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NEURAL MECHANISMS OF MOTOR PROGRAM SWITCHING IN THE MOLLUSC *PLEUROBRANCHAEA*

III. Role of the Paracerebral Neurons and Other Identified Brain Neurons[1]

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Abstract

Identified neurons in the cerebropleural ganglion (brain) of the mollusc *Pleurobranchaea* were stimulated and recorded from intracellularly while recording the identified motor program from buccal muscles (reduced preparation) or nerves (isolated central nervous system). Neurons studied included the metacerebral giant neurons (MCGs), phasic paracerebral neurons (PC_P's), polysynaptic excitors of the PC_P's (PSEs), type II electrotonic neurons (ET_{II}'s), type I electrotonic neurons (ET_I's) and several other identified neurons or neuronal classes. Intracellular stimulation of the above identified neurons generally elicited the ingestion motor program or its characteristic components, but never the egestion motor program and seldom its characteristic components. Intracellular recordings from these neurons in the isolated central nervous system preparation while eliciting the ingestion and egestion motor program generally showed cyclic membrane potential oscillations in phase with both motor programs, indicating that these neurons receive synaptic feedback from the ingestion and egestion central pattern generator(s). This study is therefore consistent with the view that an interrelated cluster of brain neurons is specialized to command the ingestion motor program. A neural model of motor program switching in the buccal motor system is formulated, comprising separate command pathways for ingestion and egestion that converge on a common central pattern generator(s).

The cerebropleural ganglion ("brain") of the mollusc *Pleurobranchaea* contains 18 identified descending interneurons that meet certain criteria for command neurons controlling feeding behavior. These interneurons include the paired metacerebral giant neuron (MCG) (Gillette and Davis, 1977) and the paracerebral neurons (PCNs) (Davis et al., 1974; Gillette et al., 1978). The PCNs are in turn subdivided into four classes: the tonic PCNs (PC_T's) (two neurons per hemiganglion; Kovac et al., 1982), the phasic PCNs (PC_P's) (two neurons per hemiganglion; Kovac et al., 1982), the polysynaptic excitors of the PC_P's (PSEs) (two neurons per hemiganglion; Kovac et al., 1983a), and the type II electrotonic neurons (or ET_{II}'s) that are electrically coupled with the PC_P's (two neurons per hemiganglion; Kovac et al., 1983a). Intracellular stimulation of the PCNs induces cyclic feeding movements in whole animal preparations, and all PCNs except the PC_T's discharge cyclically and in phase with feeding movements when feeding behavior is elicited by food stimuli (Gillette et al., 1978, 1982; Kovac et al., 1982, 1983a). Therefore, the PCNs meet two of the three general criteria (see Croll et al., 1985b, for discussion) for feeding command interneurons, namely sufficiency and appropriateness. These neurons are not individually necessary to feeding, presumably because the command role is distributed over a large population of interneurons.

The conclusion that these brain neurons are command interneurons for feeding behavior was reached before the discovery of functional subdivisions within the PCN population (Kovac et al., 1983a) and before establishing detailed criteria for distinguishing the ingestion motor program from the egestion motor program on the basis of recorded parameters of motor output (McClellan, 1980, 1982a, b; Croll and Davis, 1981, 1982; Croll et al., 1985a). In view of these recent developments, the present studies were undertaken to re-examine the hypothesis that these brain neurons serve as command interneurons for feeding, as opposed to some other buccal behavior. This re-examination has taken two forms. First, we have studied the motor effects of the identified brain neurons by stimulating them individually while recording the resultant motor output patterns in the reduced preparation and isolated central nervous system (CNS). We applied the criteria developed earlier (Croll and Davis, 1982; Croll et al., 1985a) to rigorously identify the motor program(s) elicited. Second, we have recorded the activity of these neurons individually while eliciting the motor patterns of ingestion and egestion, using techniques developed earlier (Croll and Davis, 1982; Croll et al., 1985a).

The results show that the identified members of the paracerebral population, as well as the MCG, elicit the ingestion motor

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program or its characteristic components but never the egestion motor program and seldom its characteristic components. That is, these brain neurons are individually sufficient to elicit the ingestion motor program but not the egestion motor program. These experiments thus furnish corroborative evidence for the role of these brain neurons in mediating feeding behavior (ingestion) but not other buccal behaviors (e.g., egestion). In combination with the results of the first two papers of this series (Croll et al., 1985a, b), these results permit formulation of a neurophysiological model for motor program switching in the buccal motor system of *Pleurobranchaea* based on the activation of different command pathways that converge on a common central pattern generator(s).

Materials and Methods

General methods, as well as the procedures for making the reduced and isolated CNS preparations, were identical to those described in the first paper of this series (Croll et al., 1985a). Electrophysiological methods used here include the extracellular techniques described previously (Croll et al., 1985a) and intracellular stimulation/recording from identified neurons in the cerebropleural ganglion (brain). Identification and intracellular analysis of brain neurons were achieved as described previously (Gillette and Davis, 1977; Gillette et al., 1978, 1982; Kovac et al., 1982, 1983a, b). Buccal motor programs for ingestion, egestion, and the neutral rhythm were identified using criteria developed in the first paper of this series (Croll et al., 1985a) and enumerated under "Materials and Methods" of the preceding paper (Croll et al., 1985b).

Results

The metacerebral giant neuron(s)

The MCGs are an identified pair of neurons positioned symmetrically on each side of the cerebropleural ganglion (brain). Each soma lies on the anterior edge of the brain near the midline (Gillette and Davis, 1977), and the neurons send descending axons to the buccal ganglion via the ipsilateral cerebrobuccal connective (CBC) (Gillette and Davis, 1977). Previous work (Gillette and Davis, 1977) has shown that the MCG can elicit cyclic motor output from the buccal ganglion and that the MCG fires in phase with ingestion movements in semi-intact animals. To corroborate the role of the MCG, its effect on motor output and its activity during motor output were examined here in both the reduced preparation and in the isolated CNS.

Effects of intracellular stimulation. Of four trials on two MCGs in two reduced preparations, intracellular stimulation of the MCG caused ingestion of an object placed into the buccal cavity in one trial (Fig. 1). The direction of movement of the object was monitored with a position monitor (see "Materials and Methods" in Croll et al., 1985a), providing a behavioral confirmation of the elicited motor program. In this experiment, stimulation of the MCG increased the cyclic activity of buccal constrictor m 5 and decreased the activity of protractor m 4 (Fig. 1), characteristic of the ingestion motor program. Of the remaining three trials, one showed the ingestion motor program but no net movement of an object in the buccal cavity, one showed increased motor activity but no cyclic output, and one showed no effect.

