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

II. Role of the Ventral White Cell, Anterior Ventral, and B3 Buccal Neurons¹

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Abstract

Identified buccal neurons in the mollusc *Pleurobranchaea* were stimulated and recorded intracellularly while recording the resultant identified motor program from buccal muscles (reduced preparation) or nerves (isolated central nervous system). Neurons studied included the ventral white cell (VWC), members of the anterior ventral (AV) population, and interneuron B3. Each of these neurons elicited the egestion motor program or its characteristic components when stimulated intracellularly. The characteristic prolonged plateau potential of the VWC was frequently associated with the egestion motor program but never with the ingestion motor program or its characteristic components. Intracellular recordings from these same neurons during spontaneous or induced buccal motor programs were consistent with the view that these neurons participate in production of the egestion motor program. The VWC discharged also during the neutral buccal rhythm, although in a different pattern from that seen during the egestion motor program, suggesting that it is multifunctional. Synaptic targets of the VWC are unknown, but synaptic influences of the AV and B3 neurons were found and are appropriate to their proposed role in egestion. This study therefore indicates that an interrelated cluster of buccal neurons is specialized to command the egestion motor program.

Three cyclic patterns of motor coordination (motor programs) have been characterized in the buccal musculature of the carnivorous marine gastropod *Pleurobranchaea* (Croll and Davis, 1981). The first of these motor programs is accompanied by and presumably causes ingestion of objects in the buccal cavity; the second is accompanied by egestion from the buccal cavity of unpalatable substances or objects; and the third motor program, which occurs at a slower frequency, is similar to the ingestion motor program but does not cause movement of objects in either direction through the buccal cavity and has no known behavioral correlates. This third motor program has therefore been termed the "neutral" rhythm(s). All three of these buccal motor programs share basic features in common, namely, the presence of alternate bursts of muscle potentials in the radula protractor and retractor muscles of the buccal mass. The different behavioral consequences of the three motor programs result from relative changes in the amplitude, duration, and timing of discharge in specific muscles (Croll and Davis, 1982; McClellan, 1982a).

The same three motor programs identified by electromyography in the intact animal (Croll and Davis, 1981) can be elicited and identified by several criteria in the surgically reduced preparation (Croll and Davis, 1982; McClellan, 1982a) and in the isolated central nervous system (CNS) (McClellan, 1982b; Croll and Davis, 1985a). Therefore, these three motor programs are endogenous to the CNS; i.e., they are centrally programmed. In the present work we address the issue of how the CNS mediates these different buccal motor programs, by identifying neurons in the buccal ganglion, including the previously identified ventral white cell (VWC), that are capable of eliciting the egestion motor program or its characteristic components.

The VWC has been studied extensively with regard to spike broadening (Gillette et al., 1980, 1982a, b; Gillette, 1983). A characteristic, depolarizing, prolonged plateau potential (PPP) (Russell and Hartline, 1978, 1982) can be triggered in the VWC, resulting in a train of action potentials that broaden up to 16-fold on repetition (Gillette et al., 1980). As a consequence of this spike broadening, a cyclic buccal motor program is triggered. McClellan (1980) reported that in the reduced preparation, stimulation of the VWC elicits a motor output pattern indistinguishable from the "vomiting" (egestion) motor program. Gillette and Gillette (1983), however, found that in the reduced preparation food stimuli excite (depolarize) the VWC, whereas emetic stimuli inhibit (hyperpolarize) the cell. Largely on these grounds, they suggest that the VWC is a command neuron for the feeding (ingestion) behavior (see "Discussion").

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In the present study we have applied the detailed qualitative and quantitative criteria developed in the preceding paper (Croll et al., 1985a) to the motor output patterns induced by stimulating the VWC in the reduced preparation and the isolated CNS. Stimulation of the VWC generally elicited the egestion motor program or one or more of its individual characteristic components. Moreover, the VWC is excitatorily coupled with other identified neurons in the buccal ganglion that also elicit the egestion motor program or its characteristic components. Our results therefore support the hypothesis that the VWC is one of several identified buccal ganglion neurons that together comprise a "command system" for the stereotyped egestion motor program. In the following paper (Croll et al., 1985b) we present evidence that a cluster of identified brain neurons, including the paracerebral neurons, comprises a similar command system for the ingestion motor program.

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 buccal ganglion.

Intracellular recording. The somata of neurons in the buccal ganglion were exposed for microelectrode penetration by desheathing the buccal ganglion. Individual somata were impaled under visual control with a glass capillary microelectrode filled with 3 M KCl and having a tip resistance of 10 to 20 megohms measured in sea water. Buccal motor programs were elicited either by extracellular nerve stimulation as described in the preceding paper (Croll et al., 1985a), or by intracellular stimulation of the somata of single neurons as described under "Results."

