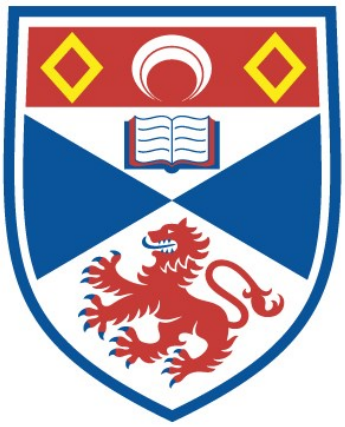
THE CONTROL OF HINDGUT MOVEMENTS IN THE LOBSTER, HOMARUS GAMMARUS (L)

William Winslow

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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by

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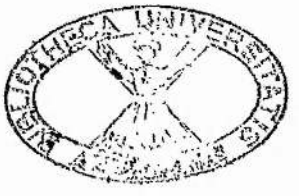

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SUPERVISOR'S CERTIFICATE.

I certify that William Winlow has fulfilled the conditions laid down under Ordinance Number 16 of the University Court, St. Andrews, and is accordingly qualified to submit this thesis for the degree of Doctor of Philosophy.

DECLARATION.

I declare that the work reported in this thesis is my own and has not previously been submitted for any other degree.

VITAE.

I was educated at King Edward VIth Grammar School, Morpeth, Northumberland. I then attended the University of Newcastle upon Tyne from which I graduated in Zoology in June 1967. I came to St. Andrews in October 1967. The work reported in this thesis was carried out between March 1968 and May 1970.

The Road goes ever on and on
  Down from the door where it began.
Now far ahead the Road has gone,
  And I must follow, if I can,
Pursuing it with eager feet,
  Until it joins some larger way
Where many paths and errands meet.
  And whither then? I cannot say.

J.R.R. Tolkien,
The "Lord of the Rings", Book 1.

Acknowledgements.

I am greatly indebted to Professor M.S. Laverack for his ready help and advice throughout the period of this work.

My thanks are also due to Mrs. Betty Wales who did a wonderful job in producing order out of chaos when typing the manuscript. This study could not have been completed without assistance at various times from Messrs. C.J. Roemmélé, J. Stevenson and R. Jack and Mrs. Eleanor Gunstone. I would also like to thank Mr. W. Wales both for reading the manuscript and for being a cheerful, helpful and rude companion during the last two years. Finally I must thank my wife, Pamela, for reading and checking the manuscript and for soothing the temper of an increasingly irascible husband.

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Summary.

1. The mechanisms underlying hindgut movements in the lobster, Homarus gammarus (L.) have been studied.

2. The hindgut is innervated from the sixth abdominal ganglion (6A.G.) by the posterior intestinal nerves (P.I.N.'s). Stimulation of any of the connectives of the ventral nerve cord (V.N.C.) will elicit hindgut and anal movements.

3. The hindgut is divisible into anterior and posterior regions, whose basic co-ordination is undisturbed by sectioning the hindgut, so long as the nerves remain intact.

4. Numerous endogenous oscillators mediating spontaneous contractions are thought to lie within the muscles of the rectum. Oscillators within the radial muscles of the anus can be activated by nervous discharge.

5. Receptors responding to anal dilation and closure have been described both anatomically and physiologically. They lie in the anal nerves. No physiological evidence exists for the presence of receptors on the rectum.

6. Hindgut and anal movements may be initiated by either 'phasic' or 'tonic' motor neurones. Bursts of tonic discharge will cause powerful hindgut movements (the defaecatory response), whilst those elicited phasically are rather weaker.

7. The form of the bursting discharge is, apparently, immutable and is unaffected by extirpation of all sensory input.

8. The structure of the 6A.G. has been determined. It is a highly complex ganglion and it is suggested that it was derived from three fused ganglia in the course of evolution.

9. The somata of neurones causing efferent discharge to the hindgut have been shown to lie in the anterior part of the posterior ventral cortical lobe of the 6A.G.

10. Some of these neurone somata have been penetrated using glass microelectrodes. Three categories of neurones, responsible for hindgut control at the level of the 6A.G., are thought to exist:- phasic neurones, tonic neurones and driver neurones.

11. The neurones within the 6A.G. represent a final motor pathway to the hindgut. These neurones are thought to be under the ultimate control of a centre lying in the tritocerebral region of the brain. Several interneurones connect the two.

PART 1. INTRODUCTION

The study of ordered networks of central neurones and their interrelations with one another and with peripheral sensory and effector organs is a highly profitable method of neurobiological investigation. Research into the properties of such discrete neurological systems (e.g. Wilson, 1964, on locust flight) has greatly furthered our knowledge of integration in the arthropod nervous system and of neuromuscular relationships. Investigations of individual systems are immensely valuable, but they can only give us an incomplete indication of their function in the intact animal, since in most neurophysiological preparations their interactions with other systems are greatly limited.

Although the majority of animals chosen for neurobiological investigations are vertebrates many workers study invertebrates, especially arthropods. This is due in part to the relatively small numbers of nerve cells in the nervous systems of lower phyla. In addition large nerve cells are often found and preparations are sufficiently durable to withstand maltreatment at the hand of the experimenter. Both insects (e.g. locusts and lepidopterans) and decapod crustacea provide favoured experimental animals.

The decapod crustacea afford excellent material for neurophysiological studies. They exhibit all the qualities mentioned above and are often large, easy to obtain and easy to maintain. Furthermore, much is already known about their anatomy and physiology. The overall anatomy of the musculature and nervous system of Astacus was described by Schmidt (1915) and Keim (1915) respectively. The postural reflexes of the macruran abdomen have been well described by Kennedy and his collaborators, whilst Atwood and his fellow-workers have studied the properties of the postural muscles. The cardiac ganglion has been studied by Maynard (1961) who has also investigated the stomatogastric ganglion and the workings of the foregut. Much of the general receptor

anatomy of reptantian decapods is also known (Laverack, 1962a,b, 1963a,b, 1964; Hámori and Horridge, 1966a,b,c,d; Clarac, 1968a,b; Bush, 1962, 1965a,b,c; Wales et al, 1970; etc.) and many other systems have received attention in recent years. One outstanding piece of work, which deserves mention here, is that carried out by Wiersma and his colleagues (Wiersma, 1958; Hughes and Wiersma, 1960; Wiersma and Bush, 1963; Wiersma and Hughes, 1961; Wiersma and Mill, 1963.). In studying the central pathways of many interneurones of the ventral nerve cord they have provided a vast amount of information relating to the central representation of numerous sensory structures.

Even though so much of the neuroanatomy and physiology of decapod crustacea is already known a great deal is still to be ascertained, especially with regard to the central co-ordination of motor output. One motor system deserving further attention is that controlling the motility of the hindgut in reptantian decapods. Both the foregut and the somatic musculature of the abdomen have been the subjects of detailed research. It therefore seems strange that the functioning of the hindgut has been neglected, from the neurophysiological aspect, since Miller's work in 1910.

The gut of reptantian decapods is innervated from two sources. Anteriorly there is the stomatogastric system (Allen, 1894; Orlov, 1926; Maynard, 1966; Dando and Laverack, 1969.) which supplies the oesophagus, stomach and, perhaps, the midgut. Posteriorly the hindgut is supplied from the sixth abdominal ganglion (Krohn, 1834; Lemoine, 1868; Police, 1908; Alexandrowicz, 1909; etc.).

The structure of the hindgut of several reptantians has been described by numerous authors (Alexandrowicz, 1909; Miller, 1910; Janisch, 1923 and Yonge, 1924.). Their findings are in substantial agreement and are discussed below (see Discussion) with reference to the hindgut of Homarus.

The sixth abdominal ganglion has been described a number of times. Both Krieger (1880) and Retzius (1890) show it to possess a pair of dorsally placed groups of cell bodies. Johanson and Schreiner (1965) confirm this finding, although they also demonstrate the presence of a third median dorsal group lying between the paired groups of neuron somata.

As mentioned above, the hindgut is supplied from the sixth abdominal ganglion by both the posterior intestinal nerves and the paired anal nerves. The posterior intestinal nerves were first described by Krohn (1834) and then by Lemoine (1868). They were apparently named by Alexandrowicz (1909). Most authors describe them as arising from a single root although Krohn mentioned that in Astacus this might sometimes be paired. They also show that this nerve divides into three, two branches passing anteriorly towards the midgut and only one branch posteriorly towards the anus. According to Alexandrowicz these nerves then give rise to a dense network of randomly distributed fibres, the 'Grundplexus', which surrounds the entire hindgut. There is then a second order plexus, the 'Endplexus'. This is not sharply delimited from the grundplexus and is in two divisions running parallel to the external circular and internal longitudinal muscle fibres of the hindgut. He also described numerous bipolar sensory cells innervating the hindgut. He noted that their axons were in direct contact with the motor fibres. However, Orlov (1926) disputed these findings and could find neither peripheral connections

between sensory and motor fibres nor the endplexus. In addition to the bipolar sensory cells he described a further group of cells innervating the hindgut, the pyloric sensory cells. Their cell bodies are reported to lie on the pylorus, whilst their axons pass to the commissural ganglion. Their dendrites ramify diffusely in the connective tissue of the midgut and to a great extent in that of the hindgut. Thus, although the exact nature of the innervation of the hindgut is disputed, it is obvious that both a rich motor and sensory nerve supply are present.

Campbell and Burnstock (1968) add weight to the implications of Orlov's work, i.e. that the hindgut is under direct central control. They make the point that the arthropod enteric plexus differs from that of other animals in being very similar to the general somatic innervation. In addition the muscles of the arthropod gut are striated and this is a condition not found in other phyla. Their main conclusion is that the major co-ordinating activities of the gut of arthropods are likely to occur in peripheral ganglia near the gut or in the central nervous system. Such a conclusion is borne out by recent studies of the stomato-gastric system.

When one considers the work of Miller (1910) it seems likely that the hindgut may be controlled from the central nervous system. He showed, in his physiological study of the rectal and anal movements of Homarus and Cambarus, that rhythmical contractions of the anus accompanied by peristaltic waves along the rectum could be elicited by several seconds stimulation of any of the connectives of the abdominal ventral nerve cord in the intact animal. Thus it seems likely that fibres in the ventral nerve cord release a neural mechanism controlling the movements of the hindgut. My initial experiments on the system were thus very simple and aimed at emulating Miller's work. I found that it was

possible to release co-ordinated hindgut movements by stimulation of the severed connectives lying between the fifth and sixth abdominal ganglia (i.e. the 5-6 connectives) of the ventral nerve cord. Thus the control mechanism, if central, must lie, at least in part, in the sixth abdominal ganglion. My work, therefore, centred round the attempt to find the location of the mechanism controlling hindgut movements, whether it was central (in the sixth abdominal ganglion) or peripheral (at the level of the plexus on the hindgut).

Several pharmacological studies have also been carried out on the rectum (ten Cate, 1924; Florey, 1954; Jones, 1962; Elofsson et al, 1962.). Florey suggested the presence of an inhibitory hindgut innervation as well as an excitatory innervation.

The aim of this thesis is to elucidate some of the nervous mechanisms underlying the control of hindgut motility in the lobster, Homarus gammarus (L.).

PART 2. EXPERIMENTAL

Animals

In all the experiments that are described below, Homarus gammarus (L.) was the species utilised. Specimens of Homarus were fished from St. Andrews Bay and kept, unfed, in a large aquarium supplied with circulating sea-water. All animals had their chelae banded with stout rubber bands and the mortality rate from all causes was less than 10% over an eight month period.

Section 2.1. Anatomy of the Sixth Abdominal Ganglion/Rectum Complex.

Introduction

The knowledge of the anatomy of any biological system is a very necessary prerequisite to the physiological study of that system. In the following section I give details of the overall anatomy of the rectum and the nerves, emanating from the sixth abdominal ganglion, which supply it.

Materials and Methods

Much of the anatomy of the posterior region of the hindgut was determined from preparations of the last two abdominal segments and the telson of the lobster which were fixed in alcoholic Bouin's solution (Pantin, 1964). These were then dissected in 70% alcohol with the aid of a Nikon SMZ2 stereomicroscope. In this way the detailed layout of the extrinsic anal musculature was determined.

Following this, many intra vitam stained methylene blue preparations of the region were made to determine the innervation of the rectum. Several approaches were tried, including the injection of leuco-methylene blue into the

heart and/or abdomen of the animal. Two methods of methylene blue staining eventually proved to be the most successful. Firstly an animal was injected with a few millilitres of Gurr's vital and fluorochrome methylene blue. It was then left for twenty minutes before being dissected. In the second method weak (very faintly blue) solutions of methylene blue in sea-water were left on the preparation and stored in a refrigerator for 12-15 hours.

Finally, serial sections of the hindgut were cut. Pieces of the rectum were fixed in alcoholic or sea-water solutions of Bouin's fixative and embedded in paraffin wax (melting point 58°C). 10µ sections were then cut with a rocking microtome and stained using either Azan or Mallory's triple stain according to the methods set down by Pantin (1964). Photographs of sections were made using a Zeiss Standard GFL Microscope with photomicroscopic attachments.

Photomicrographs of the extrinsic radial muscles of the anus and the longitudinal and circular muscles of the rectum were taken using the Nomarski interference-contrast fitment of the microscope mentioned above. From such photomicrographs the sarcomere lengths of the various muscle groups were determined.

Results

A. Relationship of the Sixth Abdominal Ganglion to the Rectum

The sixth abdominal ganglion (6A.G.) lies in the midline directly dorsal to the superficial ventral muscles of the sixth abdominal segment. It bears six paired nerve roots and innervates the hindgut, which it underlies, via the paired posterior intestinal nerves (P.I.N.'s) and the paired anal nerves. The relationship of the 6A.G. to the rectum is

summarised in Figure 2.1.1.

The P.I.N.'s divide typically into anterior and posterior branches. The anterior branches of the P.I.N.'s (i.e. the P.I.N.a.'s) supply the anterior region of the rectum - the main faecal expulsion region (see Section 2.2) - and also pass onto the midgut, whilst the posterior branches (i.e. the P.I.N.p.'s) supply the posterior region of the rectum and the extrinsic muscles of the anus as shown in Figure 2.1.1. The P.I.N.p.'s are generally much finer than the P.I.N.a.'s which also give off a series of side-branches to the mid region of the hindgut. The positions of these side-branches are rather variable as are the positions of the P.I.N.p.'s which occasionally arise from the anal nerves. All these nerves eventually come to lie below the thin tough investing connective tissue layer of the hindgut. Their size decreases as they pass distally, due to the ramifications of their axons over the gut musculature. Methylene blue staining indicates that several axons in the P.I.N.'s bifurcate many times and give off branches - dividing in the same pattern as the main nerve trunks (see Figure 2.1.2) to supply anterior, posterior and middle regions of the rectum. If the 6A.G. is desheathed dorsally and methylene blue stain allowed to run back along the axons it can be seen that axons running to supply the P.I.N.'s on either side often bifurcate and thus send a major branch to both P.I.N.'s. In one preparation these axons bifurcated outside the ganglion and a small commissure close to the base of the P.I.N.'s was found as shown in Figure 2.1.3. In this case the branches only supplied the P.I.N.a.'s, but in other preparations axons branching to the P.I.N.p.'s have also been observed. Whether single axons divide and send branches to all the divisions of both ipsilateral and contralateral P.I.N.'s is still a matter for conjecture.

The anal nerves, in the main, carry afferent information from the sensory structures of the telson. In addition they also carry some motor fibres which supply the telson flexor muscles. Further, they give off small branches medially which terminate in the region of the anus (for further details see section 2.3).

B. The Structure of the 6A.G.

The structure of the 6A.G. is outlined in Section 2.5.

C. The Structure of the Hindgut.

The anatomy of the rectum of Homarus is, with minor variations, similar to that of Nephrops (Yonge, 1924). Anteriorly, at the midgut/hindgut junction, there is a glandular swelling (see Figure 2.1.4) due to the presence of the tegumental glands which lie external to the muscles, but within the tough connective tissue sheath of the rectum.

The musculature of the rectum is arranged in an outer coat of circular muscle within which lie the separate strands of longitudinal muscle, commonly six in number. These strands of longitudinal muscle lie within the longitudinal ridges that project into the lumen of the hindgut, as shown in Figure 2.1.5. All the intestinal muscles are striated, and the lumen of both the hindgut and the foregut is invested with a layer of thin cuticle. The anterior region of the hindgut is relatively much more muscular than either the adjacent posterior midgut or the posterior hindgut. The lumen of the anterior hindgut is of much greater diameter than that of the posterior hindgut (see Figures 2.1.5 and 2.1.6). Posteriorly the floor of the hindgut is open to form the anus. Externally the anus appears as a longitudinal slit in the ventral soft cuticle of the telson. At the

sides of this slit the cuticle is invaginated to form a pair of sulci. The circular muscles around the anus continue as a layer of arched muscle fibres. The extrinsic radial muscle fibres take their proximal insertions from the proctodaeal cuticle (see Figure 2.1.7). Their distal insertions are as follows (see Figure 2.1.1):-

Muscle group R1 - the paired antero-ventral radial muscles - inserts into the ventral soft cuticle posterior to the last abdominal sternite.

R2 and R3 - the paired lateral and dorsal radial muscles - insert into the lateral and dorsal walls of the telson respectively.

R4 - the perianal radial muscles - inserts onto the islands of hard cuticle lying lateral to the anus and onto which the anal compressor and anal dilator muscles also insert.

R5 - the paired posterior oblique radial muscles are rather ill-defined. They insert mainly into the dorsal wall of the telson a few millimetres behind the anus.

There is no anal sphincter muscle present.

The sarcomere lengths of the various muscle groups have been measured and are summarised in Table 2.1.1.

Table 2.1.1.

Muscle Group	Sarcomere Length (μ)
R1	6 - 7.5
R2	6 - 8
R3	5 - 7.5
R4	7 - 8
R5	7
Circular Muscles of Rectum	7
Longitudinal Muscles of Rectum	5 - 6

Photomicrographs of some of these muscles are shown in Figure 2.1.9.

Figure 2.1.1. The isolated 6A.G./rectum complex.

A. - anal nerves
6A.G. - sixth abdominal ganglion
An. - anus
Conn - 5-6 connectives
P.I.N.a.'s - anterior branches of posterior intestinal nerves. These branches supply the anterior and middle regions of the hindgut and also run onto the midgut.
P.I.N.p.'s - posterior branches of the posterior intestinal nerves, which supply the posterior hindgut and the extrinsic radial muscles of the anus.
R1 - paired antero-ventral radial muscles.
R2 - paired lateral radial muscles.
R3 - paired dorsal radial muscles.
R4 - perianal radial muscles.
R5 - paired posterior oblique radial muscles.
Rect. - rectum.

The arrow denotes the position of a proprioceptor which lies in the hypodermis and is thought to respond to anal opening.

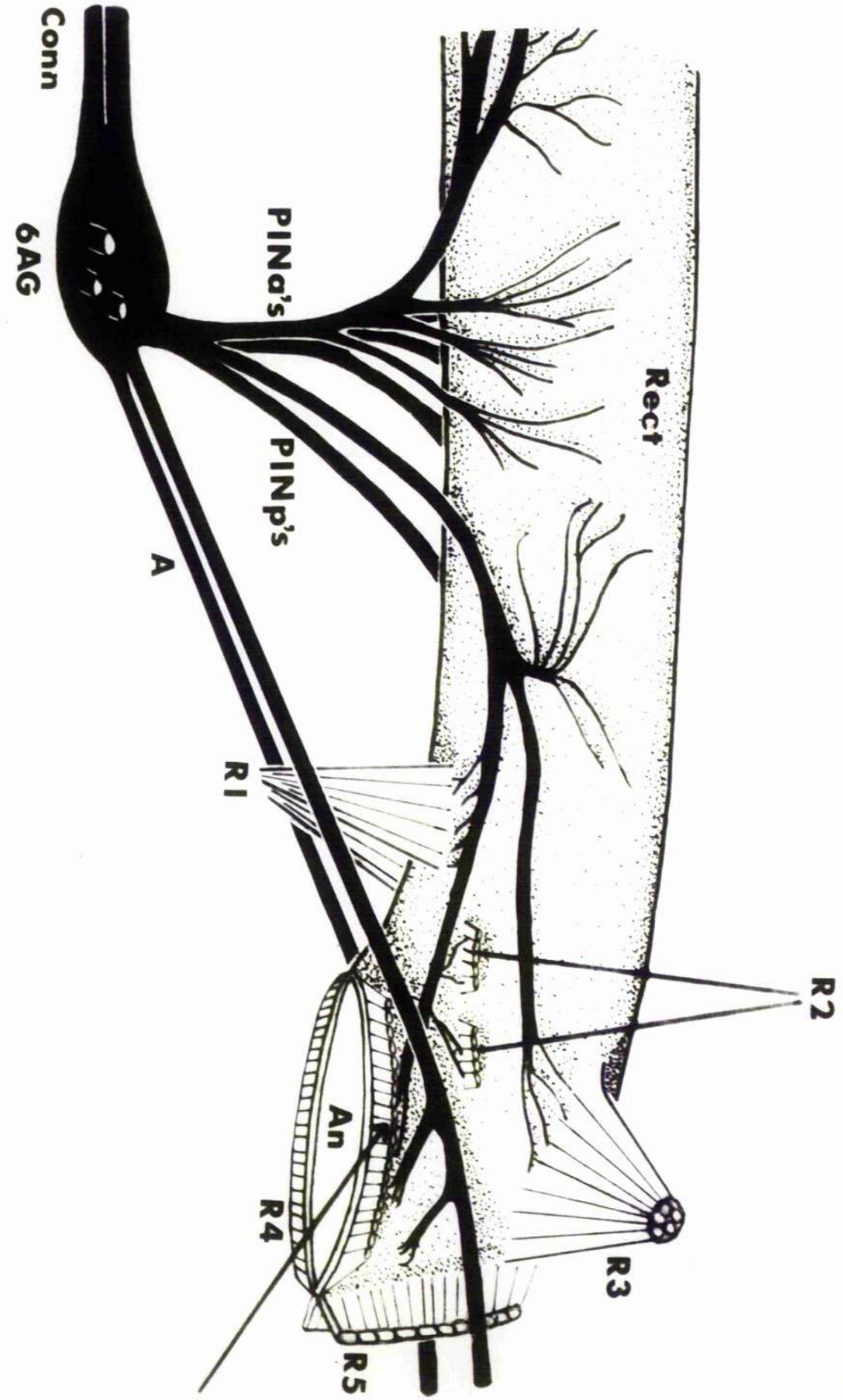

Conn
6AG
PINa's
PINp's
A
Rect
R1
R2
R3
R4
R5
An
5 mm

Figure 2.1.2. A drawing of a methylene blue stained preparation demonstrating the presence of a multibranched axon in the right P.I.N.a.

B.A. - bifurcating axon which divides at each side branch of the right P.I.N.a.

M.T. - main trunk of right P.I.N.a.

S.B. - side branch of right P.I.N.a.

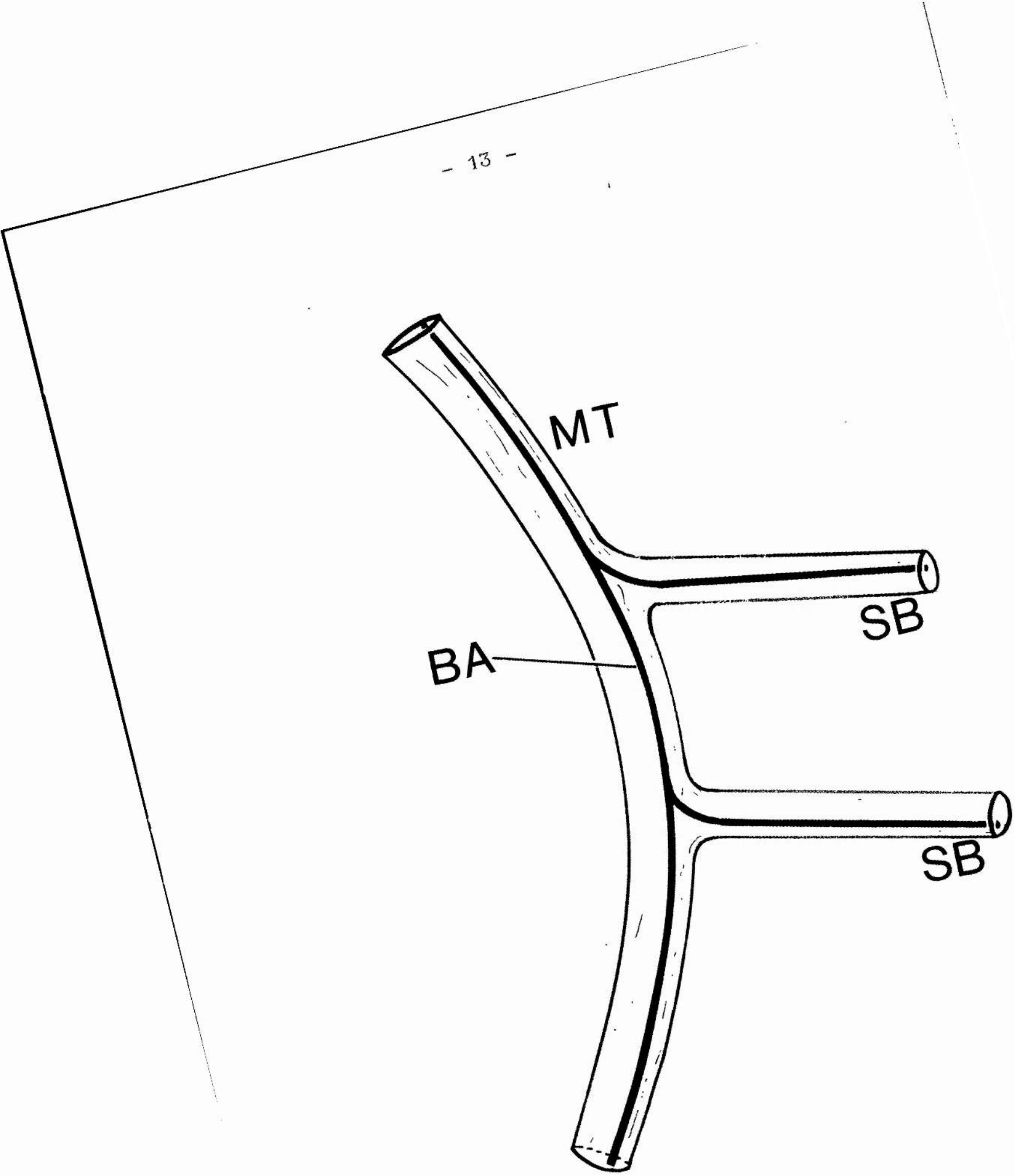

MT
SB
BA
SB

Figure 2.1.3. Axons dividing and sending branches through a commissure between left and right P.I.N.a.'s in an aberrant 6A.G., This is a semi-diagrammatic drawing of a methylene blue stained preparation.

Comm. - commissure.
Lt. D.T.N. - left dorsal telson nerve.
Ant. - anterior.
D.Ax. - dividing axons.

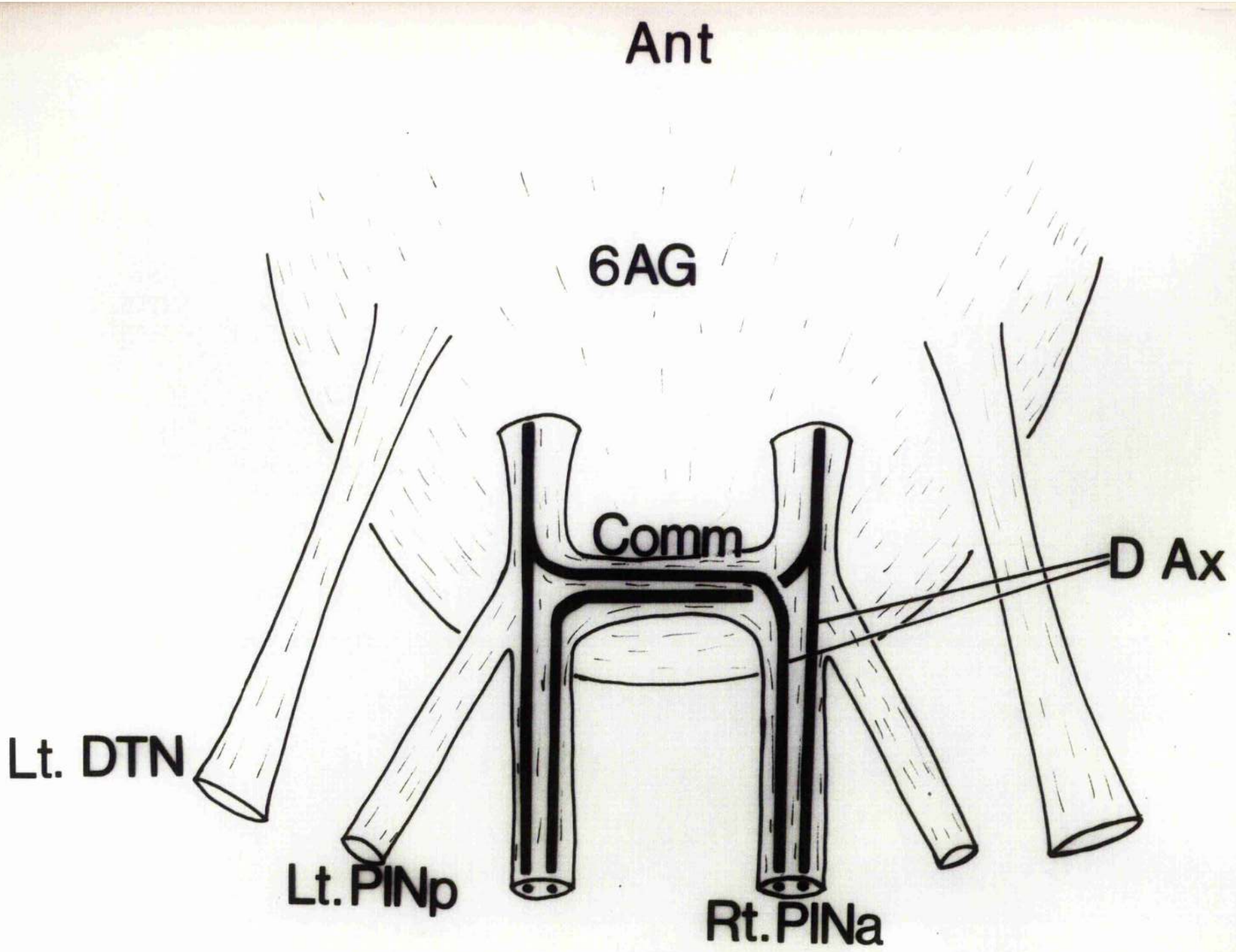

Ant
6AG
Comm
D Ax
Lt. DTN
Lt. PINp
Rt. PINa

Figure 2.1.4. T.S. of the anterior hindgut in the region of the glandular swelling.