The MCG was stimulated intracellularly in five trials in two cells in two isolated CNS preparations. Such stimulation caused cyclic motor output in three trials in two MCGs in two preparations. The salivary duct (SD) discharged cyclically in all of these trials, but SD bursts did not occur in phase with protraction, as is characteristic of the ingestion motor program. Of the remaining two trials, MCG stimulation enhanced motor output but did not cause bursting in one case, and caused no effect in the second case. From these experiments on the reduced preparation and the isolated CNS it may be concluded that the MCG can be sufficient to the characteristic components of the ingestion motor program but not the egestion motor program or its characteristic components.

Intracellular activity during buccal motor programs. Intracellular recordings from the MCG were made only in the isolated CNS preparation. In three trials in three MCGs in three preparations, the MCG showed activity during the ingestion motor program. Figure 2, for example, shows an ingestion episode elicited by tonic extracellular stimulation of the right CBC. The fast burster unit of the salivary nerve (SN) fired in phase with protraction, confirming the motor program as that of ingestion. The right MCG shows 1:1 antidromic spikes, as expected from stimulation of its large descending axon in the ipsilateral CBC (Gillette and Davis, 1977). Activity in the left MCG was converted from tonic to weakly cyclic discharge, although the phase relationship with ongoing motor activity was variable. These results were typical of all three preparations.

During the egestion motor program (Fig. 3), elicited by appropriate stimulation of the stomatogastric nerves (SGNs) and

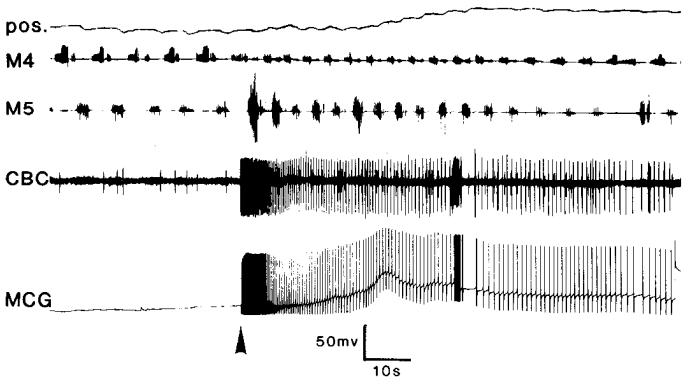

Figure 1. Effects of depolarizing (beginning with *arrowhead*) one MCG on behavior and motor output of the reduced preparation. The position trace (*pos.*) shows the output of a modified Sandeman position transducer that recorded the position of an object (a plastic worm; see Croll and Davis, 1982; Croll et al., 1985a) placed into the buccal cavity, with upward deflections signifying inward (ingestive) movements. *M4* and *M5*, extracellular activity in a buccal protractor and constrictor muscle, respectively. *CBC*, extracellular activity recorded from the ipsilateral cerebrobuccal connective. The large unit in the CBC recording corresponds to MCG spikes.

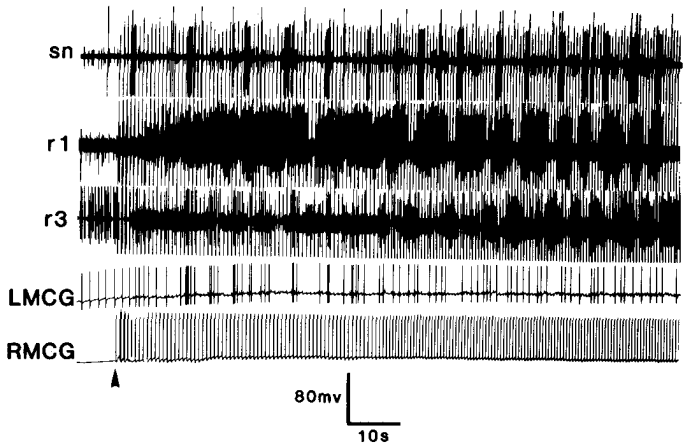

Figure 2. Intracellularly recorded activity of both the left and right MCGs (*LMCG* and *RMCG*, respectively) during production of the ingestion motor program by tonic stimulation of the right CBC (beginning with *arrowhead*) in the isolated CNS. *sn*, extracellular recording from the salivary nerve (the small unit that discharges in bursts is the salivary burster). *r1* and *r3*, extracellular recordings from buccal protractor root 1 and retractor root 3, respectively.

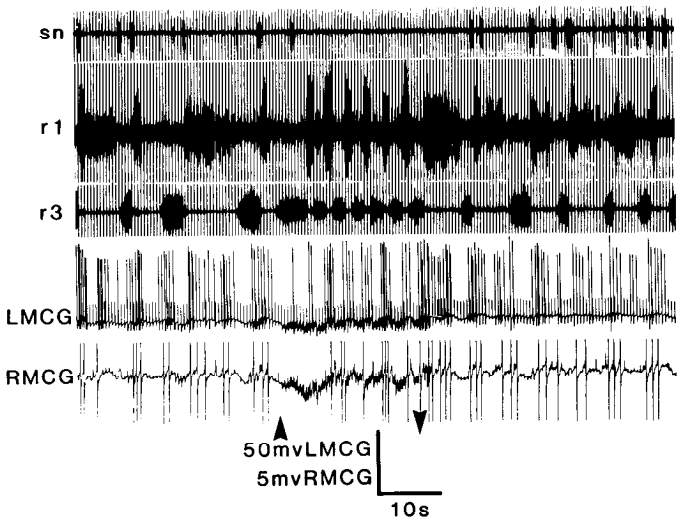

Figure 3. Intracellularly recorded activity of both the left and right MCGs (*LMCG* and *RMCG*, respectively) during production of the egestion motor program (between *arrowheads*) by tonic stimulation of the SGNs in the isolated CNS. *sn*, extracellular recording from the salivary nerve (the small unit that discharges in bursts is the salivary burster). *r1* and *r3*, extracellular recordings from buccal protractor root 1 and retractor root 3, respectively.

