

RE-EXAMINATION OF PRESUMED FEEDING MOTOR ACTIVITY IN THE ISOLATED NERVOUS SYSTEM OF *PLEUROBRANCHAEA*

BY ANDREW D. McCLELLAN*

*Department of Anatomy and Department of Biomedical Engineering,
Case Western Reserve University, Cleveland, Ohio 44106, U.S.A.*

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SUMMARY

1. In most isolated gastropod nervous systems, presumed feeding motor patterns are thought to be represented by the alternating motor activity in buccal roots, which underlies rhythmic radula movement. Since this movement accompanies several types of behaviour in the gastropod *Pleurobranchaea* (McClellan, 1979, 1980, 1982), the presumed feeding motor activity in the isolated nervous system of this animal was re-examined in more detail.

2. Alternating buccal root activity was shown here to be associated with other types of behaviour besides feeding, and is, therefore, not sufficiently unique to serve as a 'neural correlate' for feeding in *Pleurobranchaea* and presumably other gastropods.

3. Unlike the isolated nervous systems of most gastropods, which generate only one buccal rhythm, that of *Pleurobranchaea* generates two different buccal motor patterns which alternate: (1) a slow 'primary' rhythm whose behavioural identity is unclear, and (2) brief periods of a relatively fast rhythm which underlie bouts of vomiting.

4. In general, a buccal rhythm generated by an isolated gastropod nervous system can only be assigned a function if there are features of the rhythm that are unique to only one of the several behavioural responses involving the buccal mass.

INTRODUCTION

Invertebrate neuroethologists frequently examine the cellular basis of behaviour in semi-isolated or isolated nervous system preparations. The motor patterns generated by these reduced preparations are assumed to be the 'neural correlates' of behavioural responses displayed by intact animals. The association of motor patterns with behaviour must be done cautiously, particularly if an animal's behavioural repertoire includes several different responses which are expressed in part by similar motor activity.

During gastropod feeding, food is brought into the buccal cavity by rhythmic

* All correspondence to: Dr A. D. McClellan, Department of Physiology, University of North Carolina, School of Medicine, Chapel Hill, NC 27514, U.S.A.

forward and backward movements of the radula. These movements are driven by alternating motor activity in buccal roots which, in the isolated nervous system, is presumed to be the neural correlate of feeding (Berry, 1972; Kater & Rowell, 1973; Davis, Siegler & Mpitsos, 1973; Gelperin, Chang & Reingold, 1978; Benjamin & Rose, 1979; Bulloch & Dorsett, 1979*a, b*).

In the gastropod *Pleurobranchaea* rhythmic radula movement occurs not only during feeding but also during regurgitation, rejection, defensive biting and gill grooming (McClellan, 1978, 1979, 1980, 1982). These movements have also been observed during feeding and rejection in other gastropods (Bovbjerg, 1968; Kupfermann, 1974; Audesirk & Audesirk, 1979). Alternating buccal root activity is therefore not uniquely correlated with gastropod feeding.

The present study re-examines in more detail the behavioural function of buccal motor activity in the isolated nervous system which has previously been associated with feeding in *Pleurobranchaea*. This motor activity has been elicited artificially in both isolated and semi-intact preparations by electrical stimulation of oesophageal nerves, such as the stomatogastric nerve (Davis, Siegler & Mpitsos, 1973; Kovac & Davis, 1977; Siegler, 1977). The basic approach, then, was to compare this artificially elicited motor activity with the buccal motor patterns which accompany different types of naturally released behaviour in semi-intact preparations.

First, it was found that alternating motor activity in buccal ganglia roots accompanies other types of behaviour besides feeding, and thus cannot be a neural correlate for feeding. Second, the isolated nervous system of *Pleurobranchaea* generates not one but two buccal motor patterns which alternate: (1) a 'primary' rhythm which at present cannot be associated with any one particular behaviour involving the buccal mass; and (2) brief periods of a relatively fast rhythm which definitely do not underlie feeding, but instead underlie vomiting (a phase of the regurgitation behaviour).

Parts of these results have been reported previously (McClellan, 1979).

METHODS

Conventional semi-intact preparation

1. *Neural activity.* Injection of squid homogenate into the oesophagus elicits the swallowing phase of feeding (McClellan, 1978, 1980, 1982) (referred to as 'swallowing' to distinguish it from swallowing of solid food in intact preparations). Regurgitation was elicited by injecting a dilute soap solution into the oesophagus and included a writhing phase which alternated with brief bouts of a vomiting phase (McClellan, 1978, 1980, 1982). During these responses elicited by natural stimuli, the efferent pattern of activity was recorded from the cut ends of various cerebral and buccal nerves and compared with the artificially elicited motor activity in the same nerves in isolated preparations (see below).

The position of the radula was monitored by measuring the movements of the mechanically coupled radula sac with a displacement transducer (McClellan, in preparation).

2. *Muscle activity.* Muscular activity was recorded during oesophageal nerve stimulation and was compared with that previously reported during feeding, regurgitation

ation and rejection (McClellan, 1980, 1982). The oesophagus was removed, leaving the oesophageal nerves free for stimulation (see below). In some preparations, jaw and lip movements could be observed by pinning the buccal mass in a forward position to partially extend the proboscis (McClellan, 1982).

Isolated nervous system

The isolated nervous systems included all major ganglia (Fig. 7 here and Davis, Siegler & Mpitsos, 1973). The salivary duct and its bulbous enlargement (referred to as the SD) were left attached to the buccal ganglia via the salivary nerve (Fig. 7). Isolated preparations were maintained in artificial sea water containing 1% dextrose. This solution was kept at 12 °C and periodically bubbled with air.

Oesophageal nerve stimulation

Motor activity was artificially elicited in semi-intact or isolated preparations by stimulating, singly, one of two oesophageal nerves (Fig. 7): (1) the stomatogastric nerve (STGN), which connects the buccal and stomatogastric ganglia (Davis, Siegler & Mpitsos, 1973); or (2) the medial gastro-oesophageal nerve (MGON), which originates from the stomatogastric ganglia and innervates the oesophagus and possibly the gut. Constant current pulses, 10–40 μ A and 1.0 ms in duration, were delivered at 1.0 Hz through either flexible polyethylene (semi-intact preparations) or glass suction electrodes (isolated nervous systems). Since stimulation of the STGN or MGON elicited similar motor activity, the term 'oesophageal nerve' will apply to either of these nerves.