Identification of buccal motor programs. Identification of the motor programs for egestion, ingestion, and the neutral rhythm(s) was based upon the several qualitative and quantitative criteria documented in the preceding paper (Croll et al., 1985a). In many instances in the present work, some but not all of the criteria were obtained by intracellular stimulation of identified neurons. In these cases these criteria are described as "components" of the corresponding motor program.

Results

The ventral white cell(s)

Effects of intracellular stimulation

The reduced preparation. The VWC was stimulated intracellularly in 29 trials in 13 neurons in 10 reduced preparations. In 93% of these trials the VWC elicited cyclic motor output against a background of quiescence or, more often, against a background of a spontaneously occurring neutral rhythm(s). The resultant motor output resembled the egestion motor program (Fig. 1) in the following ways: (1) protractor m 4 activity was increased; (2) the amplitude of salivary duct (SD) activity was suppressed; (3) buccal constrictor m 5 was silenced or inactive

Figure 1. Effects of depolarizing (between arrowheads) the ventral white cell (VWC) on motor activity recorded from the salivary duct (sd), protractor m 4 (M4) and buccal constrictor m 5 (M5) in the reduced preparation.

or showed only occasional, small amplitude potentials; (4) retractor m 3 or r 3 activity declined relative to protractor m 4 or r 1 activity; and (5) the mean protractor duty cycle exceeded the 50% criterion.

Analysis of the entire body of data generated in all 29 trials of VWC stimulation in the reduced preparation showed that in 96% of the trials protractor activity was increased in amplitude. The mean protractor duty cycle exceeded the 50% criterion in Figure 1 and in the majority (65%) of the trials in which it could be measured accurately. In response to VWC stimulation, SD activity never increased or changed phase position with protractor activity and was suppressed in 54% of the trials. In 46% of the trials there was no effect on SD activity. The buccal constrictor m 5 activity likewise was never increased by VWC stimulation and decreased in 38% of the trials. A complete egestion motor program was elicited in three trials (11%), whereas a complete ingestion motor program was never elicited by VWC stimulation in the reduced preparation.

The isolated CNS. The VWC was stimulated intracellularly in 10 trials in five cells in five isolated CNS preparations. Such stimulation also typically elicited motor output showing several characteristics of the egestion motor program (Fig. 2). For example, activity in the smaller "fast burster" unit of the salivary nerve (SN) was suppressed. This neuron is responsible for the SD muscle potentials that are normally suppressed during the egestion motor program (Croll et al., 1985a). In addition, intense protractor discharge occurred with a high duty cycle. Finally, the somatogastric nerve (SGN) discharged tonically, which is correlated with the egestion but not the ingestion motor program (Croll and Davis, 1982; McClellan, 1982a).

Analysis of all such data on VWC stimulation in the isolated CNS revealed that SD activity was suppressed in 50% in the trials but was never increased or phase shifted to overlap with protraction. The mean protractor duty cycle was 52.3% and exceeded 50% in the majority (62.5%) of individual trials.

Figure 2. Effects of depolarizing (between arrowheads) the left VWC (LVWC) on motor output in the isolated CNS. sn, salivary nerve; r 1, buccal protractor nerve 1; r 3, buccal retractor nerve 3; lsgn, left and right SGNs, respectively. The smaller of the two units in the SN is the salivary burster neuron that is responsible for muscle potentials in the salivary duct (Croll et al., 1985a).

The "rebound" motor program

In three experiments on the reduced preparation we observed a "rebound" motor program following VWC stimulation. This motor program was highly stereotypic in form and resembled an exaggerated version of the egestion motor program typically seen in the reduced preparation (Fig. 3). During VWC stimulation prior to the rebound motor program, the motor output showed several basic features of the egestion motor program (Fig. 3). Approximately 50 sec (Fig. 3) after the termination of VWC stimulation (range for the three occurrences, 10 to 90 sec), 23 cycles of intense activity occurred in which the features of the egestion motor program were enhanced. Specifically, the amplitude and duration of retractor m 3 activity were reduced; the amplitude and duration of protractor m 4 activity increased; the mean protractor duty cycle exceeded 50% (approximately 60% over the entire episode in Fig. 3); the activity of buccal constrictor m 5 was suppressed (note the post-episodic rebound of m 5 activity in Fig. 3); and SD activity was suppressed (not shown in Fig. 3). Although these episodes of motor activity thus resemble the egestion motor program in several ways, they differ in at least two respects from a typical egestion motor program. First, the SGN discharged cyclically rather than tonically; and, second, the VWC also discharged cyclically rather than tonically. For these reasons we do not classify this program as "egestion" but instead assign it the behaviorally neutral name of "rebound motor program." As shown below, the same rebound motor program typically follows intracellular stimulation of other buccal neurons that elicit characteristic components of the egestion motor program.