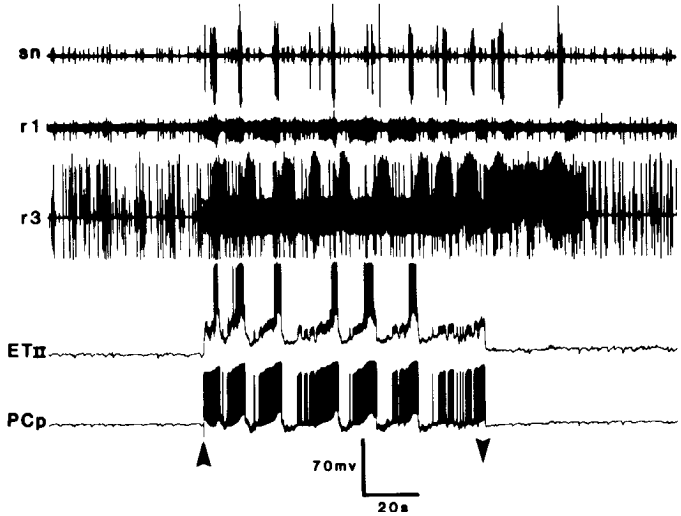

Figure 4. Effects of depolarizing (between *arrowheads*) a single PC_p on an ET_{II} and buccal nerves in the isolated CNS. *sn*, extracellular recording from the salivary nerve (the small unit that discharges in bursts is the salivary burster). *r1* and *r3*, extracellular recordings from buccal protractor root 1 and retractor root 3, respectively.

identified by suppression of the fast burster unit in the SN, MCG discharge was also slightly increased after an initial inhibition (four trials, four MCGs, three isolated CNS preparations). It may be concluded that, in the isolated CNS, the MCGs fire during both the ingestion and egestion motor programs.

The phasic paracerebral neurons.

The PC_p's are two re-identifiable neurons on each side of the brain that send descending axons to the buccal ganglion via the ipsilateral CBC (e.g., Kovac et al., 1982). Intracellular stimulation of the PC_p's elicits ingestion but not egestion behavior in the whole animal preparations (Gillette et al., 1978, 1982), and the PC_p's discharge cyclically and in phase with ingestion but not with egestion movements in the whole animal preparation (Gillette et al., 1978, 1982). In the present work we have performed experiments designed to assess the role of these neurons in the isolated CNS preparation.

Effects of intracellular stimulation. The effects of depolarizing the soma of a single PC_p were examined in 21 trials in 13 PC_p's in 12 isolated CNS preparations. Cyclic motor activity was elicited in 10 trials in 7 PC_p's in 7 preparations. Of these 10 trials, 4 showed cyclic bursts of activity in the fast burster unit of the SN in phase with protractor r 1, as is characteristic of the ingestion motor program (Fig. 4). In the remaining 6 of these 10 trials, cyclic SN activity occurred, but not in phase with protractor activity. In no case was the SN silent, as is characteristic of the egestion motor program. In 7 of the 10 trials, the duty cycle of protractor r 1 discharge was 33 to 50%, as is characteristic of the ingestion motor program. It may be concluded that intracellular stimulation of single PC_p's in the isolated CNS elicits the ingestion motor program or its characteristic components but never elicits the egestion motor program, and seldom its components.

Intracellular activity during buccal motor programs. PC_p activity was recorded intracellularly during the ingestion motor program in 23 trials in 13 PC_p's in 13 isolated CNS preparations. In these experiments the ingestion motor program was elicited by one of three different means: tonic extracellular stimulation of the ipsilateral CBC, which contains the descending axon of the PC_p (8 trials, 6 PC_p's, 6 preparations); extracellular stimulation of the contralateral CBC (12 trials, 6 PC_p's, 6 preparations); and intracellular stimulation of other command interneurons in the brain (3 trials, 1 PC_p, 1 preparation).

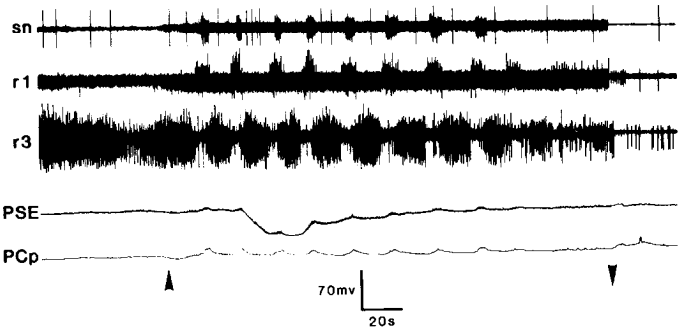

Figure 5. Intracellularly recorded activity of a PC_p and a PSE during production of the ingestion motor program by tonic stimulation of the contralateral CBC (between *arrowheads*) in the isolated CNS. *sn*, extracellular recording from the salivary nerve (the small unit that discharges in tight bursts is the salivary burster). *r1* and *r3*, extracellular recordings from buccal protractor root 1 and retractor root 3, respectively.

When the ingestion motor program was elicited by ipsilateral CBC stimulation (not shown), the stimulus pulses caused 1:1 antidromic spikes in the impaled PC_p, as well as a simultaneously impaled PSE of the PC_p (Kovac et al., 1983a). The narrow voltage window effective in eliciting the ingestion motor program by CBC stimulation (Croll et al., 1985a) usually encompassed the threshold for extracellular activation of the PC_p axon. Thus, antidromic spikes in the impaled PC_p were evident in five of the eight trials involving ipsilateral CBC stimulation.

An example of the ingestion motor program elicited by contralateral CBC stimulation is shown in Figure 5. In 10 of the 12 trials performed, oscillation of the PC_p membrane potential occurred, with depolarization simultaneous with the protraction phase of the ingestion motor program (Fig. 5). In 3 of these trials, action potentials accompanied the cyclic PC_p depolarizations (see Fig. 9). These results are similar to those reported earlier from the whole animal preparation (Gillette et al., 1978, 1982).

An example of the ingestion motor program elicited by intracellular stimulation of another command interneuron in the

brain (the PSE; see below) is shown in Figure 7. In all three of the trials performed, the PC_p showed strong oscillation of the membrane potential, and bursts of action potentials occurred in phase with protractor discharge. These data collectively demonstrate that in the isolated CNS preparation, the PC_p's receive cyclic feedback from the ingestion central pattern generator.

The activity of individual PC_p's was recorded intracellularly during the egestion motor program in 14 trials in six PC_p's in six isolated CNS preparations. In all cases the membrane potential of the PC_p showed strong oscillation, with depolarization again in phase with protractor r 1 bursts. In 5 of the 14 trials in three preparations the cyclic PC_p depolarization was accompanied by action potentials (e.g., Fig. 6). These data demonstrate that in the isolated CNS the PC_p's also receive cyclic feedback from the egestion central pattern generator, as suggested also from experiments on the whole animal preparation (Gillette et al., 1982).