For technical reasons, the long MGON was generally stimulated rather than the shorter STGN. However, all the motor activity described here was verified by stimulating each of these nerves.

Neurophysiology

Electromyograms (EMGs) and nerve activity were recorded in semi-intact preparations with flexible polyethylene suction electrodes drawn to a fine tip (100–300 μ m) in a small flame. Nerve recordings were made in the isolated nervous system with conventional glass suction electrodes. Electrophysiological instrumentation was as previously described (McClellan, 1982).

Neurophysiological data were stored and photographed as previously described (McClellan, 1982). The contrast and quality of neural activity photographed from the oscilloscope screen was greatly improved with a multichannel z-mod intensification system (McClellan, 1981).

RESULTS

Muscle activity in the semi-intact preparation: oesophageal nerve stimulation

As previously reported, muscular activity was recorded during feeding (ingestion and swallowing phases), regurgitation (writhing and vomiting phases), and rejection in response to natural stimuli (McClellan, 1978, 1980, 1982). This muscle activity was compared in the present study with the muscle activity elicited by MGON ($n = 5$) and STGN ($n = 2$) stimulation. Stimulation of either of these nerves elicits

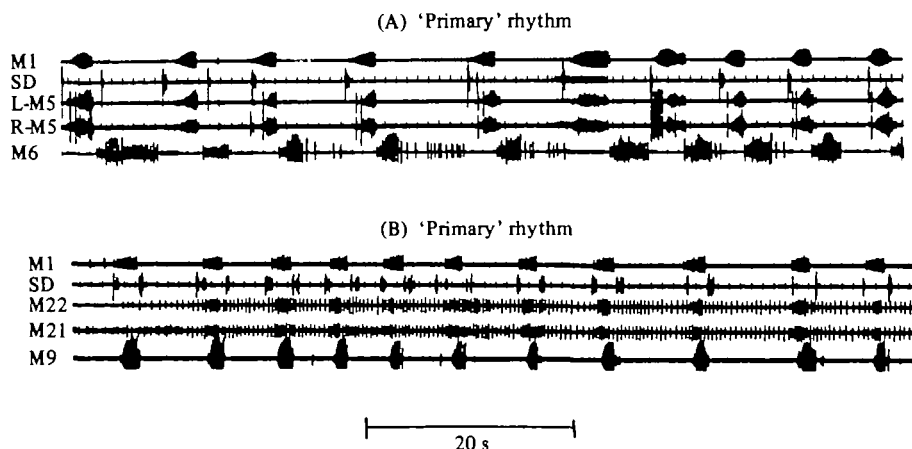


Fig. 1. Muscle activity during 'primary' rhythms in two different preparations elicited by MGON stimulation (see Methods) with 1.0 ms pulses delivered at 1.0 Hz. (A) Activity in buccal muscles (M1, M5, M6) and the salivary duct (SD) in one preparation elicited by 20.0 μ A stimulation. (B) Activity in anterior strap muscles (M22, M21) and lateral strap muscle (M9) in a second preparation evoked by 35.0 μ A stimulation.

two distinct motor patterns which alternate: (1) a slow 'primary' rhythm (Fig. 1), and (2) brief periods of a relatively fast pattern which underlie vomiting (Fig. 2, between arrows) (McClellan, 1980).

1. *Primary rhythm.* A 'primary' rhythm (Fig. 1) is elicited at the lowest effective level of stimulation current (typically in the range of 10–25 μ A). This pattern is not given a behavioural function because it is very similar to the naturally elicited pattern which accompanies feeding (ingestive and swallowing phases), rejection and the writhing phase of regurgitation (McClellan, 1980, 1982).

During primary rhythms (Fig. 1A), rhythmic radula movement is produced by alternating activity of radula retractor (M1; also M3, not shown) and protractor muscles (M6; also M2 and M4, not shown). This motor pattern consists of the biphasic cycles (i.e. retraction followed by protraction activity) which are characteristic for *Pleurobranchaea*. The salivary duct (SD) and buccal muscle five (M5) are active during each cycle of the rhythm (Fig. 1A). Anterior muscles, M22 and M21, and a lip-parting muscle, M9, are phasically active during this rhythm (Fig. 1B), with their main bursts of activity occurring during radula retraction (i.e. during M1 burst). Again, this pattern is not uniquely associated with any one particular behaviour involving the buccal mass.

The cycle time of the primary rhythm is 11.46 ± 1.1 s (mean \pm S.E., $n = 31$, 5 animals). Cyclic parting of the lips occurs reliably during radula retraction, while jaw opening occurs weakly and less frequently during the protraction–retraction transition of radula movement. These features suggest that the primary rhythm may underlie either the swallowing phase of feeding or the writhing phase of regurgitation, but other possibilities exist (see Discussion).

2. *Vomiting rhythm.* The primary rhythm is periodically interrupted at irregular intervals (e.g. 10–30 min) by a vomiting rhythm (Fig. 2, period of SD inactivity between arrows). In two experiments the lowest effective level of stimulation curr

elicited only the primary rhythm, and a slightly higher current (10–20%) was needed to elicit periodic intervals of the vomiting rhythm.

The behavioural function of the vomiting rhythm (Fig. 2, between arrows) is clear for several reasons. First, material is expelled from the buccal cavity during these artificially elicited periods of the vomiting rhythm. The lips are tonically parted and the jaws open during protraction–retraction transitions of rhythmic radula movement; these two features are characteristic of naturally elicited vomiting behaviour (McClellan, 1980, 1982). Secondly, the rhythm displays a short cycle time of 5.18 ± 0.62 s and occurs as a bout which lasts 63.54 ± 2.1 s ($n = 13$, 5 animals), again typical features of naturally released vomiting (McClellan, 1980, 1982). Finally, as previously established, the pattern of muscle activity during this rhythm (Fig. 2) is uniquely associated with vomiting: (a) the SD bursts are absent, and the M₅ bursts are either absent or markedly weaker; (b) anterior muscles, M₂₂ and M₂₁, are highly active with the main parts of their bursts shifted in phase to occur during the end of radula protractor activity; and (c) M₉, a lip-parting muscle, is highly active (McClellan, 1980, 1982). During artificially elicited vomiting rhythms, radula retractor and protractor muscles alternate in their activity (only M₁ and M₆ shown here), but this sub-pattern is not unique to any one behaviour involving the buccal mass (McClellan, 1978, 1979, 1980, 1982).