Intracellular activity during buccal motor programs

The egestion motor program. Within 10 to 50 sec after the onset of a spontaneous VWC PPP, and shortly after the previously described onset of pronounced spike broadening (Gillette et al., 1980), a vigorous and characteristic motor program was recorded from the buccal nerves and muscles (Fig. 4). The characteristic features of this motor program were: (1) suppression of SD activity; (2) reduction of the amplitude of retractor m 3 activity; (3) increase in the amplitude of protractor m 4 activity; (4) suppression of buccal constrictor m 5 activity; and (5) a protractor duty cycle in excess of 50% (in Fig. 4 the mean protractor duty cycle is approximately 66%). These five features identify the motor pattern as the egestion motor program.

The above results suggest that the VWC can cause spontaneous occurrences of the egestion motor program. Spontaneously occurring PPPs do not always cause the egestion motor program, however. In 11 instances in the present work a spontaneous PPP occurred (e.g., Fig. 4). In 4 (36%) of these cases the egestion motor program occurred within a few seconds after

Figure 3. The "rebound" motor program (second half of record) elicited by depolarization (between arrowheads) of the left VWC (LVWC) in the reduced preparation. M3 to M5, electromyograms from retractor muscle 3, protractor muscle 4, and buccal constrictor muscle 5, respectively. lsgn, extracellular recording from the left SGN (which contains the peripheral axon of the contralateral VWC).

Figure 4. Intracellularly recorded activity of a VWC before and during a spontaneous egestion bout (between arrowheads) in the reduced preparation. The motor program is identified by activity in the salivary duct (sd), and buccal muscles 3 to 5 (M3 to M5; retractor, protractor, and constrictor, respectively).

Figure 5. Intracellularly recorded activity recorded of a VWC during production of the ingestion motor program by tonic stimulation of both CBCs (between arrowheads) in the reduced preparation. sn, extracellular recording from the SN (the small unit is the salivary burster); M4 and M5, electromyograms from protractor muscle 4 and buccal constrictor muscle 5, respectively.

the PPP began. In the other 7 cases, however, there was no consistent change in the ongoing motor activity, which in most cases was a spontaneous neutral rhythm(s).

The ingestion motor program. VWC activity was recorded while eliciting the ingestion motor program by cerebrobuccal connective (CBC) stimulation in three trials in three VWCs in three reduced preparations (Fig. 5). In all of these cases the recorded ingestion motor program met at least five and usually (2 of 3) six criteria for this motor program as detailed in the preceding paper (Croll et al., 1985a). In all of these instances, the VWC was silent, showing neither the characteristic PPP nor action potentials during the motor output. The VWC membrane potential usually oscillated during CBC stimulation, but these oscillations were typically only loosely correlated with the ongoing ingestion motor program (e.g., Fig. 5). Therefore, in this limited sample, VWC activity was never associated with the ingestion motor program. The VWC thus appears to be neither necessary nor sufficient to the ingestion motor program as elicited in the present work (but see "Discussion").

The neutral rhythm(s). In many reduced preparations, the neutral rhythm(s) occurred without deliberate stimulation (e.g., Figs. 1, 2, and 4 near the beginning of the traces). The VWC was often active in cyclic bursts that were of relatively short

duration, contained up to 20 to 30 unbroadened VWC action potentials, and were locked in phase with retractor m 3 discharge. These short, rhythmic VWC bursts were clearly distinct from the PPPs that are sometimes associated with the egestion motor program. Following episodes of the egestion motor program, the neutral rhythm(s) typically resumed quickly, although the VWC was inactive for several minutes (Fig. 4). It may be concluded that VWC bursts may accompany the neutral rhythm(s) but are not necessary to this rhythm(s).

Synaptic influences on other buccal neurons

Postsynaptic influences of the VWC were sought by stimulating the VWC intracellularly while recording intracellularly from the somata of potential follower neurons in the ventral buccal ganglion. Despite an intensive search that included identified motoneurons and interneurons in both surface and subsurface soma layers, synaptic effects of the VWC were never detected, in accord with similar previous negative results (Gillette et al., 1980). The only known output target of the VWC is its contralateral homologue, with which it is electrically coupled (Gillette et al., 1980), confirmed in the present work (not shown).

