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# The Control of Hindgut Motility in the Lobster, *Homarus gammarus* (L.)

## 2. Motor Output

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1. Two types of motor activity pass from the sixth abdominal ganglion (6 A.G.) via the posterior intestinal nerves (P.I.N.'s) to the hindgut of *Homarus*. These have been designated unitary or non-repetitive and bursting or repetitive activity. They are not entirely exclusive of one another.

2. The unitary discharge is produced by at least one and possibly two interneurones (I1) whilst another class of interneurone (I2) produces bursting output.

3. Both forms of motor output are paired between branches of the P.I.N.'s. This pairing may be due to either anatomical or physiological methods of connectivity.

4. The bursting discharge is completely immutable and is not affected by extirpation of sensory input. We have attempted to explain this immutability on the basis of a fixed neuronal network in the 6 A.G. involving precise connectivity patterns.

5. Non-repetitive units produce faily weak hindgut peristalsis whilst the bursting discharge produces very powerful contractions. We suggest that burster neurones innervate both circular and longitudinal muscles whilst exclusively unitary neurones only innervate the longitudinal musculature. The role of neurones which can assume both types of discharge is not clear.

## INTRODUCTION

Stimulation of the ventral nerve cord (V.N.C.) of *Homarus* induces hindgut peristalsis co-ordinated with anal dilation and closure (Winlow and Laverack, 1972.1). We have shown this "defaecatory response" to be mediated via the posterior intestinal nerves (P.I.N.'s), but the form and

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characteristics of the motor discharge are, as yet, undescribed. In this paper we have attempted to analyse the output to the rectum and to relate this output to the hindgut movements and sensory input previously described.

## MATERIALS AND METHODS

The sixth abdominal ganglion (6 A.G.)/rectum complex was exposed by dissection as described in our earlier paper (1972.1), leaving the entire abdominal nerve cord intact. The rectum and the attached abdominal V.N.C. were then isolated and maintained in sea water at about 12°C.

The P.I.N.'s were often detached from the hindgut by fine dissection and raised on platinum wire hook electrodes into a layer of liquid paraffin for electrophysiological purposes.

In other experiments the 6 A.G./rectum complex was left *in situ* and hindgut movements were monitored concurrent with motor activity, using R.C.A. 5734 transducer valves. It is not possible to make deductions about the timing of various units from traces showing simultaneous recordings from two branches of the P.I.N.'s, since the recording position relative to the 6 A.G. varied between branches.

## RESULTS

### A. Analysis of Motor Output

There are basically two forms of nervous activity which are efferent in the P.I.N.'s to the hindgut following V.N.C. stimulation. These types are not, however, entirely exclusive of one another and both forms of activity can be elicited by stimulation of either connective in any abdominal segment (and probably by stimulation of the thoracic connectives). At low stimulus amplitudes "unitary" motor responses follow the stimulus pulses in a one to one ratio (often up to 20 or 30 Hz) as is shown in Figure 1(A). At slightly higher stimulus amplitudes pronounced bursts of activity may occur in the P.I.N.'s (Figure 1(B)). Initially the response is similar to that in Figure 1(A), but the neurones initially involved in the unitary discharge eventually start firing repetitively as do a number of smaller "burster" units. Such bursting activity may be the result of the activation of repetitively firing driver units activated by interneurones responding at higher stimulus amplitudes. The number of units involved in either form of discharge varied from preparation to preparation, but fewer units generally occurred in the P.I.N.p.'s† as compared with the P.I.N.a.'s.‡

†‡Posterior and anterior branches of the P.I.N.'s respectively.

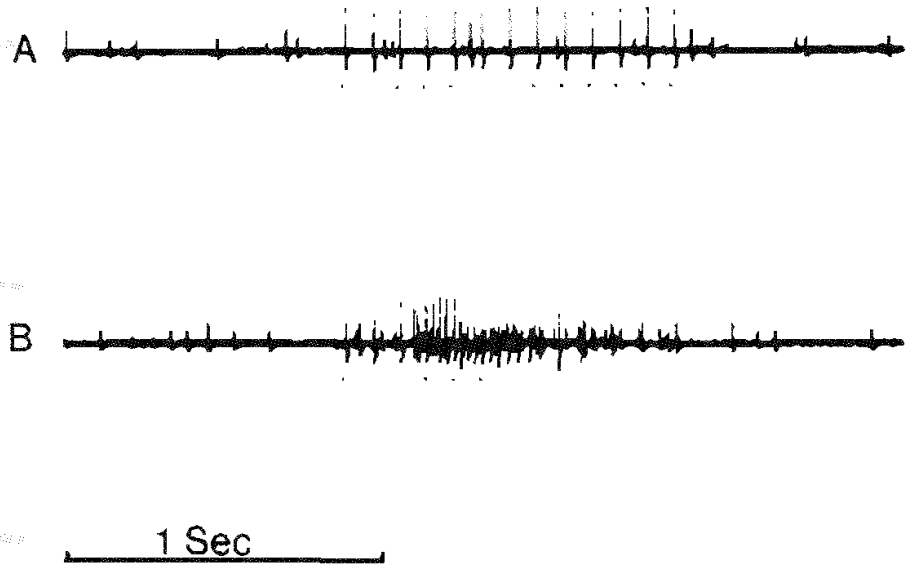


FIGURE 1 Recordings from the intact right P.I.N.p. The left 4–5 connective was stimulated at 12 Hz with 1 msec. positive going rectangular pulses. Dots indicate stimuli.

(A) Unitary responses following the stimulus marker in a one to one ratio.

(B) An increase in stimulus amplitude of 5V produces a repetitive discharge. The large units involved in this bursting discharge are the same as those which previously responded phasically.

Stimulation of either the right or left connective can elicit either unitary or bursting responses in the same axons. In Figures 2(A) and (B) the same unitary response is activated in the right P.I.N.p. by stimulation of either abdominal connective between segments 4 and 5. There is a longer delay of 5–10 msec. between delivery of the stimulus pulse and recording of the response when the right connective rather than the left connective is stimulated. Slightly increasing the stimulus amplitude as in Figure 2(C) and (D) shows that burst formation involves the same units regardless of which connective is stimulated.

Figure 3 shows recordings of the activity of both the right P.I.N.a. and P.I.N.p. during stimulation of either the left or right 4–5 connective or during stimulation of both connectives simultaneously. Burst formation takes the same form regardless of which connective is stimulated. Both bursts follow a series of paired non-repetitive units which occur with the first few stimulus pulses. In Figure 3(C), where both connectives were simultaneously stimulated above the threshold for burst generation, a burst of exactly the same duration and characteristics occurred as in 3(A) and (B), but commenced immediately after the first stimulus pulse. This indicates that the right and left interneurones mediating burst formation may both synapse onto the same units either directly or indirectly via driver units. Presumably instant burst formation is due to summation of the p.s.p.'s produced by both interneurones at near simultaneity.