Neural activity in the semi-intact preparation: natural stimuli

It is likely that oesophageal nerve stimulation elicits the same two rhythms in the isolated nervous system as occur in semi-intact preparations. However, it may be misleading to compare muscle activity from semi-intact preparations with neural activity from isolated nervous systems. Therefore, the efferent activity in various buccal and cerebral ganglia nerves (Fig. 7) was recorded in semi-intact preparations during naturally induced feeding ('swallowing' phase) and regurgitation (writhing and vomiting phases) and was compared with the neural activity in isolated nervous systems (see Methods).

1. *Buccal ganglia root activity: R₃ and R₁*. Buccal root three (R₃) primarily innervates retractor muscles (e.g. M₁, M₃, M₅) and produces radula retraction, while buccal root one (R₁) primarily innervates protractor muscles (e.g. M₂, M₄, M₆) and produces radula protraction (Davis, Siegler & Mpitsos, 1973). In all of four experiments, alternating activity in these buccal roots and rhythmic radula movement (rad) occurred during 'swallowing', the writhing phase, and the vomiting phase (Fig. 3). The buccal root activity was recorded from the cut ends of nerves on one side of the preparation, while muscle activity and radula movements were produced by the contralateral, intact buccal roots.

2. *Oesophageal nerve activity: MGON*. During the 'swallowing' phase of feeding and the writhing phase of regurgitation (when the oesophagus displays peristalsis), bursts in the medial gastro-oesophageal nerve (MGON) occur in phase with radula retractor activity (Fig. 4A, B). During the vomiting phase of regurgitation (when the oesophagus tonically contracts) the MGON is tonically active at a relatively high frequency (Fig. 4C).

3. *Brain root activity*. Several nerves originate from the cerebral ganglia (brain) and innervate various anterior structures in *Pleurobranchaea*. These include the mouth

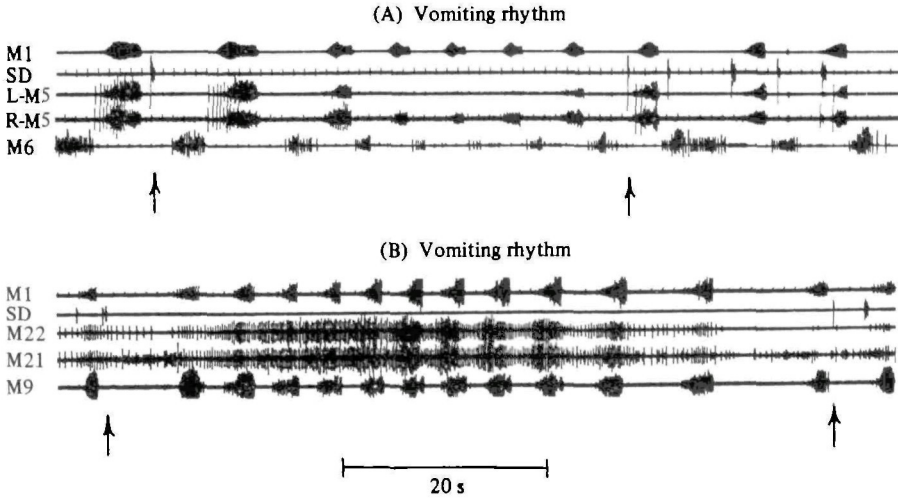


Fig. 2. Muscle activity during vomiting rhythms, which periodically interrupt the primary rhythm at irregular intervals. The A and B records are from the same two preparations and with the same stimulating parameters as in Fig. 1. (A) A clear vomiting rhythm (between arrows) is indicated by salivary duct (SD) inactivity and buccal muscle five (M5) activity which is weak or absent. (B) During the transition to a vomiting rhythm (between arrows), anterior muscle activity (M22, M21) is shifted in phase, and lateral strap muscle (M9) bursts increase in duration. Vomiting motor activity is demarcated in this and all subsequent figures by the period of SD inactivity (i.e. between arrows).

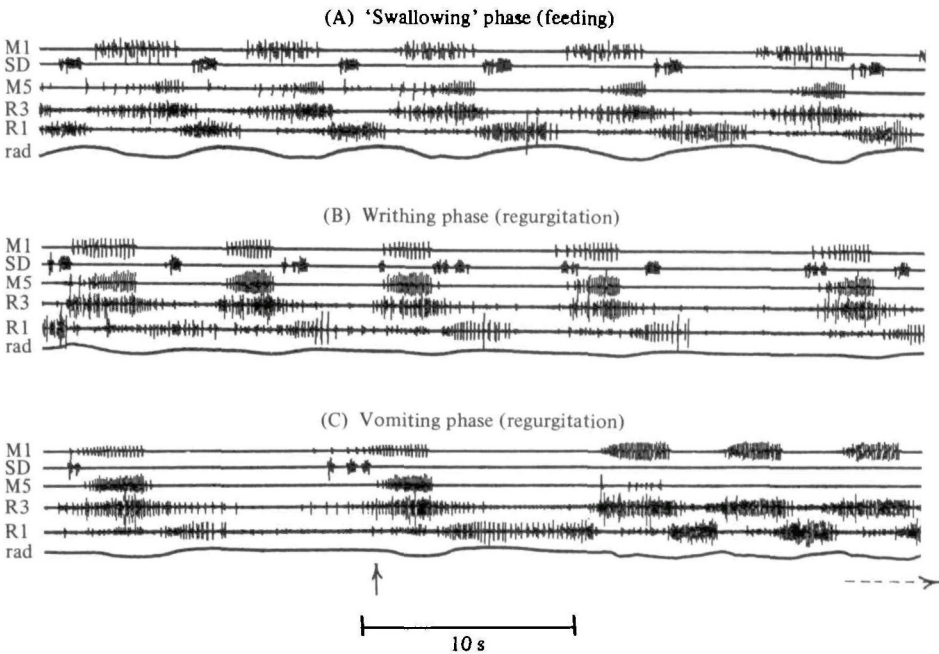


Fig. 3. Alternating activity in buccal root three (R3) and buccal root one (R1) in a semi-intact preparation during (A) the 'swallowing' phase of feeding, and (B) the writhing phase and (C) bouts of the vomiting phase (between arrows) of regurgitation. Upward deflexion of the 'rad' trace indicates radula protraction and is mediated by R1 activity. Radula retraction is produced by R3 bursts.