The following abbreviations are used in Figures 2.1.4 to 2.1.7:-

A.I.	-	Anterior edge of anal invagination.
A.M.	-	Arched muscles in region of anus.
C.M.	-	Circular muscle.
Conn. tiss.	-	Connective tissue.
Ep.	-	Columnar epithelium of hindgut.
L.M.	-	Longitudinal muscle.
L.R.	-	Longitudinal ridge.
M.D.	-	Midgut diverticulum.
P.C.	-	Proctodaeal cuticle.
R2.	-	Lateral radial muscles inserting onto the proctodaeal cuticle.
S.	-	Sulcus.
T.F.M.	-	Telson flexor muscles.
T.G.	-	Tegumental glands.
V.S.C.	-	Ventral soft cuticle of body wall.

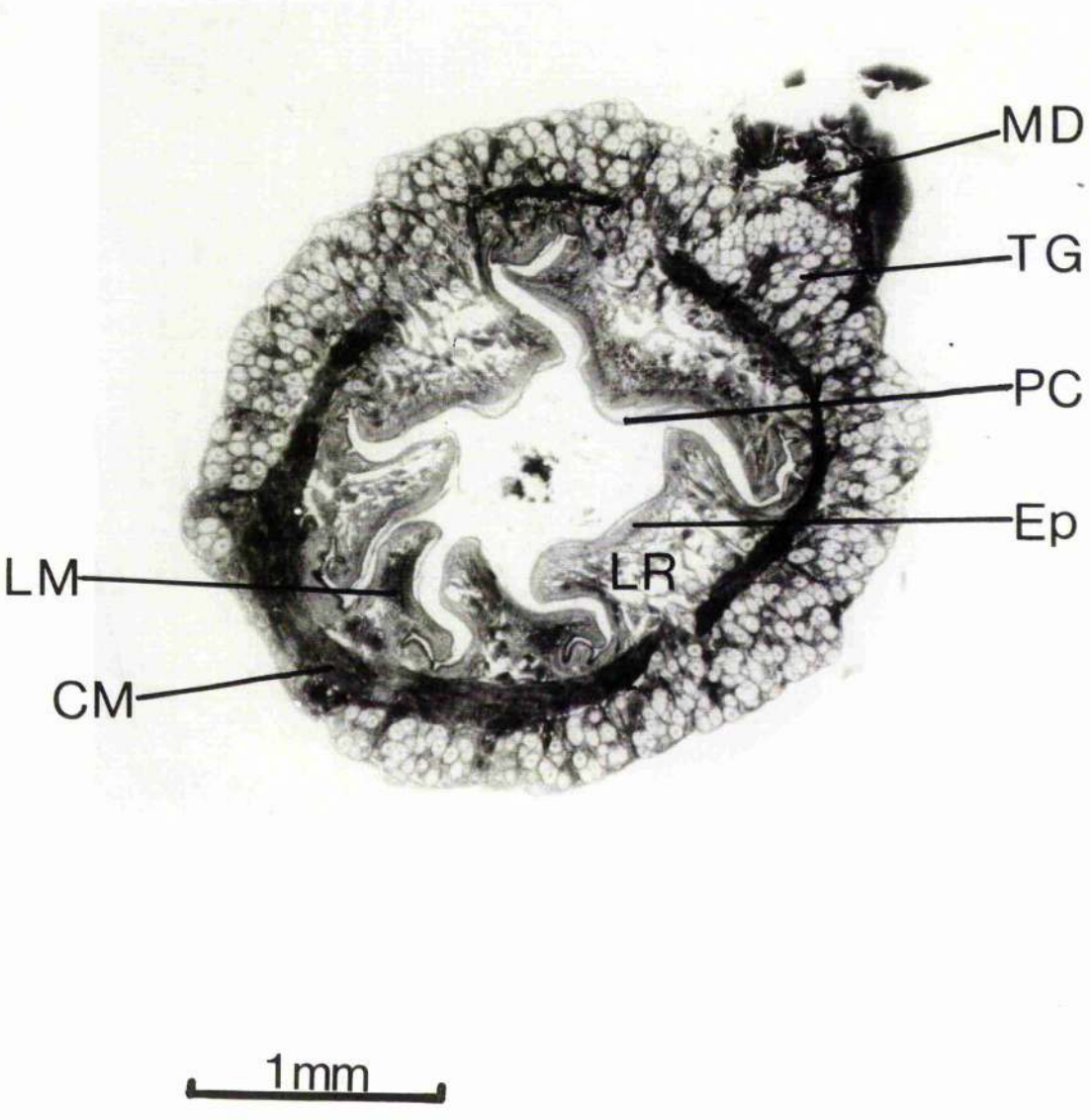

MD
TG
PC
Ep
LR
LM
CM
1mm

Figure 2.1.5. T.S. of the anterior hindgut posterior to the glandular swelling.

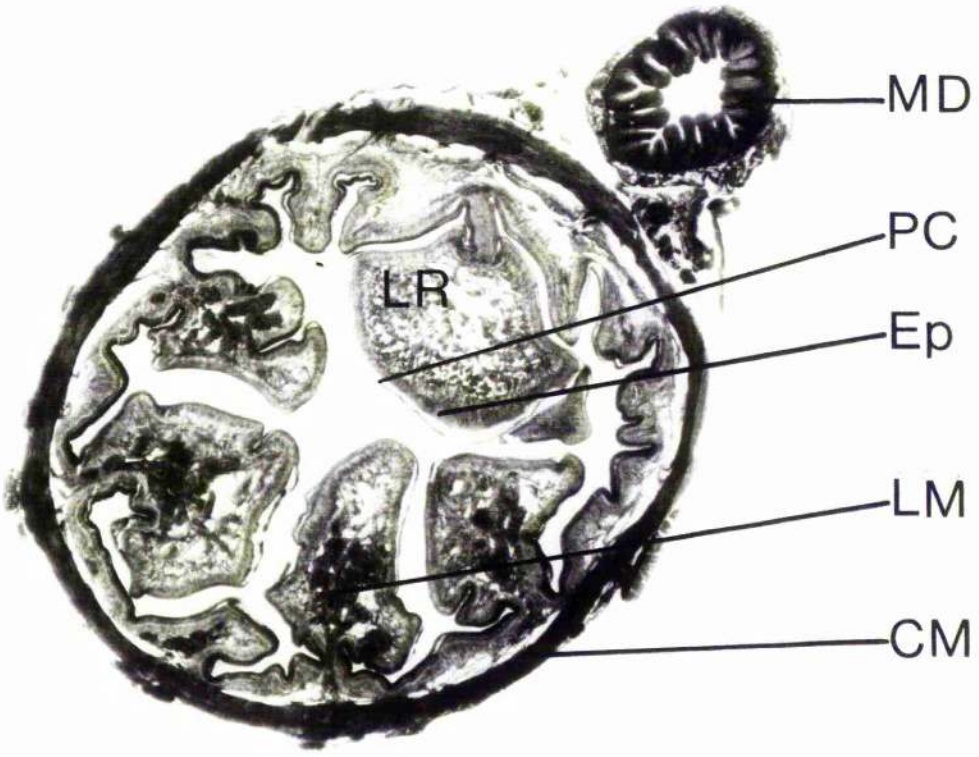

MD
PC
LR
Ep
LM
CM
1mm

Figure 2.1.6. T.S. of the posterior hindgut. The lumen is much narrower than that of the anterior region of the hindgut which is shown in Figure 2.1.5.

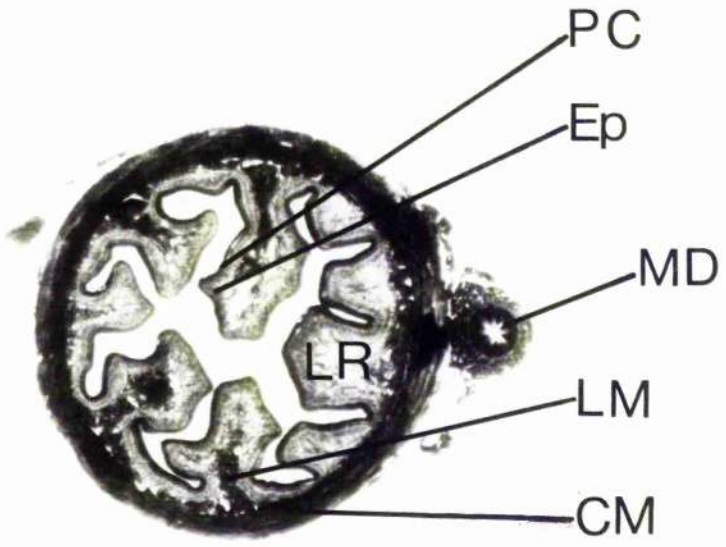

PC
Ep
MD
LR
LM
CM
1mm

Figure 2.1.7. T.S. of the hindgut in the region of the anus.

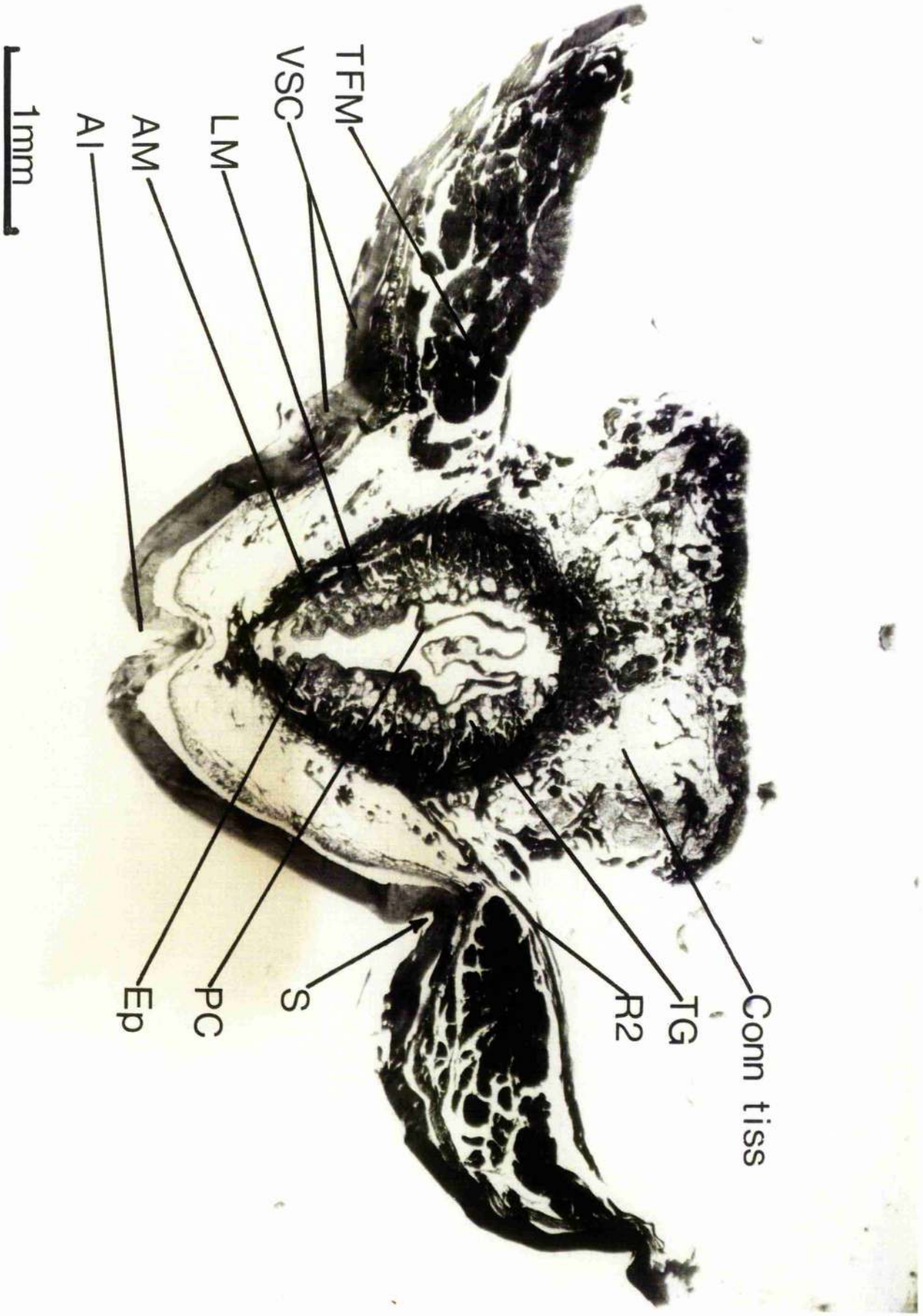
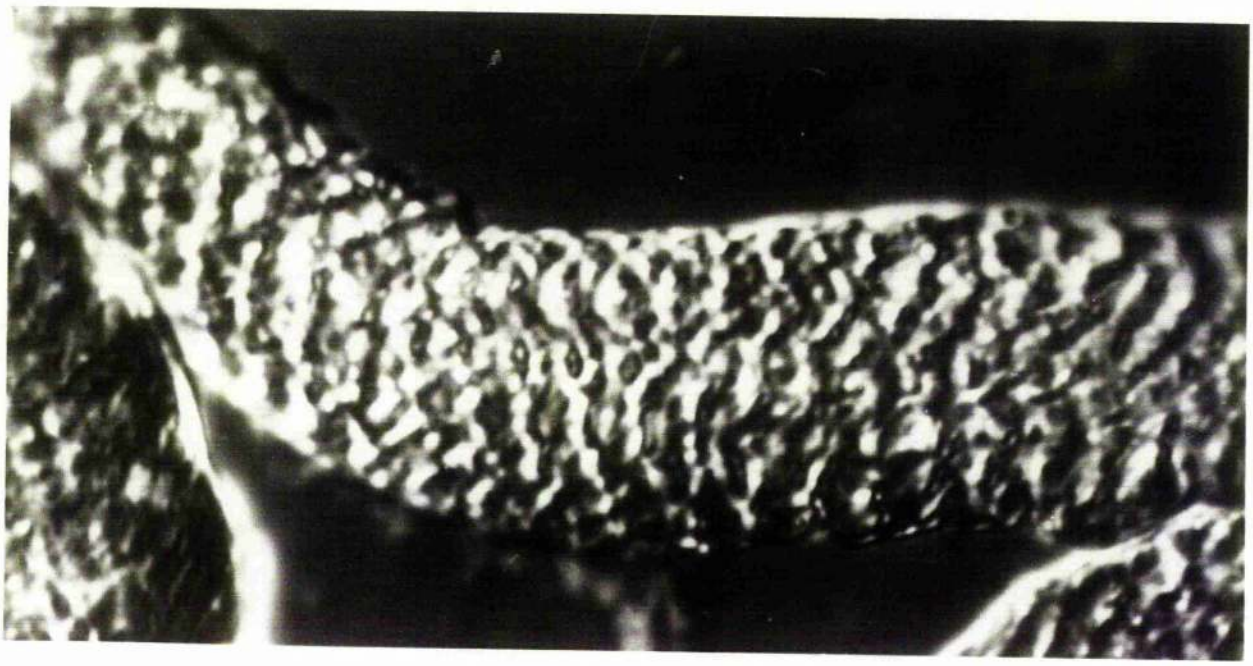

TFM
VSC
LM
AM
AI
1mm
Ep
PC
S
R2
TG
Conn tiss

Figure 2.1.8. Photomicrographs of the living, unstained R1 and R4 radial muscle groups, taken by the Nomarski interference-contrast method.

A. R1 muscle group.

B. R4 muscle group.

A



B

50 µ

Section 2.2. The Movements of the Hindgut.

Introduction

According to Miller (1910) stimulation of the ventral nerve cord (V.N.C.) of either Homarus or Cambarus produces rhythmic anal movements accompanied by peristaltic waves passing down the rectum. In this section I have, therefore, tried to correlate anal rhythmicity with hindgut peristalsis so that the whole behavioural sequence of defaecation can be better understood.

Materials and Methods

In experiments to determine the precise nature of the movements of the hindgut the last two or three abdominal segments were first severed from the animals. The uropods were removed and the preparation pinned, ventral surface uppermost, in a wax-bottomed dissecting dish filled with clean sea-water. In a number of experiments Homarus saline as recommended by Pantin (1964) was used instead of sea-water, but this made no detectable difference to the results obtained. The ventral integument was removed to expose the nerve cord, which was then freed from its connections with the somatic musculature. The somatic muscle groups lying dorsal to the V.N.C. and ventral to the hindgut were then removed leaving the 6 A.G./rectum complex intact, except for radial muscle group R1 (see Figure 2.1.1), which was invariably damaged during dissection.

The preparation was then transferred to a wax-bottomed perspex experimental dish which was cooled by tap-water running through an outer water-jacket. This maintained the preparation, which was bathed in clean sea-water or Homarus saline, at about 12°C. The movements of the rectum and anus were monitored by means of two RCA 5734 transducer

valves to the pegs of which long balsa-wood wands were attached. The distal ends of the wands, which bore blunted steel insect pins, were placed at various points along the rectum and on the anal lips. The V.N.C. was stimulated by means of rectangular d.c. pulses from a Tektronix 161 pulse generator triggered by a 162 waveform generator. The stimulus was applied through platinum wire electrodes via a stimulus isolation unit. The results were displayed on either a Tektronix 561A or 565 oscilloscope with either 3A74 or 3A3 preamplifier units. The results were photographed using a Cossor oscilloscope camera and Ilford NS6 recording paper.

A second series of experiments was carried out in which the oesophageal connectives of an almost intact animal were stimulated. In this case sea-water was passed through a perspex dish in which the animal was secured ventral surface uppermost. The chelae and all the mouthparts and appendages were then removed and the connectives exposed by cutting through the ventral soft cuticle around the mouth. Posteriorly the hindgut was exposed in the usual manner leaving the nerves intact. Stimuli were delivered to the connectives in the way described above whilst the movements of the anus and the rectum were observed.

A third series of experiments involved the measurement of tension changes in the radial muscles around the anus during stimulation of the P.I.N.p.'s. An isometric tension measuring device (Atwood et al, 1965), which involved attaching a pair of forceps to the movable peg of a transducer valve via a perspex coupling, was set up. The valve itself was mounted in a perspex tube. A heat-sink was found to be unnecessary. In most of the experiments the connective tissue at the distal end of each group of radial muscles was held in the transducer forceps whilst a

set of fixed forceps mounted on a Prior micromanipulator held their proximal ends immobile. It proved possible to immobilise the R4 muscle groups (see Figure 2.1.1) using only the transducer forceps attached to the anal lip and leaving the distal insertion of the muscle intact. Stimulation was delivered using suction electrodes.

In a fourth series of experiments the spontaneous activity of the hindgut was monitored. The rectal movements were recorded using a Devices single channel pen-recorder fed from the vertical signal output of the 565 oscilloscope using a 3A3 amplifier unit. In these experiments only one centimetre of midgut remained attached to the hindgut after dissection. As a preliminary to recording spontaneous activity the V.N.C. was always crushed at the 3-4 connectives in order to check that the hindgut gave the normal evoked response.

A fifth series of experiments was also attempted. In these it was hoped to make intracellular recordings of radial muscle membrane potentials whilst measuring tension changes in the muscles. The radial muscles were selected since they were the most easily immobilised of all the gut muscles. However, it proved impossible to penetrate the muscle fibres mainly due to the toughness of their surrounding sheath and also the fact that these muscles (except for R3) are only one or two muscle fibres thick.

Results

A. Confirmation of Miller's Work.

My findings are in broad agreement with those of Miller as is shown in Table 2.2.1. There are, however, two main discrepancies. The first is that I find the anal nerves to be devoid of any motor function with respect to the radial

TABLE 2.2.1.

Experiment	Results	
	Miller	Winlow
1. Stimulate V.N.C.	Hindgut peristalsis accompanied by rhythmic anal movements.	
2. Stimulate V.N.C. with anal nerves severed.	As above.	
3. Stimulate V.N.C. with P.I.N.'s severed, but anal nerves intact.	Single anal opening.	No movements.
4. Sever anal nerves.	As above.	
5. Stimulate anal nerves directly.	Anal opening on both sides. This is most prominent on the stimulated side.	No movements.
6. Stimulate P.I.N.'s directly.		Normal hindgut movements.
7. Stimulate hindgut directly.	Co-ordinated movements of rectum and anus.	Only localised hindgut movements.

Miller in his experiments utilized the lobster Homarus* and the crayfish Cambarus* and worked on whole animal preparations. He stimulated the animal for between 3 and 20 seconds using the 'interrupted current of an inductorium'. In my experiments I used the lobster Homarus gammarus (L.) and worked on only the last two or three abdominal segments. I applied 5-10 seconds of stimulation at 20-40 Hz and using 1-3 ms rectangular pulses.

*No further information given as to species.

muscles of the anus. Miller found that stimulation or severance of the anal nerves was sufficient to produce a single anal opening. A second point of disagreement is with Miller's assertion that direct stimulation of the hindgut "causes the passage of the usual contraction wave followed by anal opening", since in my experiments this caused only localised hindgut movements. The possible reasons for these discrepancies are considered in the Discussion.

B. Determination of the Form of the Hindgut Movements.

To determine the manner of movement of the hindgut the transducer wands were placed at various points along its length and on the lips of the anus. The distances across which their tips were deflected were determined by direct measurement. Simultaneous recordings of these movements were made and their sequential nature worked out. Figure 2.2.1. shows an oscilloscope trace typical of those obtained by this method. In this case transducers were placed on the right lip of the anus (upper beam) and immediately posterior to the midgut/hindgut junction (lower beam). The resultant trace shows that the response of the hindgut to stimulation of the V.N.C. is divisible into primary and secondary phases. These last for variable lengths of time from animal to animal and also vary with the condition of the preparation. In the primary phase the anus is held widely open, especially in fresh preparations, and exhibits only minimal closing contractions. Hindgut movements during this period are due to bursts of activity in the P.I.N's (see (Section 2.4). In the secondary phase the hindgut exhibits co-ordinated activity, whose onset is marked by a series of rhythmic peristaltic waves passing back down the rectum (see Figure 2.2.2). Accompanying the peristaltic waves is the anal rhythm. As the anus closes the anterior rectum, which

is very muscular (see Section 2.1), contracts longitudinally in a localised manner resulting in a net rearwards movement of the midgut/hindgut junction (see Figure 2.2.2.b). This longitudinal muscle contraction is then followed by a slow, powerful, posterior-going, peristaltic wave which is presumably mediated by serial contraction of the circular muscle layer. The peristaltic wave is initiated in the region of the midgut/hindgut junction (see Figure 2.2.2.d and e). During the passage of the circular muscle contraction wave the anus is held widely open (Figure 2.2.2.e and a). The anus is then kept fully open for a short time and the next cycle commences as the anus starts to close and the longitudinal muscles begin to contract. During the passage of the peristaltic wave the hindgut may elongate anteriorwards by as much as 3mm. The middle region of the hindgut exhibits very little movement during this cycle of motility and is only weakly contractile, reflecting its paucity of musculature. Following the cessation of stimulation the hindgut movements continue for a short time, but often in a less well co-ordinated manner as is demonstrated in Figure 2.2.1. Thus faecal expulsion is accomplished by contractions of the musculature of the faecal expulsion region, E (see Figure 2.2.2), of the rectum acting in synchrony with the extrinsic radial muscles which open the anus allowing the faeces to pass to the outside. Anal closure may be a function of the arched muscle fibres or may be due to elastic rebound of the ventral soft cuticle around the anus.

C. Control of the Hindgut Movements.

In a series of experiments on the intact animal it was found that stimulation of either oesophageal connective, both anterior and posterior to the commissural ganglion,

would produce the response of the hindgut described above.

A further understanding of the control mechanism for hindgut motility can be gained by sectioning the various nerves supplying the hindgut, as well as sectioning the hindgut itself.

1. Sectioning of the Hindgut.

Experiments in which the hindgut was sectioned at a position between the P.I.N.a.'s and the P.I.N.p.'s, leaving the nerves intact, do not affect the co-ordinated nature of the hindgut response (Figure 2.2.3). The form of the contraction does, however, change probably due to interference with the longitudinal muscle groups. As long as the innervation remains intact several cuts across the gut can be made without affecting the basic co-ordination of the response (Figure 2.2.4).

2. Sectioning the Nerves.

a) Anal Nerves -

Cutting the anal nerves does not disturb either the frequency of the anal rhythm or the motility of the anterior region of the rectum. The co-ordinated nature of the response is also unaffected.

b) P.I.N.A.'s -

Figure 2.2.5 shows the effects of gradual elimination of the anterior rectal nerve supply. Sectioning the main trunks of the P.I.N.a.'s whilst leaving their side-branches intact appears to prevent most of the peristaltic response and greatly reduces the longitudinal muscle contraction (Figure 2.2.5C). Cutting the side branches of both P.I.N.a.'s virtually abolishes the response of the anterior region of the hindgut to stimulation of the V.N.C. (Figure 2.2.5D). Sectioning of a single main trunk whilst leaving all its side branches intact reduces considerably the circular muscle contraction and alters the phase of the

longitudinal muscle contraction (Figure 2.2.5.B). During all these experiments the response of the anus remains totally unaffected.

c) P.I.N.p.'s -

Direct and simultaneous stimulation of both P.I.N.p.'s causes anal opening (Figure 2.2.6.A). Prolonged bursts of stimuli will cause the anal rhythm (see Figure 2.2.7.F and G). The anal response may be abolished by sectioning the P.I.N.p.'s peripheral to the stimulating electrodes (Figure 2.2.6.B).

d) The Anal Rhythm -

The anal rhythm is one facet of the highly co-ordinated movements of the hindgut (see Figure 2.2.1). In fresh preparations it occurs during the secondary phase of evoked rectal movements. Figure 2.2.7 shows the effect of increasing the duration of constant frequency stimulation of the P.I.N.p.'s on the radial musculature. The amplitude of radial muscle contraction increases in linear fashion from A to D as the number of pulses delivered increases (see graph - Figure 2.2.8). At E there is again an increase in the amplitude of contraction, but this is followed by further oscillatory contractions of the radial muscles (i.e. the onset of the anal rhythm). Figures 2.2.7.F and 2.2.7.G show the effects of prolonged periods of stimulation.

In an attempt to determine the underlying mechanisms of the anal rhythm further experiments were carried out. In these the radial muscle groups were individually immobilised and tension changes in them during stimulation of the P.I.N.p.'s were qualitatively measured using an RCA 5734 transducer valve (see Atwood et al, 1965, for

details). Figure 2.2.9 shows a trace in which tension changes in the perianal radial musculature (R4) were monitored during stimulation of the P.I.N.p.'s. The trace obtained is very similar to those recording movements of the anal lips (see upper trace of Figure 2.2.1.). There is an initial delay between stimulus and response of about 0.5 sec.. This is followed by a sustained contraction (with ripples of anal rhythm) whilst stimulus is maintained. After the stimulus ceases a further contraction (or in many cases a series of contractions) occurs. This inherent rhythmicity was found to occur in all five radial muscle groups.

D. Spontaneous Motility of the Hindgut.

It was found that the hindgut could show many forms of spontaneous activity. Such spontaneous motility was not dependent on the presence of the 6 A.G. which seemed to exert little or no influence on the rectum when not actually driving it. The most typical responses were relatively slow circular muscle contractions often associated with rather faster longitudinal muscle contractions, at least in fresh preparations (see Figure 2.2.10.A). Later in the experiments these movements often became rather desynchronised. Longitudinal and circular muscles were then found to beat at their own rate and to exhibit the phenomenon of phase drift (see Figure 2.2.10.B). In addition, the longitudinal muscle strips were also found to beat independently of one another, pulling the cut end of the midgut either to the right or left during contractions. In many cases only longitudinal muscle strips of the right or left side would contract, the other side remaining inactive. Many forms of rhythm were seen to

develop, often increasing slowly to a maximum and then gradually decaying away (Figure 2.2.10.B).

The maximal excursion made by any given point in the region of the midgut/hindgut junction was rarely more than 0.5-1mm., which is a good deal less than the movements made during evoked activity (up to 3mm.- see Figure 2.2.2). The movements of the anterior hindgut were never observed to be co-ordinated with movements of the anus (which rarely opened spontaneously during these experiments) although preliminary and post-experimental checks generally revealed the radial musculature to be in a completely functional state.

FIGURE 2.2.1. Transducer record of the movements of the hindgut occurring as a result of stimulation of the V.N.C. of a fresh preparation. Continuous trace.

First beam - transducer on right lip of anus - upward deflection denotes anal opening. Second beam - transducer at midgut/hindgut junction - upward deflection denotes posteriorward motion due to contraction of longitudinal muscles, whilst downward deflection denotes anteriorward motion due to circular muscle contraction. Third beam - stimulus marker - 40 Hz at a pulse width of 3 ms.

1st - primary phase of response. 2nd - secondary phase of response. I have arbitrarily decided that the secondary phase of the response should end after the last co-ordinated anal movement and peristaltic wave.

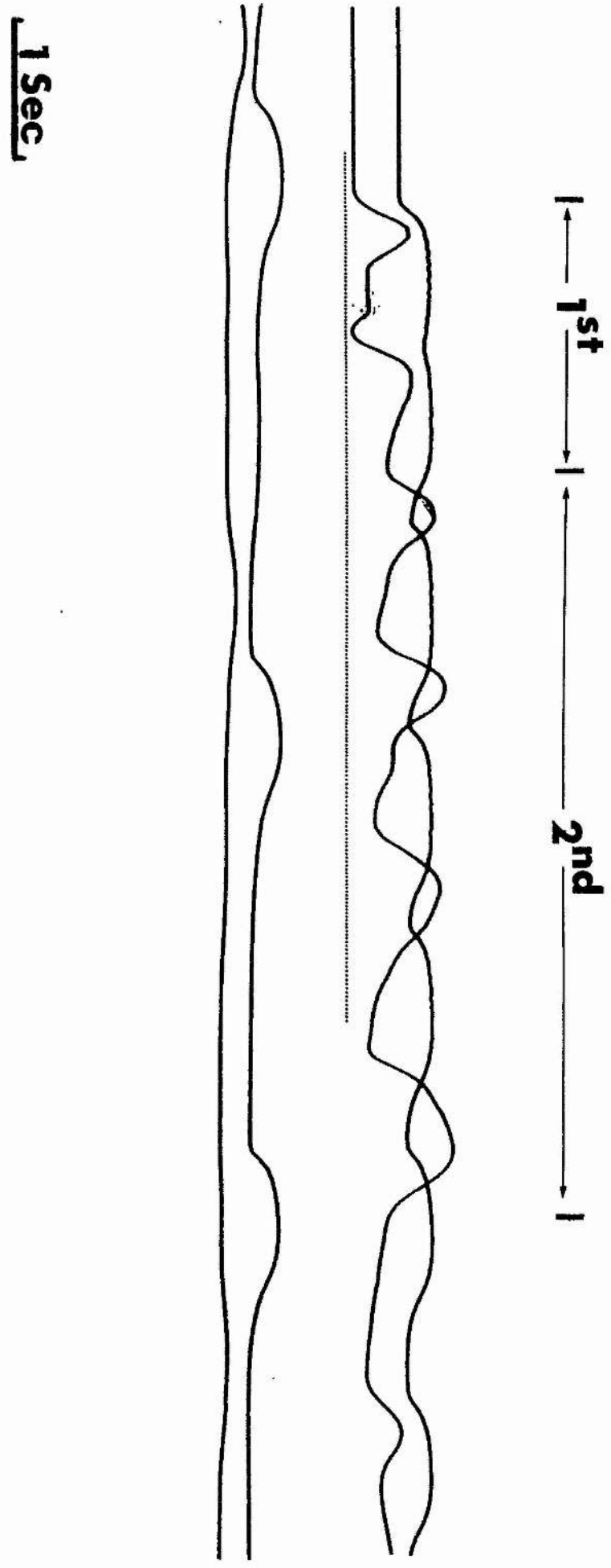

1Sec
1st
2nd

FIGURE 2.2.2. Motility of the hindgut during stimulation of the V.N.C.. One cycle of the secondary phase of the response is represented.