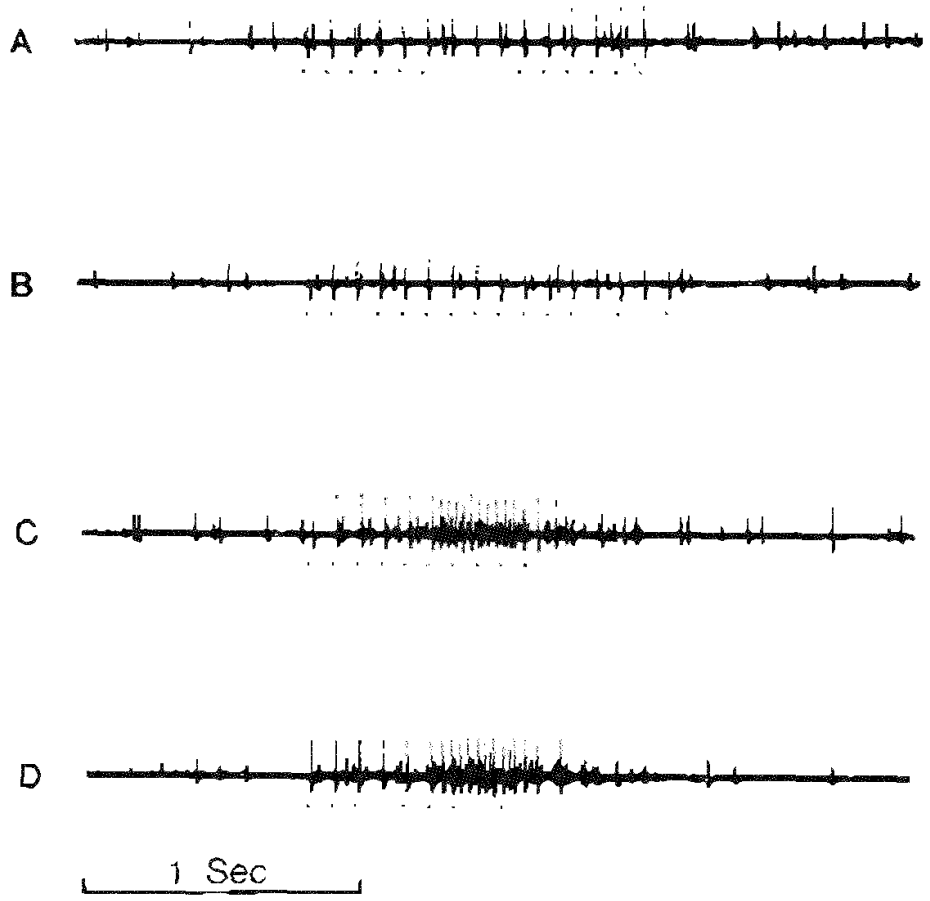


FIGURE 2 The effect of exchanging connectives on the discharge of the intact right P.I.N.p. 1 msec. pulses were delivered at 12 Hz. Dots indicate stimulus pulses.

(A) Right abdominal 4–5 connective stimulated at low stimulus amplitude.

(B) Left abdominal 4–5 connective stimulated at low stimulus amplitude.

(C) Right abdominal 4–5 connective stimulated at 5V increase in stimulus amplitude over (A).

(D) Left abdominal 4–5 connective stimulated with a 5V increase in stimulus amplitude over (B).

Note how the same units are activated by stimulation of either connective. In addition there is an increase in the delay time of about 5 msec. between the delivery of a stimulus pulse and the unitary response when the right rather than the left 4–5 connective is stimulated.

The units which respond non-repetitively in (A) and (B) discharge repetitively in (C) and (D) and a number of smaller burster units also start firing.

### 1. *Unitary responses*

As well as single non-repetitive responses to stimuli, doublets† may also occur. These non-bursting responses usually occur in both P.I.N.a.'s and P.I.N.p.'s almost simultaneously and with a constant delay time between one another.

†We have used the term doublet freely to denote either two spikes in the same axon or spikes of different magnitude occurring in a constant close relationship to one another.

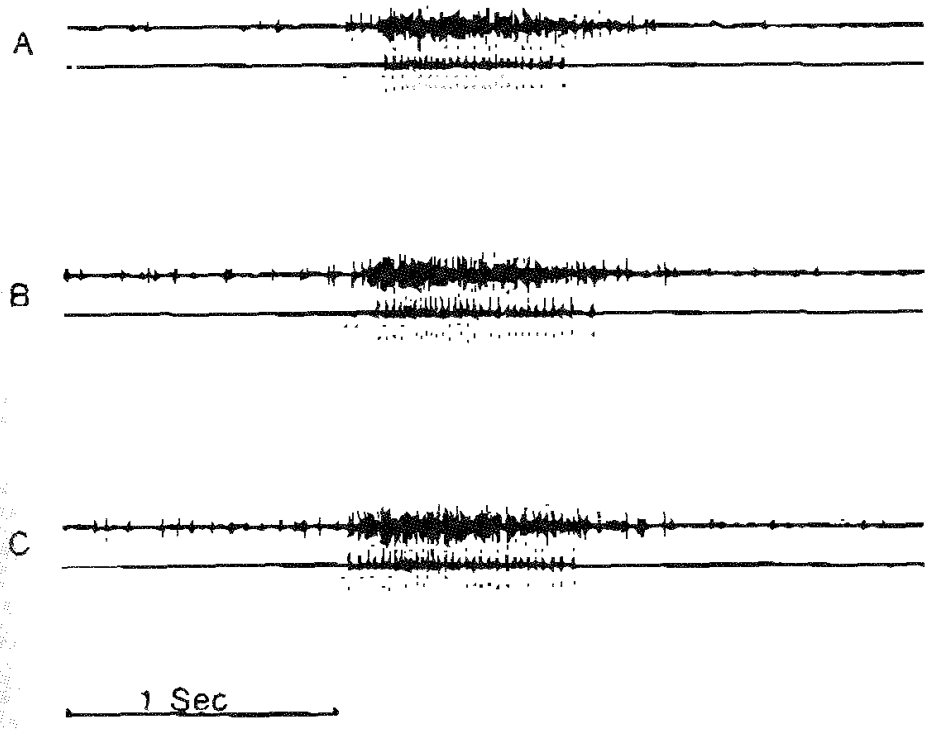


FIGURE 3 Generation of bursting activity by stimulation of either one or both 3–4 connectives.

Upper beam – intact right P.I.N.a. Lower beam – right P.I.N.p. 1 msec. stimulus pulses were delivered at 30 Hz.

(A) Stimulation of the right 3–4 connective causes bursting activity in the P.I.N.'s.

(B) Stimulation of the left 3–4 connective has the same effect, apparently on the same units.

(C) Simultaneous stimulation of *both* 3–4 connective causes a burst in the P.I.N.'s, which once initiated differs in no way from that initiated by stimulation of a single connective. The only detectable difference is in the decreased latency of the bursting response which commences at the first stimulus pulse in (C), but at the fifth in (A) and the fourth in (B). This is presumed to be due to summation of p.s.p.'s in follower cells, common to interneurones in both left and right connectives.

Figure 4(A)1 illustrates responses recorded from the P.I.N.a.'s after stimulation of the right 4–5 connectives. In the contralateral trace (upper beam) doublets occur, whilst ipsilaterally (lower beam) there are single discharges. In Figure 4(B)1 the connectives had been exchanged and the left 4–5 connective stimulated. In ipsilateral records (upper beam) the smaller unit (2) of the doublet does not discharge; a totally different unit (4) fired contralaterally (lower beam). Figures 4(A)2 and 4(A)3 show that the doublet of 4(A)1 gradually separates into its two constituent units (1+2) at 30 Hz. These units eventually come to fire alternately to one another and also on alternate stimulus pulses. The smaller unit (2) fires in synchrony with the ipsilateral (3) unit and both drop out simultaneously while the larger contralateral unit (1) continues firing for a time.