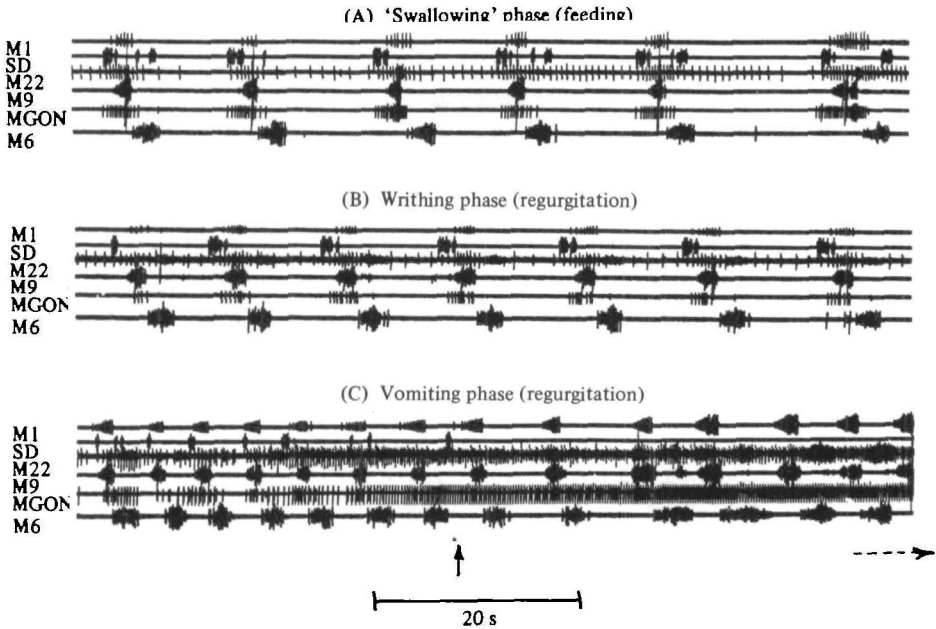


Fig. 4. Neural activity in the medial gastro-oesophageal nerve (MGON) recorded from a semi-intact preparation during (A) the 'swallowing' phase of feeding, and (B) the writhing phase and (C) bouts of the vomiting phase (between arrows) of regurgitation.

nerve (MN), the small oral veil nerve (SOVN), and the large oral veil nerve (LOVN) (Davis, Siegler & Mpitsos, 1973; Lee & Liegeois, 1974; and Fig. 7 here).

During the 'swallowing' phase of feeding and the writhing phase of regurgitation (Fig. 5A, B), MN and SOVN activity is phasic and involves high-frequency bursts occurring during radula retraction activity (i.e. M1 burst). The activity in LOVN is unpatterned or weakly in phase with radula retraction activity during these same responses.

During the transition from the writhing phase to the vomiting phase of regurgitation (Fig. 5C, see legend), the activity in MN increases and becomes largely tonic or unpatterned. In 28 of 30 bouts of vomiting (11 animals), new SOVN units were recruited which phase-shifted the bursts in this nerve to the end of radula protraction activity (Fig. 5C). In the remaining cases, SOVN activity was essentially tonic during vomiting. The activity in the LOVN could be tonic during vomiting, but in some cases this nerve displayed bursts which occurred during the end of radula protraction activity (Fig. 5C).

4. *Summary of neural activity.* Fig. 6 summarizes the naturally elicited neural activity recorded from semi-intact preparations (McClellan, 1980). The SD activity is included since it can also be recorded in the isolated nervous system (Fig. 7). The activity in Fig. 6 is compared with the motor activity elicited in the isolated nervous system by oesophageal nerve stimulation.

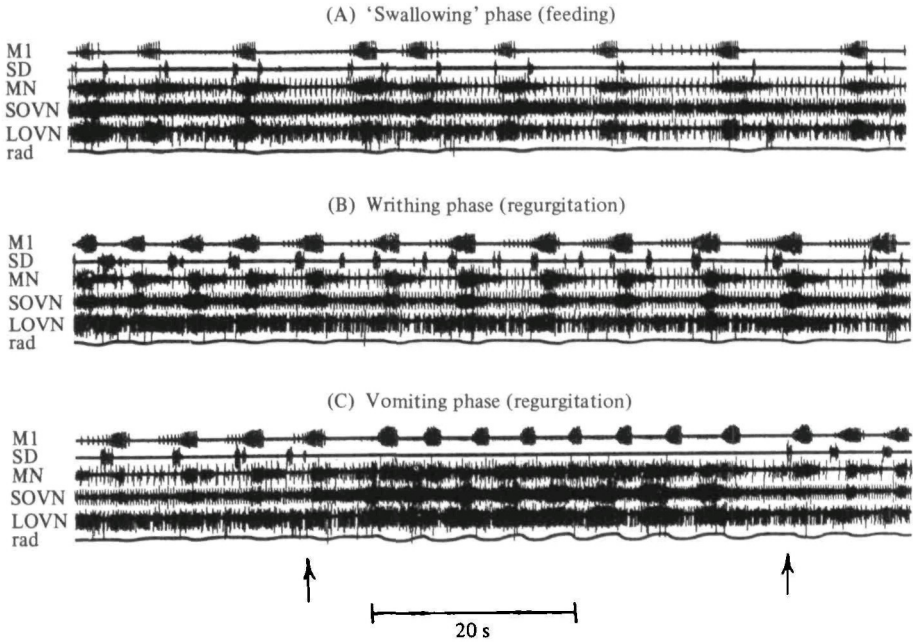


Fig. 5. Brain root activity recorded from a semi-intact preparation during (A) the 'swallowing' phase of feeding, and (B) the writhing phase and (C) bouts of the vomiting phase (between arrows) of regurgitation. During the transition to vomiting, mouth nerve activity (MN) increases and becomes largely tonic, while the burst activity of the small oral veil nerve (SOVN) switches phase. Activity in the large oral veil nerve (LOVN) can be similar to that in the SOVN, but more often is tonic or unpatterned.