The figure is drawn to scale and represents the movements of a series of points (1 to 6) along the hindgut. Anal movements are also shown. These movements were recorded using R.C.A. 5734 transducers as in Figure 2.2.1, from which some of the data was taken. Measurements of the amount of movement of the transducer wands were taken directly using a scale marker laid in the bath alongside the preparation. The timing of the various sections of the response is shown on a non-linear scale at the top of the diagram. Each cycle of movement takes 1.5 sec..

Point 1 seems to move passively following the movements of 'E', the main faecal expulsion region of the rectum, in a somewhat damped manner. Points 5 and 6 move weakly, but the peristaltic wave does pass.

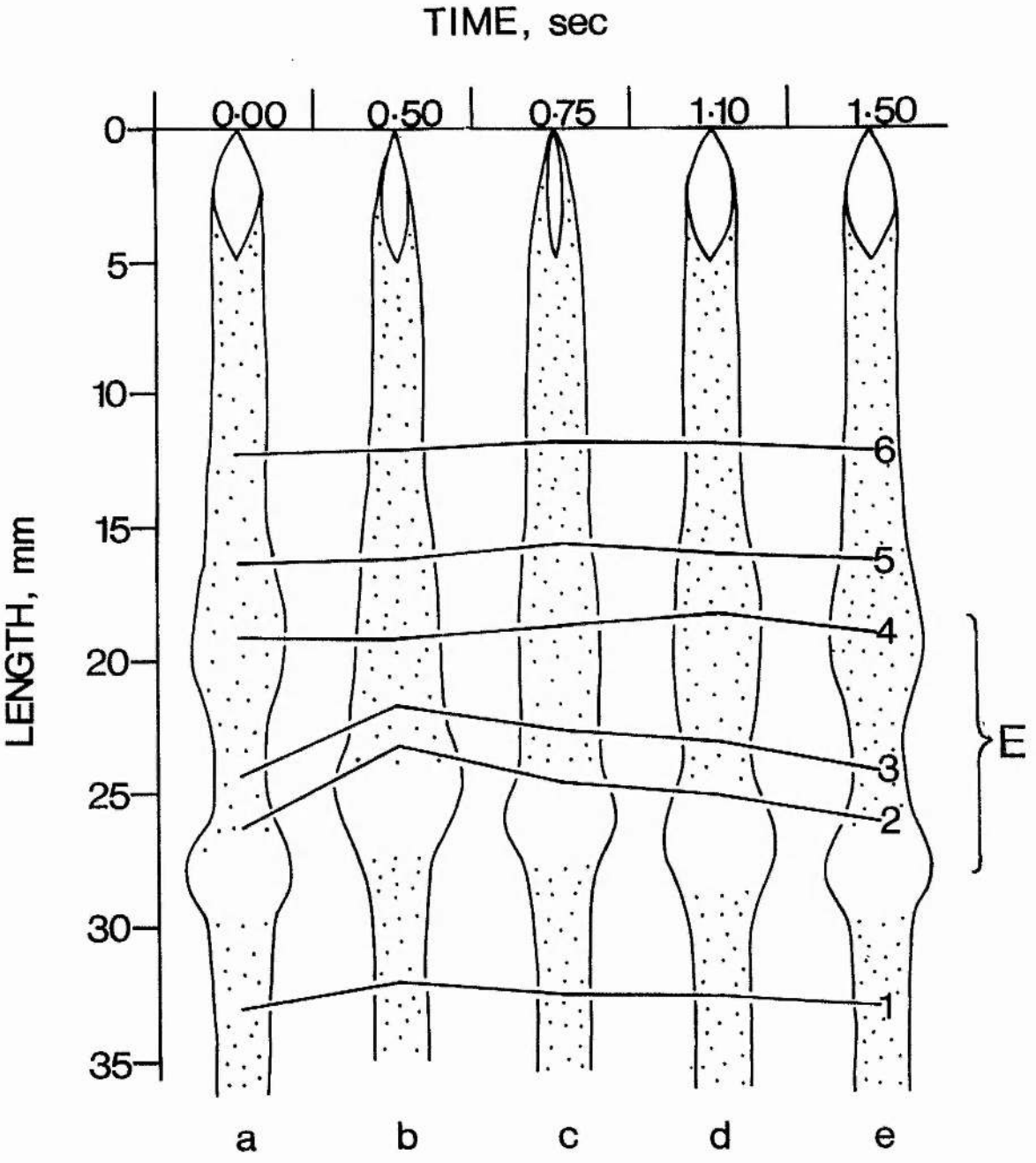

TIME, sec
0·00
0·50
0·75
1·10
1·50
0
5
10
15
20
25
30
35
LENGTH, mm
6
5
4
3
2
1
E
a
b
c
d
e

FIGURE 2.2.3. The effect of sectioning the rectum on the co-ordination of the rectal and anal responses after stimulation of the V.N.C. (40Hz, 3ms pulses).

Upper beam - transducer on left lip of anus - downward deflection denotes anal opening. Lower beam - transducer at midgut/hindgut junction - upward deflection denotes posteriorward movement and downward deflection denotes anteriorward movement. The horizontal bars are the stimulus markers.

A. - Intact preparation.

B. - Hindgut sectioned between P.I.N.a.'s and P.I.N.p.'s. The innervation remains intact.

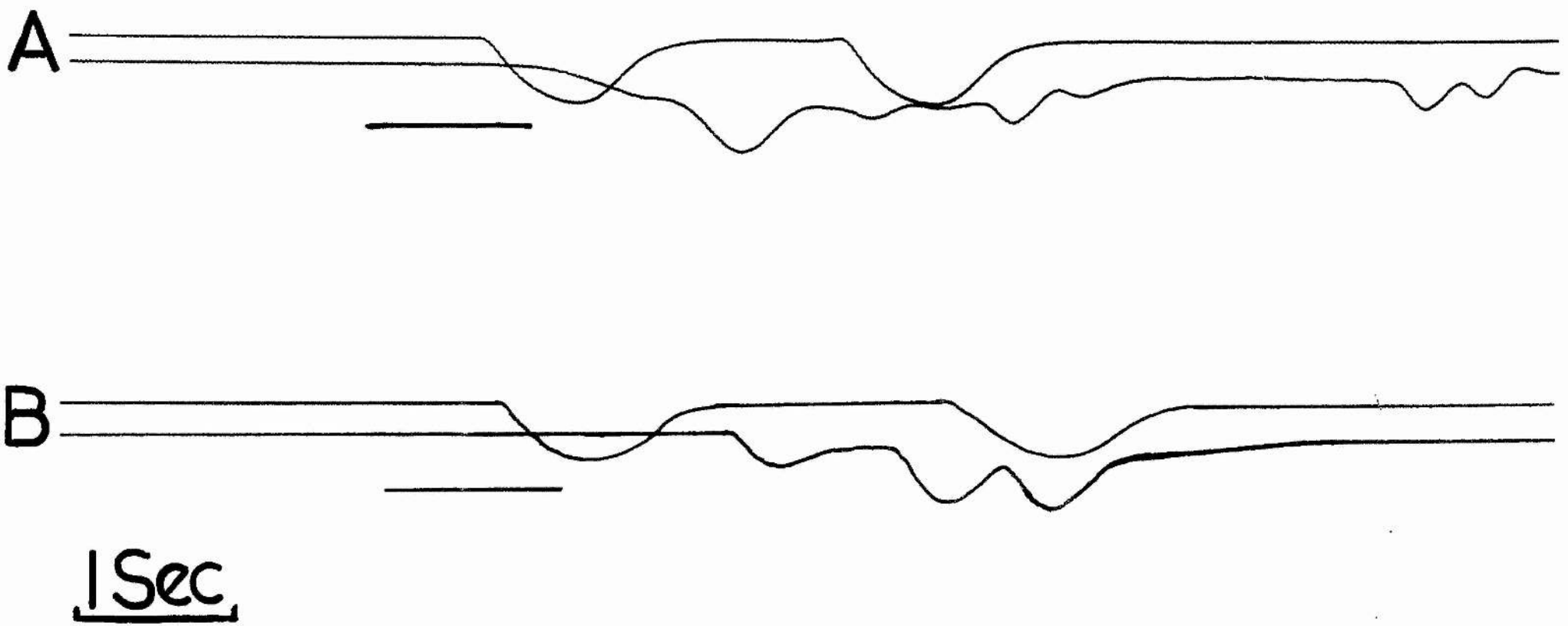

A
B
1 Sec

FIGURE 2.2.4. Responses of the hindgut elicited by stimulation of the V.N.C. (4CHz 3ms pulses) after sectioning of the hindgut at two points (the first in front of the side branches of the P.I.N.a.'s and the second behind them).

A. Illustration of points at which hindgut was sectioned.

B. Transducer traces of the movements of the various regions of the hindgut. The dotted lines are the stimulus markers.

i. Transducers placed on right anal lip (upper beam) and on middle section of hindgut (lower beam).

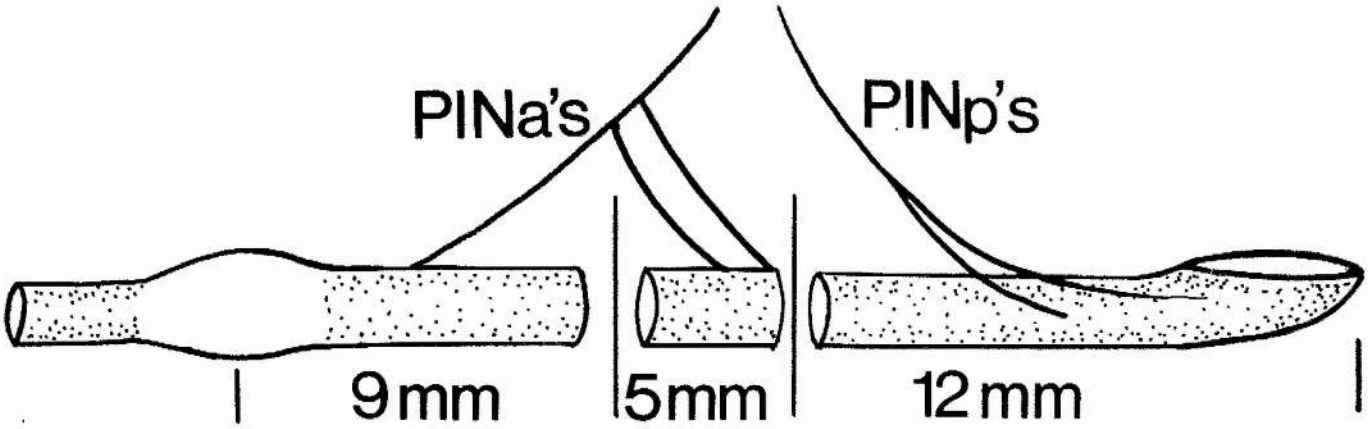
ii. Transducers placed on posterior section of hindgut (upper beam) and on middle section of hindgut (lower beam).

iii. Transducers placed at midgut/hindgut junction (upper beam) and on middle section of hindgut (lower beam).

i (upper beam) - upward deflection denotes anal opening.

i (lower beam), ii and iii (both beams) - upward deflection denotes posteriorward movement and downward deflection denotes anteriorward movement.

The partial lack of posteriorward movement is thought to be due to the disruption of the longitudinal muscle strands. The remaining response is due to the passage of the peristaltic wave by the circular muscles. The basic co-ordination of the response is maintained. Thus the middle portion of the hindgut is controlled by the side branches of the P.I.N.a.'s.



A

B

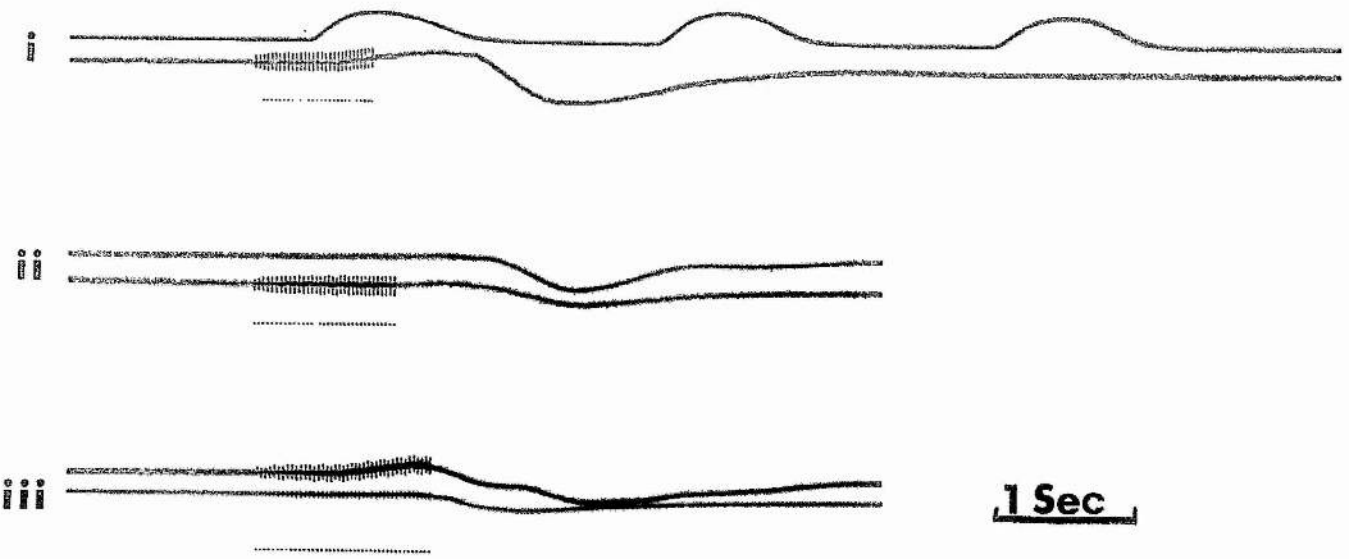


FIGURE 2.2.5. The effect of progressive extirpation of the P.I.N.a.'s and their side-branches on the movements of the hindgut when the V.N.C. is stimulated (40Hz, 3ms pulses). Horizontal bars are stimulus markers. Transducer placements as in Figure 2.2.3.

A. - System intact. Normal response.

B. - Main trunk of right P.I.N.a. sectioned peripherally leaving side branches intact.

C. - Main trunk of left P.I.N.a. sectioned peripherally leaving side branches intact.

D. - Side branches of both P.I.N.a.'s sectioned.

For further details see text.

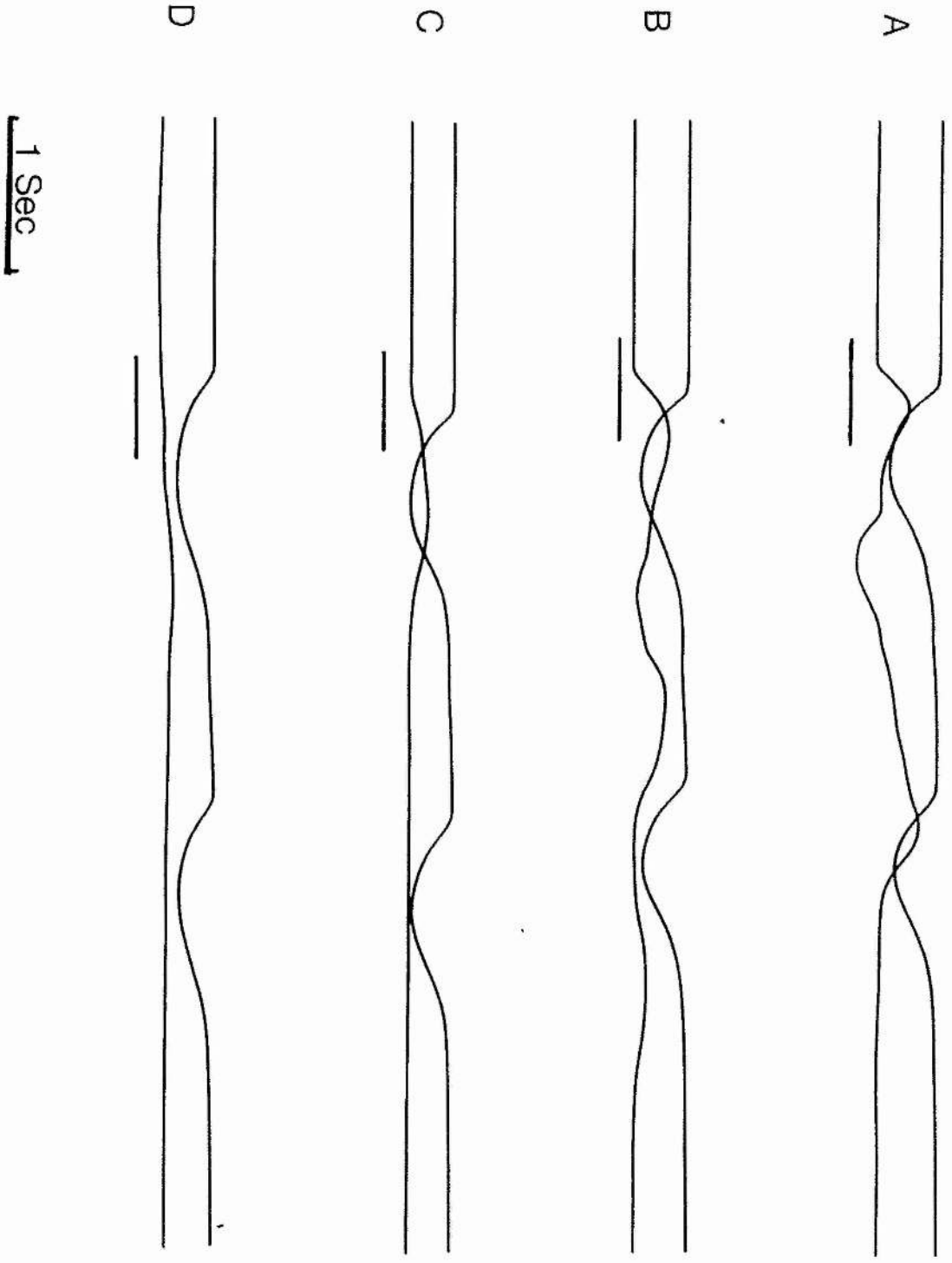

A
B
C
D
1 Sec

FIGURE 2.2.6. Simultaneous stimulation of both P.I.N.p.'s and monitoring of the movements of both anal lips. Upper beam - left anal lip. Lower beam - right anal lip.

A. Both P.I.N.p.'s intact.

B. Right P.I.N.p. severed peripheral to the stimulating electrodes.

Displacement of either beam indicates anal opening.

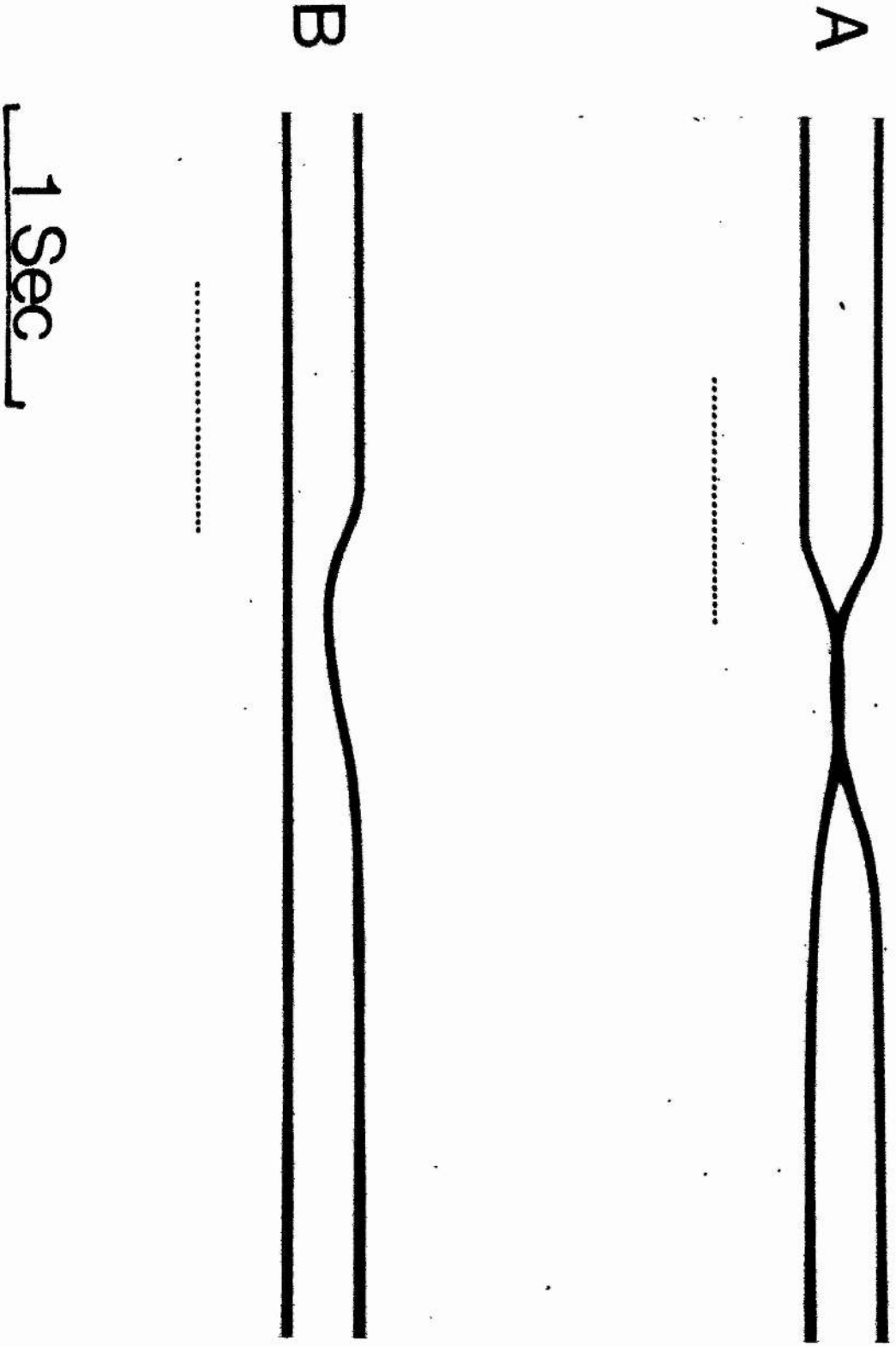

A
B
1 Sec

FIGURE 2.2.7. Movements of left anal lip in response to bursts of stimuli at constant frequency (20Hz) but of gradually increasing duration. Stimuli were delivered down the intact P.I.N.p.'s. The numbers of pulses per burst were as follows:-

A, 10; B, 13; C, 15; D, 18; E, 20; F, 180; G, 410.

Figures F and G show the effects of prolonged periods of stimulation. The decrease in contraction amplitude towards the end of stimulation may be due to fatigue.

For further details see text and Figure 2.2.8.

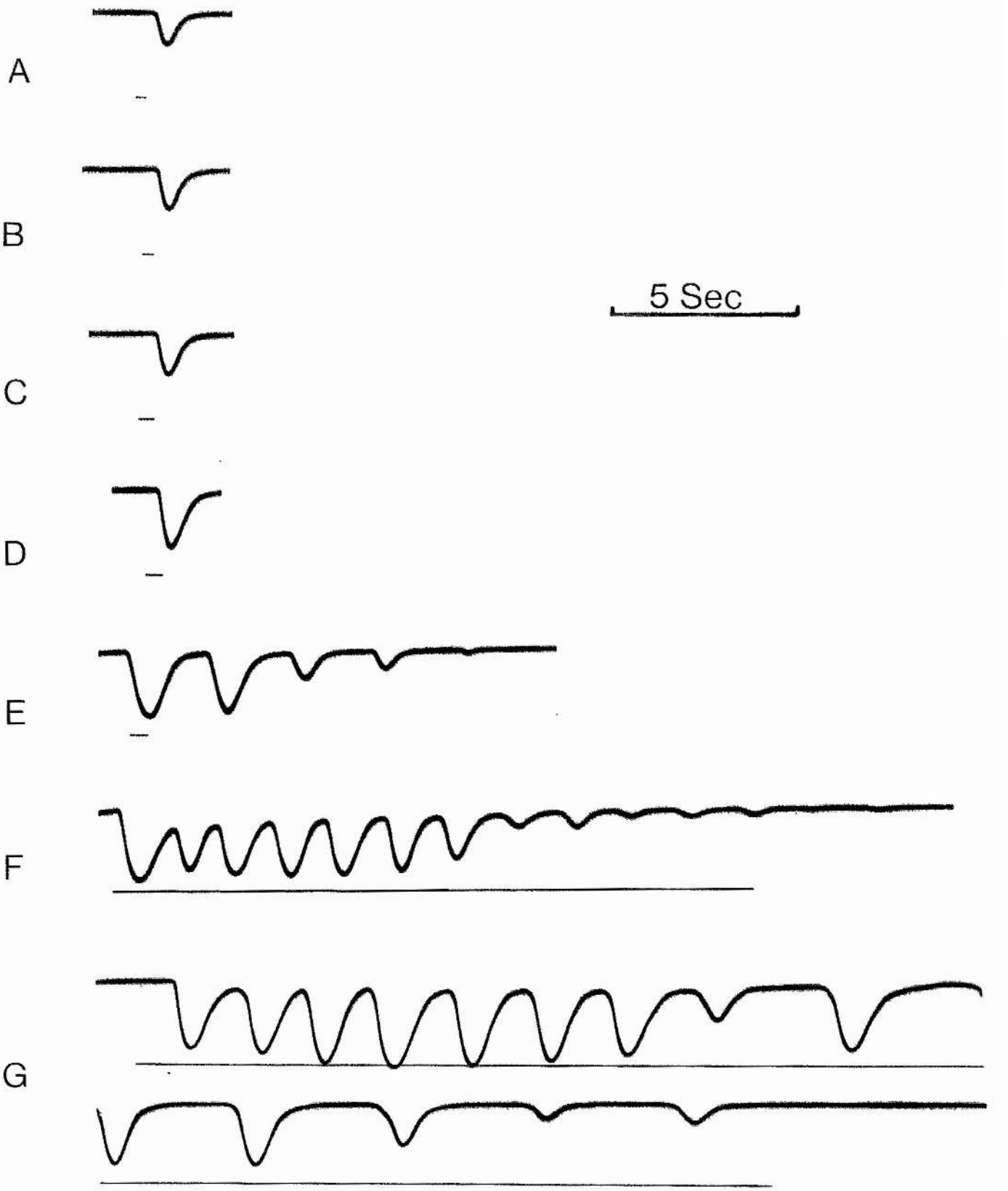

A
B
5 Sec
C
D
E
F
G

FIGURE 2.2.8. Graph relating the number of impulses delivered down the P.I.N.p.'s to the amplitude of contraction of the perianal radial muscles (i.e. to the amount of movement of the left anal lip expressed as a percentage of maximum). The values plotted were determined from Figure 2.2.7 and calculations show that the R.C.A. 5734 transducer valve was functioning in the linear part of its range during these experiments.

Vertical axis - contraction of anal muscles measured as a percentage of maximum. Maximum contraction was taken as the mean of the first contractions in Figures 2.2.7.E, F and G.

Horizontal axis - number of pulses delivered down P.I.N.p.'s at constant frequency.