The anterior ventral cells

The anterior ventral (AV) cells are a population of several neurons whose somata are located on the ventral surface of the buccal ganglion anterior to the transganglionic commissure on each side. Although they have not previously been studied extensively, it has been shown that they are exceptionally effective in driving other buccal and brain neurons. A single AV spike can trigger intensive and widespread motor effects (Gillette et al., 1978). The AV cells also adapt rapidly in response to injected current, making it impossible to drive more than a few action potentials in response to a prolonged pulse of DC current (Gillette et al., 1978). Finally, the AV cells possess peripheral axons in the contralateral SGN (not shown).

In the present studies we have worked primarily with a single, identified AV neuron whose soma is positioned posterior and slightly medial to the VWC. We also separately report results from other neurons whose somata are located immediately lateral or medial to the soma of the identified AV neuron. These also show current inactivation and are presumed to belong to the same functional population of AV neurons.

Effects of intracellular stimulation

The reduced preparation. The aforementioned identified AV neuron was stimulated intracellularly in 25 trials in six cells in five reduced preparations. The cell was usually stimulated during the spontaneously occurring neutral rhythm(s) and it was therefore usually difficult to ascertain whether this single AV neuron alone is capable of inducing the cyclic motor program. The AV neuron, however, was nearly always effective in modifying an ongoing motor program (e.g., Fig. 6). The modifications induced by AV stimulation almost always resembled components of the egestion motor program. That is, SD activity was suppressed or eliminated; retractor m 3 activity was reduced in amplitude; protractor m 4 activity was increased in amplitude; buccal constrictor m 5 activity was suppressed; and the mean protractor duty cycle, when accurately measurable, was generally increased above the 50% criterion for the egestion motor program. In Figure 6, the protractor duty cycle is >60% for the three middle cycles.

Analysis of the entire body of data generated by AV stimulation in the reduced preparation showed that in 64% of the trials, the activity of protractor m 4 increased in amplitude, characteristic of the egestion motor program, whereas in 36% of the trials there was no change. Reduction of protractor m 4

Figure 6. Effects of depolarizing an AV neuron with brief (10 msec) depolarizing pulses (visible on the lowest trace) in the reduced preparation. The top four traces are electromyograms made, respectively, from the salivary duct (sd), and buccal retractor muscle 3, protractor muscle 4, and constrictor muscle 5 (M3 to M5, respectively). The variable amplitude of the AV stimulus pulses was caused by variable invasion of the soma by the spike, as simultaneously confirmed on the oscilloscope.

activity was never observed. Stimulation of the identified AV neuron reduced or eliminated retractor m 3 activity in 40% of the trials and had no effect on retractor m 3 discharge in the remaining 60% of the trials; and SD activity was reduced or eliminated in 64% of the trials and unchanged in 36%. Buccal constrictor m 5 activity was never increased by AV stimulation but was reduced or eliminated in 48% of the trials and unchanged in 52%. A motor program in which all of these parameters clustered together to form the characteristic egestion motor program was observed in a half-dozen (~25%) instances (e.g., Fig. 6). The complete ingestion motor program was never elicited by AV stimulation in the reduced preparation.

The isolated CNS. The identified AV neuron was stimulated in 15 trials in four cells in four preparations. In these cases intense motor output was usually elicited, and during the stimulus this motor output showed characteristic features of the egestion motor program in the majority of cases, including tonic CBC and SGN discharge (e.g., Fig. 7). In three trials a vigorous motor program was elicited similar to the "rebound" motor program described for VWC stimulation. As developed below, this motor program exhibited the characteristic of an exaggerated egestion motor program.

The "rebound" motor program

A "rebound" motor program was elicited from the isolated CNS by stimulation of the identified AV neuron in 47% (7 of 15) of all trials (e.g., Fig. 7). This motor program was initiated 10 to 60 sec after the termination of AV stimulation. It exhibited the characteristics of the egestion motor program, including the episodic nature, suppression or elimination of the fast burster unit in the SN, and a protractor duty cycle in excess of 50%. This rebound motor program differed from the typical egestion motor program, however, in that it was characterized by cyclic rather than tonic discharge in the CBCs and SGNs (Fig. 7). This "rebound" motor program therefore ap-

Figure 7. The "rebound" motor program (second half of record), elicited by depolarizing the left anterior ventral (LAV) neuron in the isolated CNS (between arrowheads). LCBC, left CBC; lsgn and rsgn, left and right SGNs rsd, electromyogram from right salivary duct; rr3, right buccal retractor nerve root 3.

pears to be identical to that induced in the reduced preparation by VWC stimulation (cf. Figs. 7 and 3).