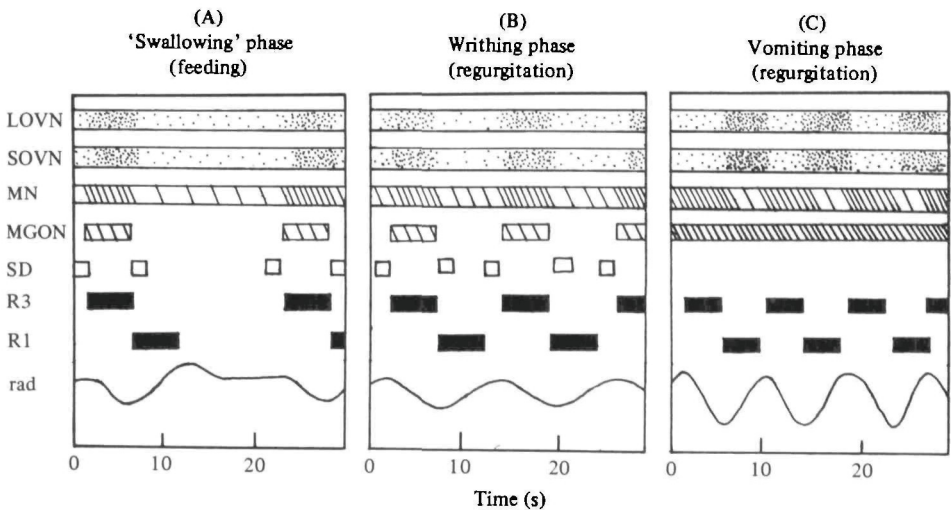


Fig. 6. Summary of motor activity in cerebral nerves (LOVN, SOVN), buccal nerves (R3, R1), oesophageal nerve (MGON) and the salivary duct (SD) recorded from semi-intact preparations (Figs. 3-5) in response to natural stimuli during feeding ('swallowing' phase) and regurgitation (writhing and vomiting phases). Upward deflexion of 'rad' trace indicates radula protraction. The naturally released pattern of activity in the above components forms the basis for comparing the artificially elicited motor activity in the same components in the isolated nervous system. The activity in LOVN is quite variable and is often unpatterned.

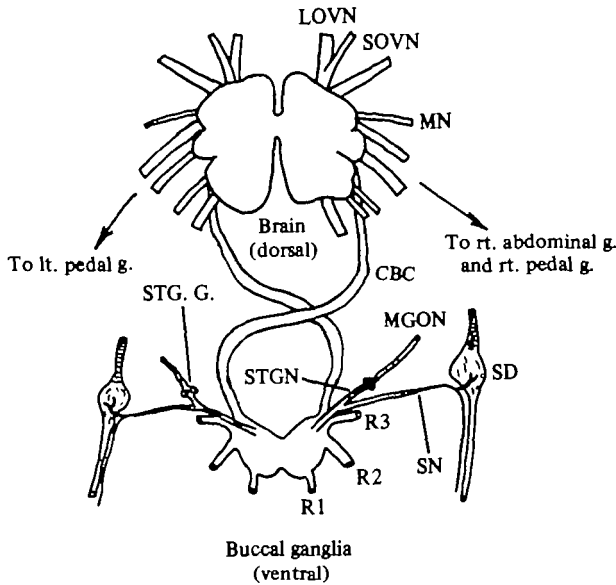


Fig. 7. Illustration of the isolated nervous system preparation. All the major ganglia are included in this preparation, but only the cerebral (i.e. brain) and buccal ganglia are shown here. The connectives between the two pedal ganglia are normally cut. Abbreviations: LOVN, large oral veil nerve; SOVN, small oral veil nerve; MN, mouth nerve; CBC, cerebral buccal connective; R1, R2, R3, buccal ganglia roots one, two and three; SD, the bulbous part of the salivary duct; STGN, stomatogastric nerve; STG. G., stomatogastric ganglia; MGON, medial gastroesophageal nerve; SN, salivary nerve (some terminology adapted from Davis, Siegler & Mpitsos, 1973).

Motor activity in the isolated nervous system: oesophageal nerve stimulation

In 15 of 22 preparations (69%), the lowest effective levels of MGON stimulation (see Methods) elicited a single type of motor pattern (Fig. 8A) which resembled the activity during the 'swallowing' phase of feeding or the writhing phase of regurgitation (Fig. 6). Since the behavioural rhythm of this artificially elicited pattern is unknown, it will be referred to as a 'primary' rhythm as in the semi-intact preparation (Fig. 1).

At the lowest current threshold, I_1 (usually 10–20 μA), a primary rhythm is elicited with a cycle time of 21.3 ± 1.3 s ($n = 31$, 5 animals). A second current threshold, I_2 , is reached by increasing the MGON stimulating current above I_1 by 2.5 μA increments (the limits of the stimulator). At I_2 , a faster primary rhythm is produced (Fig. 8B, C; left and right parts of records) which has a cycle time of 13.9 ± 0.6 s ($n = 69$, 9 animals). In addition, this primary rhythm is periodically interrupted at irregular intervals by relatively short periods of a vomiting rhythm (Fig. 8B, C). I_2 is only 14% \pm 2.6% (22 animals) greater than I_1 , but this value could be slightly higher or lower due to the limit in current increments allowed by the stimulator. In 31% of the preparations (7 animals), an I_2 threshold did not exist, and I_1 elicited both primary and vomiting rhythms.