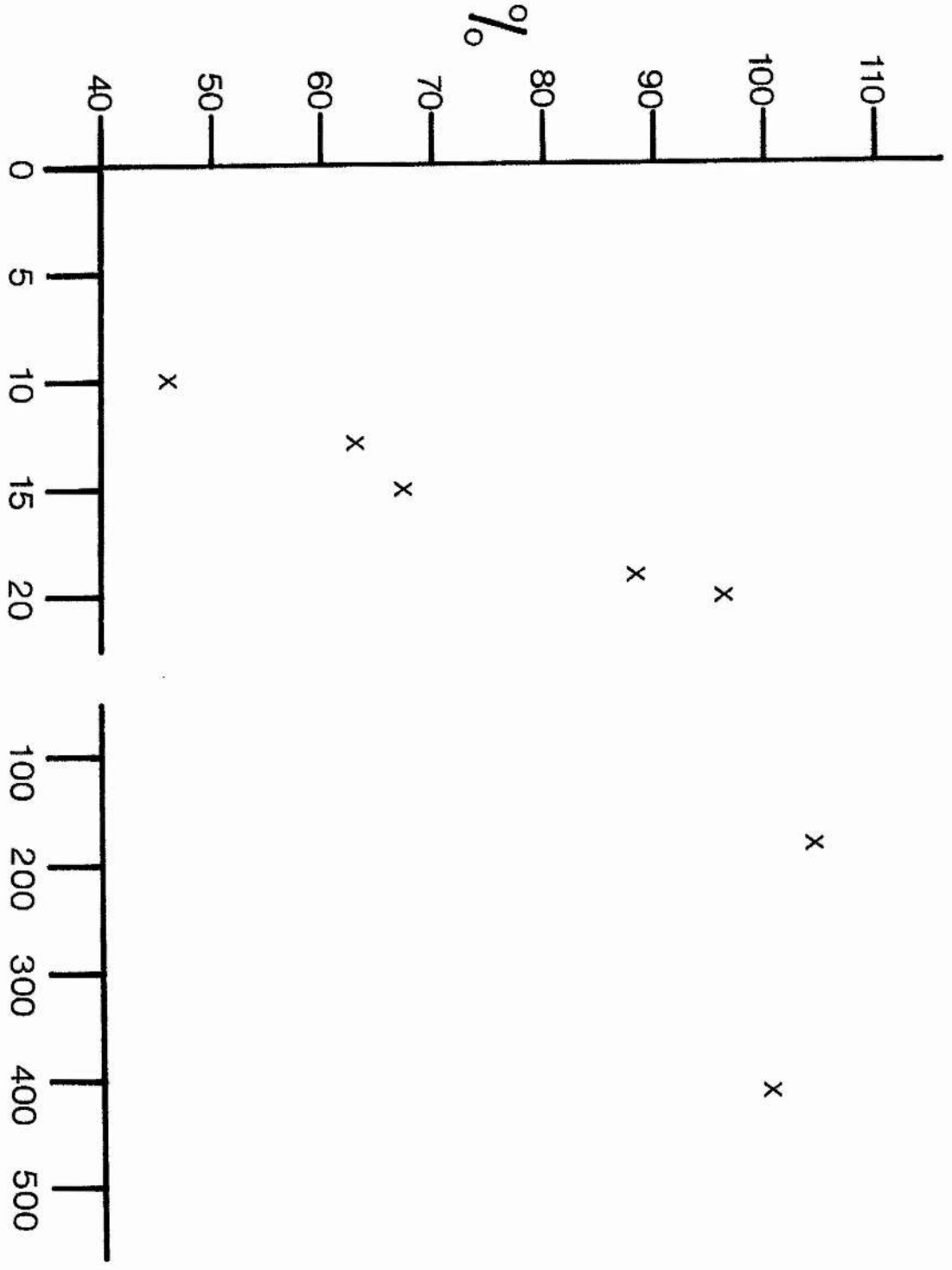

%
110
100
90
80
70
60
50
40
0
5
10
15
20
100
200
300
400
500
No. of pulses

FIGURE 2.2.9. Tension response of immobilised right anal lip (i.e. R4 radial muscle group) due to stimulation of cut peripheral ends of P.I.N.p.'s using a suction electrode. Stimulus characteristics:- 25Hz, 0.1ms pulses. Horizontal bar is stimulus marker.


1 Sec.

FIGURE 2.2.10. Spontaneous hindgut contractions recorded directly posterior to the midgut/hindgut junction using an RCA 5734 transducer diode. In this case the 6 A.G. was intact. Upward deflection denotes posteriorward movement. Downward deflection denotes anteriorward movement.

A.) Longitudinal muscle contractions directly preceding circular muscle contractions during the first 10 minutes of the experiment. The circular muscle contractions become more complex at the right.

B.) Continuous trace starting 50 minutes after initiation of the experiment.

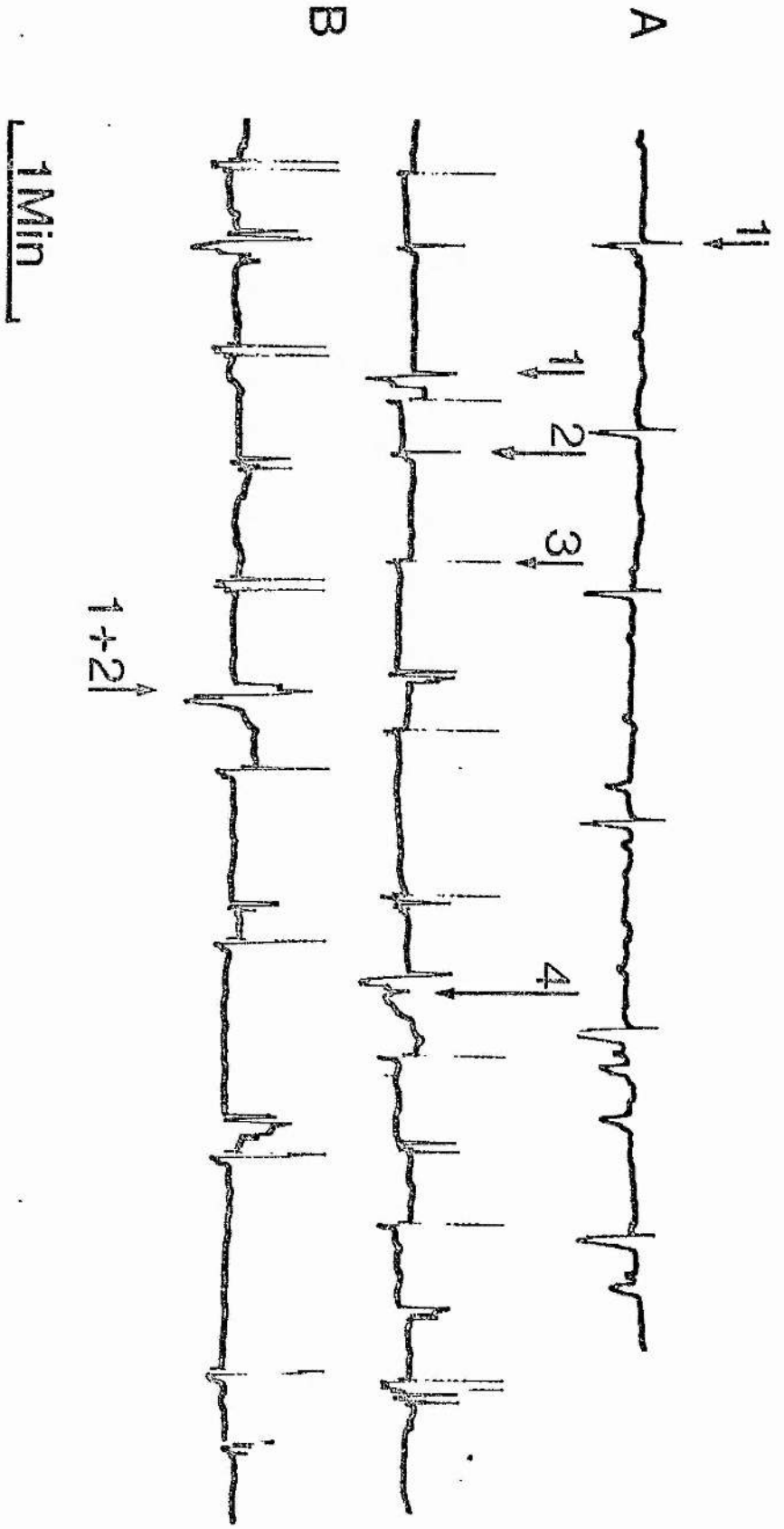
1. Rhythmic longitudinal and circular muscle contractions as in A.

2. Longitudinal muscle contractions pulling the cut end of the midgut to the left. These become double contractions before returning to single contractions.

3. Longitudinal muscle contractions to the right. These also double up.

4. Longitudinal muscle contraction to the right.

1 + 2 - A fusion of 1 and 2. Contractions gradually slowed until 15 minutes after the end of the trace, when all activity ceased. Crushing the V.N.C. still elicited normal hindgut movements, however.


A
B
1
1
2
3
4
1+2
1 Min

Section 2.3. Receptors Responding to Hindgut Movements.

Introduction

In recent years receptors have been shown to serve two functions. The first and most obvious of these is that they provide information about the external or internal environment of the organism. In addition to this they are now thought to have the more subtle function of setting and/or maintaining the central excitatory state of the organism. Thus they may influence an animal's behaviour over a fairly long time course, as has been shown by Wilson (1965) in the locust flight system and by Cohen (1965) for the crustacean myochordotonal organ proprioceptive system. Therefore, in the analysis of any behavioural sequence it is necessary to know
a.) whether any receptors are involved or not, and
b.) whether their presence or absence affects the co-ordinated nature of the response.

In this section I have attempted to confirm, by physiological means, the presence of the receptors which were described by Alexandrowicz (1909) and Orlov (1926) as lying on the hindgut. I have also searched for other possible receptors.

Materials and Methods

Methylene blue preparations of the hindgut and the anal region were produced according to the methods outlined in Section 2.1.

Oscilloscope recordings were made using the methods and equipment described in Section 2.2. In these experiments only the last two abdominal segments were utilised. Initial experiments on the hindgut and anal

soft cuticle were carried out by touching these regions with a camel hair brush whilst recording from the appropriate nerves with platinum wire electrodes. In the case of the hindgut attempts to record from the P.I.N.p.'s were also made. In these experiments the hindgut was artificially distended with air. This was achieved by injecting air into the hindgut down a fine polythene tube. The rectum was ligatured posteriorly using nylon thread. The tube was inserted anteriorly and tied in. Several methods of stimulating receptors around the anal lips were tried. In many cases one lip of the anus was passively deflected using a set of forceps attached distally to a Prior micromanipulator. The micromanipulator was fitted with a potentiometer which monitored its horizontal movements (see Shelton and Laverack, 1968). In other experiments the forceps were attached to a Southern Instruments type 940 A pen unit driven at low frequencies by a Servomex, type LF 51 (Mark 2), wave-form generator.

In all recordings the anal nerves were first cut centrally and then teased into fine bundles. In this way clear records of receptor responses in the anal nerves were made possible.

The records for Figure 2.3.1 were made according to the methods described in Section 2.4.

Results

A. Proprioceptors on the Hindgut

Under no circumstances has it proved possible to record receptor activity in the P.I.N.'s. Neither hindgut movements (see Section 2.4) nor artificial distension of the hindgut elicited any recordable

receptor activity. However, stimulation of one P.I.N.a. will often produce an output in the contralateral P.I.N.a. as is shown in Figure 2.3.1. Three sizes of single spikes are displayed, the fastest having conduction velocity of 5 - 7.5 metres/sec., whilst the slowest has a conduction velocity of only 2 - 3.5 metres/sec.

B. Anal Proprioceptors

Although it has not been possible to demonstrate the presence of mechano-receptors on the hindgut, a number of fibres responding to anal movements have been found in the anal nerves.

1. Anatomy

Methylene blue staining of the anal nerves in the region of the anus indicates that in at least one ventrally going branch on each side there is one large bipolar sensory cell. The sense cell body lies deep alongside the anus and the dendrite passes posteroventrally to lie on a portion of the hypodermis below the soft cuticle of the anus (see Figures 2.3.2 and 2.3.3). The dendrite then loops to run anteriorly and finally ramifies in the hypodermis on the same side of the anus.

2. Physiology.

It was possible to elicit non-specific receptor responses in the anal nerves by touching the soft cuticle around the anus with a soft camel-hair brush.

More precise information was obtained by making recordings of the responses of the anal receptors in teased anal nerves whilst driving the radial muscles of the anus by stimulation of the P.I.N.p.'s. Figure 2.3.4 demonstrates the activity of receptors discharging during anal opening, at which time the soft cuticle surrounding

the anus was deformed. Removal of the overlying cuticle does not interfere with this response, but cutting the underlying hypodermis abolishes it. The receptor cell described above is thought to generate this response. However, in a number of preparations a second receptor responding to anal closure has also been found. This often occurs in the same teased bundle as the dilation receptor (see Figure 2.3.5). The anatomical position of the proprioceptor responding to anal closure is unknown.

Passive opening of the anal lip revealed the dilation receptor to be phaso-tonic. In Figure 2.3.6A the frequency of the response is shown to be dependent on the extent of anal opening. During rapid cyclical movements of the anal lip, as in Figure 2.3.6B, only the phasic portion of the response can be recorded due to the short time over which the stimulus is applied. Movement of the anal lip close to its maximum degree of opening (Figure 2.3.7A) gives a typical, slowly adapting response, whose frequency can be further increased by additional opening of the anal lip. If the anal lip is only moved to 80% of its natural open position the response totally adapts, but moving the lip to the fully open position again increases the tonic frequency of the receptor output with a resultant increase in the adaptation time.

The evidence presented in Figure 2.3.7 suggests that both phasic and tonic components of the receptor response are positionally sensitive with respect to anal dilatation. The receptor is not thought to be velocity or movement sensitive, although there is no conclusive evidence on either of these points. In Figure 2.3.7 the receptor is only shown to respond when passively opened almost to its natural maximally opened position. Figures 2.3.4 and 2.3.5, however, show that the response to active anal

opening is initiated at the beginning of the active opening cycle. This apparent discrepancy may well be due to the fact that when the anal lip is passively moved, the forceps attached to it at one point tend to pull the parts of the anal lip, anterior and posterior to the attachment of the forceps, into a sharply angled shape. Thus the soft cuticle at the extreme anterior and posterior ends of the anal lip is unlikely to be deformed to quite the same extent as when active anal movements occur. In such cases contractions of the R4 radial muscles pull the anal lip open in a smooth arc, thus deforming the hypodermis and the receptor endings associated with it, in a different manner.

Further analysis of the receptor responding to anal dilatation was attempted using a variety of movements at different frequencies and amplitudes. The results obtained proved inconclusive due to the imprecise nature of the response from cycle to cycle. The main conclusions reached were much as has already been mentioned. Wider opening of the anus causes a higher degree of both tonic and phasic discharge. Figure 2.3.8 illustrates this point and shows that above 70-80% of natural anal opening there is little increase in the discharge frequency of the phasic component of the receptor.

In many cases more than one unit was found to discharge during anal closure or anal dilation. Figure 2.3.9.A and B shows several units responding to active anal closure. In Figure 2.3.9.C a two unit response to passive anal opening is demonstrated. Thus it seems that a number of units, which discharge during anal movements, can be isolated from the anal nerves. No evidence of any complex form of stretch receptor has as yet been discovered. It is, therefore, assumed that these

receptors are probably all rather unspecialised mechanoreceptors associated with the hypodermis underlying the soft cuticle of the anal lips.

Figure 2.3.1. Response elicited in right P.I.N.a. by stimulation of the left P.I.N.a. at 6Hz and with 1 msec pulses. Suction electrodes were utilised for both recording and stimulating purposes.

Three spikes (a, b and c) per stimulus pulse were elicited. The distance apart of the recording and stimulating electrodes was 12.5mm. ± 2.5mm. and the conduction velocities of the spikes between stimulus and recording electrodes have been calculated. They are as follows:-

a) 5 - 7.5 metres/sec.

b) 2.5 - 3.7 metres/sec.

c) 2 - 3.5 metres/sec.

Dots indicate stimulus pulses.

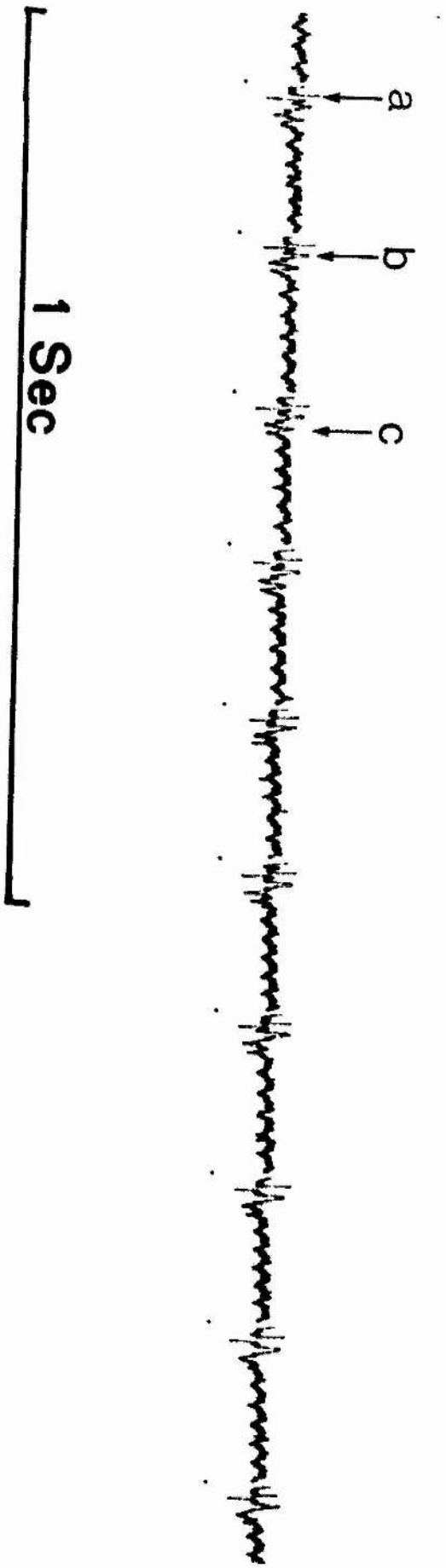

a
b
c
1 Sec

Figure 2.3.2. Diagrammatic representation of the position of the bipolar sensory cell postulated as responding to anal dilatation.

R_1 to R_5 - Radial muscle groups as shown in Figure 2.1.1.

An. - left anal nerve

Ax. - Axon of receptor cell.

C.B. - Receptor cell body.

Hy. - Hypodermis around anal arifice.

P.I.N.p. - Posterior branches of posterior intestinal nerves.

R.D. - Ramifying dendrites of receptor cell lying in the hypodermis.

The ventral soft cuticle is not represented.

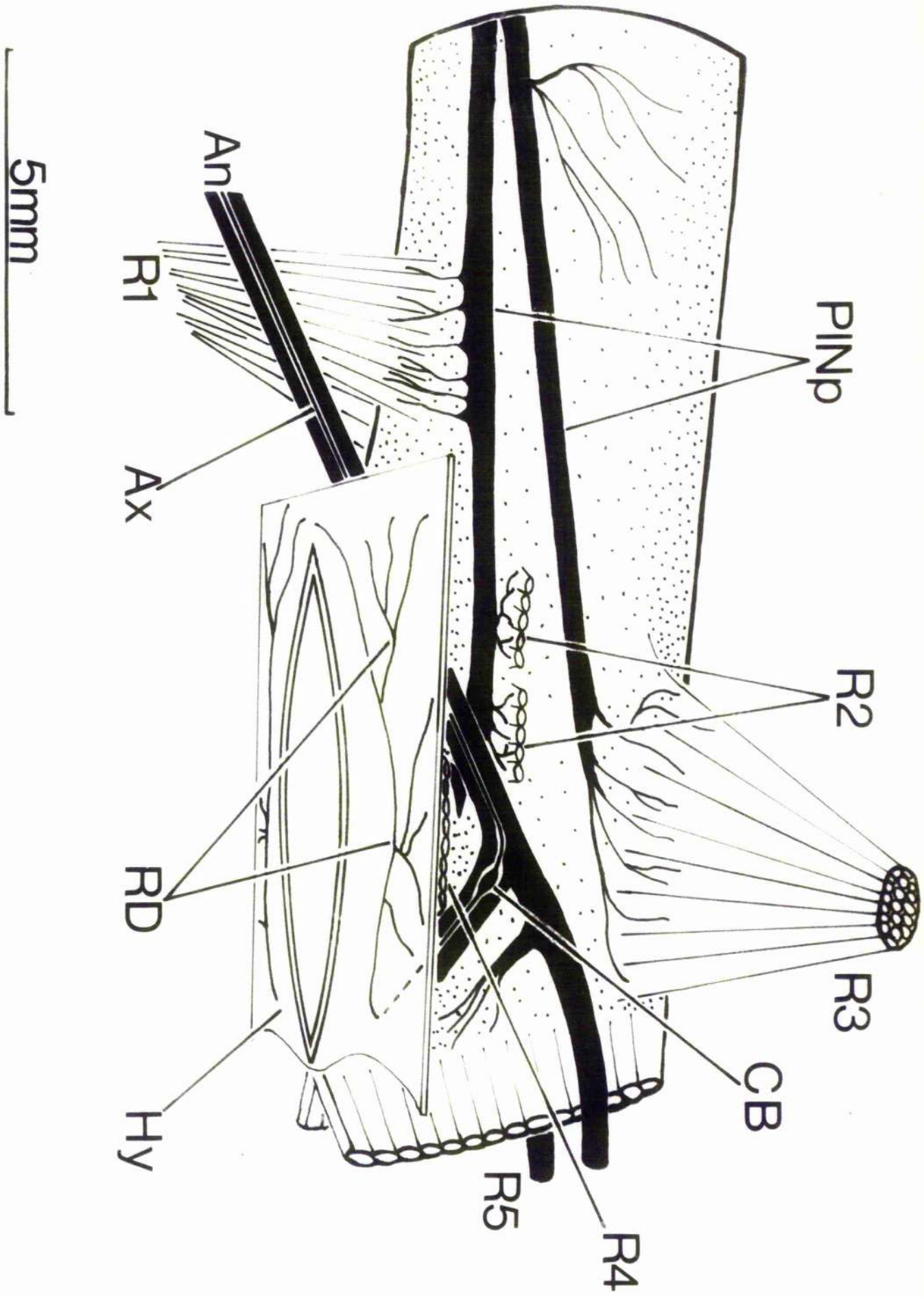
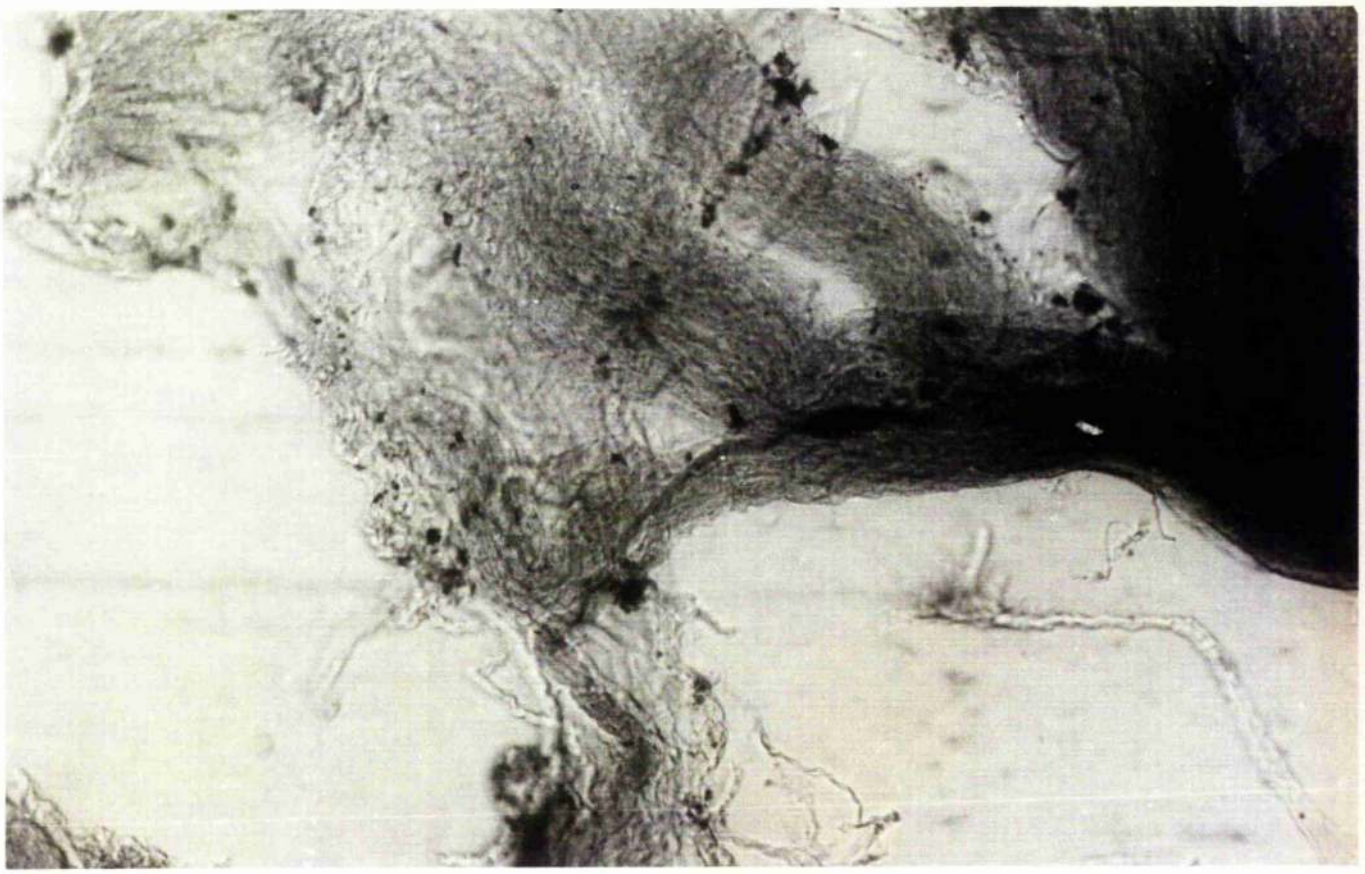

An
R1
Ax
PINp
R2
R3
CB
RD
Hy
R5
R4
5mm

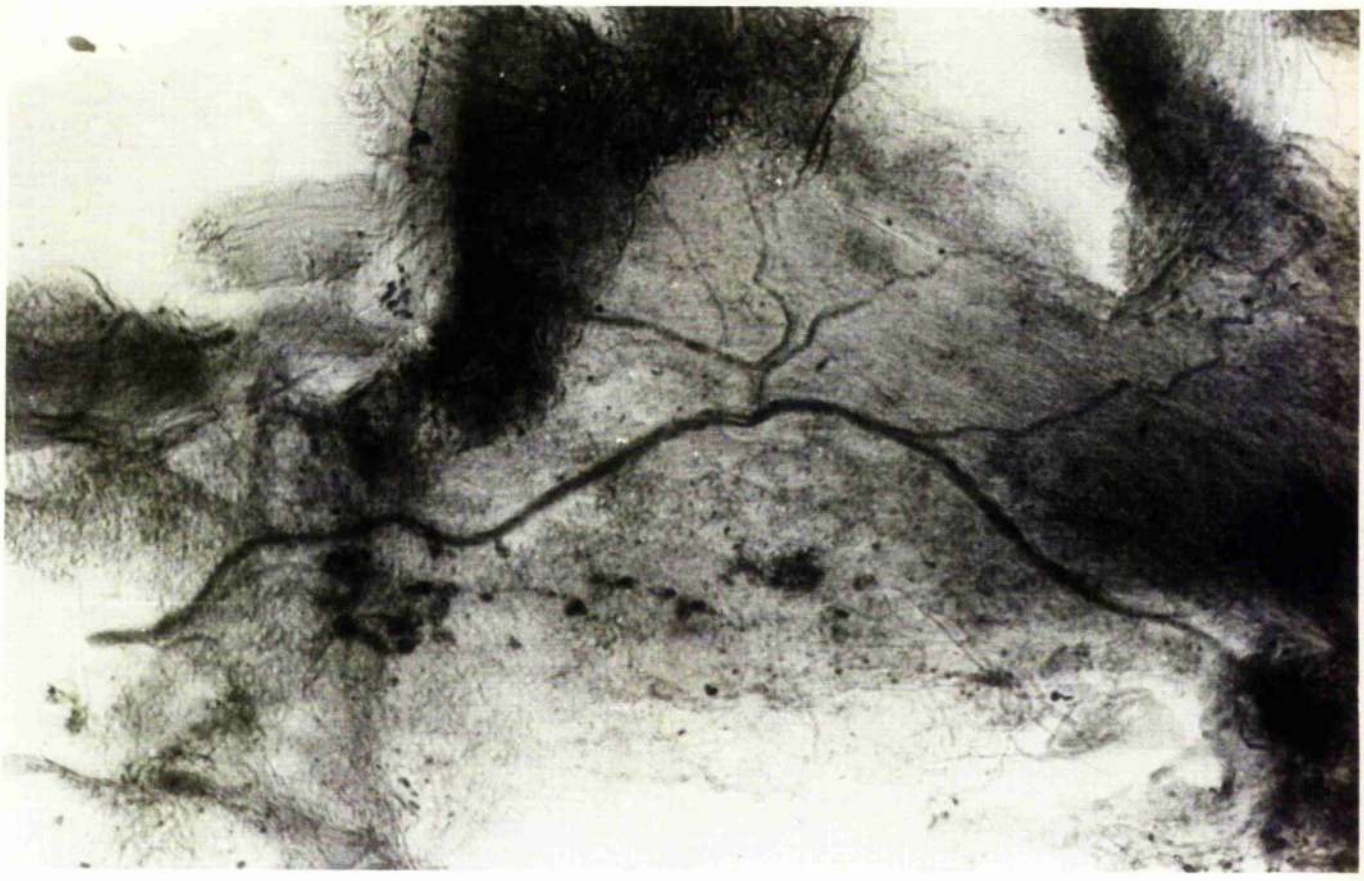
Figure 2.3.3. Photographs of:-

A. Methylene blue preparation of a receptor cell body in the ventral going branch of the left anal nerve.

B. Methylene blue preparation of dendrites ramifying in the hypodermis of the left anal lip.



A



B

1mm

Figure 2.3.4. Response of a soft cuticle receptor of the anal lip during anal opening.
Upper beam - Activity of the receptor in the teased left anal nerve.
Lower beam - Record of the movements of the left anal lip recorded with an R.C.A. 5734 transducer valve (downward deflection denotes anal opening).

A. Spontaneous activity of the receptor with the anus in the closed position. Mean frequency of output is approximately 3Hz.

B. Activity of the receptor during anal opening elicited by simultaneous stimulation of both P.I.N.p.'s with 3 msec. pulses at 50Hz for approximately 1.5 sec. (dotted line indicates stimulus pulses).

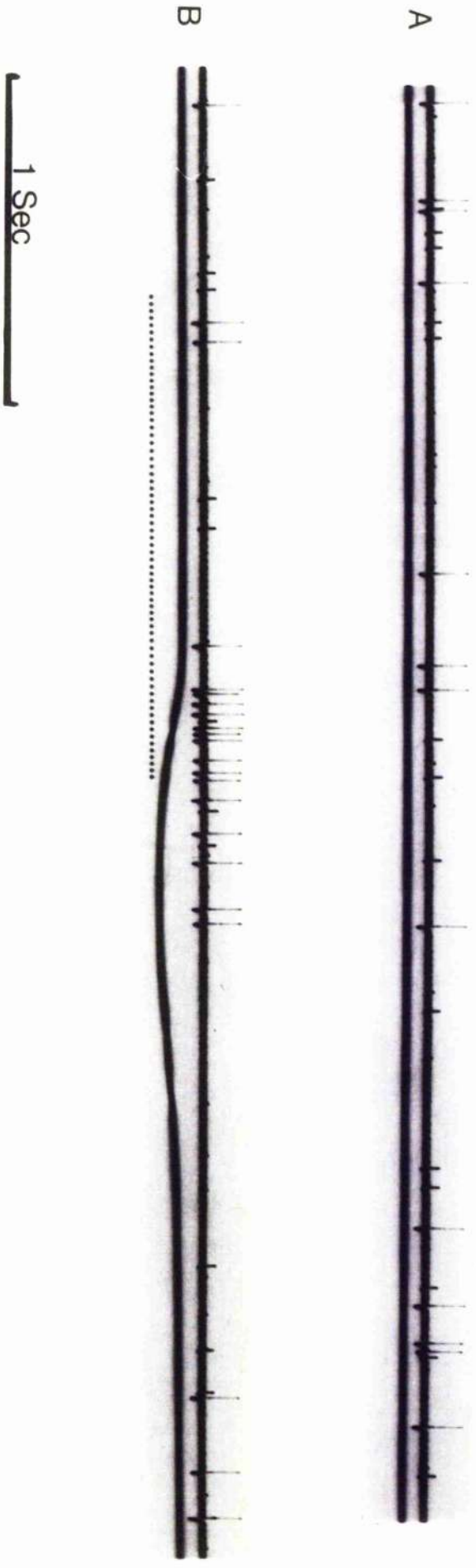

A
B
1 Sec

Figure 2.3.5. Opening and closing responses of receptors on the left anal lip.