The polysynaptic excitors

The PSEs are two identified interneurons on each side of the brain that deliver chemical polysynaptic inputs to the PC_p's (Kovac et al., 1983a). The PSEs share in common with the PC_p's the presence of a descending axon in the ipsilateral CBC, and also the capacity to elicit cyclic motor output that exhibits at least some of the characteristics of the ingestion motor program (Kovac et al., 1983a). In the present work we have performed experiments on these neurons in the isolated CNS preparation.

Effects of intracellular stimulation. The effects of depolarizing the soma of a single PSE were examined in nine trials in four PSEs in four preparations. Cyclic motor activity was elicited in seven of these trials in three PSEs in three preparations. Of these seven trials, six showed cyclic bursts of activity in the fast burster unit of the SN in phase with protractor r 1 discharge, as is characteristic of the ingestion motor program (Fig. 7). In the remaining one of these seven trials, cyclic SN activity also occurred, but not in phase with protractor activity. In no case was the SN silent, as is characteristic of the egestion motor program. In six (86%) of the seven trials the duty cycle of

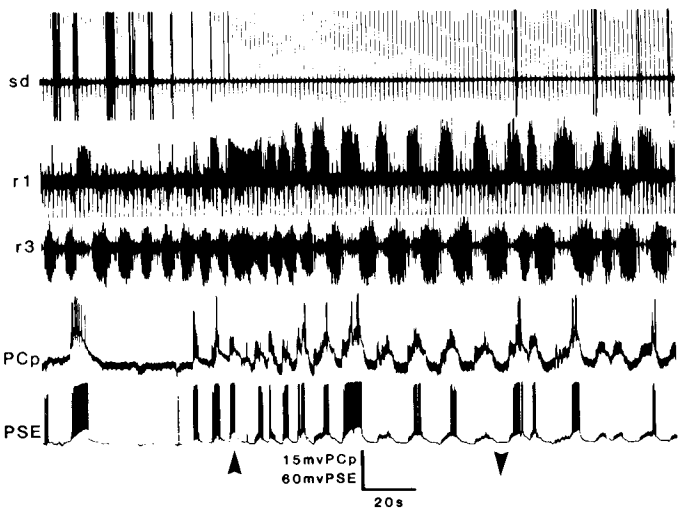

Figure 6. Intracellularly recorded activity of a PC_p and a PSE during production of the egestion motor program (indicated by suppression of SD activity, between *arrowheads*) by tonic stimulation of both SGNs in the isolated CNS. The egestion motor program occurs in the middle of an ongoing neutral rhythm(s) elicited by the stimulus and is identified by suppression of extracellularly recorded activity from the salivary duct (*sd*). *r1* and *r3*, extracellular recordings from buccal protractor root 1 and retractor root 3, respectively.

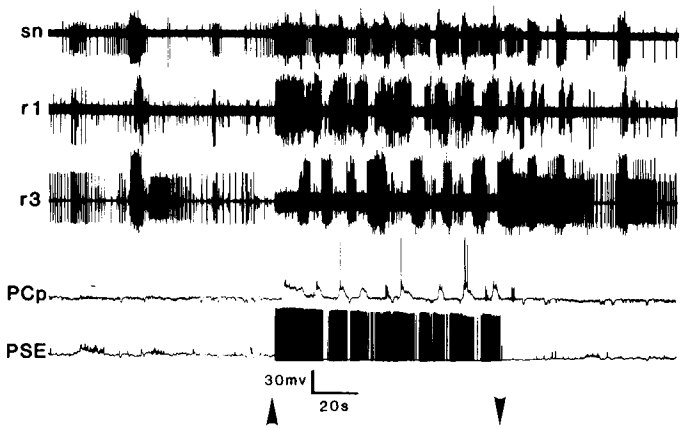

Figure 7. Effects of depolarizing (between *arrowheads*) a single PSE on a PC_p and buccal nerves in the isolated CNS. *sn*, extracellular recording from the salivary nerve (the small unit that discharges in tight bursts is the salivary burster). *r1* and *r3*, extracellular recordings from buccal protractor root 1 and retractor root 3, respectively.

protractor r 1 discharge was less than 50%, as is characteristic of the ingestion motor program. In the seventh trial the protractor duty cycle was 52%, but all other parameters of motor output were characteristic of the ingestion motor program. PSE stimulation never elicited the egestion motor program and seldom elicited individual characteristic components of the program. It may be concluded that intracellular stimulation of a PSE induces the ingestion motor program or its characteristic components but not the egestion motor program and seldom its characteristic components.

Intracellular activity during buccal motor programs. PSE activity was recorded intracellularly during the ingestion motor program in four trials in four PSEs in four isolated CNS preparations. The ingestion motor program was elicited by tonic extracellular stimulation of the ipsilateral (two trials, two PSEs, two preparations) or contralateral (two trials, two PSEs, two preparations) CBC. In the former case (ipsilateral CBC stimulation), the threshold for the ingestion motor program was near the threshold for PSE activation, and in both trials 1:1 antidromic action potentials were recorded from the PSE in association with each stimulus pulse (not shown). Thus, PSE activation may contribute to elicitation of the ingestion motor program by CBC stimulation. In the latter case (contralateral CBC stimulation), the membrane potential of the PSE fluctuated in phase with the ingestion motor program, with depolarizations occurring during protractor discharge (Fig. 5). These results indicate that PSEs receive synaptic feedback from the central pattern generator that underlies the ingestion motor program, as suggested earlier (Kovac et al., 1983a).

The activity of the individual PSEs was recorded intracellularly during the egestion motor program in a single trial, in which the egestion motor program was elicited by high voltage tonic extracellular stimulation of the SGNs. The membrane potential of the PSE oscillated in phase with the egestion motor program, with action potential bursts coincident with the protractor activity (Fig. 6). Therefore, the PSEs also receive cyclic feedback from the egestion central pattern generator.

Class II electrotonic neurons

The ET_{II}'s are two re-identifiable neurons on each side of the brain that are electrically coupled with the PC_p's (Kovac et al., 1983a). One of the two identified ET_{II}'s also delivers chemical polysynaptic inputs to the PC_p's. In the present experiments we did not differentiate between the two ET_{II}'s. The ET_{II}'s share in common with the PC_p's and the PSEs the presence of a descending axon in the ipsilateral CBC, and they

exhibit a weak capacity to elicit cyclic buccal motor output showing at least some characteristics of the ingestion motor program (Kovac et al., 1983a). In the present work we have studied these neurons in the isolated CNS preparation.