A behavioural function can be associated with the vomiting rhythm (Fig. 8B, C) for several reasons: (a) the rhythms occur periodically as bouts which last 90.5 ± 3.9 s ($n = 40$, 9 animals), a typical characteristic of naturally released bouts of vomiting (McClellan, 1978, 1980, 1982); (b) the SD is inactive; (c) activity in the MGON

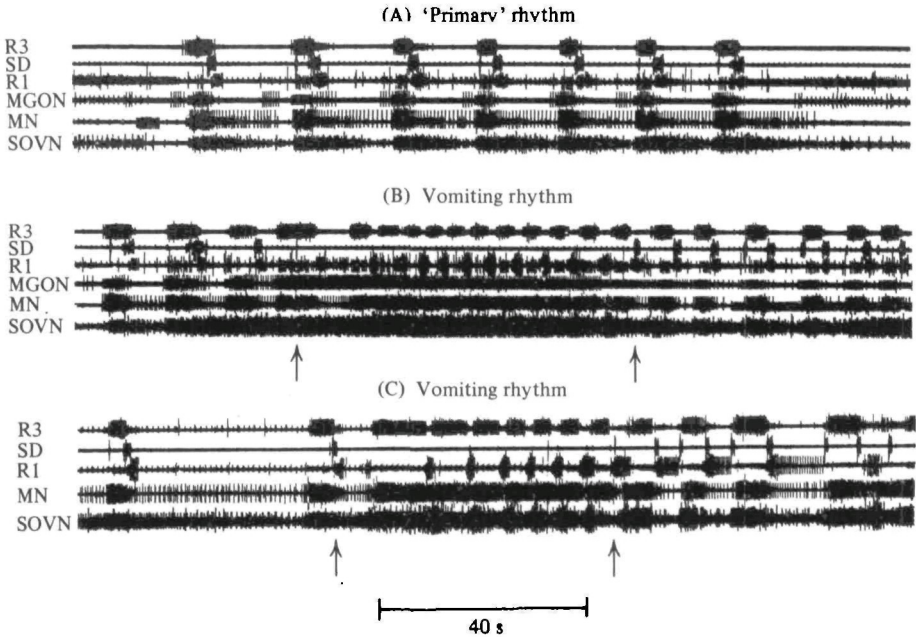


Fig. 8. Motor patterns elicited in the isolated nervous system by 1.0 ms current pulses delivered at 1.0 Hz to the MGON (see Methods). (A) Pure 'primary' rhythm elicited by the lowest current threshold level, I_1 (17.5 μ A). (B) Higher-frequency 'primary' rhythms which are periodically interrupted by relatively short bouts of a vomiting rhythm (between arrows) are elicited by a slightly higher current threshold level, I_2 (20 μ A). In this particular preparation and in 21% of the bouts of vomiting motor activity (7 bouts, 9 animals), the SOVN activity became tonic. (C) In a different preparation and in 79% of the bouts of vomiting motor activity (28 bouts, 9 animals), the burst activity in the SOVN was shifted in phase (between arrows). In this preparation $I_1 = 15 \mu$ A and $I_2 = 17.5 \mu$ A. Other features of vomiting motor activity, such as a relatively high rhythm frequency for several cycles, SD inactivity, tonic MGON activity, and relatively tonic MN activity occur in all preparations. In the few experiments that LOVN activity was recorded, it was found to be essentially unpatterned.

and MN is largely tonic, while the SOVN activity is either tonic (Fig. 8 B) or shifted in phase to occur during the end of radula protractor activity (Fig. 8 C); (d) buccal root activity (R3 and R1) alternates with a relatively short cycle time of 7.9 ± 0.5 s ($n = 40$, 9 animals). The above pattern of motor activity in the isolated nervous system correlates well with the activity that occurs in the same components during vomiting released by natural stimuli in semi-intact preparations (Fig. 6; McClellan, 1980, 1982).

The motor activity in Fig. 8 was elicited by stimulating the MGON. Presumed feeding motor patterns have usually been activated in the isolated nervous system of *Pleurobranchaea* by stimulation of the stomatogastric nerve, STGN (Davis, Siegler, & Mpitsos, 1973). Stimulation of the STGN was, therefore, tested in five preparations and was found to elicit activity similar to that produced by MGON stimulation. That is, STGN stimulation at I_1 elicited a primary rhythm (Fig. 9 A), while stimulation at I_2 elicited a slightly faster primary rhythm (Fig. 9 B, right section of record) which was periodically interrupted by a vomiting rhythm (Fig. 9 B). I_2 was $9.0\% \pm 1.0\%$ greater than I_1 (5 animals). Vomiting motor activity is clearly identified by SD inactivity and tonic oesophageal nerve activity (STGN in this case) which accompanies a relatively fast rhythm.

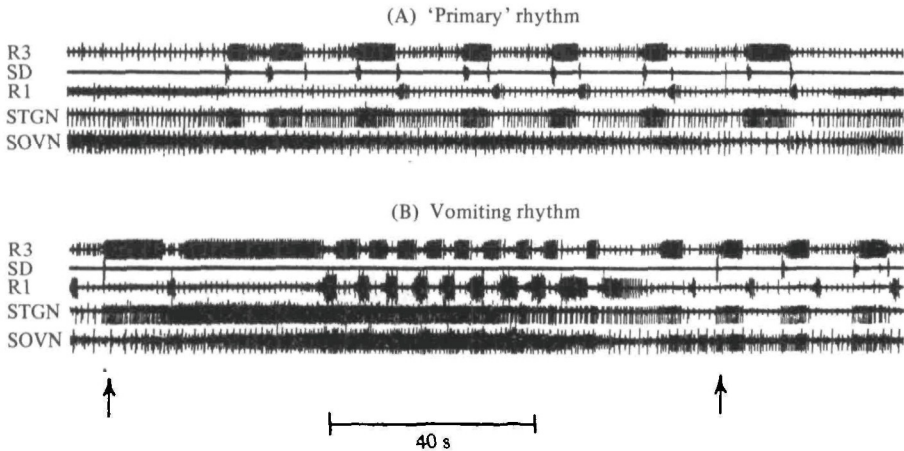


Fig. 9. Motor patterns elicited in the isolated nervous system by 1.0 ms current pulses delivered at 1.0 Hz to the STGN (see Methods). (A) Pure 'primary' rhythm elicited by current threshold level I_1 ($25 \mu\text{A}$). (B) Higher-frequency 'primary' rhythms which are periodically interrupted by short bouts of a vomiting rhythm (between arrows) are elicited by current threshold level I_2 ($27.5 \mu\text{A}$). It appears that SOVN activity is more variable with STGN stimulation than with MGON stimulation. For example, in the preparation here SOVN activity was largely unpatterned during the 'primary' rhythm (A), while during the vomiting rhythm (B) the burst activity in the SOVN was, at best, only weakly in phase with protraction activity.