Upper beam and lower beam as in Figure 2.3.4. Anal movements were produced by stimulation of the V.N.C. (40Hz and 3 msec. pulses).

Note the increased amplitude of movement in the second cycle, producing greater separation of the responses in time. The dilation receptor responds over most of the range of anal opening, whilst the closing receptor responds only when the anal orifice is almost closed.

Horizontal bar denotes stimulus pulses.

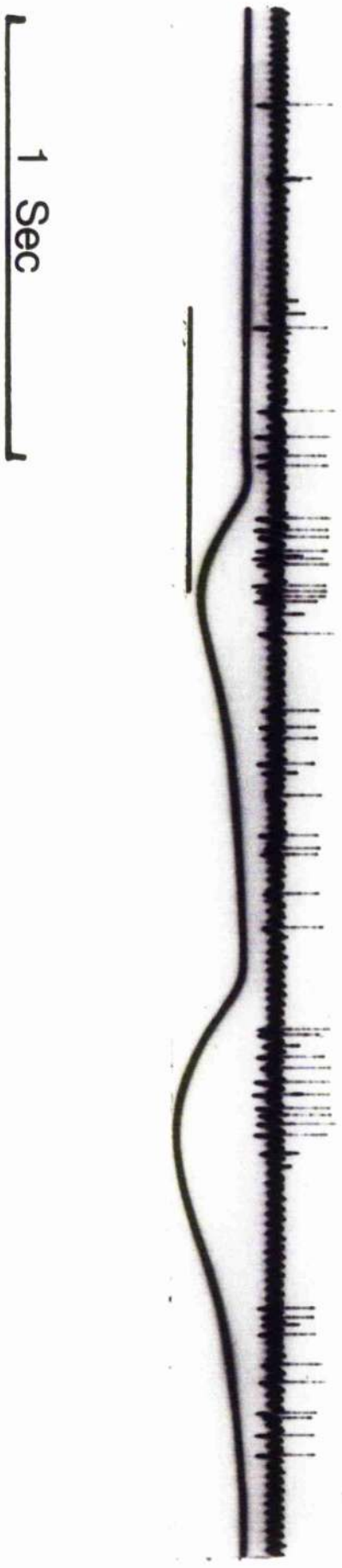

1 Sec

Figure 2.3.6. Passive movements of the anal lip producing responses of the opening receptor. In all cases the right anal lip was moved by a pair of forceps mounted on a Prior micromanipulator.

A. Arrows pointing upwards denote the onset of the stimulus whilst those pointing downwards denote its cessation. In both cases there is a phaso-tonic response. In the second case the anus was opened more widely, thus causing greater hypodermal deformation and giving rise to a higher frequency of both phasic and tonic output from the receptor.

B. Rapid cyclical movements of the anal lip monitored with a transducer valve. Only the phasic component of the response was recorded, due to the rapidity of the movements. Upward deflection indicates anal opening.

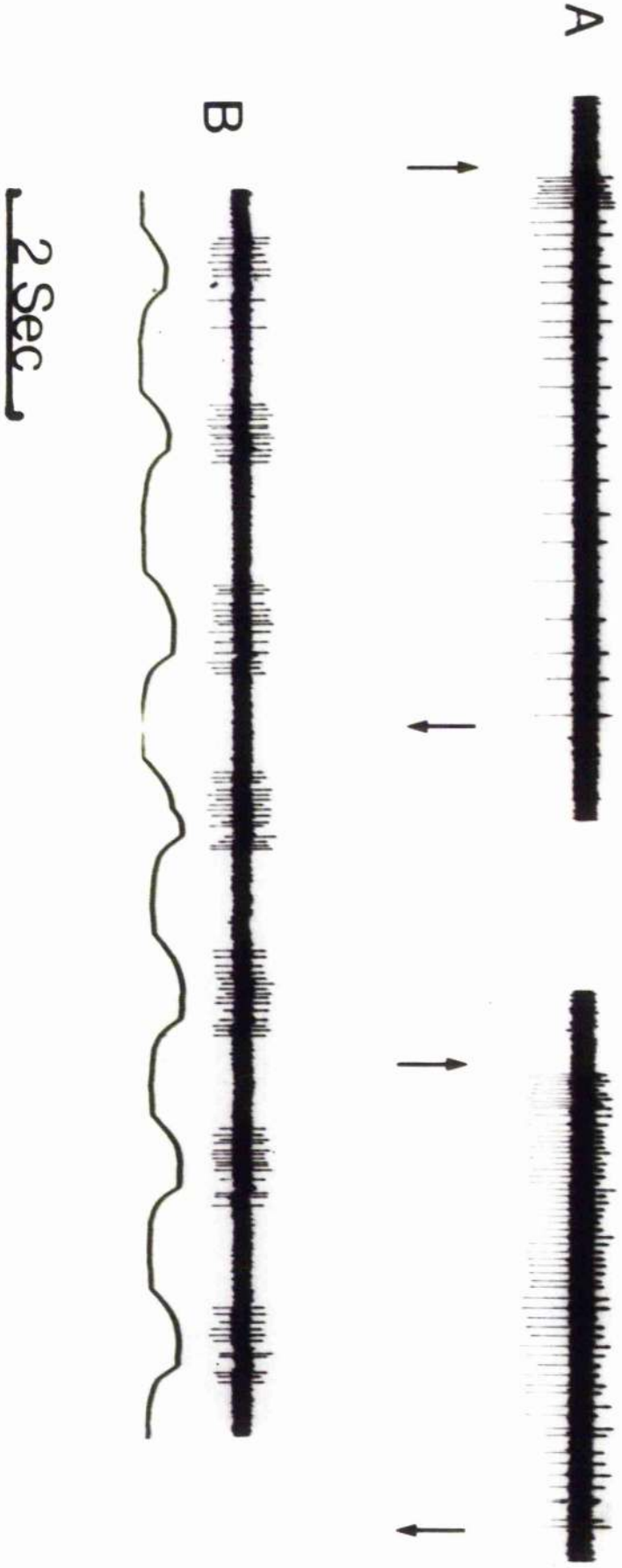

A
B
2 Sec

Figure 2.3.7. Responses of the dilation receptor to imposed opening of the anal lip monitored by a potentiometer mounted on a Prior micromanipulator.

A. The anus was initially opened to 95% of its natural maximum and was then opened wider to 110%.

B. The initial opening was 80% of the natural maximum and this was later increased to 100%.

In both A and B the initial opening caused a phaso-tonic output and wider opening caused a similar response, but at a higher frequency. The final phasic burst as the anus was closed is thought to have been due to rebound of the forceps. Two units may have been involved in both A and B.

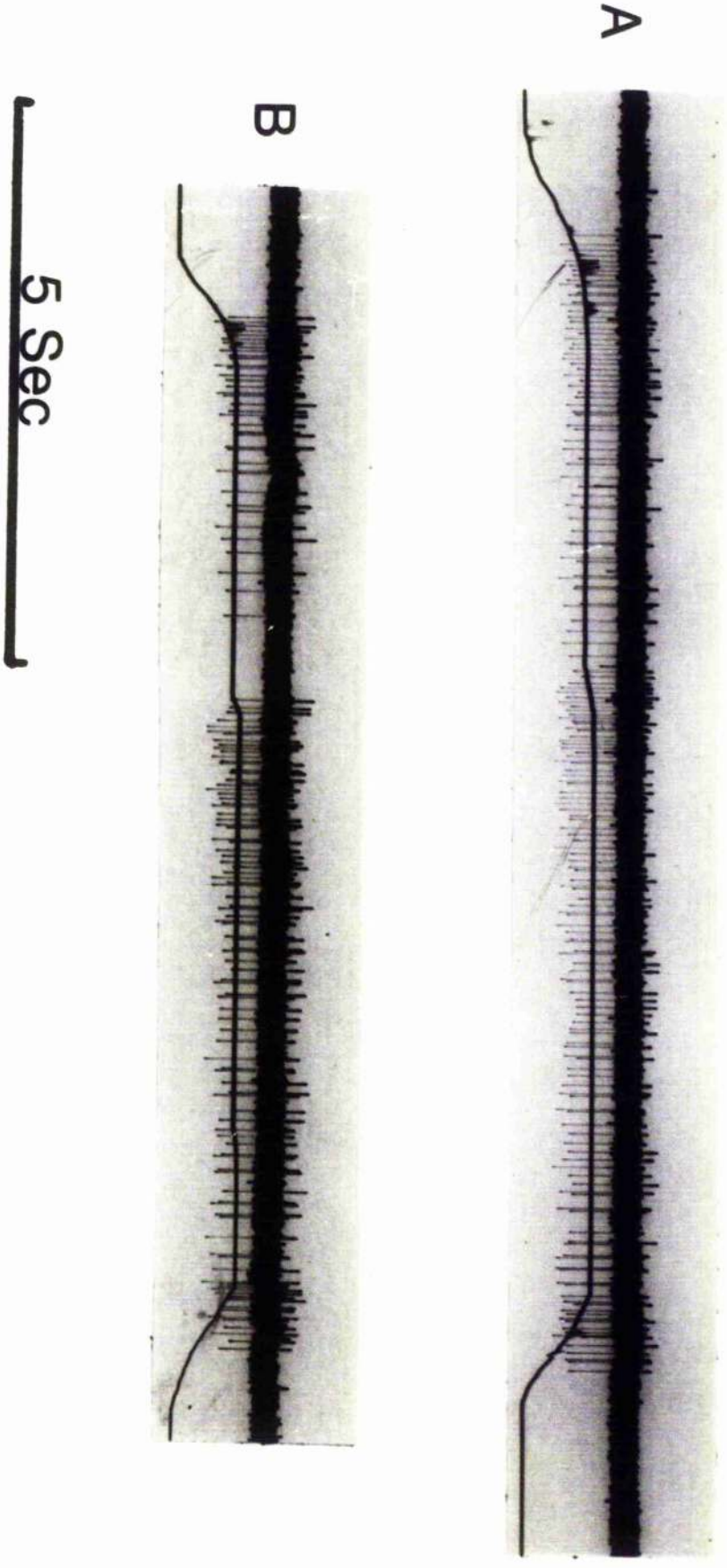

A
B
5 Sec

Figure 2.3.8. Triangular movements at constant frequency (0.7Hz) imposed on the right anal lip and responses recorded from the right anal nerve. The movements were produced using a waveform generator to drive a pen arm on which the forceps, attached to the anal lip, were mounted. The amplitude of the movements was gradually increased.

Upper beam - activity recorded in the teased right anal nerve. Lower beam - monitor of the imposed waveform, upward deflection denotes anal opening.

A - anal lip opened to 40% of its natural maximum.
B - 70%; C - 80%; D - 120%; E - 160%.

The frequency of the receptor discharge remains fairly constant above about 70% of natural anal opening.

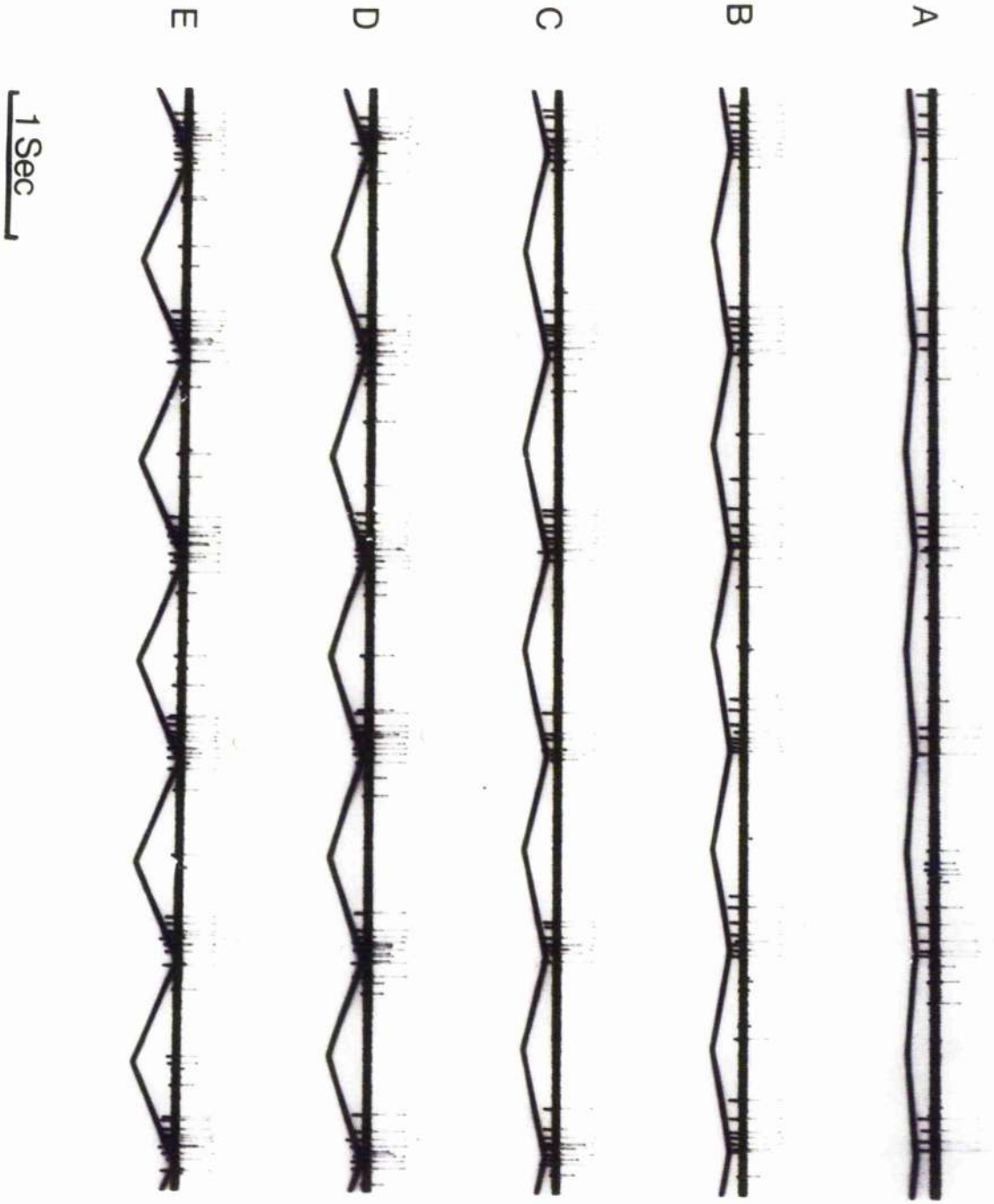
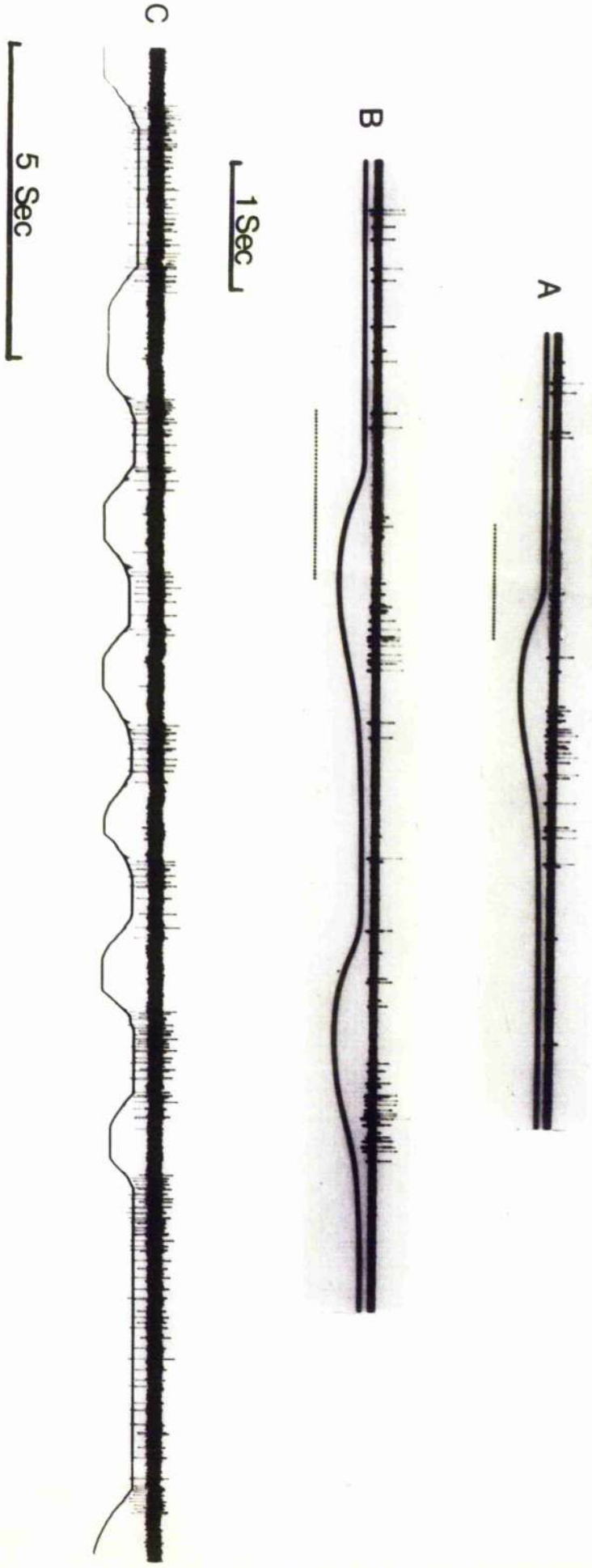

A
B
C
D
E
1 Sec

Figure 2.3.9. Several units responding to both anal opening and anal closure. In A and B the lower beam monitored active movements of the left anal lip as in Figure 2.3.4, whilst in C imposed movements of the left anal lip were monitored as in Figure 2.3.7. The movements in A and B were produced by stimulation of the V.N.C. at 40Hz with 3 msec. pulses. Dots indicate stimulus pulses.

A and B. Three separate units responded to anal closure. The two traces demonstrate the variability of the response.

C. Two units were involved. They discharged phaso-tonically.


A
B
C
1 Sec
5 Sec

Section 2.4. Motor Output from the Sixth Abdominal Ganglion to the Hindgut.

Introduction

In a previous section (2.2) the mode of action of the hindgut due to stimulation of the intact V.N.C. was studied. Also the presence of recep ors responding to anal movements was demonstrated (Section 2.3). It now becomes necessary to examine more closely the neural mechanisms underlying this pattern of behaviour.

I have already determined that the hindgut is exclusively controlled via the P.I.N.'s. In this section I shall demonstrate the activity in the P.I.N.'s evoked by stimulation of single abdominal connectives and attempt to correlate this activity with the movements of the hindgut.

Materials and Methods

In order to record motor activity from the P.I.N.'s the abdominal nerve cord and the rectum were dissected from the animal. The nerve cord was then placed ventral surface uppermost in a perspex dish with a plasticine bottom and an outer water jacket. Plasticine proved to be a much better conductor of heat than did the paraffin wax of earlier experiments. The preparation was bathed in sea-water maintained at 12-14^{o}C.

In this series of experiments the P.I.N.'s were often detached from the hindgut by fine dissection, thus severing their peripheral ends. Nervous activity in the P.I.N.'s was initially recorded using suction electrodes made up with platinum wire. These were found to be very sensitive to hum and most experiments were carried out

using platinum wire hook electrodes with which the nerves were raised into a layer of liquid paraffin.

In a second series of experiments the dissection was carried out as in Section 2.2 and hindgut movements were correlated with motor activity using an R.C.A. 5734 transducer valve.

In both series of experiments stimuli were delivered to single abdominal connectives. In some of the first series it was necessary to deliver precise numbers of stimulus pulses. In these cases an inverter amplifier was driven from the right hand horizontal shift out of the Tektronix 565 oscilloscope (see Figure 2.4.1).The inverter supplied a negative-going sawtooth waveform to a Tektronix 161 pulse generator, which then fed pulses to a 162 waveform generator. The pulses so produced were then passed to a second 161 which produced pulses of the correct dimensions. For the purposes of pulse selection the output of the 161 was displayed through the right hand 3A3 oscilloscope amplifier, whilst the input from the differential amplifiers (and hence the preparation) and the stimulus marker were displayed through the left hand 3A3 amplifier. Time base A (left hand side) was then adjusted to give a stationary spot on the screen. Time base B (right hand side) was independently adjusted to single sweep and the intensity of its beam turned to zero. Precise numbers of stimuli could then be delivered by manual depression of the single sweep button which caused the right hand time base unit to drive the 160 series of stimulator units.

In other cases two 161 pulse generators were driven from a single 162 waveform generator in order to stimulate a pair of desheathed abdominal connectives simultaneously.

In experiments in which the activities of the P.I.N.'s

were recorded, it was not possible to determine which unit of a pair was activated first, since the recording position relative to the 6A.G. varied between branches.

Results

A. Analysis of Motor Output

There are basically two forms of efferent activity along the P.I.N.'s to the hindgut when the V.N.C. is stimulated. They are not, however, mutually exclusive of one another and both types can be elicited by stimulation of either connective in any abdominal segment (and presumably by stimulation of thoracic connectives - see Section 2.2). At low stimulus amplitudes *phasically responding units follow the stimulus pulses in a one to one ratio (often up to 40 or 50Hz) as is shown in Figure 2.4.2.A. At slightly higher stimulus amplitudes pronounced bursts of activity may occur in the P.I.N.'s following stimulation as in Figure 2.4.2.B. Initially the response is similar to that in Figure 2.4.2.A but the units which initially responded phasically now begin to fire tonically. A number of smaller units all of which are tonic also became active. Perhaps tonic driver units may be activated by interneurones responding at higher stimulus amplitudes. The number of units involved in either form

* I have used the terms phasic and tonic in a rather different way than is usual. I have defined a phasic response as a response which follows the stimulus pulses in a one to one manner and adapts out at higher frequencies. A tonic or 'bursting' response is a response in which the output of the unit exceeds the number of stimulus pulses. Such tonic responses generally occur below about 50Hz.

of activity varies from preparation to preparation, but generally fewer units occur in the P.I.N.p.'s than in the P.I.N.a.'s.

It was found that stimulation of either the right or left connective could elicit either tonic or phasic responses in the same units. In Figures 2.4.3.A and B the same phasic response is activated in the right P.I.N.p. by stimulation of either 4-5 connective. Additionally there is a greater delay between delivery of the stimulus pulse and recording of the response when the right connective rather than the left connective is stimulated. This difference is in the order of 5-10 msec. Slightly increasing the stimulus amplitude in Figure 2.4.3.C and D shows that burst formation involves the same units regardless of which connective is stimulated.

These findings are further borne out in Figure 2.4.4 where recordings of the activity of both the right P.I.N.a. and P.I.N.p. were made during stimulation of either the left or right connectives or during simultaneous stimulation of both connectives. The results show that burst formation takes the same form regardless of which connective is stimulated. Both bursts are delayed (until the fifth stimulus pulse in A and the fourth stimulus pulse in B) although a number of pre-burst phasic units fire in the right P.I.N.a. (upper beam) which might be paired with very small units in the P.I.N.p. (lower beam). The units of the lower beam are assumed to be tonic, since they do not fire at the first stimulus pulse. In Figure 2.4.4.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 Figure 2.4.4.A and B. 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. Further evidence of this situation lies in the fact that in Figure 2.4.4.C tonic activity is initiated at the first stimulus pulse. This is presumably due to summation of the p.s.p.'s produced by both interneurones, at near simultaneity, moving the membrane potential of the tonic units, or driver units, immediately above threshold. Bearing this in mind it is now possible to go on to study the effects of stimulation of the abdominal connectives on impulse formation in the P.I.N.'s.

1. Phasic Responses

As well as single phasic responses to stimuli, doublets* may occur. Figure 2.4.5 shows an example of doublets firing in the normal frequency range. 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. Figure 2.4.6 illustrates responses recorded from the P.I.N.a.'s after stimulation of the right 4-5 connectives. The contralateral unit (upper beam) is a doublet - this becomes obvious in Figure 2.4.7 - whilst the ipsilateral unit (lower beam) is only single. In Figure 2.4.6.B the connectives were exchanged and the left 4-5 connective stimulated. Ipsilaterally (upper beam) the smaller unit of the doublet

* I 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.

now dropped out and a totally different unit came in contralaterally (lower beam). In Figure 2.4.7 these units are further analysed. Figure 2.4.7.A.2 shows that the doublet of 2.4.6A gradually separates into its two constituent units at 20Hz. These units fire alternately with one another and also on alternate stimulus pulses. The smaller unit fires in synchrony with the ipsilateral unit and both drop out simultaneously while the larger contralateral unit continues firing for a time.

In Figure 2.4.7.B.2 the ipsilateral unit again drops out first, but additional complications occur:- on the whole the ipsilateral and contralateral units fire alternately, but a smaller ipsilateral unit comes to fire almost simultaneously with the contralateral unit. The small ipsilateral unit and the contralateral unit eventually drop out together. In Figure 2.4.7.B.3 (preparation fatiguing) the small unit eventually replaces the large ipsilateral unit as the partner to the contralateral unit. Highly complex neuronal circuits would be required to explain this activity (see Discussion). A much simpler firing pattern is shown in Figure 2.4.8 where the same units are apparently activated by each interneurone and both contralateral and ipsilateral units drop out simultaneously.

2. Tonic Responses.

In Figure 2.4.9 a typical burst involving several units in the right P.I.N.a. is shown. This output was elicited by stimulation of the ipsilateral 5-6 connective. Several units follow each stimulus pulse and the stimulus train is eventually succeeded by bursting activity involving many units. In Figure 2.4.10 the activity elicited in both P.I.N.a.'s following stimulation of the right 4-5

connective is exhibited. Bursting activity occurs in both nerves simultaneously and several units in each may be paired (see Figure 2.4.10, arrows marked 1) as were the phasically responding units mentioned above. These units must therefore be either anatomically or physiologically connected. Further evidence of this is shown in Figure 2.4.11 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.

Characteristics of the Bursting Activity:-

a.) Response to Stimulation Frequency.

The frequency of stimulation of the interneurones activating tonic units is important in the build up of a burst. Bursting activity is normally initiated when the stimulus frequency reaches or exceeds 10Hz, although there is some individual variation. Figure 2.4.12 illustrates this point. Below 10Hz stimulus pulses begin to entrain 4 or 5 spontaneously active units. At 7.7 and 10Hz these units do not produce a fully formed burst but some post-stimulatory activity does occur. At 20Hz and 40Hz fully formed bursts occur. Above 40Hz the burst is abolished and only 1:1 firing of otherwise tonic units takes place. Overall the most affective frequencies of stimulation lie between 10Hz and 40Hz. In most of the following work stimuli were generally delivered in the lower part of this range to prevent undue fatigue of the preparation.

b.) Response to Variation in Number of Stimulus Pulses

As shown in Figure 2.4.12 stimulus pulses delivered at 1Hz have little effect on tonic entrainment, but they

generally elicit a phasic response. In Figure 2.4.13 a single stimulus pulse will only cause a single phasic response. When two or three stimulus pulses at 10Hz are delivered as in Figure 2.4.13.B and C these cause an abortive short burst. Delivery of 4 or more stimulus pulses results 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 2.4.13.D, E and F. 1,2 & 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 2.4.14). Figure 2.4.15 illustrates that the efficiency with which four stimulus pulses may drive a burst decreases with increasing frequency of stimulation.

B. Correlation of Motor Output with Hindgut Motility.

Both phasic and tonic activity in the P.I.N.'s may elicit hindgut movements as is demonstrated in Figure 2.4.16. The type of response elicited is very different in each case. A short burst of units driven phasically will release only a single rather weak longitudinal muscle contraction of the hindgut and a weak peristaltic wave, which merely returns the hindgut to its resting position and does not cause it to elongate (see Figure 2.4.16.A). A single tonic burst will, however, elicit a single cycle of powerful hindgut movements as demonstrated in Figure 2.4.16.B. The onset of the response is much more rapid than that in A and the longitudinal and circular muscle

contractions are much more pronounced. The succeeding peristaltic contraction causes elongation of the hindgut as shown in Section 2.2. Longer periods of stimulation involving phasically driven units (see Figure 2.4.16.C) cause numerous weak hindgut movements. At higher stimulus amplitudes (Figure 2.4.16.D) the initial tonic response is exactly as in B, but once the burst is completed it is succeeded by a series of phasic responses producing rather weaker contractions. Such a neural response as occurs in Figure 2.4.16.D presumably underlies the hindgut movements of Figure 2.2.1.

Thus a single burst down the P.I.N.'s can give rise to very complex hindgut movements (see also Figure 2.4.17) and to the anal rhythm (Figure 2.4.18). In a few preparations phasic responses have not occurred (see Figure 2.4.19) and prolongation of the stimulus makes little difference to the form of the response since bursting activity once completed cannot be reinitiated by continuation of the stimulus.

It must be stated here that although all units which respond phasically also have a tonic response, not all units responding tonically have a phasic response.

Figure 2.4.1. Circuit diagram of equipment used to produce precise numbers of stimulus pulses.