Effects of intracellular stimulation. The effects of depolarizing the soma of a single ET_{II} were examined in 14 trials in four ET_{II}'s in four preparations. Cyclic motor output was elicited in only 1 of these 14 trials. In this single case (Fig. 18 of Kovac et al., 1983a), the SN discharged intense bursts of activity, but these occurred in phase with retractor discharge, characteristic of the neutral rhythm(s). More typically, in 5 of the 14 trials stimulation of an ET_{II} increased the extracellular discharge recorded from buccal nerve roots but did not induce obvious oscillatory motor activity (Fig. 8). In all of these trials protractor and retractor activity appeared equally activated, and in no case was SD or SN activity suppressed as is characteristic of the egestion motor program. The available data therefore indicate that the ET_{II} neurons alone play a weak role in eliciting motor output, as previously reported (Kovac et al., 1983a). In the rare cases when oscillatory output results from ET_{II} stimulation, the pattern is that of the neutral rhythm(s).

Intracellular activity during buccal motor program. ET_{II} activity was recorded intracellularly during the ingestion motor program in eight trials in six ET_{II}'s in six isolated CNS preparations. The ingestion motor program was elicited by tonic extracellular stimulation of the ipsilateral CBC, stimulation of the contralateral CBC, and intracellular stimulation of another command interneuron in the brain.

When the ingestion motor program was elicited by ipsilateral CBC stimulation (three trials, three ET_{II}'s, three preparations), the ET_{II} was activated antidromically at approximately the same voltage threshold as the motor rhythm, resulting in 1:1 antidromic spikes recorded from the ET_{II} soma (two of three preparations; not shown). In the third trial, antidromic spikes were absent, but the ET_{II} membrane potential oscillated, with depolarization occurring in phase with protraction activity (not shown). When the ingestion motor program was elicited by stimulation of the contralateral CBC (four trials, two ET_{II}'s, two preparations), the membrane potential typically oscillated in phase with protractor activity (three of four trials), with action potentials occurring during the depolarizing phase (one of four trials; Fig. 9). When the ingestion motor program was elicited by stimulating a PC_p (one trial, one ET_{II}, one preparation), the ET_{II} showed cyclic bursts of excitatory polysynaptic potentials (EPSPs) in phase with protractor discharge (Fig. 4). These results collectively indicate that in the isolated CNS preparation, the ET_{II}'s receive cyclic feedback from the ingestion central pattern generator.

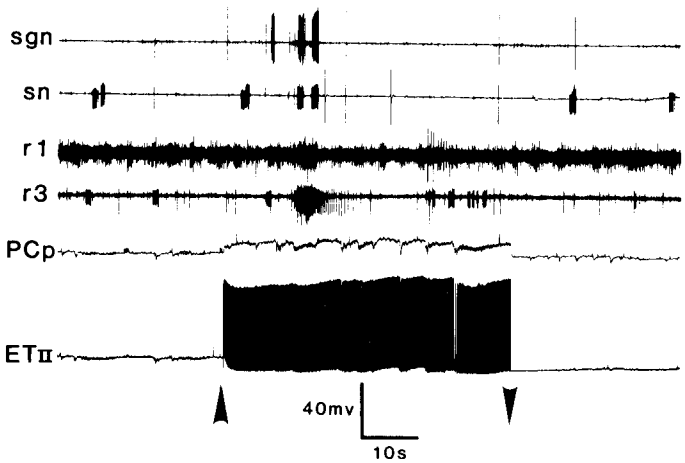

Figure 8. Effects of depolarizing (between *arrowheads*) a single ET_{II} on a PC_p and buccal nerves in the isolated CNS. *sgn*, extracellular recording from the ipsilateral stomatogastric nerve; *sn*, extracellular recording from the ipsilateral salivary nerve (the small unit that discharges in tight bursts is the salivary burster). *r1* and *r3*, extracellular recordings from buccal protractor root 1 and retractor root 3, respectively.

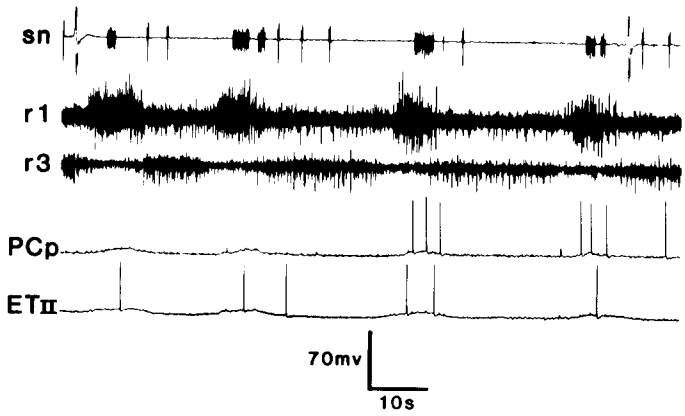

Figure 9. Intracellularly recorded activity of an ET_{II} and a PC_p during production of the ingestion motor program by tonic stimulation of the contralateral CBC (throughout record) in the isolated CNS. *sn*, extracellular recording from the salivary nerve (the small unit that discharges in bursts is the salivary burster). *r1* and *r3*, extracellular recordings from buccal protractor root 1 and retractor root 3, respectively.

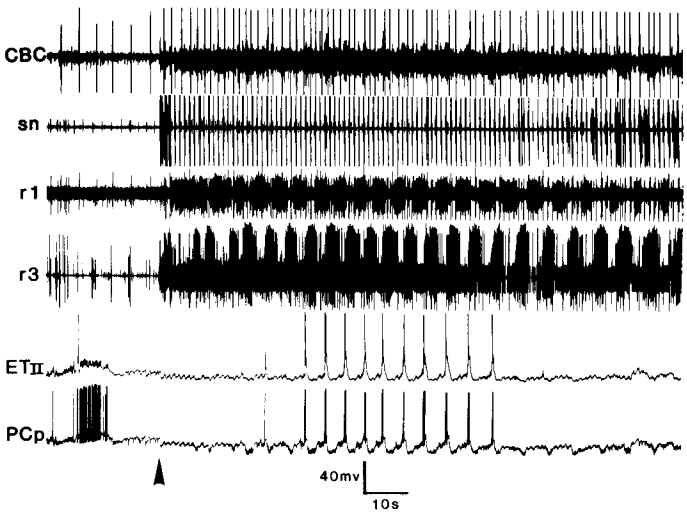

Figure 10. Intracellularly recorded activity of a PC_p and an ET_{II} during production of the egestion motor program (indicated by r 1 duty cycle >50% and suppression of SN bursts) by tonic stimulation of both SGNs (beginning at the *arrowhead*) in the isolated CNS. *CBC*, extracellularly recorded activity from the ipsilateral cerebrobuccal connective; *sn*, extracellular recording from the salivary nerve (the small unit that discharges in bursts is the salivary burster). *r1* and *r3*, extracellular recordings from buccal protractor root 1 and retractor root 3, respectively.