DISCUSSION

Motor activity elicited by oesophageal nerve stimulation

Electrical stimulation of oesophageal nerves in *Pleurobranchaea* presumably activates afferent axons which originate from chemoreceptive or tactile sensory neurones in the oesophagus. Only four neurones in the buccal ganglia and one neurone in the cerebral ganglia (the metacerebral giant) have efferent axons in the STGN (McClellan, 1980; Gillette & Davis, 1977). Three of the buccal neurones do not elicit motor activity, even when stimulated at high frequencies. Neither the remaining buccal neurone (the ventral white cell) nor the cerebral neurone elicits rhythmic motor activity when stimulated at 1.0 Hz (A. D. McClellan, in preparation).

1. *Limitations of electrical stimulation.* The motor activity elicited here by electrical stimulation of oesophageal nerves is well within normal limits for *Pleurobranchaea*. Nonetheless, the burst duration of M9 activity is often slightly shorter during artificially elicited vomiting rhythms than during naturally released bouts of vomiting. Also, the structure of the bursts in some muscles is sometimes slightly different qualitatively between artificially and naturally evoked patterns. However, since the above differences are minor and within the normal limits of variability, the artificially elicited rhythms can be considered to underlie normal behavioural responses.

2. *Variability of elicited motor activity.* It is implied here that oesophageal nerve stimulation elicits the same two rhythms in both semi-intact and isolated preparations, and also in different animals. This seems true for the vomiting rhythm. However, during the primary rhythm the phasing of anterior strap muscle activity and the amplitude of lip and jaw movements are often slightly different in different animals. This variability may result from small differences in the types of oesophageal nerve

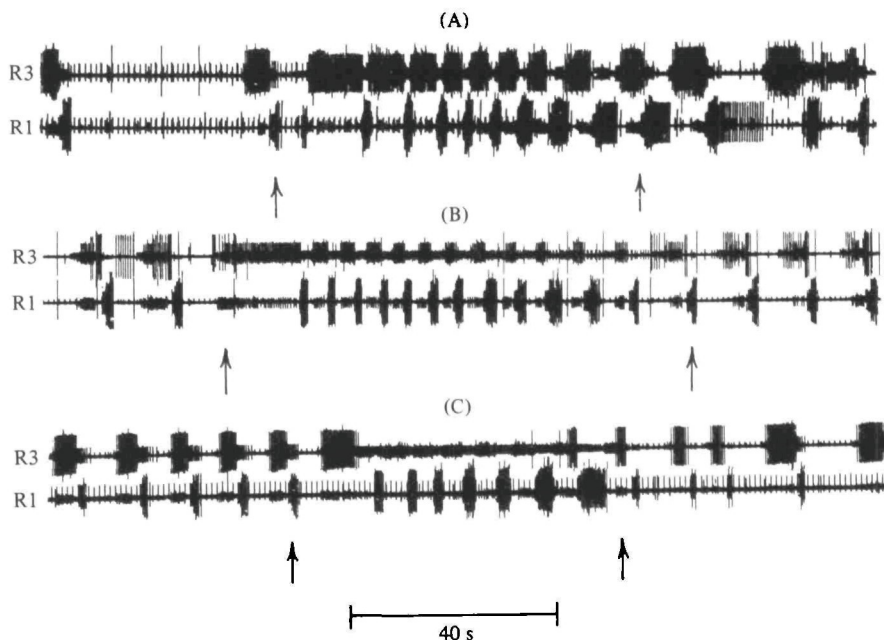


Fig. 10. Buccal root activity which undergoes (A) small, (B) moderate, and (C) large changes in amplitude during the transition to vomiting motor activity (between arrows) elicited by MGON stimulation in three different isolated nervous systems. The majority of preparations displayed changes in R3 and R1 activity which were intermediate between A and B. Less than 5% of the preparations ($n = 30$) displayed the large changes in buccal root activity shown in C. The burst durations of R3 and R1 activity are both increased during vomiting, and the increased intensity of R1 bursts accounts for the powerful radula protraction movements which occur during vomiting in intact animals. In general, vomiting motor activity cannot be reliably identified by buccal root activity alone and was identified here on the basis of other features of the motor pattern, such as SD inactivity, which were purposely omitted from the above records.

axons being stimulated or from differences in the behavioural states of the preparations. The primary rhythm must be analysed in more detail to determine if it truly underlies only a single response (see below).

Comparison with other gastropod studies

Oesophageal nerve stimulation was originally adopted as a technique for artificially eliciting presumed feeding motor activity in the isolated nervous system of *Pleurobranchaea* (Davis, Siegler & Mpitsos, 1973). Since other gastropod behavioural responses involving the buccal mass had received little attention it was logical to associate this motor activity with the feeding behaviour.

It is now known, however, that the same buccal structures are involved in similar, but not identical movements during feeding, rejection, regurgitation, defensive biting and gill grooming in *Pleurobranchaea* (McClellan, 1978, 1980, 1982). Since some of these functionally different behavioural responses are partly expressed by similar motor patterns, the correlation between motor activity and behaviour must be made cautiously. The complete behavioural repertoire of an animal must therefore be considered, even to study only a single behaviour. This is particularly true for m

activity which is generated by isolated nervous systems, because the mechanical consequences of a motor pattern cannot be observed.