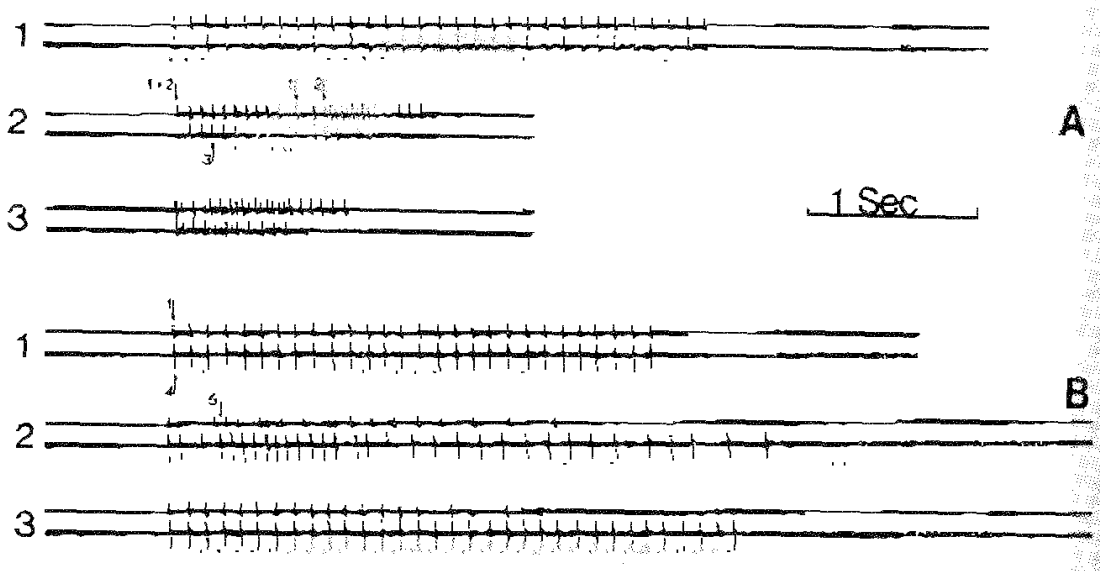


FIGURE 4 Unitary responses recorded from the cut central ends of both P.I.N.a.'s. Upper beam – left P.I.N.a. Lower beam – right P.I.N.a.

(A) Stimulation of right 4–5 connective.
(1) Stimuli delivered at 10 Hz. The spikes in the upper beam are doublets.
(2) Stimuli delivered at 30 Hz. Doublets eventually separate and fire on alternate stimulus pulses. The smaller unit (2) drops out with the ipsilateral unit (3).
(3) Repetition of 2 showing an earlier alternation of large and small pulses.

(B) Stimulation of left 4–5 connective. Only the larger of the two upper units (1) remains and the lower unit (4) is different from that in (A).
(1) Stimuli delivered at 10 Hz.
(2) Stimuli delivered at 30 Hz. The large ipsilateral unit (1) eventually lags behind a smaller unit (5) which fires in simultaneity with the contralateral unit (4). Unit 1 drops out first and unit 5 is lost in simultaneity with 4.
(3) Stimuli delivered at 10 Hz later in experiment. The system is fatiguing and unit 1 initially fires in simultaneity with unit 4 but later drops out leaving units 4 and 5 firing in synchrony.

In Figure 4(B)2, in which stimuli were delivered at 30 Hz, the ipsilateral unit (1) drops out first, but additional complications occur. On the first stimulus pulse units 1 and 4 fire synchronously and 1 then drops out for a few pulses unmasking a much smaller unit, 5. Unit 5 fires synchronously with unit 4. They initially fire spasmodically and then on alternate stimulus pulses. Later their discharge frequency decreases and both units drop out together. Unit 1 reappears and fires alternately, but erratically, to units 4 and 5. It then drops out independently. Similar events take place in Figure 4(B)3 at 10 Hz as the preparation fatigues. A much simpler firing pattern is shown in Figure 5 in which the same units are apparently activated by each interneurone and both contralateral and ipsilateral units drop out simultaneously.

2. *Repetitive responses*

The activity elicited in both P.I.N.a.'s following stimulation of the right

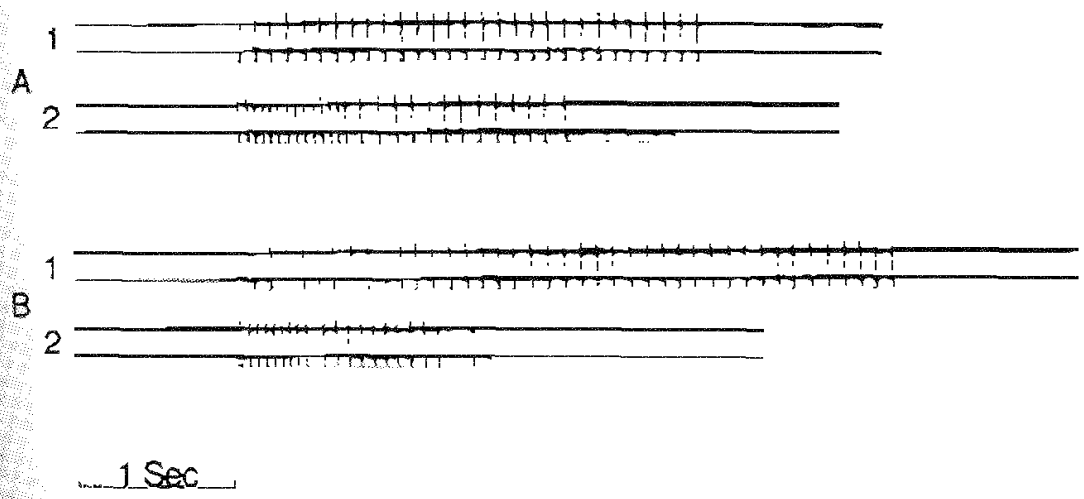


FIGURE 5 Non-repetitive units recorded from the cut central ends of the P.I.N.a.'s. Upper beam – left P.I.N.a. Lower beam – right P.I.N.a. 1 msec. stimulus pulses were delivered to the 4–5 connectives at 10 Hz in (A)1 and (B)1 and at 40 Hz in (A)2 and (B)2. Dots indicate stimulus pulses.

(A) Stimulate right 4–5 connective.

(B) Stimulate left 4–5 connective.

The same units are apparently elicited in both P.I.N.a.'s by stimulation of either connective. In addition both ipsilateral and contralateral units always drop out simultaneously.

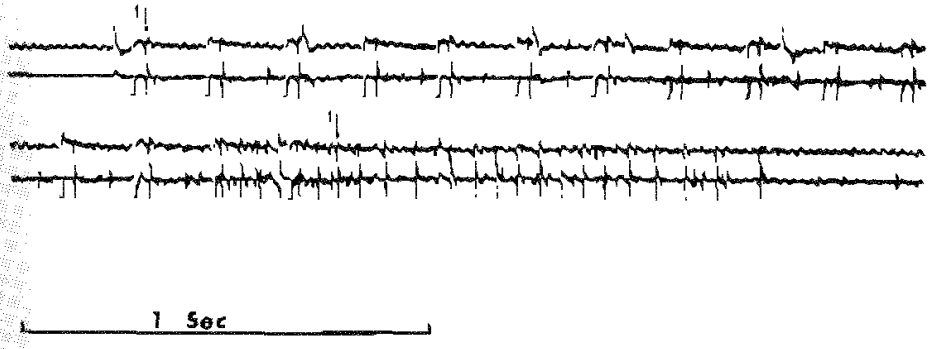


FIGURE 6 Bursting activity recorded from the cut central ends of both P.I.N.a.'s. Upper beam – right P.I.N.a. Lower beam – left P.I.N.a. 1 msec. stimulus pulses were delivered at about 6 Hz to the right 5–6 connective. Dots indicate stimulus pulses. The arrows labelled 1 denote a unit in the right P.I.N.a. which always occurs at near simultaneity and constant delay with a larger unit in the left P.I.N.a. Records continuous.