The activity of individual ET_{II}'s was recorded intracellularly during the egestion motor program in six trials in three ET_{II}'s in three preparations. In all cases the ET_{II} received cyclic bursts of EPSPs or produced bursts of action potentials (Fig. 10) that occurred in phase with protractor activity. Therefore, the ET_{II}'s also receive cyclic feedback from the egestion central pattern generator.

The class I electrotonic neuron

The class I electrotonic neuron (ET_I) is a re-identifiable neuron on each side of the brain that is electrically coupled

with the PC_P's (Kovac et al., 1983a). Unlike the ET_{II}'s, ET_I lacks a descending axon in the CBC and does not alone elicit cyclic motor output from the buccal ganglion or the brain (Kovac et al., 1983a). In the present work we have studied this neuron in the isolated CNS.

Effects of intracellular stimulation. The effects of depolarizing the soma of a single ET_I were examined in 13 trials of six ET_I's in five preparations. Cyclic motor output was never elicited, corroborating previous findings (Kovac et al., 1983a). Usually (6 of 13 trials), there was no visible effect of stimulation. In some cases (5 of 13 trials), however, ET_I stimulation caused a single burst of motor output from the buccal ganglion, with SD activity in phase with retractor r 3 discharge, as is characteristic of the neutral rhythm(s) (Fig. 11). In a few cases (2 of 13 trials), stimulation of the ET_I accelerated an ongoing rhythm, but in these cases recordings were not made from the SD. On these grounds it appears that ET_I may weakly bias the motor output toward the neutral rhythm(s), which is similar to the ingestion motor program (Croll et al., 1985a).

Intracellular activity during buccal motor program. ET_I activity was recorded intracellularly only during the ingestion motor program (seven trials, four ET_I's, four preparations) (Fig. 12). In all cases the membrane potential of the neuron oscillated in phase with the motor output, with depolarizations occurring during retractor activity, as reported earlier (Kovac et al., 1983a). Therefore, the ET_I neuron receives synaptic feedback from the ingestion central pattern generator, but the sign of the feedback is paradoxically opposite to that received by PC_P's, PSEs, and ET_{II}'s, resulting in oscillations in antiphase with these other brain neurons.

Other identified brain neurons

The effects on motor output in the isolated CNS of depolarizing several additional identified neurons that are associated with the paracerebral command system have been examined in the course of the present studies. Tonic paracerebral neurons (PC_T's) (Kovac et al., 1982), for example, were studied in eight trials on five PC_T's in five preparations. In seven of these trials there was no effect on motor output, whereas in one trial PC_T stimulation accelerated an ongoing neutral rhythm(s). Dorsomedial polysynaptic inhibitors (Kovac et al., 1983a) were stimulated in three trials in one MSE in one preparation with no effect. Monosynaptic inhibitors (MSIs) (Kovac et al., 1983b) were stimulated in seven trials in three MSIs in three preparations with no effect on motor output. Therefore, these additional identified brain neurons appear to play little or no direct role in causing ingestion or egestion motor output, although these neurons presumably modulate the respective motor programs by their documented actions on the brain command interneurons.

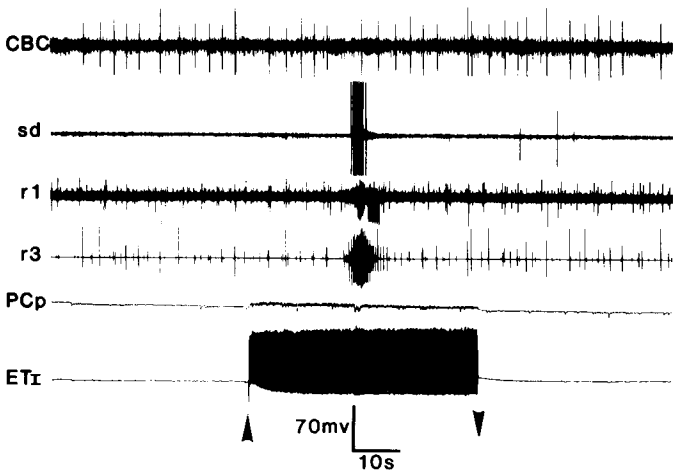

Figure 11. Effects of depolarizing (between *arrowheads*) a single ET_I on a PC_P and buccal nerves in the isolated CNS. *CBC*, extracellularly recorded activity from the ipsilateral cerebrobuccal connective; *sd*, extracellular recording from the ipsilateral salivary duct; *r1* and *r3*, extracellular recordings from buccal protractor root 1 and retractor root 3, respectively.

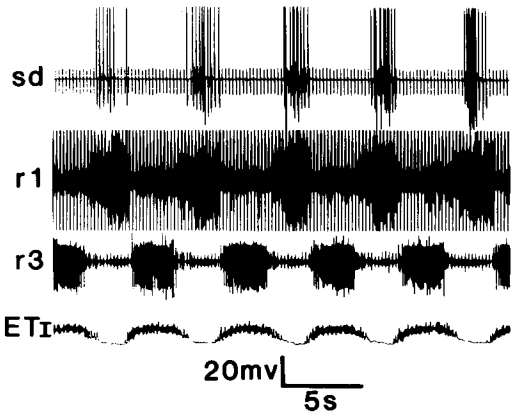

Figure 12. Intracellularly recorded activity of an ET_I during production of the ingestion motor program (throughout record) by tonic stimulation of the contralateral CBC in the isolated CNS. *sd*, extracellular recording from the ipsilateral salivary duct; *r1* and *r3*, extracellular recordings from buccal protractor root 1 and retractor root 3, respectively.