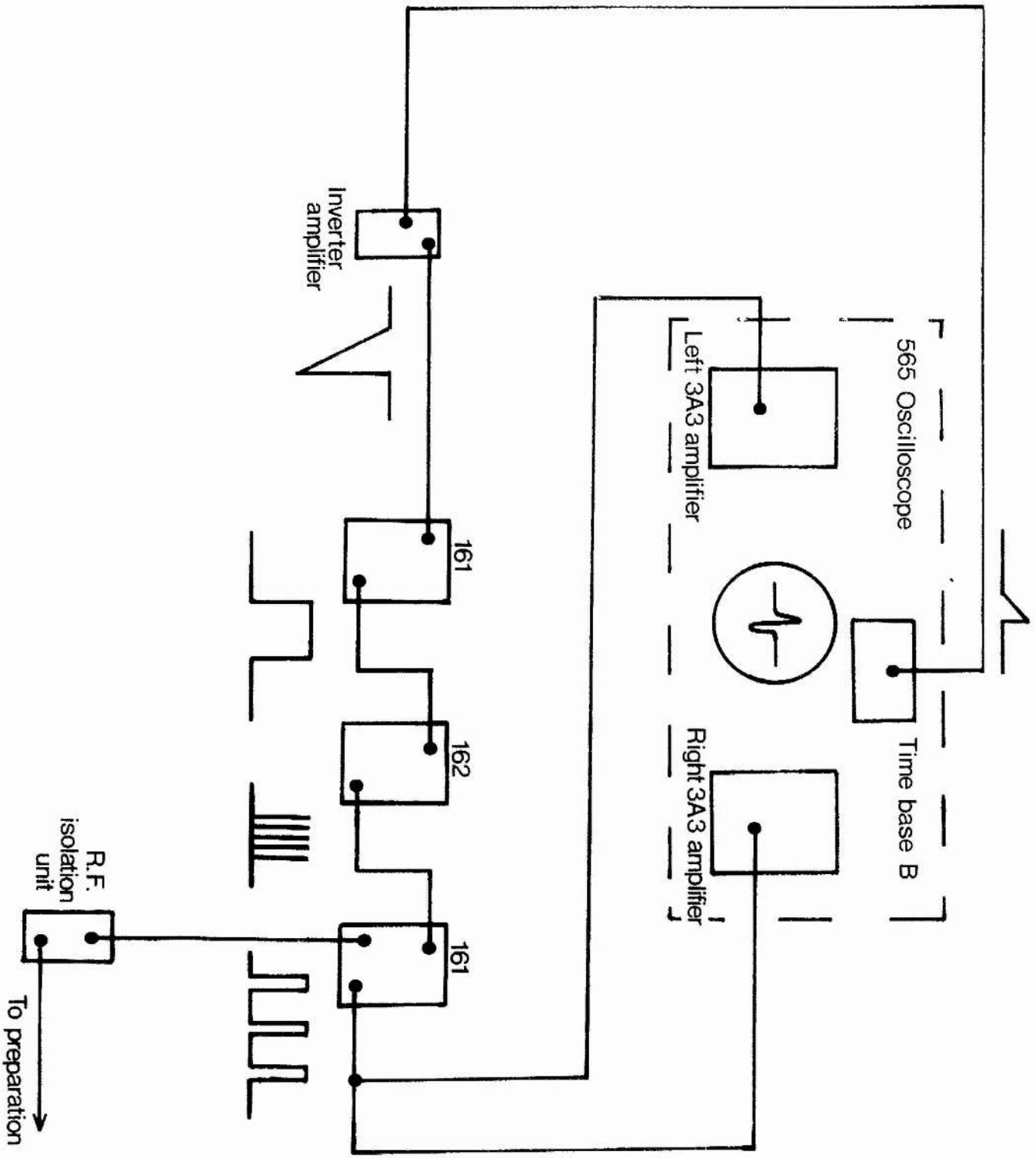

565 Oscilloscope
Time base B
Left 3A3 amplifier
Right 3A3 amplifier
Inverter amplifier
161
162
161
R.F. isolation unit
To preparation

Figure 2.4.2. Recordings from the intact right P.I.N.p. The left 4-5 connective was stimulated at 12Hz with 1 msec. positive going rectangular pulses. Dots indicate stimuli.

A. Phasic responses following the stimulus marker in a one to one ratio.

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

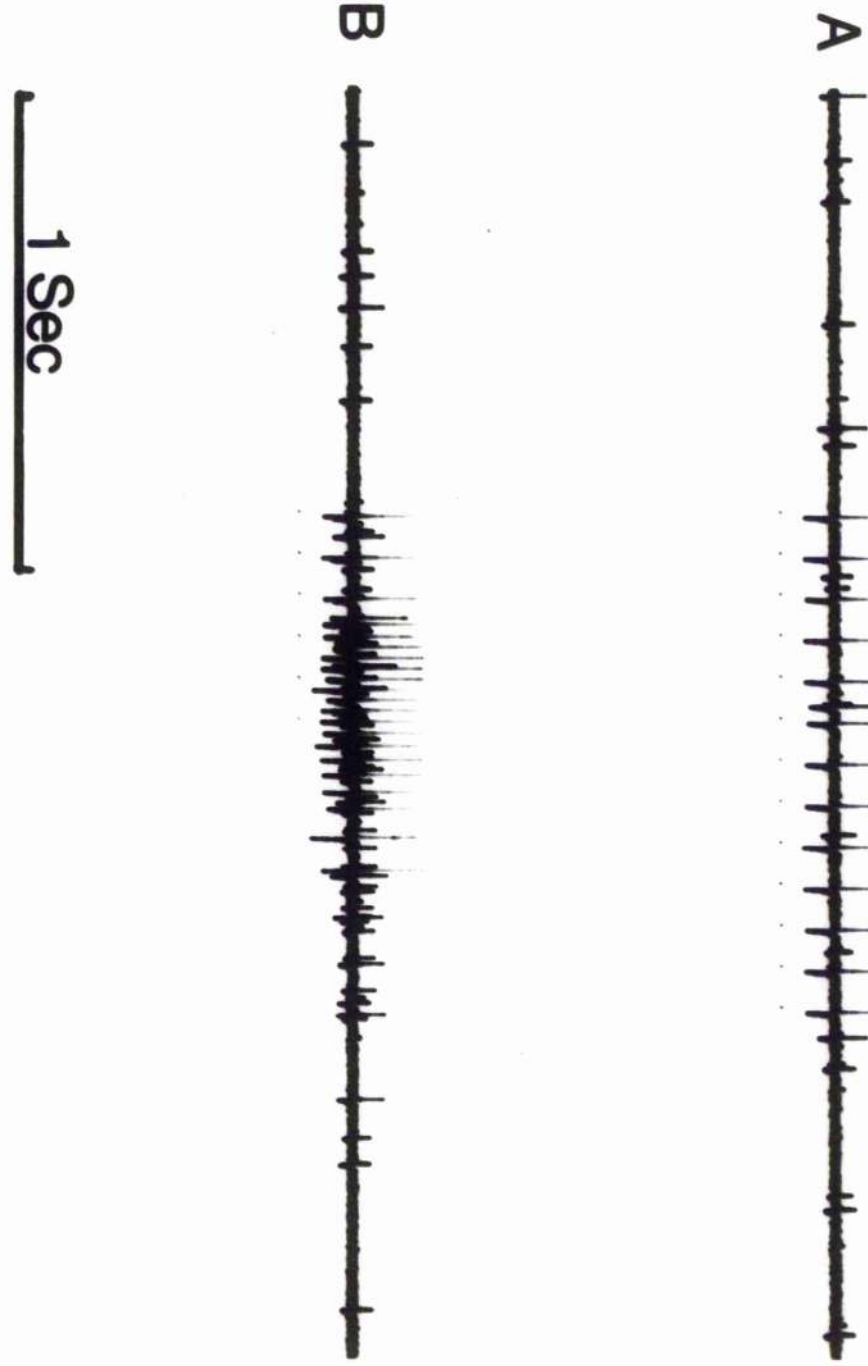

A
B
1 Sec

Figure 2.4.3. The affect of exchanging connectives on the discharge of the intact right P.I.N.p.. 1 msec. pulses were delivered at 12Hz. Dots indicate stimulus pulses.

A. Right 4-5 connective stimulated at low stimulus amplitude.

B. Left 4-5 connective stimulated at low stimulus amplitude.

C. Right 4-5 connective stimulated at 5V increase in stimulus amplitude over A.

D. Left 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 phasic response when the right rather than the left 4-5 connective is stimulated.

The units which respond phasically in A and B discharge tonically in C and D and a number of smaller tonically responding units also start firing.

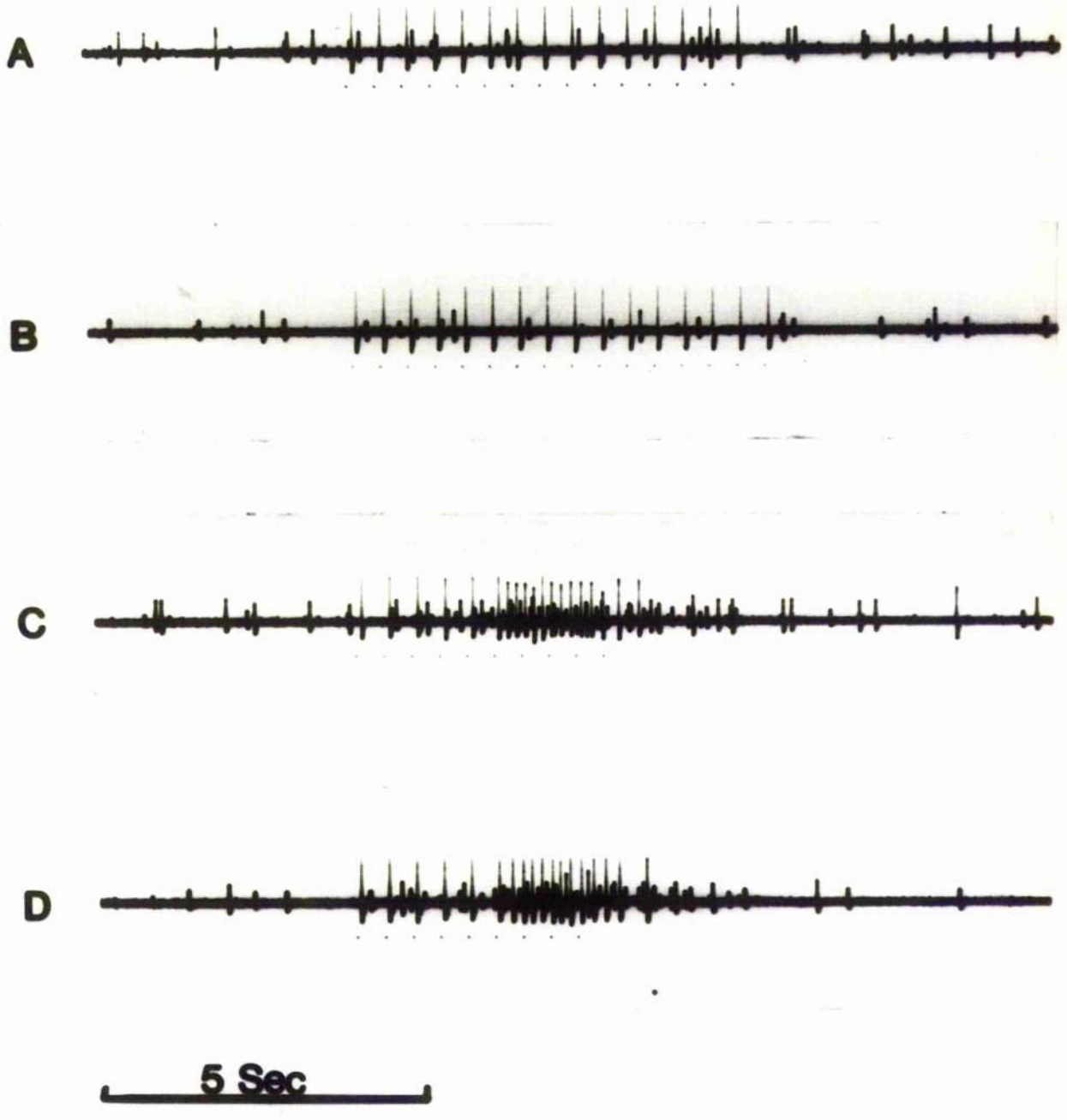

A
B
C
D
5 Sec

Figure 2.4.4. 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. stimulates pulses were delivered at 30Hz.

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 connectives 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 tonic 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.

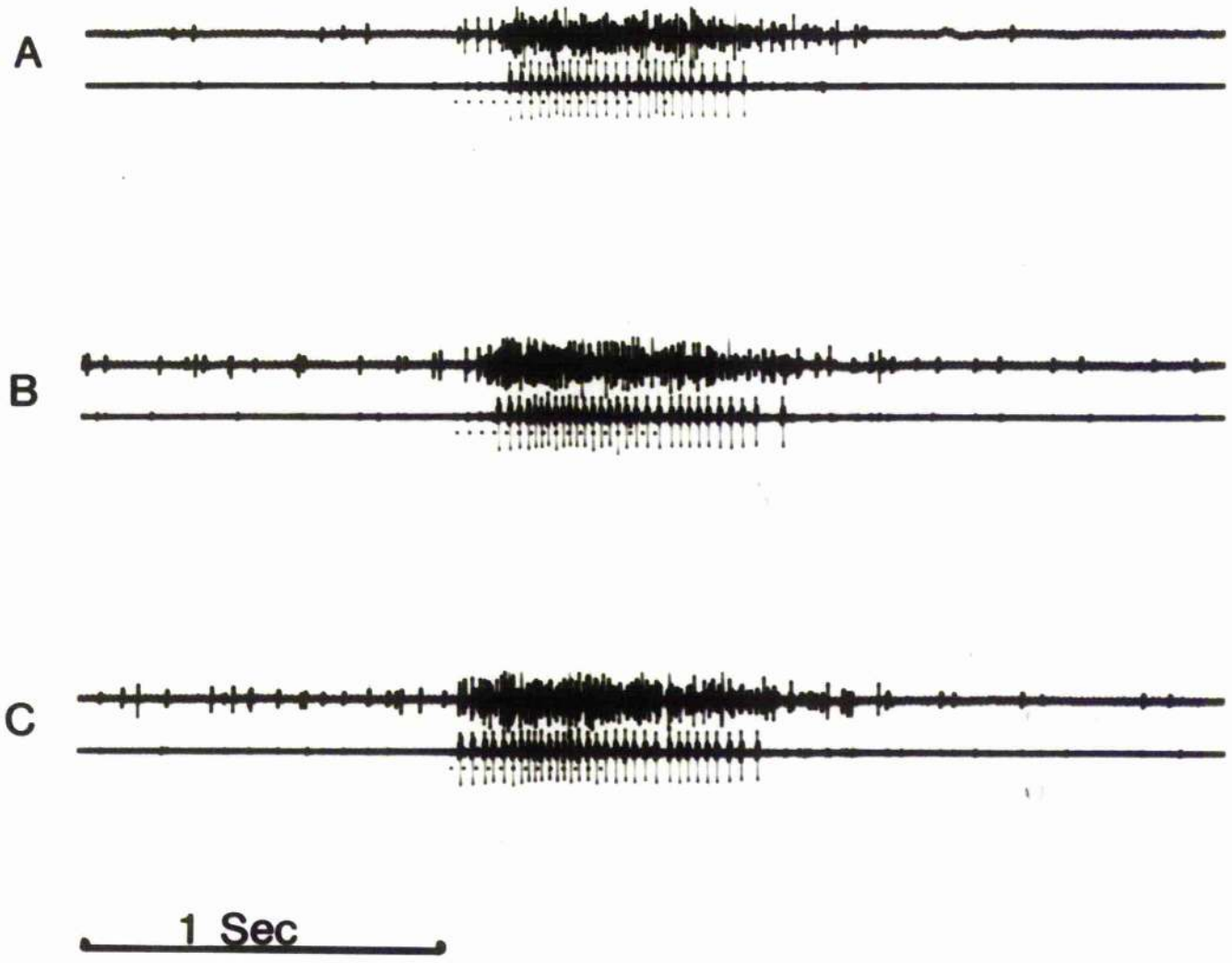

A
B
C
1 Sec

Figure 2.4.5. Phasic doublets recorded from the cut central end of the left P.I.N.a. and elicited by stimulation of the left 4-5 connective with 1 msec. pulses. Dots indicate stimulus pulses.

A. Left 4-5 connective stimulated at 10Hz.

B. Left 4-5 connective stimulated at 20Hz.

The doublets are possibly made up of two separate units.

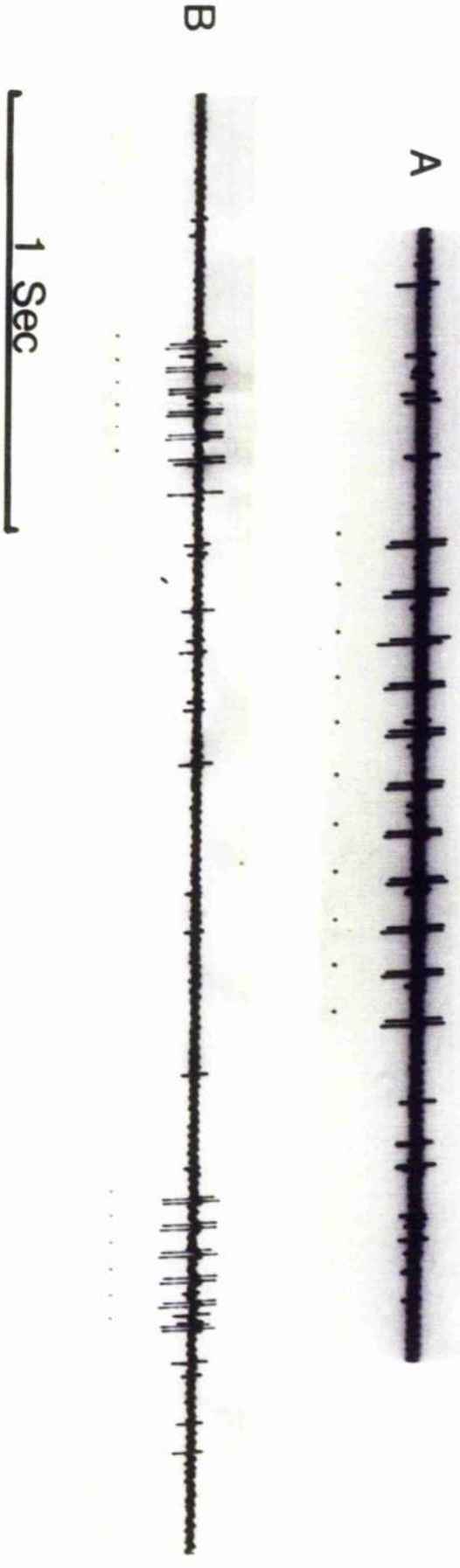

A
B
1 Sec

Figure 2.4.6. Phasic 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. Stimulate right 4-5 connective.
B. Stimulate left 4-5 connective.

Stimuli were delivered at 10Hz with 1 msec. pulses. Dots indicate stimuli. For further explanation see text.

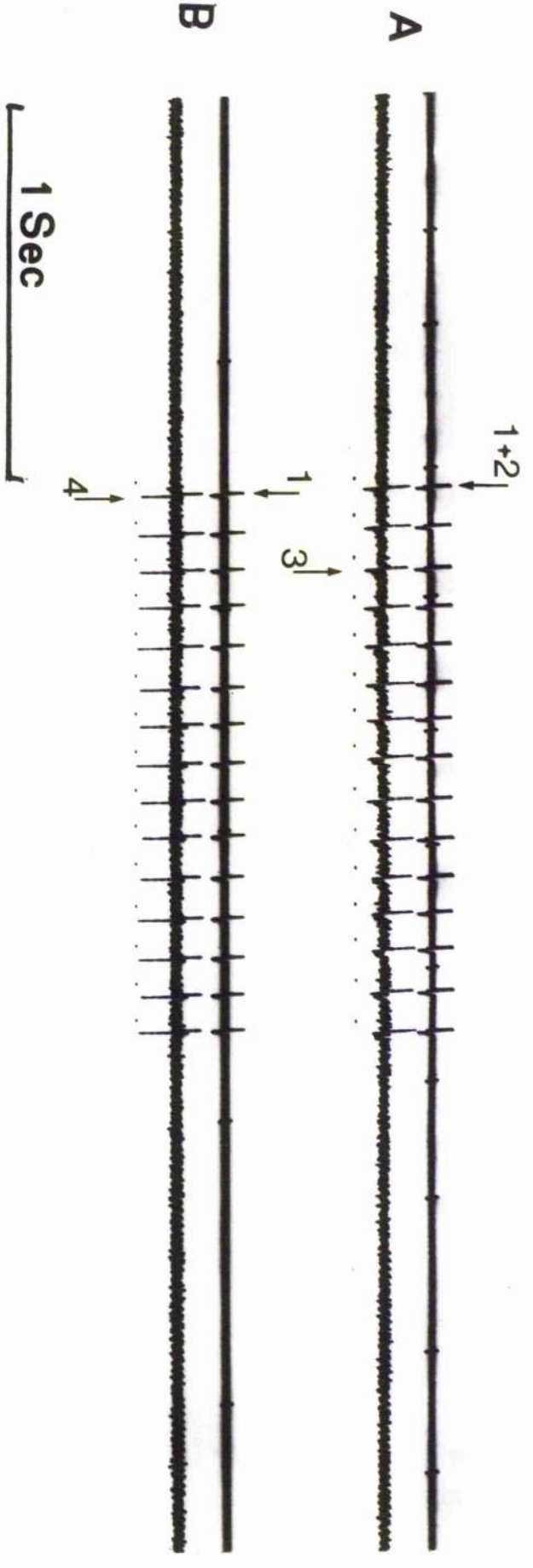

A
B
1+2
1
3
4
1 Sec

Figure 2.4.7. Phasic responses recorded from the same preparation and in the same manner as those in Figure 2.4.6. 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 10Hz. The spikes in the upper beam are doublets.

2. Stimuli delivered at 30Hz. Doublets eventually separate and fire on alternate stimulus pulses. The smaller unit drops out with the ipsilateral unit.

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 remains and the lower unit is different from that in A.

1. Stimuli delivered at 10Hz.

2. Stimuli delivered at 30Hz. The large ipsilateral unit lags behind a 'new' smaller unit which fires in simultaneity with the contralateral unit. The ipsilateral large unit drops out first and the small unit is only lost when the contralateral unit drops out.

3. Stimuli delivered at 10Hz later in experiment. The system is fatiguing and the ipsilateral large unit initially fires in simultaneity with the contralateral unit but is later replaced by the small ipsilateral unit.

Arrows numbered 1 to 5 indicate the various units described above. These numbers are used in the Discussion (Section 3.2).

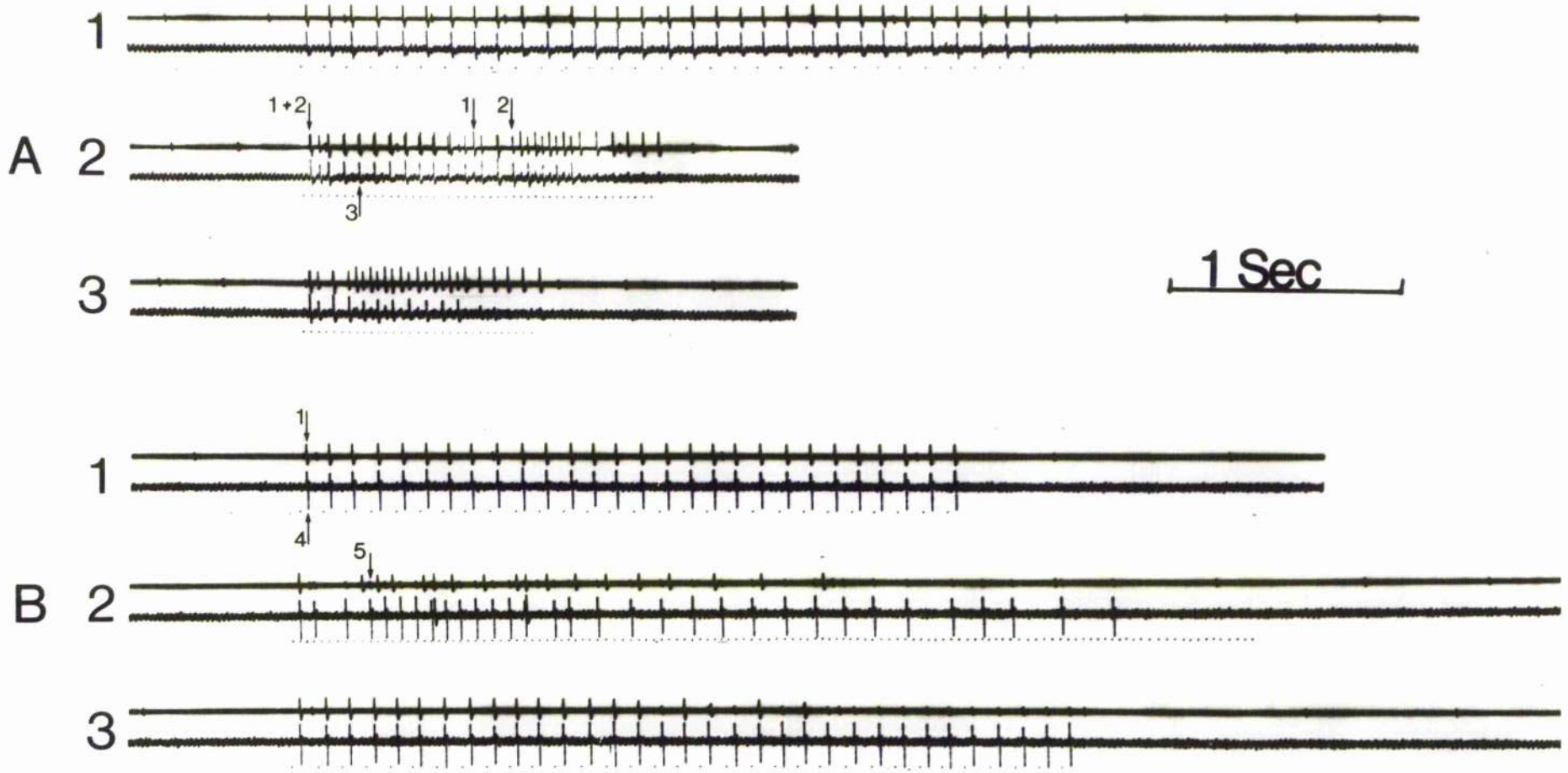

1
A 2
1+2
1
2
3
3
1 Sec
1
1
4
5
B 2
3

Figure 2.4.8. Phasic 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 10Hz in A1 and B1 and 40Hz in A2 and B2. 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.

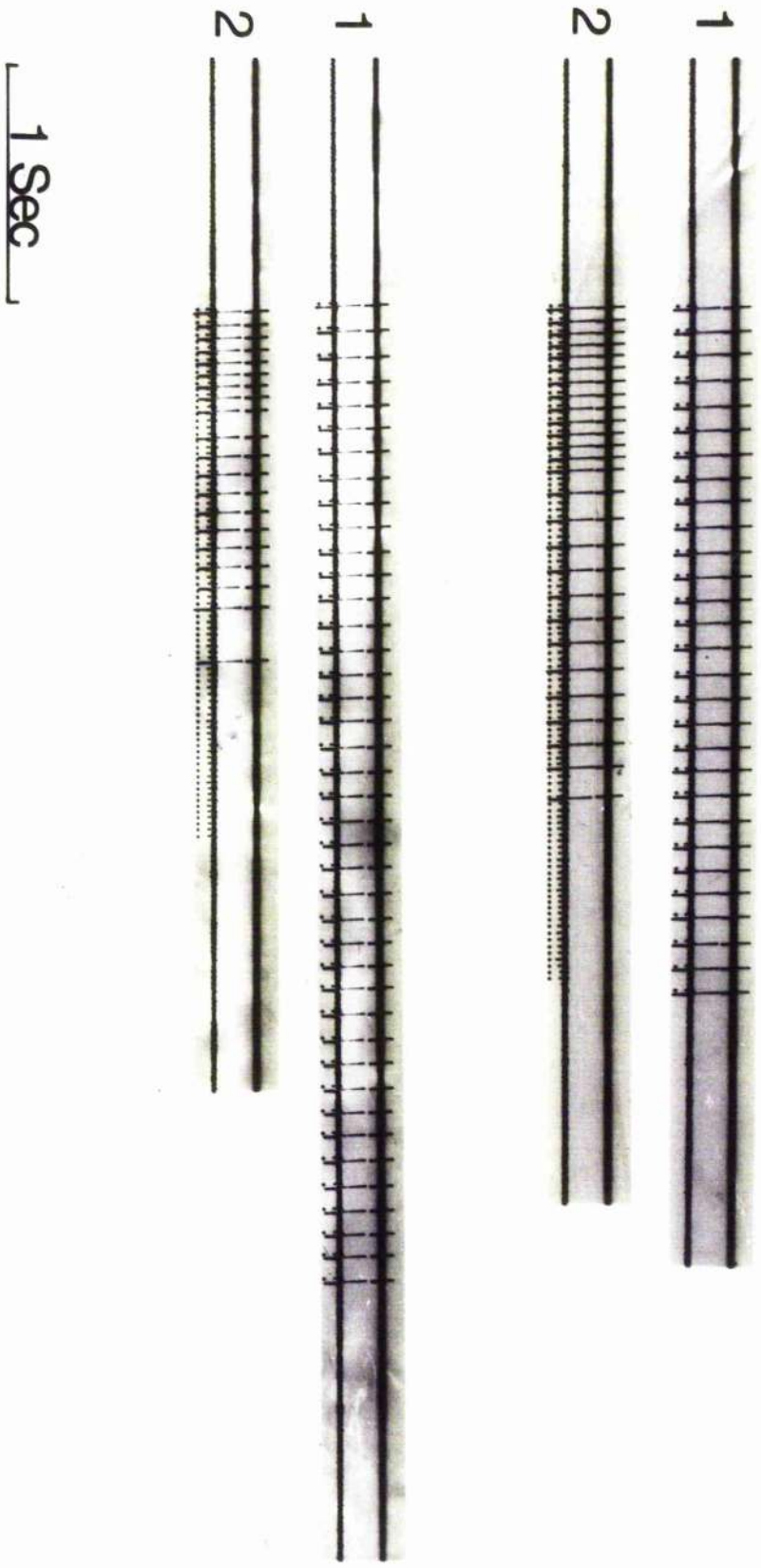

A
1
2
B
1
2
1 Sec

Figure 2.4.9. 'Bursting' activity recorded from the central end of right P.I.N.a. using suction electrodes. 1 msec. stimulus pulses at about 7Hz were delivered to the right 5-6 connective. Many units are involved in the burst.