Intracellular activity during buccal motor programs

Experiments in which the activity of the identified AV neuron was recorded during induced or spontaneous motor programs seldom revealed AV action potentials (e.g., Fig. 6). Perhaps this reflects, in part, the rapid adaptation of active electrical soma membrane properties revealed by the foregoing current injection experiments. The discharge of action potentials by the AV neuron is therefore not necessary to the occurrence of the egestion motor program. The AV membrane potential nonetheless showed a characteristic step-like oscillation during the egestion motor program, with depolarization in phase with the protractor activity (Fig. 6). The prolonged hyperpolarization of the AV neuron before and during the rebound motor program shown in Figure 7 was atypical; in most such cases the AV neurons showed the same rectangular oscillation in membrane potential occurred during the neutral rhythm(s) (Fig. 6, before and after the egestion motor program is elicited by intracellular stimulation).

Synaptic influences on other buccal neurons

In contrast to the VWCs, the AV neurons exhibit widespread and powerful synaptic effects in the buccal ganglion, in keeping with their potent functional effects (Fig. 8). For example, the identified AV neuron described above caused a large excitatory postsynaptic potential (EPSP) in the contralateral VWC (Fig. 8A). Another AV neuron just medial to the individual, identified AV showed electrical coupling with a neuron having an axon in buccal root 1, i.e., a probable protractor motoneuron (Fig. 8B). The same AV neuron produced a large inhibitory postsynaptic potential (IPSP) in an identified retractor r 3 motoneuron (Fig. 8C). The same AV neuron produced a small IPSP (Fig. 8D) in a member of the corollary discharge population (CD; Davis et al., 1974). This inhibitory influence was highly effective inasmuch as it suppressed CD action potentials driven by injected current (Fig. 8D). Thus, although the present survey of postsynaptic effects is incomplete, the synaptic connections identified to date are all appropriate to the hypothesized role of the AV neuron in mediating the egestion motor program (see "Discussion").

Figure 8. Synaptic influences of AV neurons on other buccal neurons. A, Excitatory effect of the identified right AV (RAV) neuron on the contralateral (left) VWC (LVWC). B, Electrical coupling with a probable protractor (buccal root 1) motoneuron (r1mn). C, Inhibition of an identified retractor (buccal root 3) motoneuron (r3mn). D, Inhibition of a member of the previously identified corollary discharge (cd) population.

B3 interneuron

In a limited number of reduced preparations we have studied an identified neuron in the buccal ganglion previously termed B3 interneuron (Gillette et al., 1980). The soma of this cell is typically located two cells posterior to the identified AV neuron described above, and just anterior to the interganglionic commissure in the region of the ganglion corresponding to rectangular coordinates B3 of Siegler (1977). In the present experiments this neuron alone never elicited cyclic motor output, although it was always effective in modifying a neutral rhythm(s). Of six such trials in three cells in two reduced preparations, the amplitude of SD activity was reduced or eliminated in 83% of the trials and unchanged in the remaining 17%. In 50% of the trials protractor m 4 activity was increased in amplitude. The amplitude of retractor m 3 activity was reduced or eliminated in 83% of the trials and never increased. In 83% of the trials the amplitude of buccal constrictor m 5 was reduced or eliminated. All of these characteristics are indicative of the egestion, and not the ingestion, motor program. In 33% of the trials all of the components of the egestion motor program were observed together, but in no trial was the complete ingestion motor program observed.

Discussion

The data presented here show that three classes of identified buccal neurons—the ventral white cell(s) (VWC), the anterior ventral (AV) neurons, and interneuron(s) B3—meet some criteria normally ascribed to "command" neurons. Three criteria for such assignment are now generally accepted, namely, sufficiency, necessity (Kupfermann and Weiss, 1978), and appropriateness (Parsons et al., 1983). A neuron is sufficient if it causes the behavior or recognizable components when stimulated to fire at physiological discharge frequencies. It is necessary if the behavior or its components are blocked when the neuron is silenced. The neuron is appropriate if it fires during the execution of the behavior (motor appropriateness) and/or in response to the appropriate eliciting stimuli (sensory appropriateness).

In the present study we have applied, in various degrees, all three criteria to single neurons in the reduced preparation and isolated CNS. There are, however, at least two limitations in our approach. The first relates to the "sufficiency" criterion. When complex motor output is mediated by several command neurons, as indicated in the present studies (and probably most

motor systems), stimulation of single neurons in the command system is unlikely to cause complete motor patterns. This may explain why stimulation of single buccal neurons often did not elicit the complete egestion motor program but only some of its components.