Discussion

The ingestion command system. Previous work on the PCNs in the brain of *Pleurobranchaea* has shown that in each hemiganglion these neurons are subdivided into four classes of two identified neurons each: the PC_T's, the PC_P's, the PSEs, and the ET_{II}'s (Kovac et al., 1982, 1983a). Each of these categories of brain neurons, as well as the previously studied MCGs (Gillette and Davis, 1977) is capable of eliciting cyclic motor output from the buccal ganglion when intracellularly stimulated, although as reported previously (Kovac et al., 1983a), some classes are more efficacious than others. In previous studies on the PCNs in whole animal preparations, it was found that these neurons are capable of inducing cyclic ingestion movements when depolarized, and that the same neurons discharge in cyclic bursts of action potentials that are phase locked with ingestion (but not egestion) movements in response to presentation to the animal of food stimuli (Gillette et al., 1978, 1982). Those studies were performed before the functional subdivisions within the PCN population had been established, however (Kovac et al., 1982, 1983a), and before criteria for rigorously distinguishing the different buccal motor patterns had been developed (McClellan, 1980, 1982a, b; Croll and Davis, 1981, 1982; Croll et al., 1985a).

In the present study we have reassessed the functional roles of each of these neurons in light of the new information available on their functional subdivisions, and in light of newly developed criteria for distinguishing the buccal motor programs in the reduced preparation and in the isolated CNS (Croll et al., 1985a). This study has confirmed that each of the above classes of brain neurons is sufficient to induce the ingestion motor program or its individual characteristic components but is never sufficient to induce the egestion motor program and seldom its characteristic components. These findings are subject to the qualifications detailed in the preceding paper (Croll et al., 1985b) regarding the unavoidable limitations of drawing behavioral inferences from analysis of single neurons in dissected preparations. This study therefore corroborates our previous conclusions regarding the role of the paracerebral neurons

and supports the view that the ingestion behavior of *Pleurobranchaea* is controlled by an interrelated population of command interneurons located in the brain.

In contrast to our conclusions, McClellan (1983) recently reported that MCG stimulation elicits a relatively weak motor pattern "of uncertain function," and that stimulation of PCNs during ongoing motor activity sometimes (20%) elicited "vomiting-like motor patterns." However, examination of the published records on which McClellan's (1983) conclusions were based shows that MCG stimulation never resulted in the suppression of SD discharge that is characteristic of the egestion motor program. Instead, in McClellan's single published record of continuous MCG stimulation (McClellan, 1983, Fig. 1*B*), the SD discharges biphasically or monophasically near the end of retractor (r 3) discharge, characteristic of the neutral rhythm(s) (Croll and Davis, 1982; Croll et al., 1985a). In McClellan's nine published recordings of motor patterns elicited during stimulation of PCNs (McClellan, 1983, Figs. 2, *A* to *D*), and 3, *A* to *E*), the SD shows bursting activity in eight of nine (89%) cases, indicative of the ingestion motor program or the neutral rhythm(s), but not the egestion motor program (Croll and Davis, 1982; Croll et al., 1985a). Of these eight cases the motor pattern elicited during PCN stimulation can be positively identified as the ingestion motor program in four (50%) cases, on the basis of the concurrence of SD discharge with protractor (r 1) activity (McClellan, 1983, Figs. 2, *A* and *B*, and 3, *A* and *C*). Therefore, although McClellan's (1983) conclusions differ from ours, his published data are in accord with the interpretations presented here.

Activity in the command system during motor activity. Intracellular recordings from these same command neurons have shown that they oscillate in phase with both the ingestion and egestion motor programs elicited from the reduced preparation and/or isolated CNS. However, both the ingestion and egestion motor programs can occur in the absence of action potentials in any individual command neuron, showing that no individual command cell is necessary for the expression of the corresponding motor program. This result is consistent with the finding that the command role is distributed over a relatively large population of interneurons.

As noted previously, however (Croll et al., 1985b), the activity of neurons in dissected preparations during artificially elicited motor programs may be expected to vary significantly from the activity that accompanies the same motor program in the intact animal. In the reduced preparation, the only "normal" input to the neurons during motor pattern is the feedback received from the central pattern generator(s). The finding that brain neurons in the dissected preparation oscillate during both ingestion and egestion means either that these neurons receive feedback from both pattern generators, or that both behaviors are generated by a common pattern generator. We propose the latter hypothesis, largely because the motor programs are similar, based upon recordings from muscles in the intact animals and from single neurons in the reduced preparation and isolated CNS. In all cases the different motor programs entail cyclic alternation between antagonistic motor pools, with biases toward ingestion or egestion furnished by separate command populations.

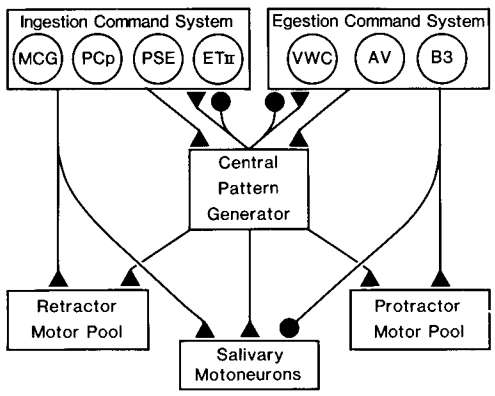

Figure 13. A partial, hypothetical neural model for how motor program switching is accomplished in the buccal motor system of *Pleurobranchaea*. According to this model, separate command systems for ingestion (*top left*) and egestion (*top right*) converge on a common central pattern generator, which provides oscillatory feedback (excitation and inhibition) to the command neurons. The command pathways also provide appropriate biases to the motor pools. *Solid triangles*, excitatory connections; *solid circles*, inhibitory connections. *VWC*, ventral white cell; *AV*, anterior ventral neuron. See the text for further description.

Specialization within the command population. Previously we showed that the PCNs are specialized to perform different functions correlated in part with differences in neuronal size. Thus, the smaller PC_T's have a relatively weak effect upon the ingestion motor output compared to the larger PC_P's, which have a much stronger effect on ingestion motor output (Kovac et al., 1982). The present experiments corroborate and partially quantify our previous qualitative impressions regarding the relative efficacy of the different elements of the ingestion command system. Based upon the frequency of eliciting cyclic ingestion motor output in the present experiments, the command efficacy within the population of identified brain neurons declines in the sequence $PSE > PC_P > ET_{II} > MCG > PC_T$.