4–5 connective is exhibited in Figure 6. Bursting activity occurs in both nerves simultaneously and several units in each may be paired as were the non-repetitive units mentioned above. Further evidence of pairing is demonstrated in Figure 7 in which spontaneously active units were recorded from each P.I.N.a. Only four units were active in each P.I.N.a. and these were all paired with one another, but it is not known whether they were unitary or burster neurones, or whether the pairing was anatomically or physiologically achieved.

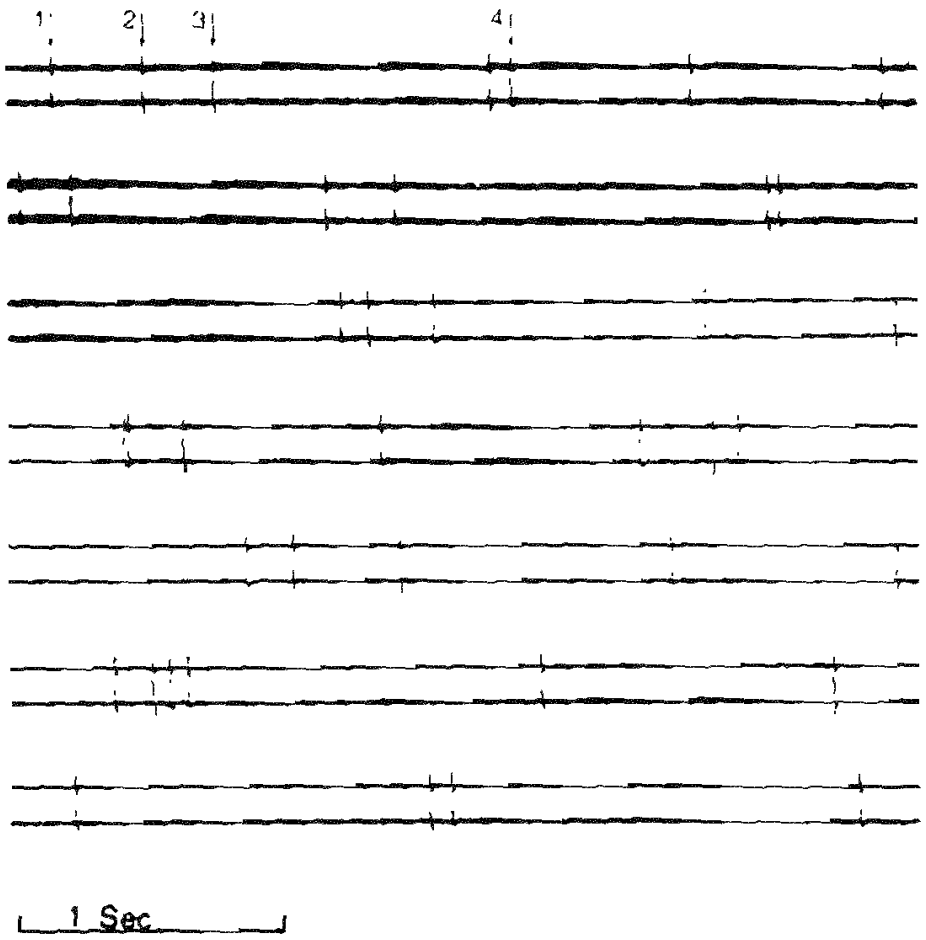


FIGURE 7 Spontaneous activity of paired units recorded from the intact P.I.N.a.'s. Upper beam – left P.I.N.a. Lower beam – right P.I.N.a. Arrows labelled 1 to 4 indicate four pairs of units. Continuous trace.

*Characteristics of the bursting activity*

a) *Response to stimulation frequency* The frequency of stimulation of the interneurones activating bursting units is important in the genesis of a burst. Bursting activity is normally initiated when the stimulus frequency reaches or exceeds 10 Hz, although there is some individual variation (Figure 8). Below 10 Hz stimulus pulses begin to entrain 4 or 5 spontaneously active units. At 7.7 and 10 Hz these units do not produce a fully formed burst but some post-stimulatory activity does occur. At 20 Hz and 40 Hz fully formed bursts occur. Above 40 Hz the burst is abolished and only 1 : 1 firing of otherwise repetitive units takes place. Overall the most effective frequencies of stimulation lie between 10 Hz and 40 Hz.

b) *Response to variation in number of stimulus pulses* Stimulus pulses delivered at 1 Hz have little effect on tonic entrainment (Figure 8(B)), but they generally elicit a unitary response. In Figure 9 a single stimulus pulse elicited

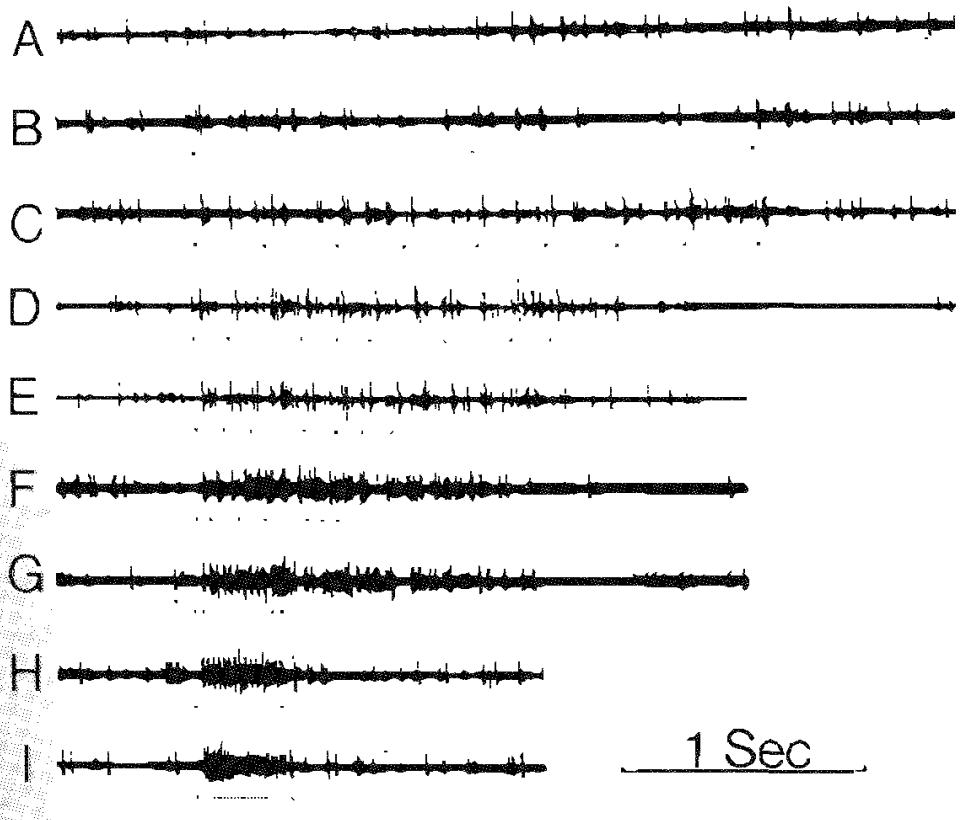


FIGURE 8 Effect of increasing stimulation frequency on burst formation at constant stimulus amplitude. In all but (A) which is a recording of spontaneous activity, 1 msec, stimulus pulses were delivered to the left 3–4 connective. Recordings were made from the cut central end of the right P.I.N.a. The frequencies of stimulation were as follows:

(B) 1 Hz; (C) 4 Hz; (D) 7.7 Hz; (E) 10 Hz; (F) 20 Hz; (G) 40 Hz; (H) 50 Hz; (I) 77 Hz.