The second limitation of our approach relates to the "necessity" criterion. When motor patterns are elicited "artificially" by electrical stimulation of mixed nerves, as in the present study, major normal inputs associated with the sensory releaser for the movement are absent. Moreover, neurons not normally active during the motor output are probably stimulated. Thus, the activity recorded from command elements under these conditions will at most reveal only one normal component of activity, namely, feedback from central pattern generator(s), and the recorded activity may contain spurious components not normally associated with the motor pattern. For example, when we elicited the ingestion motor program by tonic CBC stimulation, we probably did so via the paracerebral command neurons (PCNs) and metacerebral giant cells (MCGs) that send descending axons to the buccal ganglion via the CBCs. In the process, however, we undoubtedly stimulated other neurons, and we may have disrupted the normal cyclic discharge pattern of the PCNs, which in turn supply synaptic inputs to the VWCs and other buccal neurons (Gillette et al., 1982a, b).

These two limitations emphasize the need for studies during more normal behavior for assessing the role of a neuron, as noted previously by other investigators (e.g., Camp and Pinsker, 1979; Parsons et al., 1983; Pinsker, 1980; Pinsker and Eberly, 1982). The data presented here nonetheless furnish suggestive evidence. We have shown that intracellular stimulation of single buccal neurons in the reduced preparation and isolated CNS causes motor output that is similar or identical to the egestion motor program and specifically dissimilar from the ingestion motor program and/or the neutral rhythm(s). These results are consistent with the view that these neurons comprise a command system for egestion.

The ventral white cell(s)

Role in buccal motor programs. The VWCs have been studied extensively with regard to membrane properties and related cAMP metabolism (Gillette et al., 1980, 1982a, b; Calhoun and Gillette, 1983). In addition to normal action potentials, the VWCs often exhibit a spontaneous depolarization, 1 to 4 min in duration, that recurs on a regular period (Gillette et al., 1980). These depolarizations can also be triggered by injected current or synaptic inputs (Gillette et al., 1980) and, thus, resemble the "plateau potentials" described by Russell and Hartline (1978, 1982) in the lobster stomatogastric ganglion. Therefore, we term these sustained, recurrent depolarizations of the VWCs "prolonged plateau potentials" (PPPs). During the PPPs, VWC action potentials broaden from approximately 15 msec in duration to up to 250 msec. This broadening is, in turn, causal to a stereotyped, vigorous, and episodic cyclic motor output recorded from buccal nerves (Gillette et al., 1980).

Independently from the above studies on the VWC, we found that the stereotyped, episodic egestion motor program shows similar spontaneous recurrence in many preparations, and that the time course of the motor program episodes (1 to 4 min), as well as their rate of repetition, is similar to the duration and frequency of the PPP. This correspondence suggests, circumstantially, that the VWCs serve as command neurons for the egestion motor program (Croll and Davis, 1982), as proposed by McClellan (1980). The present studies lend support to this hypothesis by demonstrating that intracellular stimulation of a single VWC elicits the egestion motor program or its components in reduced preparations and the isolated CNS, but never the ingestion motor program and seldom its components. Moreover, the spontaneous occurrence of the PPP is sometimes

associated with the egestion motor program, but never with the ingestion motor program. We conclude that a single VWC is sufficient and sometimes appropriate (from the motor aspect) but not necessary to induction of the egestion motor program and/or its characteristic components in the reduced preparation. We found no evidence to suggest that the VWCs are necessary, appropriate, or sufficient to the ingestion motor program or its components. Within the limitations discussed above, these and previous (Gillette et al., 1980) findings suggest that the VWCs serve as elements of the egestion command system, and that this capacity is conferred by the PPP and the resultant spike broadening.

In addition to this command role for the egestion motor program, the VWCs may play a motor role in the neutral rhythm(s). In both the reduced preparation and the isolated CNS, the VWCs discharge cyclic bursts of unbroadened action potentials during the neutral rhythm(s). Gillette and Gillette (1983) reported that food stimuli excite the VWCs, suggesting a possible role in feeding behavior (but see below). Previously it was shown that each VWC sends a peripheral axon out of the contralateral SGN to innervate the musculature of the esophagus (Gillette et al., 1980), and that stimulation of the VWCs at physiological discharge frequencies comparable to those seen here during the neutral rhythm(s) causes strong contraction of the esophagus (Gillette et al., 1980; Gillette and Gillette, 1983). The observed tonic discharge of the VWCs during the egestion motor program might be expected to aid in the expulsion of material from the esophagus. The available data on the VWC thus suggest the possibility that the VWCs are multifunctional neurons, playing different roles in different buccal motor programs.