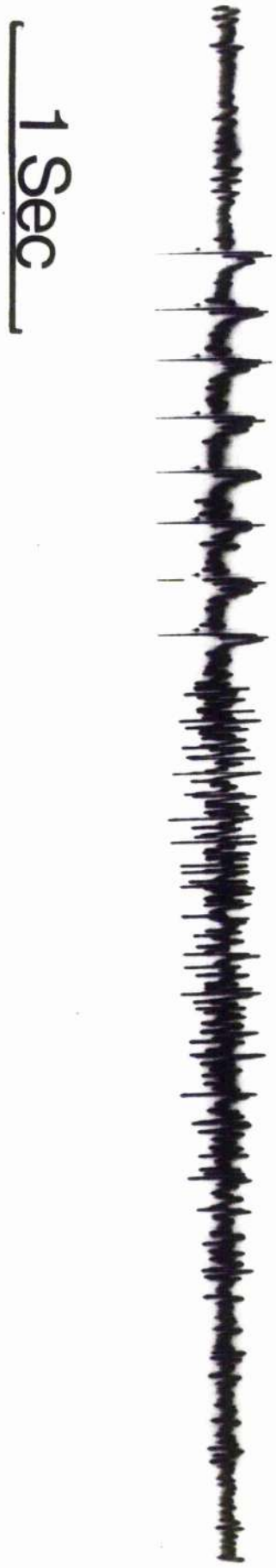

1 Sec

Figure 2.4.10. 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 6Hz 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.

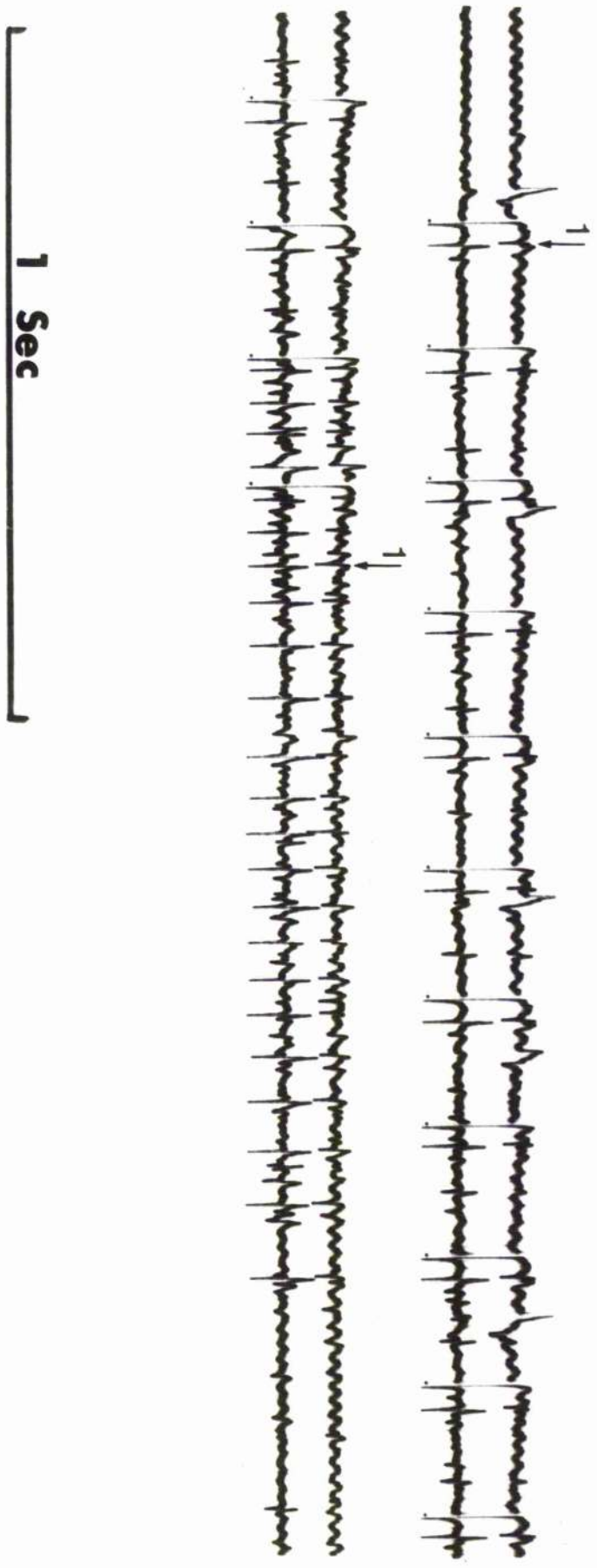

1 Sec

Figure 2.4.11. 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.


1
2
3
4
1 Sec

Figure 2.4.12. 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 - 1Hz; C - 4Hz; D - 7.7Hz; E - 10Hz; F - 20Hz; G - 40Hz; H - 50Hz; I - 77Hz.

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 both phasic and tonic responses) drop out, leaving the small ones which would normally only participate in burst formation to follow the stimuli in a 1:1 ratio.

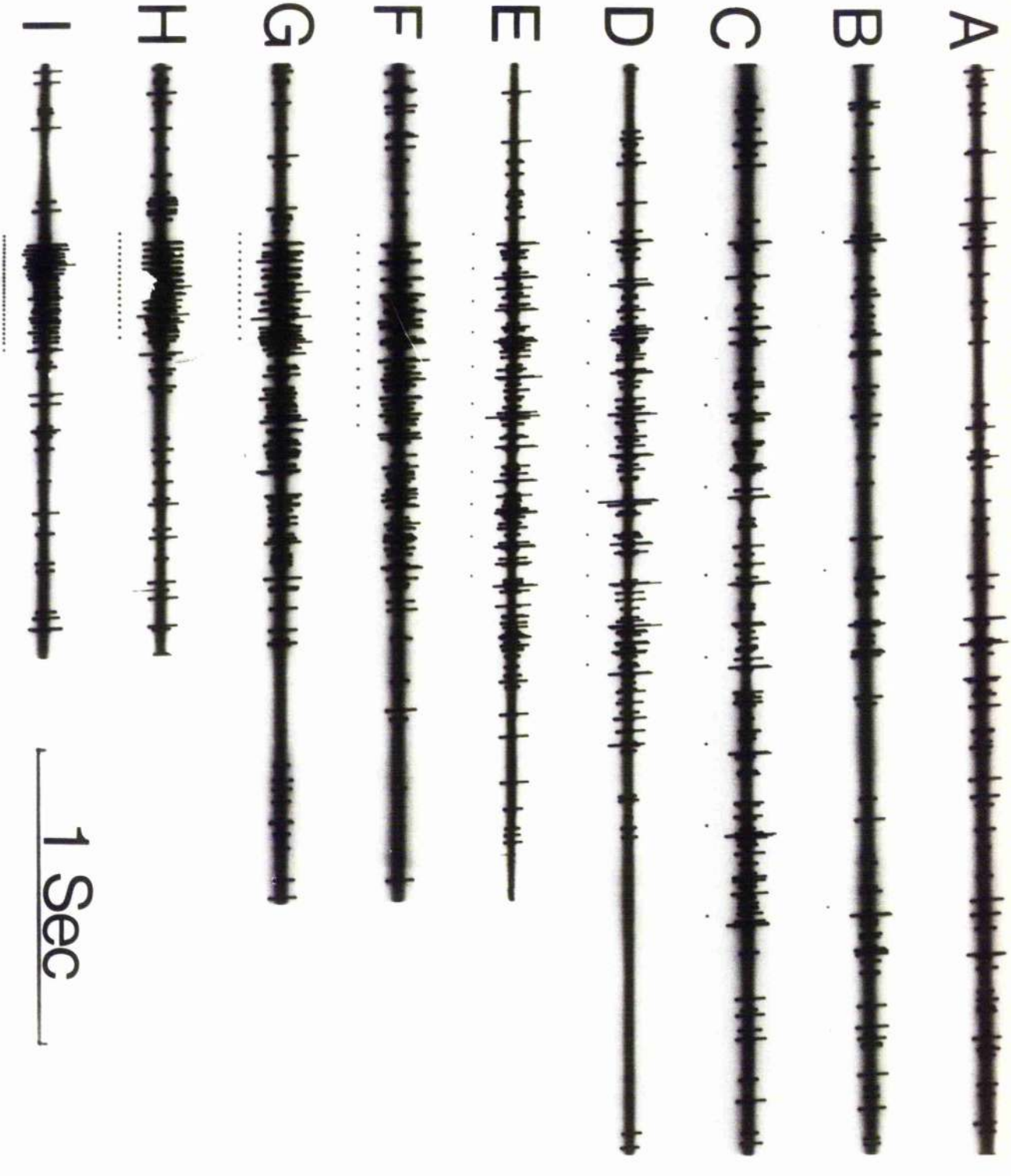

A
B
C
D
E
F
G
H
I
1 Sec

Figure 2.4.13. The effect of increasing the number of pulses at constant frequency on burst formation. In all cases 1 msec. pulses were delivered at 10Hz 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.

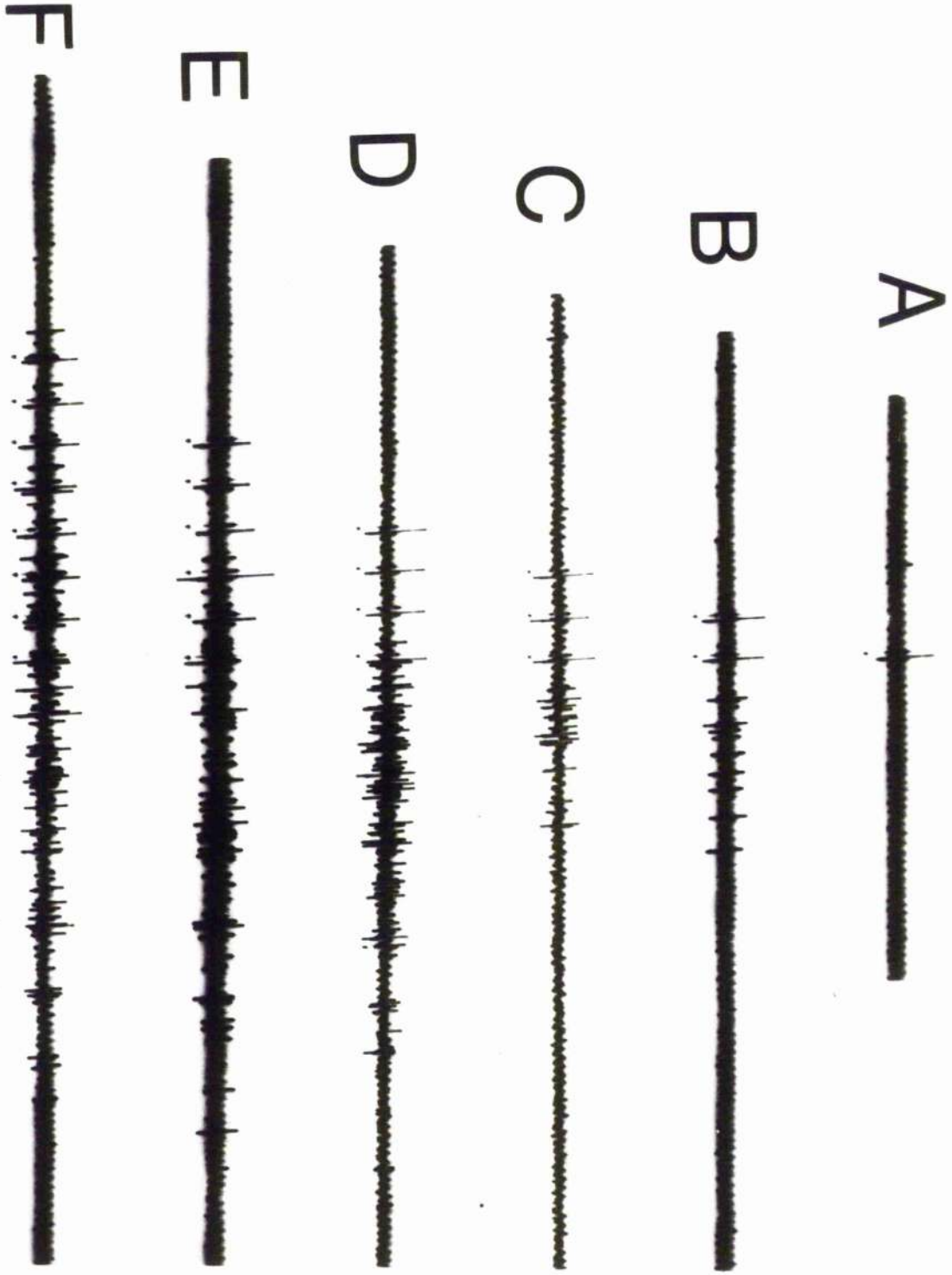

A
B
C
D
E
F
1 Sec
1
2
3

Figure 2.4.14. Facilitation of burst formation by 2 or 3 pulses by previous stimulation of the system using 2 or 3 pulses a few seconds earlier. In both cases 1 msec. pulses at 10Hz were delivered to the left 3-4 connective and recordings were made from the cut central end of the right P.I.N.a. Dots indicate stimulus pulses.

A. Continuous trace. Burst formation by 2 stimuli is facilitated by 2 stimuli occurring 2.6 secs. earlier.

B. Continuous trace. Burst formation by 3 stimuli is facilitated by 3 stimuli occurring 1.75 secs. earlier.

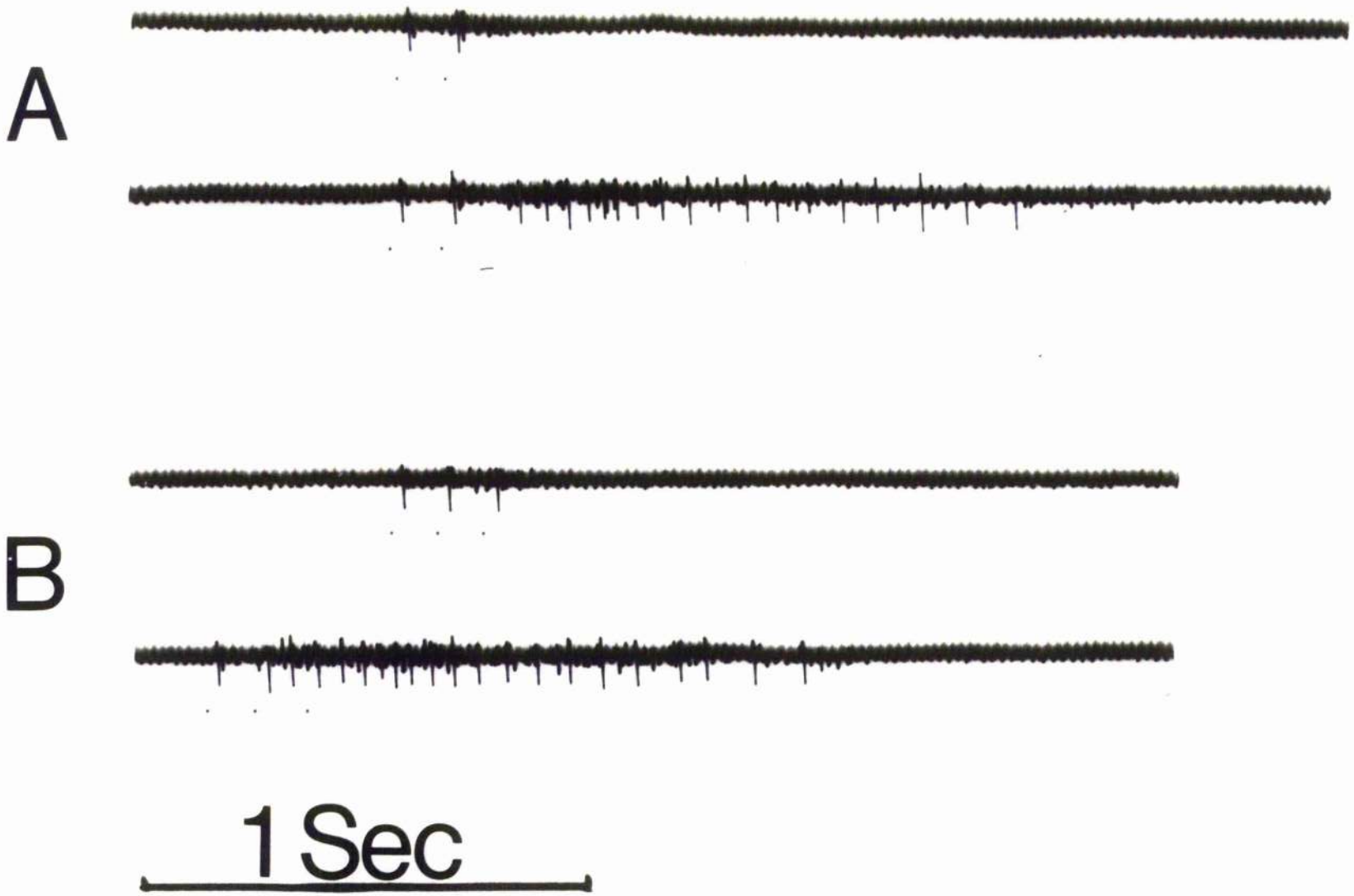

A
B
1 Sec

Figure 2.4.15. 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 10Hz and normal burst formation ensued.
B. Continuous trace. Stimuli were delivered at 25Hz.
C. Stimuli were delivered at 50Hz.

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

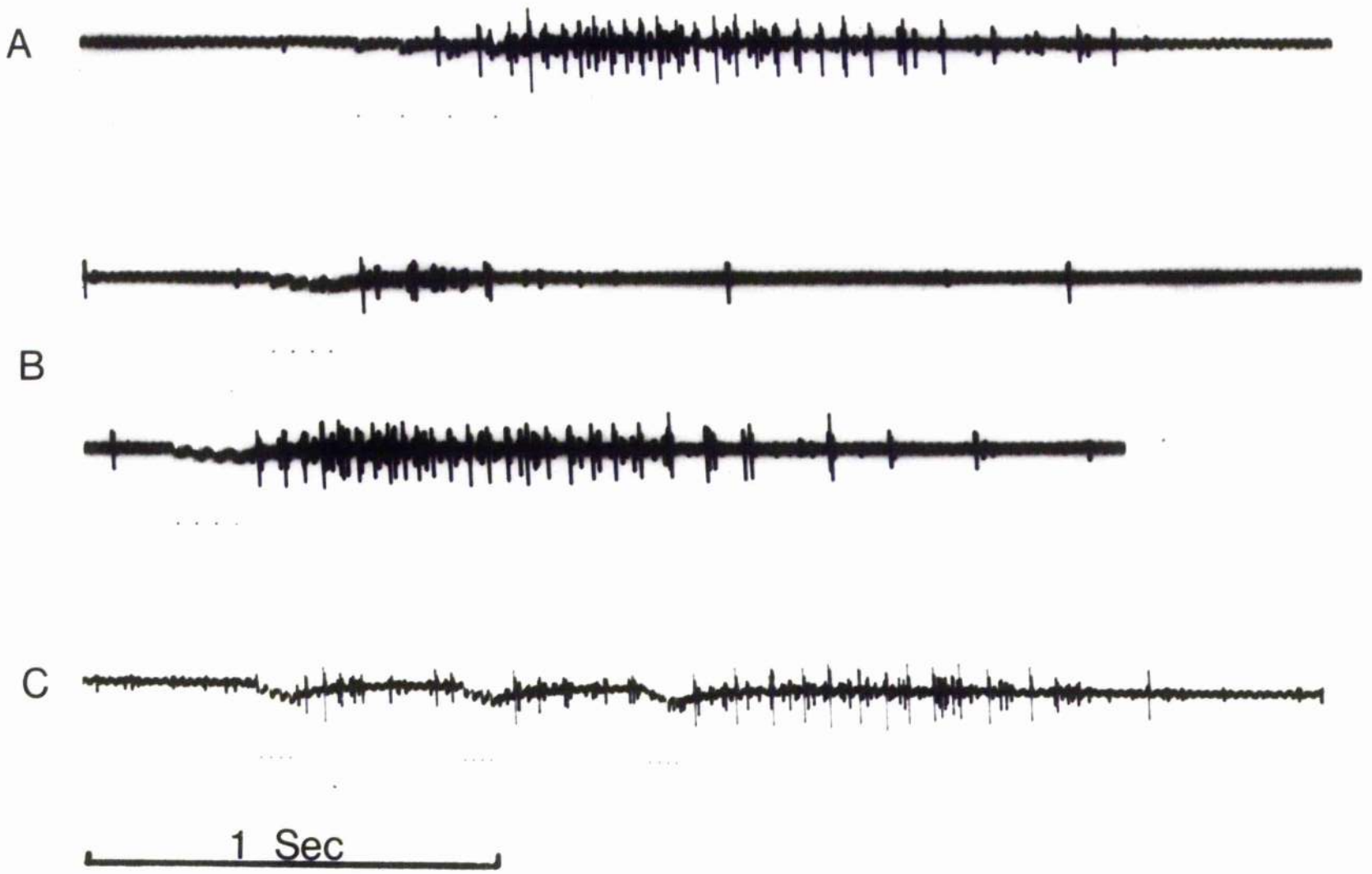

A
B
C
1 Sec

Figure 2.4.16. Correlation of hindgut movements with activity in the P.I.N.'s. Stimuli were delivered to the left 4-5 connective at 25Hz 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. Short burst of stimuli at low amplitude gives rise to a phasic output in P.I.N.'s and weak hindgut movements.

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

C. Long burst of low amplitude stimuli causes a series of weak hindgut movements.

D. Long burst of higher amplitude stimuli causes burst formation in the P.I.N.'s succeeded by phasic responses. The initial burst causes hindgut movements as in B and the phasic responses drive the hindgut rather more weakly.

All records from same preparation.

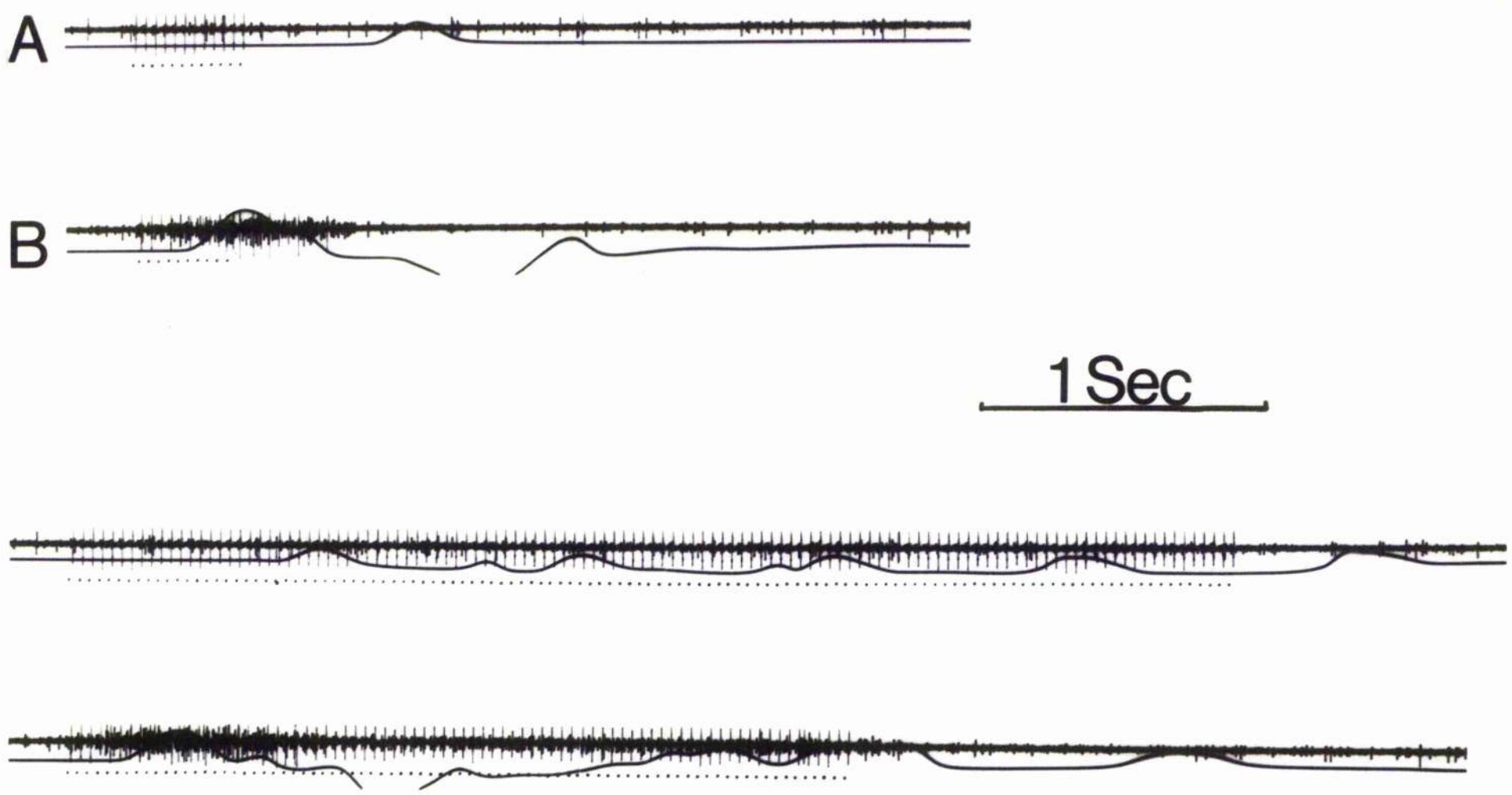

A
B
1 Sec
C
D

Figure 2.4.17. A highly complex series of hindgut movements produced by a single burst in a fresh preparation. Continuous trace.

Upper beam - intact right P.I.N.a. Middle beam - intact right P.I.N.p. Lower beam - transducer at midgut/hindgut junction; upward deflection denotes posteriorward movement. 1 msec. stimulus pulses at 10Hz were delivered to the right 3-4 connective. Dots indicate stimuli. Some small spontaneous units occur in the P.I.N.a. both before and after the burst.


1 Sec

Figure 2.4.18. 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 10Hz were delivered to the left 3-4 connective. Dots indicate stimulus pulses.

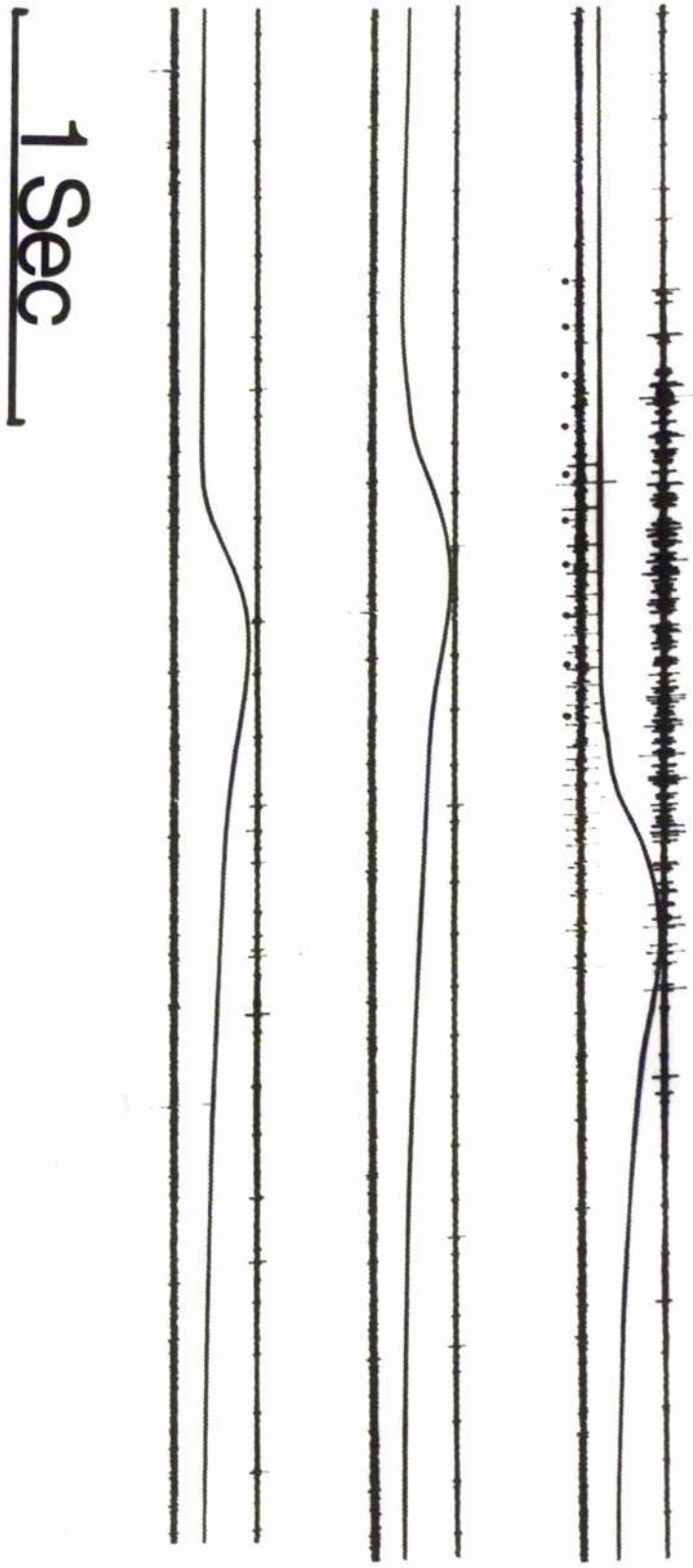
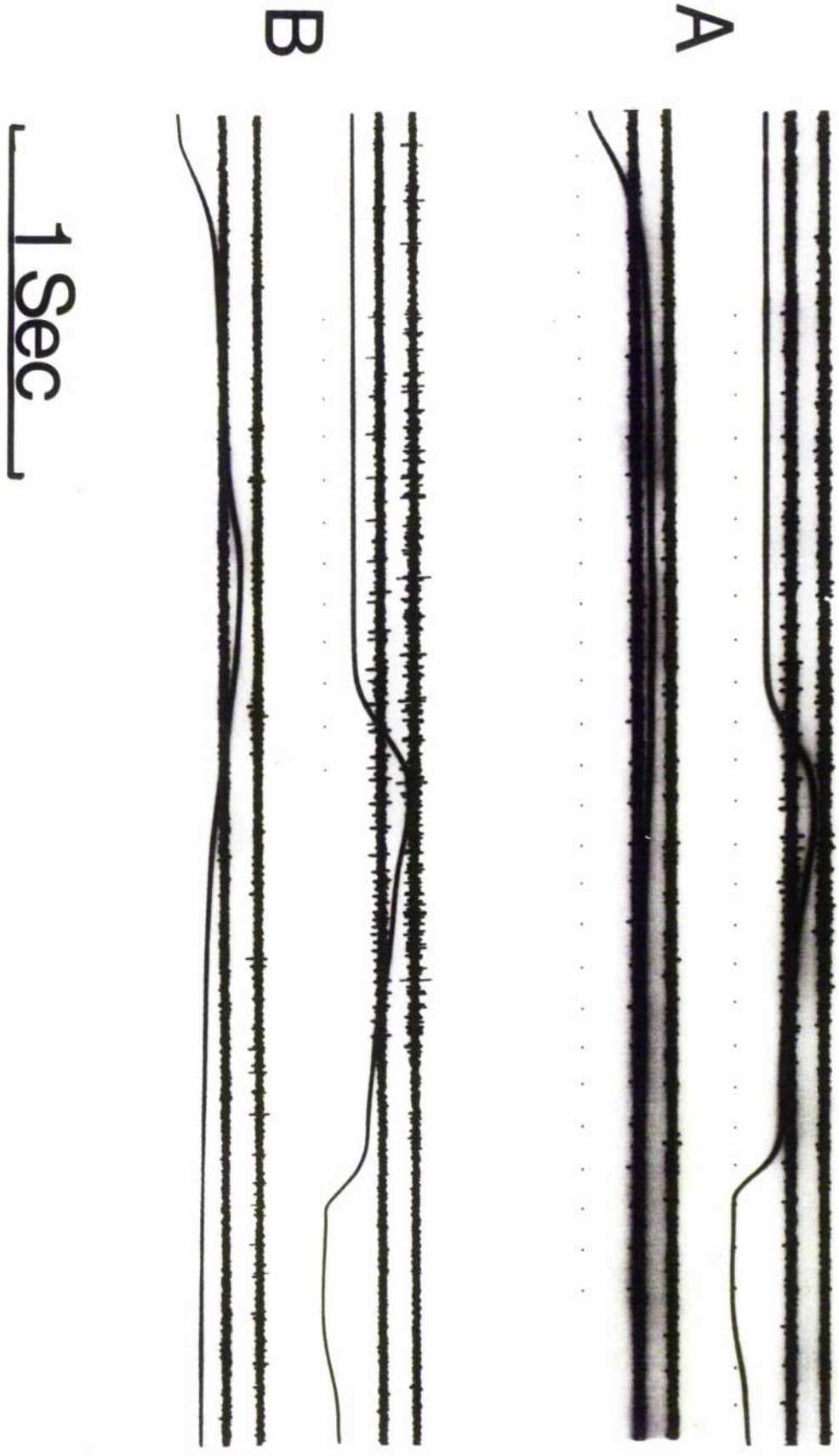

1 Sec

Figure 2.4.19. Constancy of response with different stimulus durations. Stimuli were delivered at 10Hz and 1 msec. pulses to the right 3-4 connective. Dots indicate stimulus pulses.