From (B) to (D) the response is gradually being entrained whilst between (E) and (G) there is the normal range of stimulus frequencies which produce bursting activity. In (H) and (I) there is no post-stimulus burst and the large units (which normally exhibit unitary and bursting responses) drop out, leaving the small ones which would normally only participate in burst formation to follow the stimuli in a 1:1 ratio.

only a single unitary response. When two or three stimulus pulses at 10 Hz were delivered as in Figure 9(B) and (C) a short burst followed. Delivery of 4 or more stimulus pulses resulted in normal burst formation. It is interesting to note that within limits (up to 10 or 12 stimulus pulses at constant frequency) the duration and form of the burst following the final stimulus pulse is fairly constant. This is well shown in Figure 9(D), (E) and (F). Arrows labelled 1, 2 and 3 indicate similar "sub-bursts" within the burst. Bursting activity may be elicited by delivery of two or three stimulus pulses provided that a set of facilitating pulses is delivered in the preceding two or three seconds. Figure 10 illustrates that the efficiency with which four stimulus pulses may drive a burst decreases with increasing frequency of stimulation.

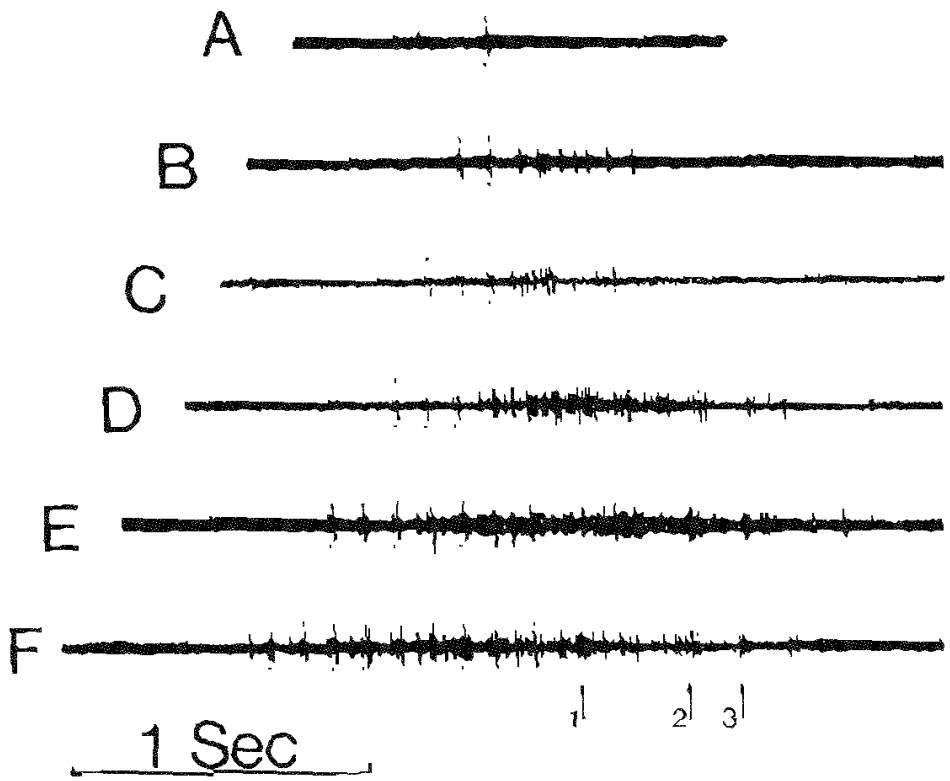


FIGURE 9 The effect of increasing the number of pulses at constant frequency on burst formation. In all cases 1 msec. pulses were delivered at 10 Hz to the left 5–6 connective and recordings were made from the cut central end of the left P.I.N.a.

A minimum number of 4 stimulus pulses is required for burst formation although abortive short bursts may occur with 2 or 3. Within limits, the burst carries on for the same length of time after the last stimulus pulse in all cases and has the same basic characteristics as denoted by arrows 1, 2 and 3. These arrows denote 3 "sub-bursts" which occur at the same positions within bursts (D), (E), and (F) after the cessation of stimulation. The various bursts are lined up so that the final stimulus pulses lie below one another to illustrate this point.

## B. Correlation of Motor Output with Hindgut Motility

Both unitary and bursting activity in the P.I.N.'s may elicit hindgut movements (Figure 11). The type of response shown is very different in each case. A short term activation of units driven non-repetitively will release only a single rather weak longitudinal muscle contraction of the hindgut and a weak peristalic wave, which merely returns the hindgut to its resting position and does not cause it to elongate (Figure 11(A)), whilst a single burst elicits a single cycle of powerful hindgut movements (Figure 11(B)). The onset of the response is much more rapid in (B) than in (A) and the longitudinal and circular muscle contractions are much more pronounced. The circular muscle contraction wave causes normal elongation of the hindgut as previously described (Winlow and Laverack, 1972.1). Longer periods of stimulation involving non-repetitive discharge (Figure 11(C)) cause numerous weak hindgut movements. At higher stimulus amplitudes (Figure 11(D)) the initial bursting response is exactly as in

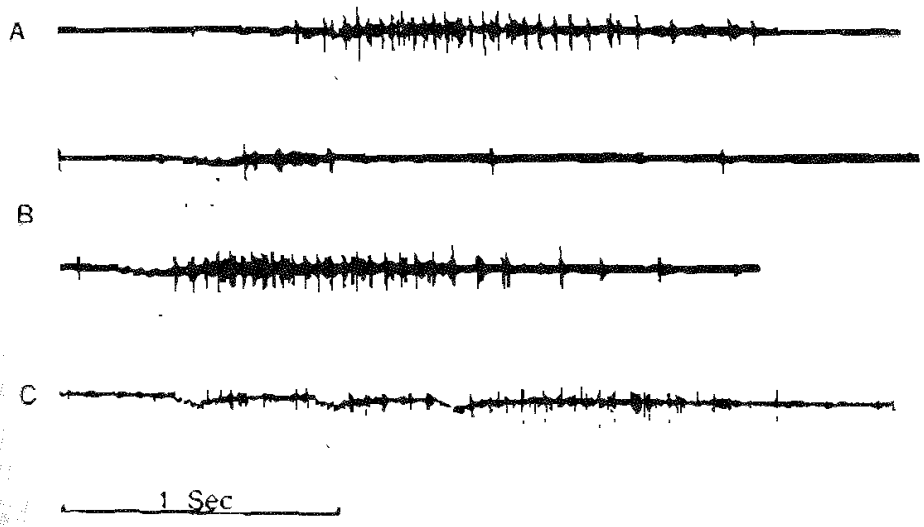


FIGURE 10 Efficiency with which 4 stimulus pulses can cause burst formation decreases with frequency. 1 msec. pulses at various frequencies were delivered to the left 3–4 connective and recordings were made from the right P.I.N.a.

(A) Stimuli were delivered at 10 Hz and normal burst formation ensued.

(B) Continuous trace. Stimuli were delivered at 25 Hz.

(C) Stimuli were delivered at 50 Hz.

In both (B) and (C) facilitating bursts of stimuli were necessary. Non-bursting units are lacking in this case. All records from same preparation.