Given the potent effect of the VWCs in eliciting the egestion motor program or its components, the paucity of their synaptic outputs in the buccal ganglion is puzzling. There are at least two alternative explanations for this result (see also Gillette et al., 1980). First, it is possible that the VWCs exercise their motor influence via nonsynaptic cellular interactions that would not have been detected with the techniques used here. Second, it is possible that the motor effects of the VWCs are confined to neurons that have not been sampled in our experiments.

Comparisons with previous studies. McClellan (1980) has also reported that the VWC elicits the egestion ("vomiting") motor program when stimulated intracellularly. Inasmuch as the present studies employed qualitative criteria similar to McClellan's for identifying the egestion motor program and utilized newly available quantitative criteria as well (Croll et al., 1985a), the present results confirm and extend McClellan's (1980) findings. Gillette and Gillette (1983), however, have concluded that the VWCs serve as command neurons for ingestion ("feeding") behavior. They based this conclusion on two findings. First, food stimuli applied to the oral veil of the reduced preparation depolarize the VWC and generate the PPP. Emetic stimuli also frequently depolarize the VWC, but this depolarization is weaker than that induced by food stimuli and does not trigger the PPP. They also found that emetic stimuli hyperpolarized the VWC in the reduced preparation. Second, Gillette and Gillette (1983) report that intracellular stimulation of the VWC caused inward movement of food strips. In other words, McClellan's (1980) and our studies support an egestion command function for the VWC based on stimulation of the VWC (the sufficiency criterion) and recording during the occurrence of the motor programs in the reduced preparations (the motor aspect of the appropriateness criterion), whereas the study of Gillette and Gillette (1983) supports a feeding role for the VWC based largely on recording from the cell during sensory stimulation (the sensory aspect of the appropriateness criterion).

What, then, is the normal behavioral role of the VWC? The

evidence offered by Gillette and Gillette (1983) may be questioned on two grounds. First, McClellan (1980, 1982a) has reported that, in reduced preparations similar to those used by Gillette and Gillette (1983), food stimuli can cause egestion ("vomiting") rather than ingestion ("feeding"). In intact animals we have observed vigorous ingestive biting in response to injecting ethanol (normally at emetic stimulus) into the buccal cavity (unpublished observations). Therefore, the response of the VWC to sensory stimuli in the reduced preparation, one criterion employed by Gillette and Gillette (1983) to infer the behavioral role of the VWC, does not enable unambiguous conclusions regarding the normal behavioral role of the VWC.

Second, the data published by Gillette and Gillette (1983) in support of their hypothesis that the VWC induces ingestion consist of tracings from a movie of a single buccal movement cycle during VWC stimulation. From this figure (Fig. 5), it appears that the squid strip in the buccal cavity moved alternately in both directions. We have frequently observed similar behavior in reduced preparations in the absence of stimulation, i.e., spontaneously, and hence from the data shown in Gillette and Gillette (1983) it is not clear that VWC stimulation was causal to whatever movements are shown. Simultaneous recordings from the VWC were not presented, nor were recordings made from the muscles; therefore, it is impossible to identify the accompanying motor patterns using criteria developed by McClellan (1980, 1982a) and us.

If the VWC were a command neuron for ingestion, as concluded by Gillette and Gillette (1983), we would expect some component of the ingestion motor program to result from stimulating it in the reduced preparation. Instead, we find here that only the egestion motor program or its characteristic components result from VWC stimulation. Therefore, pending the study of VWC in the intact animal using techniques such as those developed by Pinsker and colleagues (Camp and Pinsker, 1979; Pinsker, 1980; Pinsker and Eberly, 1982; Parsons et al., 1983), we conclude that the VWCs play a command role for the egestion motor program and a motor role in the neutral rhythm(s) (by virtue of their discharge pattern and their innervation of the esophagus) and perhaps the ingestion motor program, which is similar to the neutral rhythm(s).

