intact animals, and stimulates fictive feeding activity in the isolated CNS (Gillette et al. 1997). The arousal actions of 5-HT in the molluscs partly resemble those in the mammalian CNS, where descending serotonergic fibers from the raphe nuclei stimulate the excitability of motor networks in motor nuclei and spinal cord. However, the molluscs appreciably contrast with mammals in that their serotonergic neurons are embedded in the various motor networks as intrinsic elements (Gillette and Davis 1977; Katz and Frost 1996; McPherson and Blankenship 1991; Weiss et al. 1982), such that neuromodulatory motor arousal could be affected through activity in the various sub-systems serving feeding, locomotor, and defensive behaviors. This study partly defines and elucidates the organization of a CNS-wide serotonergic arousal system in which the As1–4 may play a central role.

The results suggest that the serotonergic arousal system comprises distributed but coupled arousal subsystems for defensive reactions and feeding and show how they are linked together (Fig. 13). Highlighted in particular are dual roles for As1–4 as 1) intrinsic neuromodulatory components of the swim pattern generator and as 2) hierarchic central organizers of a neural network that we expect contributes to general arousal. As1–4, acting largely as excitatory neuromodulators, participate in neural networks for multiple defense-related behaviors including escape swimming, locomotion, and avoidance turning (Jing 1998; Jing and Gillette 1996), and perhaps reflexive withdrawal as do the homologous neurons of Tritonia (Getting and Dekin 1985). As well, As1–4 provide direct excitation to serotonergic elements of the feeding arousal system, the MCGs and the adjacent serotonergic cluster. The MCGs are embedded in the feeding motor network where they act as intrinsic neuromodulators; the adjacent, coupled serotonergic cluster may have a similar role, because these cells are likewise activated during feeding motor output (Gillette and Davis 1977).

The serotonergic As1–4 provide intrinsic neuromodulatory excitation to the swim CPG that sustains the burst episode through multiple swim cycles (Jing and Gillette 1999) like the DSI neurons in the homologous escape swimming network of Tritonia (Katz and Frost 1997; Katz et al. 1994; Lennard et al. 1980). We have also shown that As1–4 drive the serotonergic locomotor G neurons of the pedal ganglion. The G neurons correspond to serotonergic locomotor neurons in pedal ganglia of other species: excitors of ciliary locomotion in Tritonia (Audesirk et al. 1979) and Lymnaea (Syed and Winlow 1989), of both parapodal swimming and pedal muscular wave locomotion in Aplysia (McPherson and Blankenship 1991, 1992), and of parapodal locomotion in the pteropod Clione (Satterlie 1995). The combined phasic and tonic excitation of the serotonergic locomotor cells by As1–4 resembles the premotor neuromodulatory excitation of the locomotor cells in Clione noted by Satterlie and Norekian (1996) and Panchin et al. (1996) and may represent evolutionary conservation of neural circuit and function in the face of changing structure and function in the periphery, which was suggested for evolution of leech feeding mechanisms (Lent et al. 1989). The premotor role of the As1–4 in locomotion may be manifested in their prolonged activity after a swim, a period during which Pleurobranchaea displays rapid creeping locomotion (Gillette et al.
1991). The homologous DSI neurons of Tritonia show similar activity (Getting et al. 1980; Katz et al. 1994) and can indeed initiate locomotion (W. Frost and I. Popescu, personal communication).

It was notable to find that the As1–4 provide potent phasic and tonic excitation to serotonergic cells of the feeding network. The MCGs, known mediators of feeding arousal in diverse gastropods (Gillette and Davis 1977; Granzow and Fraser-Rowell 1981; Rosen et al. 1989; Weiss et al. 1982; Yeoman et al. 1996), innervate Pleurobranchaea’s buccal ganglion, buccal musculature and lip area, and stimulate the feeding oscillator network of the buccal ganglion (Gillette and Davis 1977). The As1–4 similarly drive the adjacent and coupled anterior serotonergic cerebral cluster which innervates the oral veil musculature and chemosensory epithelium (Moroz et al. 1997), both of which are involved in motor and sensory aspects of feeding behavior. Thus the neuromodulatory role of As1–4 may extend across defensive and locomotor behaviors to feeding.

Also consistent with neuromodulatory stimulation of the feeding network was the finding that As1–4 caused slow excitation of the major I2 retractor interneurons of the feeding network. Cyclic activity in the I2s is necessary to the feeding motor pattern and tonic activity underlies the suppression of feeding in food-avoidance trained and satiated animals (see the first section of DISCUSSION). Because the As1–4 are excited by electrical shocks like those used in the food-avoidance learning paradigm, we speculate that through their potentiation of I2 activity they might have roles in the elevation of feeding thresholds in food-avoidance conditioned animals (Mptisos and Davis 1973). The I2s also directly phasically excite the MCGs (London and Gillette 1984), thus adding another feed-forward path in the putative serotonergic arousal system.

The model diagram of Fig. 13 summarizes our observations to distinguish the distinct contributions of the pattern-generating and serotonergic cells of the swim CPG to suppression of feeding (behavioral switching) and neuromodulatory stimulation of the feeding and locomotor networks (putative behavioral arousal). In it, corollary outputs from the pattern-generating cells inhibit critical neurons of the feeding motor network. The serotonergic cells, themselves embedded in the swim CPG, distribute output to other serotonergic neurons embedded in feeding CPG and composing the locomotor network, thereby enhancing the potential for those behavioral expressions. The cells As1–4, potentially contribute to the general level of network activity in feeding and locomotion whether or not the escape swim occurs, because their prolonged activity following noxious stimulation is not dependent on the occurrence of the swim (Jing 1998). These observations may provide a useful example of the coordination of mechanisms of behavioral switching and distributed network neuromodulation that potentially underlies general behavioral arousal.

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