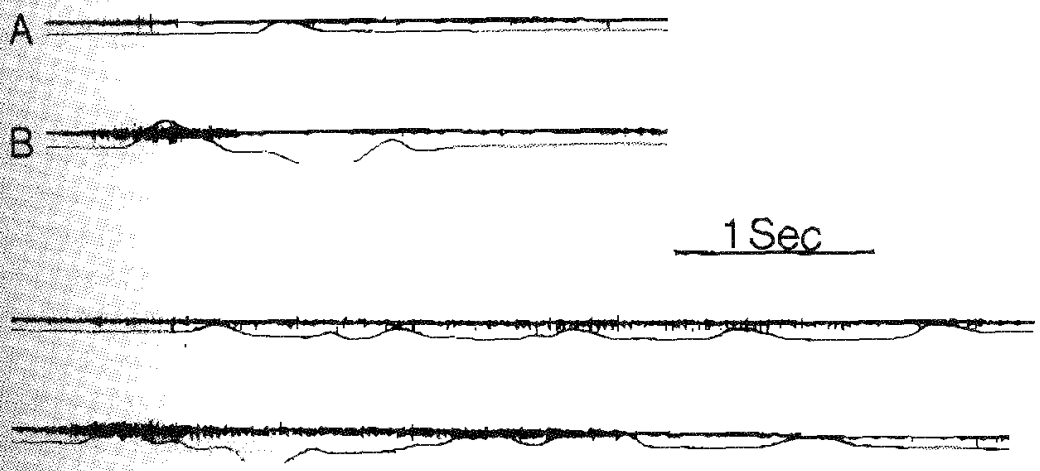


FIGURE 11 Correlation of hindgut movements with activity in the P.I.N.'s. Stimuli were delivered to the left 4–5 connective at 25 Hz with 1 msec. pulses. Stimulus duration and amplitude were varied.

Upper beam – intact right P.I.N.a. Lower beam – transducer at midgut/hindgut junction Upward deflection denotes posteriorward movement.

(A) A short burst of stimuli at low amplitude gives rise to a unitary output in the P.I.N.'s and weak hindgut movements.

(B) A short burst of stimuli at 6V higher amplitude than in (A). This causes burst formation and a single cycle of powerful hindgut movements.

(C) A long burst of low amplitude stimuli causes a series of weak hindgut movements.

(D) A long burst of higher amplitude stimuli causes burst formation in the P.I.N.'s succeeded by unitary responses. The initial burst causes hindgut movements as in (B) and the subsequent unitary discharge drives the hindgut rather more weakly.

Figure 11(B), but once the burst is completed it is succeeded by a series of unitary responses producing rather weaker contractions. Thus a single burst is followed by a complex of hindgut movements that are co-ordinated with the anal rhythm (Figure 12).

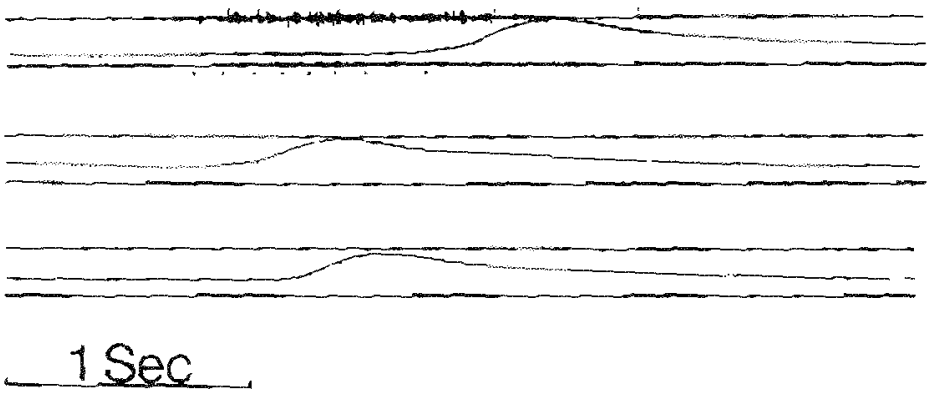


FIGURE 12 Rhythmic anal movements produced by a single burst in the P.I.N.'s. Continuous trace. Upper beam – intact right P.I.N.a. Middle beam – transducer on right anal lip; upward deflection denotes anal opening. Lower beam – intact right P.I.N.p.

1 msec. stimuli at 10 Hz were delivered to the left 3–4 connective. Dots indicate stimulus pulses.

### C. Sensory Modulation of Bursting Discharge

No differences in the form of the bursting discharge were detectable prior to and following anal nerve section so the anal receptors (Winlow and Laverack, 1970, 1972.1) do not modulate it directly. In addition the bursting motor discharge recordable from completely isolated preparations (Figure 9) as compared with that from the intact 6 A.G./rectum complex (Figure 11(B)) shows no differences in form whatever. Thus modulation of the bursting pattern does not occur, at least not at the level of the 6 A.G.

Although many of the units so far described as having a non-repetitive discharge could also fire repetitively many others appeared exclusively repetitive or non-repetitive.

## DISCUSSION

The two types of motor discharge shown in Figures 1 and 2 may be elicited by stimulation of any segmental connective of the V.N.C. Thus two types of interneurone would appear to elicit the output and hence control the defaecatory response. The interneurone (I1) which drives the unitary discharge consistently fires at lower stimulus amplitudes than that (I2) producing

repetitive discharge, so that both interneurones must be activated at stimulus amplitudes sufficient to excite I2. Since many "non-repetitive units" also fire repetitively at higher stimulus amplitudes both types of interneurone, or associated driver cells, must impinge upon these units, whilst only I1 or I2 respectively impinge on exclusively unitary or burster neurones. This division into unitary and burster neurones is real, since further experiments involving intracellular stimulation of neurone somata (Winlow and Laverack, 1972.3) indicate that some cells may be driven non-repetitively and others repetitively by direct depolarisation.

With regard to the two types of motor output it is seen that firing in one branch of the P.I.N.'s is always paired with firing in other branches both from anterior to posterior and from side to side. The basis of these linked outputs may be due to either lateral excitation between neurones or neuronal groups, or to individual multibranched or bifurcating neurones sending axons down several branches of the P.I.N.'s. We have previously reported neurones with these latter features (1972.1). The presence of neurones causing and receiving lateral excitation is suspected on consideration of Figure 4, in which most ipsilateral non-repetitive responses drop out during stimulation at high frequencies leaving mainly contralateral output. This suggests a breakdown of information flow across the ganglion probably at the level of individual synapses. In addition the loss of the ipsilateral rather than the contralateral response suggests that I1 interneurones must decussate in the 6 A.G. This is borne out by the traces shown in Figure 2(A) and (B) where there is an increase in delay between stimulus pulse and unitary reponse when ipsilateral rather than contralateral interneurones are stimulated.

In some cases (Figure 5) neurones in both right and left P.I.N.a.'s discharge with a fixed temporal relationship both to one another and to the stimulus pulse regardless of whether the right or left connective is stimulated. What is more, at high stimulus frequencies, both ipsilateral and contralateral units drop out simultaneously. The implication of such responses is that both interneurones may either synapse onto some sort of medial driver unit which then drives both motor units, or onto a medial branching motor unit. However, it is our view that multibranched motor units are not involved in the unitary discharge (Winlow and Laverack, 1972.3). Therefore it is possible that a second class of interneurone producing non-repetitive discharge occurs, so that three classes of interneurone controlling defaecation may exist. George Wolfe (personal communication) of the University of Texas at Austin supports this proposition and states that from one to five interneurones of the second and third abdominal connectives affect the hindgut of the crayfish, *Procambarus clarkii*.