The anterior ventral neurons

Unlike the VWCs, which require numerous broadened action potentials to elicit motor output, single action potentials in the AV neurons induce widespread motor effects. Such functional effects are similar to those reported for the trigger neurons in the buccal ganglion of the snail *Planorbis* (Berry, 1972). In common with the VWC(s), stimulation of the AVs invariably elicited the egestion motor program or its components, but never elicited the ingestion motor program and seldom its components. During the spontaneous or induced expression of the egestion motor program, however, the AV neurons generally did not discharge action potentials, perhaps reflecting in part their rapid inactivation as revealed by current injection experiments. The results of intracellular stimulation suggest that the AV neurons also comprise part of the egestion command system, although clearly they are not necessary for the egestion motor program. It is also possible that the AVs are sensory neurons presynaptic to command elements, since they possess peripheral axons in the SGN. Complex integrative activity comparable to that seen in the AV neurons occurs in complex mechanoreceptors having central somata in *Tritonia* (Audesirk and Audesirk, 1979).

In contrast to the paucity of synaptic effects of the VWCs, single AV neurons displayed synaptic connections with a variety of identified postsynaptic target neurons in the buccal ganglion. AV neurons strongly excited the contralateral VWC, were electrically coupled with a probable protractor mononeu-

ron, and strongly inhibited an identified retractor motoneuron. The egestion motor program is characterized by activity in the VWCs enhancement of protractor activity, and suppression of retractor activity (Croll and Davis, 1981, 1982; Croll et al., 1985a). Therefore, the synaptic effects of the AV neurons are consistent with their hypothesized role in egestion and inconsistent with a role in the ingestion motor program. If the AVs are in fact sensory neurons, as suggested by their peripheral axons, then these data would imply sensory biasing of the motor pools as a mechanism of motor program switching, in addition to the hypothesized egestion command role performed by the VWC and the B3 interneuron.

Interneuron B3

Although the B3 interneuron has been studied less extensively than the VWCs or the AVs, all data obtained from this neuron are consistent with the view that it, too, contributes to the production of the egestion motor program. Intracellular stimulation of the B3 interneuron usually elicited either the egestion motor program or its characteristic components and never elicited the ingestion motor program and seldom its characteristic components. Previous work has shown that this interneuron produces a large, long duration EPSP in the VWC (Gillette et al., 1980), a synaptic effect that is consistent with the hypothesized role of the B3 interneuron in the egestion motor program.

The rebound motor program

Intracellular stimulation of either the VWC or AV neurons was frequently followed within 10 to 90 sec by a stereotyped, vigorous motor program that we term the "rebound" motor program. In hundreds of preparations studied in this and previous studies, we have never observed this motor program except in association with previous VWC or AV activity. The rebound motor program resembles in several respects an extreme form of the egestion motor program. In three respects, however, this motor output program differed from the egestion motor program described previously in this preparation (Croll and Davis, 1982; McClellan, 1982a, b). First, the VWC discharged cyclically, rather than tonically. Second, the SGN also discharged cyclically rather than tonically, presumably reflecting the cyclic activity of the VWC, which sends a peripheral axon out of the contralateral SGN. Third, the CBCs showed cyclic rather than tonic discharge.

It is possible that this rebound motor output represents a new motor program that previously has not been recorded in the intact animal (Croll and Davis, 1981) or in the reduced preparation (Croll and Davis, 1982; McClellan, 1982a, b). Alternatively, this motor program may have been recorded previously in the intact animal and in the reduced preparation but may not have been recognized as a distinct motor program, since it is similar to the identified motor program for egestion and since, in the intact animal, recordings have not been made from the VWC, SGNs or CBCs during the egestion motor program. In the reduced preparation, Gillette and Gillette (1983, Fig. 9) show records from the VWC of similar cyclic activity during an episode of egestion, but in that case the activity is accompanied by tonic discharge in the SGN. Deciding whether the rebound motor program is an extreme form of the egestion motor program or a separate motor program that is closely related to the egestion motor program will probably require recording from the VWC in the intact, behaving animal. The observation that both the VWC and the AV neurons elicit this same "rebound" motor program is further evidence of the functional synergy of these identified neurons.

Relation of cellular properties to behavior

In several instances it has been possible to relate properties of behavior to neuronal membrane properties in molluscs (Blankenship and Haskins, 1979; Byrne et al., 1979; Haskins and Blankenship, 1979; Shapiro et al., 1979; Byrne, 1980, 1981; Getting, 1983a, b). The present study suggests that the temporal properties of the PPP and accompanying spike broadening previously identified by our laboratory in the VWC (Gillette et al., 1980) are responsible for the stereotyped, episodic nature of the egestion motor program. This and previous work also support the hypothesis of multifunctionality of neurons (Davis, 1976), by showing that the VWCs can play both a command and a motor role and may participate in more than one buccal motor program. These interpretations, however, depend upon the interpretation of the VWC as playing a role in initiating the egestion motor program. The conflicting interpretations regarding this issue, discussed above, indicate the need for studies on the VWCs of the behaving organism.

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