In the isolated nervous system of *Pleurobranchaea*, oesophageal nerve stimulation was shown to elicit a primary rhythm of uncertain behavioural identity which was periodically interrupted by a clear vomiting rhythm. In the isolated nervous systems of other gastropods, presumed feeding motor activity is released spontaneously rather than by electrical stimulation (Berry, 1972; Kater & Rowell, 1973; Gelperin, Chang & Reingold, 1978; Benjamin & Rose, 1979; Bulloch & Dorsett, 1979*a, b*). These studies are thus not directly comparable to the present results, because the isolated nervous system of *Pleurobranchaea* does not normally generate spontaneous motor activity. Nevertheless, some gastropods do display at least feeding and rejection (Bovbjerg, 1968; Kupfermann, 1974; Audesirk & Audesirk, 1979), which are undoubtedly expressed by similar but not identical motor activity. Thus the presumed feeding motor activity generated by the isolated nervous systems of these other gastropods could underlie other responses and should be re-examined in more detail.

The presumed feeding rhythms in isolated gastropod nervous systems have rarely been compared with the pattern of neural activity that accompanies feeding in more intact preparations. During presumed feeding motor activity in isolated *Pleurobranchaea* preparations, SOVN bursts were previously reported to occur during radula protraction (Davis, Siegler & Mpitsos, 1973), a phase relationship shown here to be associated with vomiting (Fig. 6).

Unique versus non-specific motor activity

Since each cerebral and buccal ganglia nerve in *Pleurobranchaea* innervates more than one muscle, the function of the units recorded in these nerves is not always clear. Nonetheless, the neural activity recorded from semi-intact preparations in response to natural stimuli (Fig. 6) allows a comparison of the motor activity generated in the isolated nervous system. This motor activity includes both unique and non-specific features.

1. *Non-specific features.* Alternating buccal root activity underlies rhythmic radula movement and has traditionally served as a neural correlate for gastropod feeding. This activity is, however, not unique to feeding, because rhythmic radula movement accompanies five behavioural responses in *Pleurobranchaea* (McClellan, 1978, 1979, 1980, 1982) and at least two responses in other gastropods (Bovbjerg, 1968; Kupfermann, 1974; Audesirk & Audesirk, 1979).

In Fig. 3, alternating buccal root activity was shown to occur during both feeding and regurgitation. These responses cannot, of course, be directly observed in isolated nervous systems, and the common practice of making behavioural correlations solely on the basis of buccal root activity is, therefore, unsupported. Vomiting, and presumably other types of behaviour involving the buccal mass, cannot usually be reliably identified on the basis of buccal root activity alone (Fig. 10A, B; between arrows).

2. *Unique features.* The primary and vomiting rhythms are clearly distinguished in the isolated nervous system by recording features of the pattern in addition to buccal root activity. SD inactivity and tonic MGON activity, when accompanied by a fast rhythm, are reliable 'neural correlates' for vomiting (Figs. 6, 8 and 9). In contrast,

the behavioural identity of the primary rhythm is unclear, partly because the differences between feeding (ingestion and swallowing), the writhing phase of regurgitation, and rejection are accounted for by differences in the activity of muscles which are at present unidentified or difficult to record from (McClellan, 1980, 1982).

Role of the primary rhythm

The primary rhythm presumably underlies a behavioural response, other than vomiting, which is activated by sensory input from the oesophagus. Defensive biting and gill grooming are very unlikely candidates, because they are not activated from the oesophagus and are difficult to elicit even in intact animals. The ingestive phase of feeding is elicited by food near the anterior part of the buccal mass and is, therefore, also unlikely. Rejection results from tactile stimuli within the buccal cavity, but probably cannot be ruled out because it can be initiated from the oesophagus in some gastropods (Kupfermann, 1974). The mechanics of the response and the frequency of the pattern in the semi-intact preparation suggest that the primary rhythm most probably underlies either the swallowing phase of feeding or the writhing phase of regurgitation. In addition, the primary rhythm involves phasic oesophageal nerve activity (Fig. 8A) and presumably peristalsis of the oesophagus, which are characteristics of the above two behavioural responses. The primary rhythm may even represent two responses, since the two current thresholds, I_1 and I_2 , elicit activity which differs in intensity (Figs. 8, 9).

The above 'process of elimination logic' does not, of course, rigorously establish the identity of the primary rhythm. More exact methods are needed. First, it would be useful simply to place an object in the buccal cavity of a semi-intact preparation and observe the consequences of oesophageal nerve stimulation during the primary rhythm. Secondly, the motor patterns underlying different behavioural responses may appear similar when comparing only the qualitative features of whole nerve recordings. It would, therefore, be useful to compare quantitatively, in the same animal, the neural activity released from a relatively intact preparation during feeding, regurgitation and rejection with the neural activity which occurs in the same nerves during patterns generated by isolated nervous systems.

Semi-isolated preparations

A recently developed semi-isolated gastropod preparation leaves part of the lips attached to the isolated nervous system. Presumed feeding motor activity can thus be elicited by presenting food substances to the lips (Gelperin, Chang & Reingold, 1978; Senseman, 1977). The interpretations concerning this motor activity are unclear. First, reduced gastropod preparations may not always feed in response to food because of an altered motivational state. Semi-intact *Pleurobranchaea* preparations, for example, occasionally vomit when presented with food (McClellan, 1980, 1982). Secondly, the presumed feeding motor activity should be compared in more detail to the motor activity underlying all behavioural responses involving the buccal mass. Nonetheless, the semi-isolated preparation is a very promising technical advance.

Central programming

Because presumed feeding motor patterns have been generated in the isolated nervous systems of several gastropods, the feeding behaviour has been assumed to be controlled by a centrally programmed network that does not require sensory input. The present study with the isolated nervous system of *Pleurobranchaea* only provides evidence that vomiting is centrally programmed. However, the other behavioural responses involving the buccal mass (see Introduction) are probably also centrally programmed by a single motor pattern generator which controls all of these functions (McClellan, 1978, 1980, 1982). This, however, remains to be shown.

CONCLUSIONS

The common assumption that gastropod buccal rhythms underlie feeding was shown here to be incorrect or at least oversimplified. This should not be considered as a negative finding. On the contrary, the challenge now is to understand how the neural circuits generate several functionally different gastropod behavioural responses which involve the same buccal structures. The isolated nervous system of *Pleurobranchaea* produces at least two different buccal motor patterns, and thereby serves as a model system for multibehavioural pattern generation (A. D. McClellan, in preparation).

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