In other cases the relationships of paired non-repetitive units may be rather more obscure. An example of the complications faced is shown in Figure 4

which we have attempted to analyse in the terms of a neural model as an indication of the many possible connectivity patterns of these neurones. In the absence of sufficient information recorded from individual units using microelectrodes such models must be incomplete. In Figure 4 differences in output from the P.I.N.a.'s are detectable according to which connective is stimulated. Stimulation of the right 4–5 connective (Figure 4(A)) initiates a discharge by two contralateral units (1 and 2 – see arrowed units on Figure 4) firing as a doublet and one ipsilateral unit (3). On stimulation of the left 4–5 connective (Figure 4(B)1) unit 2 does not discharge leaving only unit 1 (now ipsilateral) and unit 3 is replaced by unit 4 (contralateral). There are also additional complications as shown in Figure 4(B)2 and 3. At higher frequencies of stimulation of the left 4–5 connective unit 1 fires spasmodically unmasking a further ipsilateral unit (5). It is thought that this small unit always fires in synchrony with unit 1 during stimulation of the left connective. The neuronal network shown in Figure 13 would account for these phenomena. In this network units 1, 2 and 3 are coupled by two driver cells (D1 and D2) which are activated by the right I1. At low stimulus frequencies these units fire in

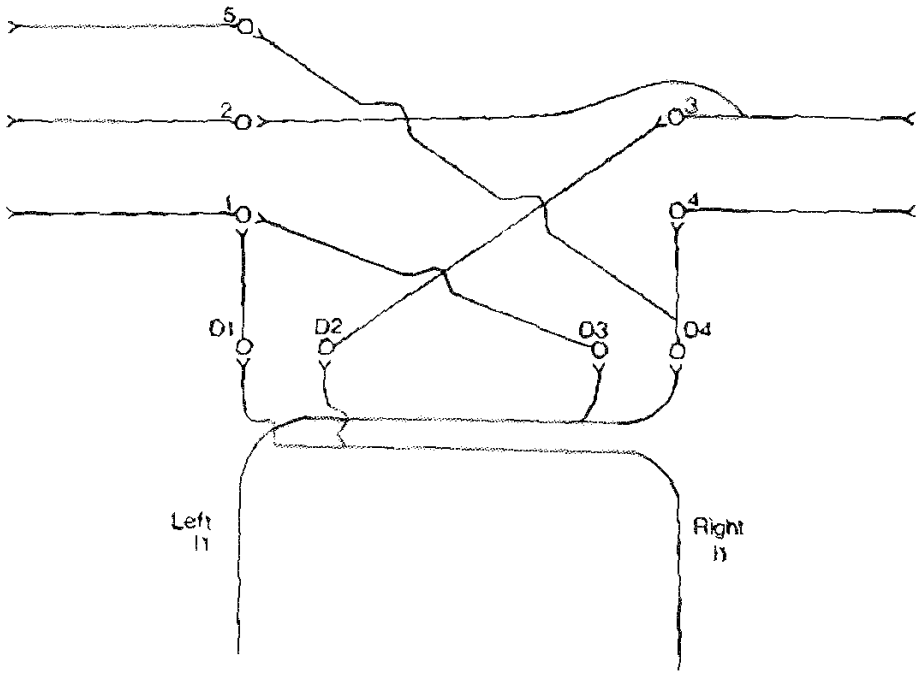


FIGURE 13 Possible neural connections to account for the outputs shown in Figure 4.

The right I1 stimulates two driver cells (D1 and D2) coupling units 1, 2 and 3 at 10 Hz. At 30 Hz units 2 and 3 drop out simultaneously which suggests breakdown of a single synapse. These units may therefore be linked by a unilateral driver (D2) and one unit may laterally excite the other. Unit 1 gradually separates from unit 2 till it fires alternately with it. This suggests activation by a separate driver cell (D1).

On stimulation of the left I1 units 1, 4 and 5 are usually coupled at 10 Hz. At 30 Hz units 4 and 5 are strongly coupled, but unit 1 fires independently. Units 4 and 5 are therefore shown to be driven by a bifurcating driver cell, D4, whilst the erratic unit 1 is probably activated by a separate driver neurone, D3, which eventually fatigues even at low frequencies (Figure 4(B)3).

synchrony, but at slightly higher frequencies units 2 and 3 fail simultaneously and it is thought that stimulation of one or other rather than both units would be most likely to produce this phenomenon than if two synapses were to fail simultaneously. The excited unit, which we arbitrarily selected as 3, would then excite its contralateral homologue (2). A further possible explanation is that units 2 and 3 are driven by a bifurcate driver cell. Another result of high frequency stimulation is that units 1 and 2 gradually separate and come to fire on alternate stimulus pulses. In the first part of Figure 4(A)2 unit 1 gradually slows with respect to unit 2 probably due to decreasing synaptic efficiency of D. Unit 1 then fires erratically on several stimulus pulses and misses altogether on some before its output is restored. Missing of stimulus pulses may account for the abrupt shift of phase of unit 1 so that in the latter part of the trace it is uncoupled from and fires alternately with units 2 and 3. It has been assumed that the maximal frequency of sustained discharge of the driver units is less than 30 Hz, so that each selects to fire on every second stimulus pulse at this frequency. This would allow the fortuitous phase shift mentioned above. In trace 4(A)3 units 1 and 2 again come to fire on alternate stimulus pulses after an uncertain start.

Stimulation of the left I1 at 10 Hz causes units 1, 4 and 5 to fire simultaneously. At 30 Hz 4 and 5 are strongly coupled and it seems reasonable to assume either that unit 4 is itself laterally linked to unit 5 or perhaps both are driven by a bifurcating driver unit, D4, as shown in Figure 13. Unit 1 fires very sporadically in traces 4(B)2 and 4(B)3 and is uncoupled from 4 and 5. We have therefore assumed that it is driven by a separate driver, D3.

If our view, that the unitary responses recorded in the P.I.N.'s are not due to bifurcating motor neurones, is correct, then these units might be expected to be symmetrically paired with either lateral connections (e.g. between units 2 and 3) or bilateral (e.g. D4) and unilateral (e.g. D2 and D3) driver cells crossing the mid-line. Figure 13 goes some way towards providing a model on which to base such a network. However extracellular recordings are unlikely to give a true picture of the output to the rectum due to the lack of resolution between units (e.g. 1 and 5) inherent in this method. Intracellular microelectrode studies on this system should eventually help to determine the connectivity patterns of the various neurones involved.

The pairing of units shown to occur for unitary discharges is also maintained in the bursting responses elicited by the high threshold I2 interneurones (Figure 6). In addition stimulation of either right or left connectives at high stimulus amplitudes induces a bursting discharge of fixed pattern in the P.I.N.'s. The immutability of this discharge with respect to the connective stimulated is demontrated in Figure 3. On simultaneous stimulation of both I2's (Figure 3(C)) the burst is initiated at the first stimulus pulse rather than after several stimulus pulses after which unitary responses occur (Figure 3(A)

and (B)). This may be because both I2's synapse either onto the same units or onto the driver cells of these units and this results in a rapid shift of membrane potential above threshold, on simultaneous stimulation. From other investigations (Winlow and Laverack, 1972.3) we know that penetration and stimulation of the somata of certain units of the 6 A.G. will produce bursting activity of several neurones in both ipsilateral and contralateral P.I.N.'s. Thus a network of bursting neurones is implied in which individual units may mutually excite one another or be excited by driver cells. As yet we are not certain if either or both processes take place, but the immutability of the discharge suggests that certain units may be utilised exclusively to disseminate the interneuronal input to the system. We tentatively suggest the sort of situation shown in Figure 14 in which all units are excited via a median driver cell along a fixed pathway. At least some of these fibres might be bifurcate or multibranched. Their lateral branches could then serve to induce some form of cascading group discharge which would be self-generating over a short