A neural model of motor program switching. A primary purpose in undertaking this study was to determine how the same muscles can be used in different patterns of coordination or motor programs. The data now available permit formulation of a hypothetical model that can account for motor program switching in this "model" system. According to this model (Fig. 13), food stimuli activate exteroreceptors in the oral veil, rhinophore, and tentacles (Davis and Matera, 1982; Matera and Davis, 1982) and are conveyed via partially understood chemosensory pathways (Bicker et al., 1982a, b) to the brain. Emetic stimuli activate interoreceptors, including presumed chemoreceptors in the buccal cavity and presumed stretch receptors in the esophagus (Croll and Davis, 1982), both of which areas are innervated by buccal nerves. The corresponding ingestion command system is located in the brain, while the egestion command system originates in the buccal ganglion (Croll et al., 1985b).

The corresponding ingestion and egestion command pathways are hypothesized to converge upon a common pattern generator in the CNS. This is the simplest testable hypothesis consistent with presently available data; direct evidence for it is lacking, inasmuch as the pattern-generating system has not yet been identified. The present data indicate that command neuron collaterals (or interposed interneurons) bias the protractor and retractor motor pools as appropriate to the respective motor output. Thus, the MCG biases for retractor activity (Gillette and Davis, 1977), characteristic of the ingestion motor program (Croll and Davis, 1981, 1982; Croll et al., 1985a), whereas buccal command interneurons bias for protractor motor activity (Croll et al., 1985b). By means of these synaptic influences, the changes in amplitude, duration, and timing of motor activity that have been documented to underlie motor program switching in the buccal motor system (Croll and Davis, 1981, 1982; Croll et al., 1985a) could be realized.

The hypothetical model proposed here for motor program switching in the buccal motor system of *Pleurobranchaea* is consistent with most available data on this motor system. A possible exception is the synaptic organization of PC_P's, which generally excite protractor motoneurons and inhibit retractor motoneurons (Gillette et al., 1982). This model is similar to

one proposed to account for different kinds of limb movements in cats (Berkinblit et al., 1981). According to that model, stepping and scratching are produced by the same central pattern generator but are elicited by different command systems. Rigorously testing this model in *Pleurobranchaea* will require elucidating the buccal pattern generator(s), which has not yet been identified.

References

Berkinblit, M. B., T. G. Deliagina, A. G. Feldman, I. M. Gelfand, and G. N. Orlovsky (1981) Generation of scratching. II. Non regular regimes of generation. J. Neurophysiol. *41:* 1058–1069.

Bicker, G., W. J. Davis, E. M. Matera, M. P. Kovac, and J. Stormo-Gipson (1982a) Mechano- and chemoreception in *Pleurobranchaea californica.* I. Extracellular analysis of afferent responses. J. Comp. Physiol. *149:* 221–234.

Bicker, G., W. J. Davis, and E. M. Matera (1982b) Mechano- and chemoreception in *Pleurobranchaea californica.* II. Neuroanatomical and intracellular analysis of centripetal pathways. J. Comp. Physiol. *149:* 235–250.

Croll, R. P., and W. J. Davis (1981) Motor program switching in *Pleurobranchaea.* I. Behavioral and electromyographic study of ingestion and egestion in intact specimens. J. Comp. Physiol. *145:* 277–287.

Croll, R. P., and W. J. Davis (1982) Motor program switching in *Pleurobranchaea.* II. Ingestion and egestion in the reduced preparation. J. Comp. Physiol. *147:* 143–154.

Croll, R. P., W. J. Davis, and M. P. Kovac (1985a) Neural mechanisms of motor program switching in the mollusc *Pleurobranchaea.* I. Central motor programs underlying ingestion, egestion, and the "neutral" rhythm(s). J. Neurosci. *5:* 48–55.

Croll, R. P., M. P. Kovac, and W. J. Davis (1985b) Neural mechanisms of motor program switching in the mollusc *Pleurobranchaea.* II. Role of the ventral white cell, anterior ventral, and B3 buccal neurons. J. Neurosci. *5:* 56–63.

Davis, W. J., and E. M. Matera (1982) Chemoreception in gastropod mollusks: Electron microscopy of putative receptor cells. J. Neurobiol. *13:* 79–84.

Davis, W. J., G. J. Mpitsos, M. V. S. Siegler, J. M. Pinneo, and K. B. Davis (1974) Neuronal substrates of behavioral hierarchies and associative learning in the mollusk *Pleurobranchaea.* Am. Zool. *14:* 1037–1050.

Gillette, R., and W. J. Davis (1977) The role of the metacerebral giant neuron in the feeding behavior of *Pleurobranchaea.* J. Comp. Physiol. A *116:* 129–159.

Gillette, R., M. P. Kovac, and W. J. Davis (1978) Command neurons in *Pleurobranchaea* receive synaptic feedback from the motor network they excite. Science (NY) *199:* 798–801.

Gillette, R., M. P. Kovac, and W. J. Davis (1982) Control of feeding motor output by paracerebral neurons in the brain of *Pleurobranchaea california.* J. Neurophysiol. *47:* 885–908.

Kovac, M. P., W. J. Davis, E. M. Matera, and R. Gillette (1982) Functional and structural correlates of cell size in paracerebral neurons of *Pleurobranchaea californica.* J. Neurophysiol. *47:* 909–927.

Kovac, M. P., W. J. Davis, E. M. Matera, and R. P. Croll (1983a) Organization of synaptic inputs to paracerebral feeding command interneurons of *Pleurobranchaea californica.* I. Excitatory inputs. J. Neurophysiol. *49:* 1517–1538.

Kovac, M. P., W. J. Davis, E. M. Matera, and R. P. Croll (1983b) Organization of synaptic inputs to paracerebral feeding command interneurons of *Pleurobranchaea californica.* II. Inhibitory inputs. J. Neurophysiol. *49:* 1539–1556.

Matera, E. M., and W. J. Davis (1982) Paddle cilia (discocilia) in chemosensitive structures of the gastropod mollusk *Pleurobranchaea californica.* Cell Tissue Res. *222:* 25–40.

McClellan, A. D. (1980) Feeding and regurgitation in *Pleurobranchaea californica*: Multibehavioral organization of pattern generation and higher order control. Ph.D. thesis, Case Western Reserve University, Cleveland, OH.

McClellan, A. D. (1982a) Movements and motor patterns of the buccal mass of *Pleurobranchaea* during feeding, regurgitation and rejection. J. Exp. Biol. *98:* 195–211.

McClellan, A. D. (1982b) Re-examination of presumed feeding motor activity in the isolated nervous system of *Pleurobranchaea.* J. Exp. Biol. *98:* 213–228.

McClellan, A. D. (1983) Higher order neurons in the cerebral ganglia of *Pleurobranchaea* have diverse effects on buccal motor patterns. J. Comp. Physiol. *153:* 533–541.