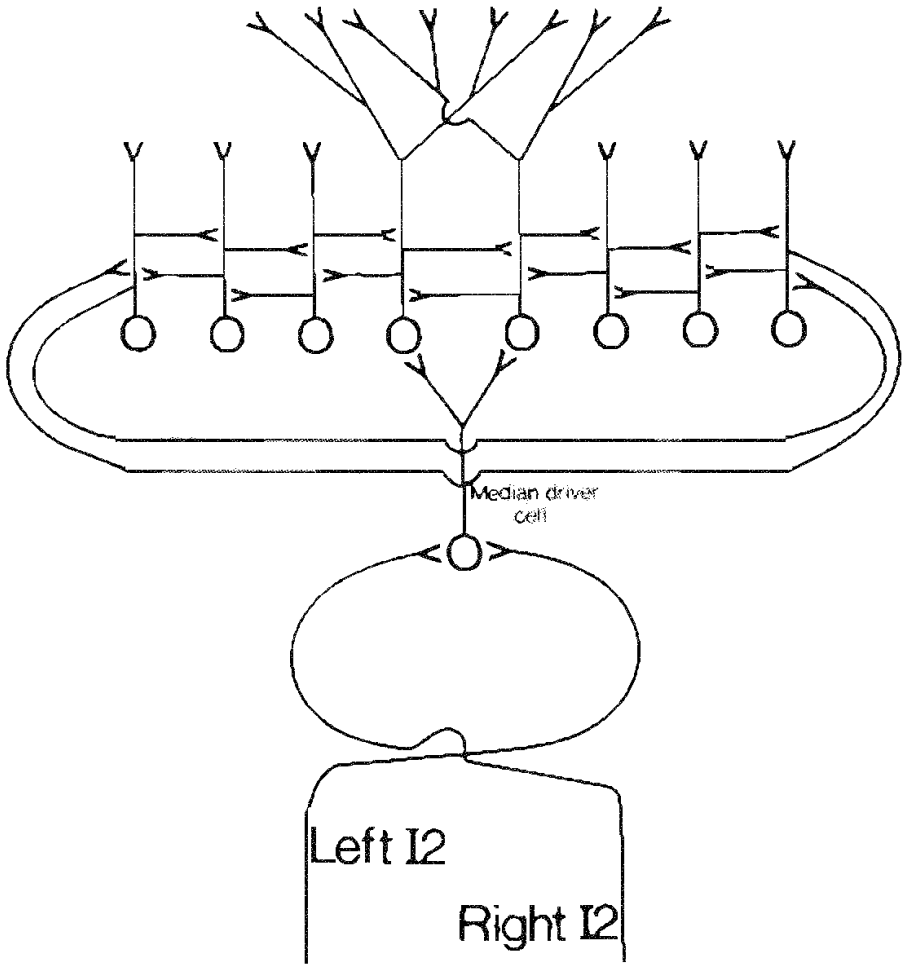


FIGURE 14 Convergence of the left and right I2 onto a median driver cell driving a network of burster motor neurones, some of which are multibranched.

period, rather like the cascading output demonstrated in the brain of the nudibranch mollusc, *Tritonia*, by Willows and Hoyle (1969). The interneurones producing the bursting discharge are presumed not to be in contact distally since the latency between stimulus onset and burst discharge varies according to whether one or both neurones are activated. However, they could well be simultaneously activated in the brain of the intact animal.

All this suggests a single, probably median, network of repetitively discharging units which drives the hindgut but, the general situation in arthropods is that the ganglia are symmetrically arranged (Kennedy *et al*, 1969; Cohen and Jacklet, 1967; Bentley, 1970) and the partner units may be coupled to one another in some way. However, in all the cases cited above the systems innervated were themselves bilaterally symmetrical, whereas the gut is a median tubular organ rather like the heart. The cardiac ganglion of the lobster, *Homarus americanus* contains a group of non-symmetrical interacting cells which produce synchronised bursts of activity to the heart muscle at regular intervals. These bursts produce tetanic contractions of the myocardium (Maynard, 1966; Horridge, 1968). The heart is controlled in its rate of discharge by symmetrically arising inhibitory and excitatory neurones from the central nervous system.

The stomatogastric ganglion lies within the anterior aorta on the anterodorsal surface of the stomach. It is the seat of patterned sequences of motor activity which control the quite complex actions of the gastric muscles, which are inserted extrinsically, like the radial muscles around the anus (Winlow and Laverack, 1972.1). According to Maynard (1966) it is quite common for axonic processes to divide soon after leaving the ganglion and "it is characteristic for the paired stomach muscles to receive innervation from a single biaxonic neurone". The output from the ganglion is thought to be modulated from higher centres (Morris and Maynard, 1970).

Good evidence, therefore, exists to show that median collections of nerve cells control organs lying in the midline. It is our proposition that the hindgut is partly controlled by a medial group of repetitively discharging cells which may laterally excite or inhibit one another in ways which are not yet fully understood. In addition, some of these cells are thought to be multibranched and to send major axonal branches to both the right and left P.I.N.'s.

As mentioned in our previous paper (1972.1) sectioning of the anal nerves does not alter the form or co-ordination of the movements of the hindgut. In addition we have now shown that the usual bursting discharge remains completely unaltered in that situation. Indeed it seems that the bursting output once initiated is completely immutable (Figures 8 and 9) which reinforces the theory that fixed neural pathways involving precise connectivity patterns and numbers of neurones form the basis of the defaecatory behavioural sequence. All the interneurones previously implicated as evoking

motor discharge may be concerned and it is conceivable that the II's merely act on selected regions of the burster network.

In Figure 11 the unitary and repetitive units are shown to produce rather different muscle responses. Non-repetitive units will often produce weaker rhythmical contractions of the hindgut, although these movements are of much greater amplitude than the spontaneous hindgut contractions we previously described (1972.1). The major point of difference in the response of the hindgut is that repetitive units produce a massive peristaltic movement which considerably elongates the hindgut, whilst this elongation is not so marked with non-repetitive units. It is thought that exclusively unitary neurones may only innervate the longitudinal muscles. The weaker peristaltic contractions caused by unitary neurones may be produced as a result of stretching of the circular muscles during longitudinal muscle contractions. The circular muscles may then contract against the imposed load.

The response initiated by repetitive units is usually of large amplitude and a neurally evoked circular muscle contraction is thought to be involved so that burster units apparently innervate both longitudinal and circular muscles. The function of the unitary discharge may be, in part, to "prime" the longitudinal muscles and thus facilitate the defaecatory response. The primary phase of hindgut movements (Winlow and Laverack, 1972.1) may be induced by repetitive units and the subsequent rhythmic contractions may depend solely on a unitary discharge. The role of units which fire both repetitively and non-repetitively is unknown.

The origins of the radial and longitudinal muscles may be similar (Winlow, 1970). It is interesting to note that both can be driven rhythmically by non-repetitive units. It is also of interest, in this context, to consider the pharmacological investigations of Florey (1954) and Elofsson *et al* (1968). Florey found that both noradrenaline (and adrenaline) and acetylcholine increased the frequency of spontaneous contractions of the hindgut. Acetylcholine considerably increased the muscle tone of a longitudinally suspended hindgut, but noradrenaline did not. Thus acetylcholine may have specifically affected only the longitudinal muscles, whilst noradrenaline affected both longitudinal and circular groups. Elofsson *et al* show noradrenaline to be distributed among both longitudinal and circular muscle groups. Thus it is possible that the non-repetitive units may utilise a different transmitter from repetitive units. A basis for a pharmacological study of the neurones is thus provided and the two different types of neurone would be expected to be segregated into groups dependent on their biochemical properties (Otsuka *et al*, 1967). The response of both radial and longitudinal muscles to non-repetitive units may reflect similarities in their pharmacological properties.

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