Upper beam - intact right P.I.N.a. Middle beam - intact left P.I.N.p. Lower beam - transducer at midgut/hindgut junction; upward deflection denotes posteriorward movement.

A. Continuous trace. Long term stimulation produces initial burst and no further tonic or phasic activity.

B. Continuous trace. Short term stimulation produces a very similar response to that in A.


A
B
1 Sec

Section 2.5. The Structure of the Sixth Abdominal Ganglion.

Introduction

In previous Sections (2.2 and 2.4) the 6A.G. was clearly implicated as exerting a great measure of control over the functioning of the hindgut, with respect to defaecation. In order to study more closely the neuronal interactions of those units comprising the 'hindgut control centre' it will be necessary to determine their precise location. To do this the structure of the 6A.G. must first be known in detail. Many authors (Krieger, 1880; Retzius, 1890; Johansson and Schreiner, 1965) have previously described the ganglion, but none of them has given a particularly lucid or full account of the complete ganglionic structure. In this section I have, therefore, attempted to describe the positions of major nerve tracts, commissures and groups of neurone somata.

Materials and Methods

Specimens of the 6A.G. were dissected out from Homarus. They were then fixed, embedded, sectioned (in all three major planes), stained and photographed as in Section 2.1. A series of photographs of sections in T.S. was mounted on thin card, cut to shape and built up into a model of the ganglion. In this manner the major details of ganglionic structure were determined and a series of diagrammatic drawings was produced.

The blood supply of the 6A.G. was displayed by injection of Gunther-Wagner Pelikan ink (No. C11/1431a), or strong methylene blue solutions, into the heart. Methylene blue solution was also injected into the abdomen

through the ventral soft cuticle at about the level of the fourth abdominal segment.

Results

The last of the abdominal ganglia is the most complex in Homarus. Its general structure is shown in Figure 2.5.1. There are six pairs of nerves emanating from the ganglion. These are, from anterior to posterior:-

N1	-	anterior nerves (to swimmerets)
N2	-	uropod nerves
N3	-	ventral telson nerves
N4	-	dorsal telson nerves
N5	-	anal nerves
N6	-	posterior intestinal nerves.

This nomenclature is derived from Keim (1915) except for N6 which as mentioned in Part 1 was named by Alexandrowicz (1909). The structure of the ganglion as seen in T.S. is summarised in Figures 2.5.2 to 2.5.8. Each figure is given a scale number to coincide with the scale on Figure 2.5.1. The zero mark for this scale is taken as the point at which the 5-6 connectives become fused into the sheath of the 6A.G. The 200 mark is taken as the most posterior part of the ganglionic sheath.

A. Non-nervous Components of the Ganglion

Externally the 6A.G. is bounded by a tough connective tissue sheath directly beneath which is a layer of highly vacuolated tissue of unknown function. This tissue is 100-200μ deep and completely packs the space between the external connective tissue sheath and the nervous elements of the ganglion, except in the region of the

non-vascular space which lies in the anterior dorsal region of the ganglion. This space is large, extending into the connectives and partially surrounding the anterior neuropile (see Figures 2.5.2 to 2.5.4). Surprisingly, this space is in no way connected with the blood system of the ganglion, as has been demonstrated by injection of indian ink or methylene blue into the heart. In such cases all the fine capillaries and sinuses of the ganglion were permeated by the injected fluids whilst the non-vascular space remained devoid of colouration.

The blood supply to the 6A.G. takes the form of several major sinuses from which are derived numerous capillaries that run through the neuropile and supply the neurone somata. Figure 2.5.10 shows the capillary network of the ventral cell bodies visualized by indian ink injection. The sinuses arise from a large median dorsal blood vessel which passes into the 6A.G. from the anterior and then gives off a ventral going vessel (see Figure 2.5.2). This vessel then bifurcates and the branches come to lie dorsal to the main mass of ventral cell bodies, forming the ventral sinus. This ventral sinus extends over the entire dorsal surface of the ganglionic cortex and even wraps around the dorsal groups of cell bodies (see Figure 2.5.7). It is perforated at many points by neurone tracts passing from the cortex (Figures 2.5.2, 2.5.5 and 2.5.6). In the anterior region of the ganglion there is a major dorsal sinus, interposed between the non-vascular space and the neuropile, arising directly from the median dorsal blood vessel. This sinus eventually peters out between scale marks 140 and 150 (see Figure 2.5.1), at the posterior limit of the neuropile. At several points the sinuses almost come to surround the neuropile (see Figures 2.5.3, 2.5.4 and 2.5.6). Many capillaries ramify

throughout this region and appear to originate from the two major sinuses. The ventral sinus very obviously gives off branches to the ganglionic cortex and these branches can often be seen to supply individual cell bodies. Posteriorly the dorsal vessel is reconstituted and leaves the ganglion. The direction of blood flow is thought to be from anterior to posterior.

B. The Ganglionic Cortex.

For the most part the cortical region of the 6A.G. lies ventral to the neuropile and nerve tracts of the ganglion. It is divided into anterior and posterior collections of neurone somata as is shown in Figures 2.5.1 and 2.5.9. A narrow 'waist' of cell bodies connects these two major ventral lobes. Dorsal to the anterior ventral cortical lobe and lying between the dorsal sinus and the neuropile is a pair of superficial median dorsal cell bodies (see Figure 2.5.1 and 2.5.3) lying one behind the other. A further single isolated cell body, the deep dorsal cell body, lies ventral to the more superficial pair, directly below the most ventral division of the first commissure. It is about 10μ in diameter.

From the posterior ventral lobe there arises a symmetrically arranged pair of dorsal lobes as is shown in Figures 2.5.6 and 2.5.7. These groups of cell bodies are found in the most posterior region of the ganglion. They lie lateral to the origins of the P.I.N.'s and wrap over their fibre tracts dorsally.

There are many large neurone somata in the cortex, some of which may reach 80-90μ in diameter (see Figure 2.5.5.A). The diameter of the majority of cell bodies

lies between 10μ and 50μ. A transversely ordered group of somata of very uniform diameter (30μ) lies ventral to the fourth commissure (Figure 2.5.6). The significance, if any, of such uniformity, which does not occur elsewhere in the ganglion, is unknown.

C. The Neuropile

Most of the neuropile proper lies between the first and fourth commissures (scale marks 100 to 155). Anterior to the first commissure (Figure 2.5.2) the 5-6 connectives enter the ganglion and break up into tracts many of which pass ventrally to the anterior ventral lobe, whilst a smaller number travel dorsally into the posterior region of the ganglion. Posterior to the fourth commissure (Figures 2.5.7 and 2.5.8) the neuropile gives way to the various nerve tracts which run out posteriorly. Many nerve tracts and several commissures can be discerned within the neuropile, as can the origins of the major nerve trunks.

1. Commissures and Tracts.

Four groups of commissures lie within the neuropile and these are divisible into eight individual groups of fibres traversing the ganglion. The positions of these commissures are summarised in Table 2.5.1.

In addition to the commissures there are numerous tracts running within the neuropile. The most obvious of these are shown in Figures 2.5.3 and 2.5.4. They are on the whole symmetrically arranged and those in Figure 2.5.3 appear to run into the anterior nerve (N1) to the swimmeret musculature, whilst those in Figure 2.5.4 are thought to be involved in the formation of the uropod

TABLE 2.5.1.

Com-missure	Fibre tracts	Position relative to regions of neurone somata	Scale mark w.r.t. Figure 2.5.1.	See Figure
1st	a. Superficial dorsal b. Deep dorsal	Dorsal to waist	105	2.5.3.
2nd	a. Superficial dorsal b. Deep dorsal	ditto	120	2.5.4.
3rd	a. Superficial dorsal b. Deep dorsal c. Ventral	Dorsal to anterior region of posterior ventral lobe.	140	2.5.5.
4th	Ventral	ditto	155	2.5.6.

nerves (N2). Two major median tracts also occur; the anterior one ascending at the level of the third commissural group (Figure 2.5.5). The posterior median tract lies posterior to the fourth commissure (Figure 2.5.6) and many of its fibres ascend towards the origins of the P.I.N.'s which are already well formed.

2. The Origins of the Nerve Trunks within the 6A.G.

The derivation of the main trunks is summarised in Table 2.5.2. The anterior nerves divide into posterior and anterior branches soon after leaving the ganglion. The dorsal telson nerve is, however, more unusual in that it arises from two different roots which pass out separately from the posterior dorsal surface of the ganglion and then fuse. The P.I.N.'s arise medial to the anal nerves and from much the same region of the neuropile. They accompany the anal nerves for some way before passing from the posterior dorsal surface of the ganglion to supply the hindgut.

Many of the external features of the ganglion outlined here are quite variable especially the positions of exit of the dorsal telson nerves and the P.I.N.'s. In addition, many minor variations of the P.I.N.'s may occur as has been mentioned in Section 2.1. In all other respects the structure of the 6A.G. is constant from animal to animal, especially with regard to the position of the neurone somata and the various commissures.

TABLE 2.5.2.

Nerve trunk	Origin and scale mark	Direction and point of exit (scale mark)	Figure
Anterior N. (N1)	1st commissure 95 - 110	Laterally 95 - 120	2.5.3.
Uropod N. (N2)	2nd and 3rd commissure. Mainly in lateral ventral neuropile. 120 - 140	Obliquely posteriorwards. 150 - 160	2.5.4. and 2.5.5.
Ventral Telson N. (N3)	3rd commissure. Derived from lateral dorsal neuropile ventral to the anterior root of N4. 135 - 140.	Very oblique 170 - 180	2.5.5. to 2.5.8.
Dorsal Telson N. (N4) :- Anterior Root.	3rd commissure. Arises from lateral dorsal neuropile. 135 - 140.	Posteriorly 200	2.5.5. to 2.5.8.
Posterior Root.	4th commissure. Rudiments lie in dorsal neuropile medial to anterior root. 145 - 155.	Posteriorly 200	2.5.6. to 2.5.8.

TABLE 2.5.2. (cont'd.)

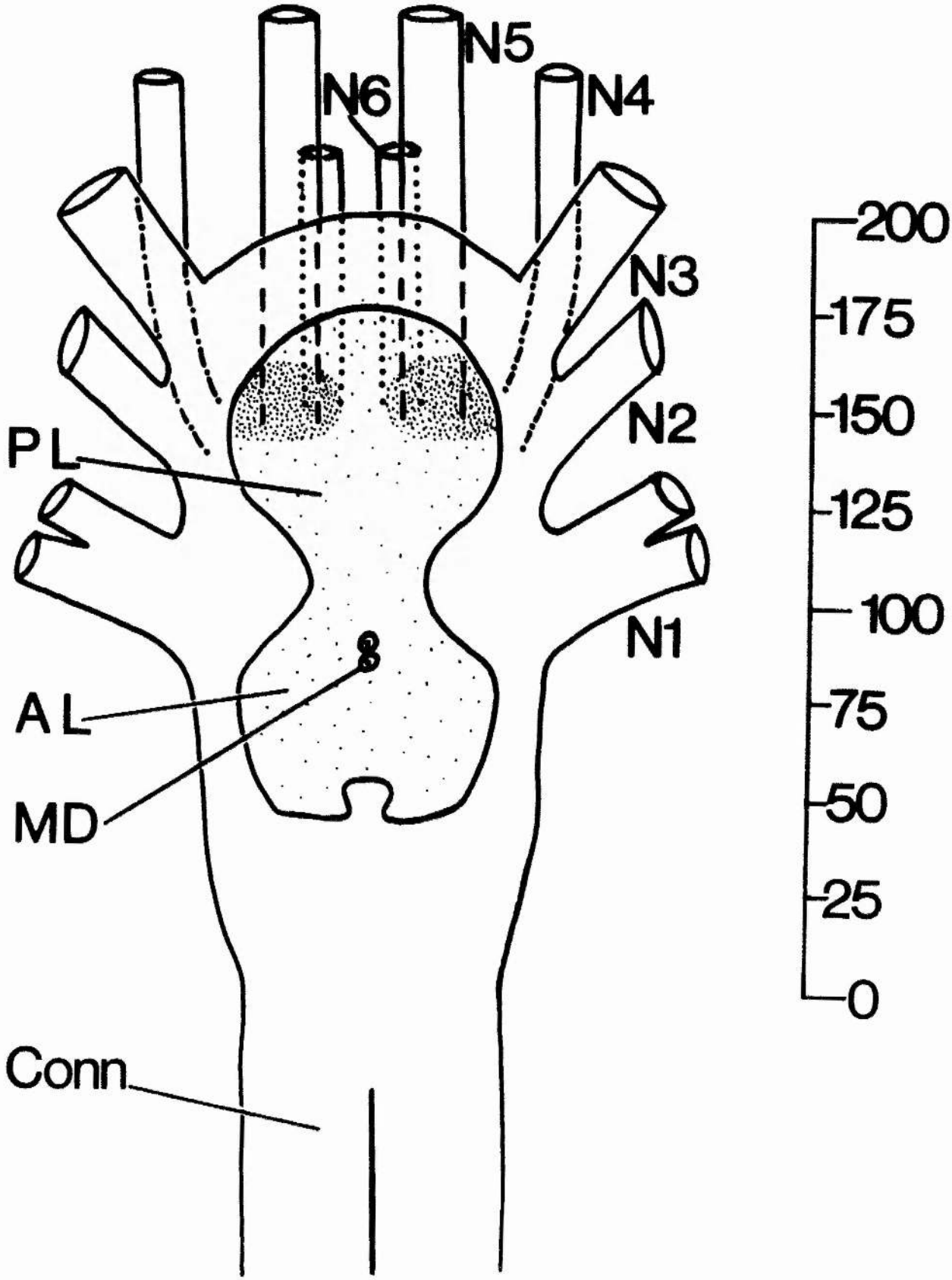
Anal N. (N5)	4th commissure. Arises in ventral neuropile lateral to P.I.N.'s. 150 - 155	Posteriorly 200	2.5.6. to 2.5.8.
Posterior Intestinal N. (N6)	4th commissure. Originates in ventral neuropile medial to anal nerves. 150 - 155.	Posteriorly 200	2.5.6. to 2.5.8.

Figure 2.5.1. Diagrammatic view of the ventral surface of the 6A.G.

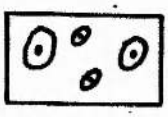
The region of light strippling denotes the ventral cell bodies, whilst the heavy strippling indicates the position of the dorsal cell bodies.

A.L. - anterior lobe of ventral cell bodies.
P.L. - posterior lobe of ventral cell bodies.
M.D. - median dorsal cell bodies.
Conn - connectives.
N1 - N6 - major nerve trunks.

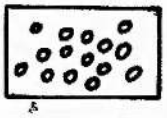
The scale marker is equivalent to 200 x 10μ transverse sections so that 200 = 2mm. It is used as a reference scale in Figures 2.5.2 to 2.5.8.


N5
N6
N4
N3
N2
N1
PL
AL
MD
Conn
200
175
150
125
100
75
50
25
0

Figures 2.5.2. to 2.5.9. In all these figures a standard form of presentation is utilised. Part A of each figure is a transverse section (except for 2.5.9. which is a longitudinal section) of the ganglion, stained with Mallory's Triple Stain, in approximately the same position as Part B which is a diagrammatic representation of a T.S. through the ganglion in the position indicated by the scale number (corresponding to the scale in Figure 2.5.1). Different tissues are represented as follows:-

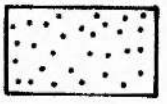

Neurone somata.

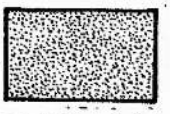
Tracts and commissures:-


a) In T.S.


b) In L.S.


Vacuolated tissue.


Non vascular space.


Blood vessels.

Standard abbreviations used are as follows:-

Neurone Somata

A.L.	-	anterior lobe of ventral cell bodies.
P.L.	-	posterior lobe of ventral cell bodies.
D.L.	-	dorsal lobes of cell bodies arising from the posterior ventral cortex.
deep M.D.	-	deep median dorsal cell body.

sup. M.D.	-	superficial median dorsal cell bodies.
Waist	-	narrow waist of neurone somata connecting anterior and posterior ventral ganglion cortices.

Tracts

conn	-	connectives.
N1	-	anterior nerve
N2	-	uropod nerve
N3	-	ventral telson nerve
N4	-	dorsal telson nerve
A.R. N4	-	anterior root of N4.
P.R. N4	-	posterior root of N4.
N5	-	anal nerve
N6	-	posterior intestinal nerve
T. N1	-	tracts to N1.
T. N2	-	tracts to N2.
A.M.T.	-	anterior median tract
P.M.T.	-	posterior median tract.

Commissures.

deep D.F.T.	-	deep dorsal fibre tract.
sup. D.F.T.	-	superficial dorsal fibre tract.
V.F.T.	-	ventral fibre tracts.

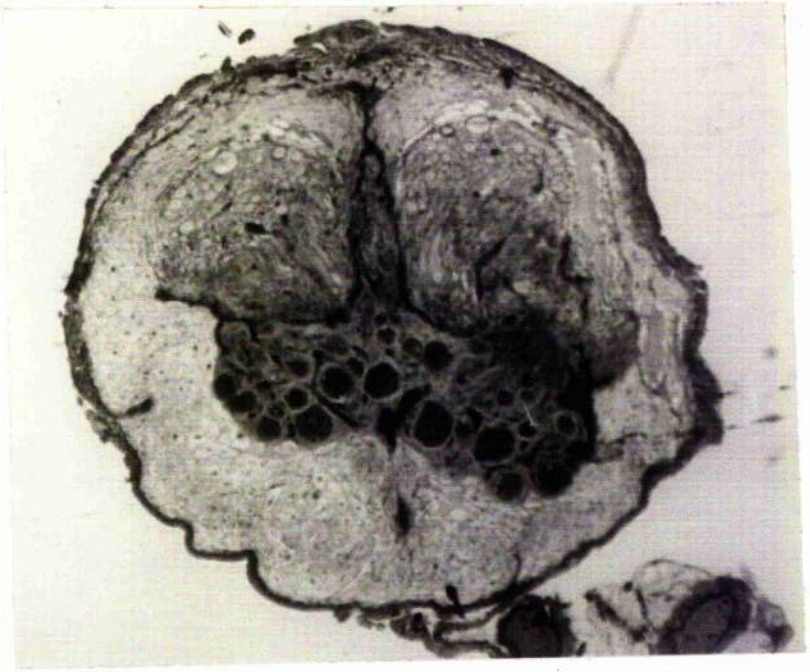
Blood Vessels.

D.S.	-	dorsal sinus
V.S.	-	ventral sinus
V. go. S.	-	ventral going sinus derived from median dorsal blood vessel.

Figure 2.5.2. T.S. of 6A.G. through anterior ventral lobe of cortex.

Scale mark 95.

A



B

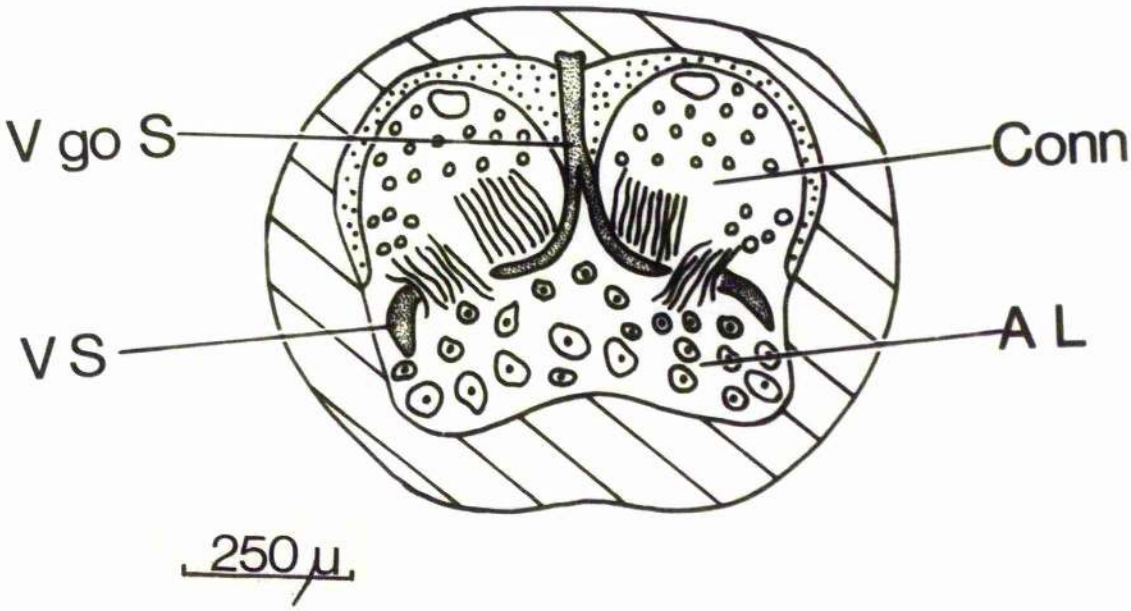
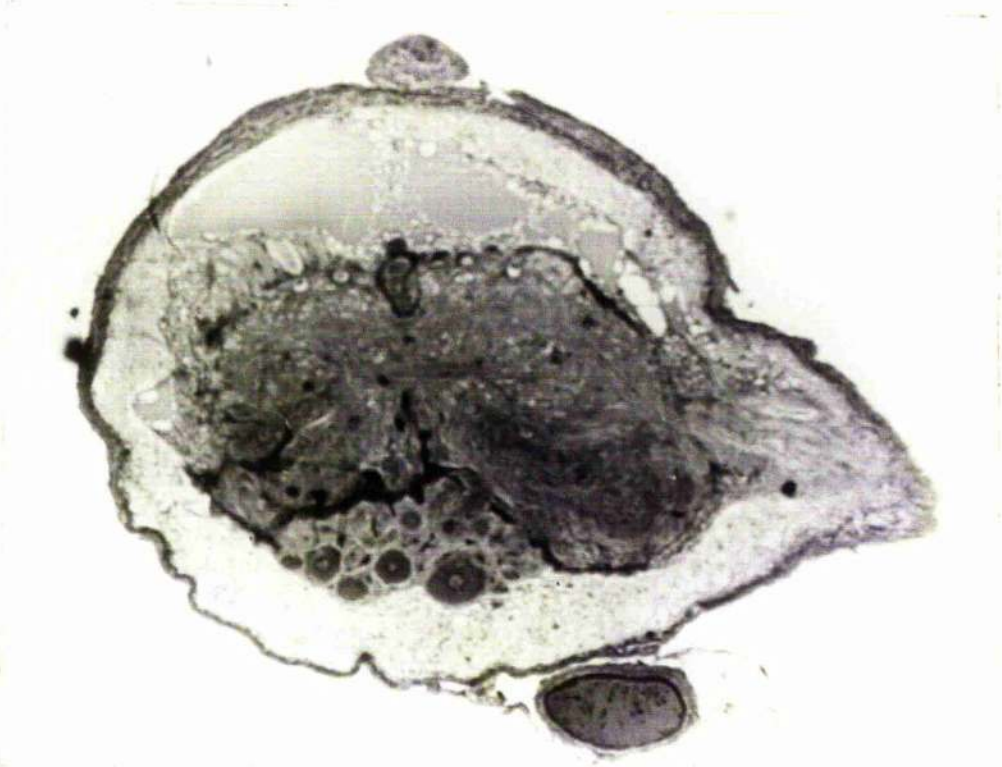
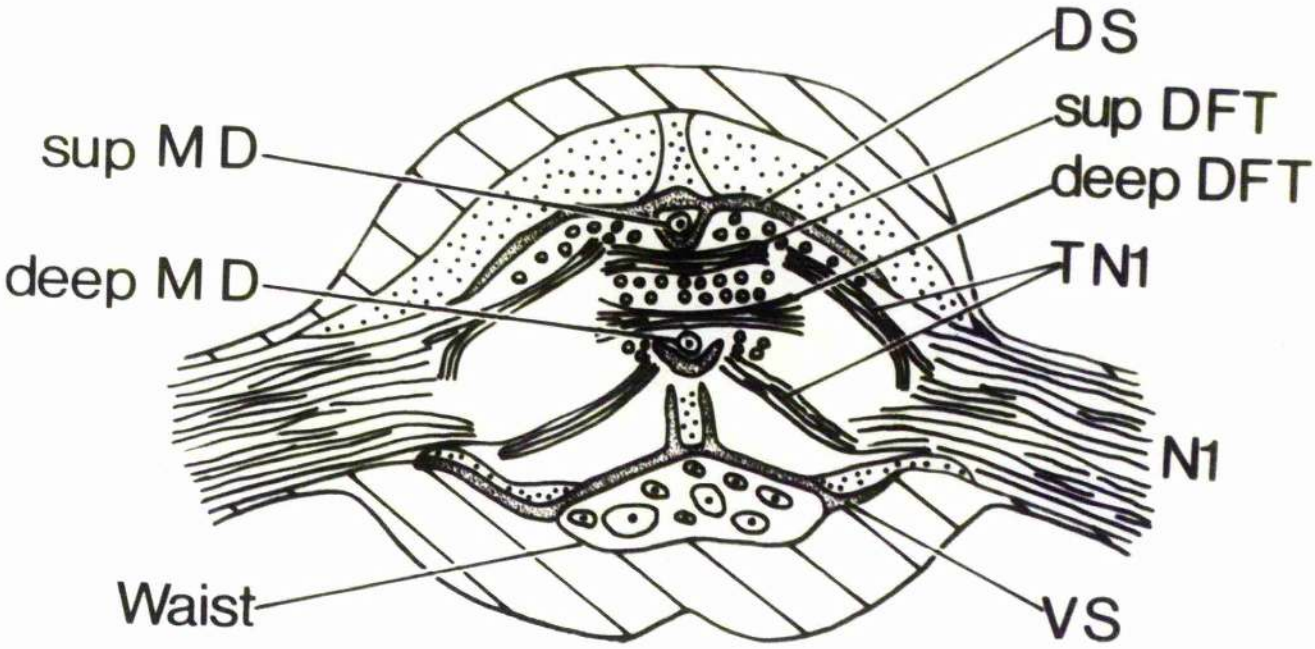


Figure 2.5.3. T.S. of 6A.G. in region of first commissure.

Scale mark 105.



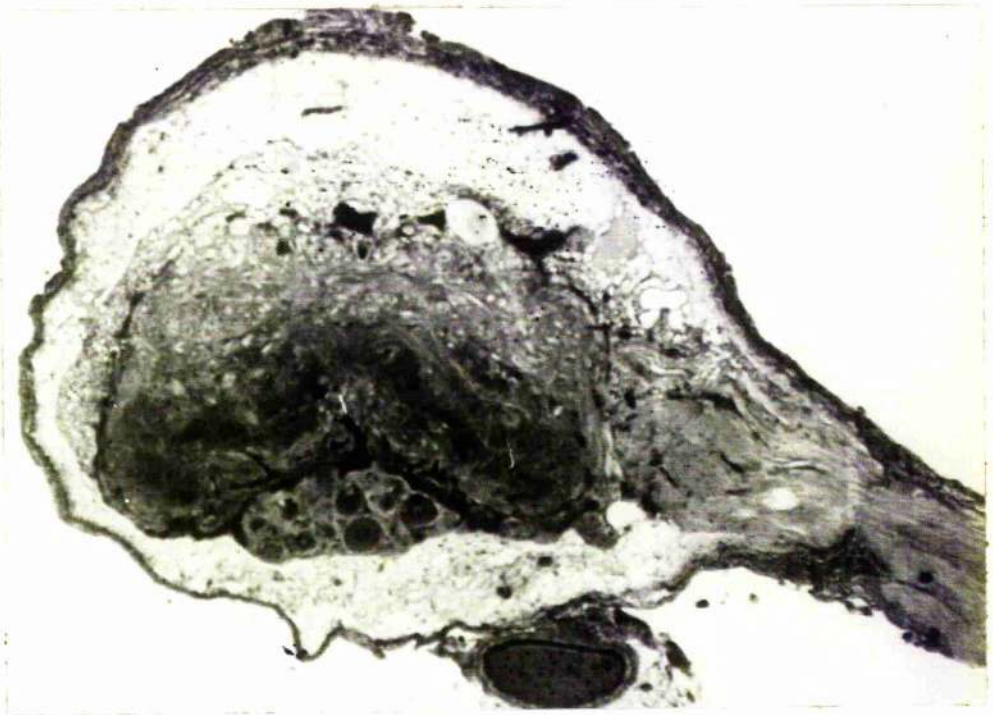
A



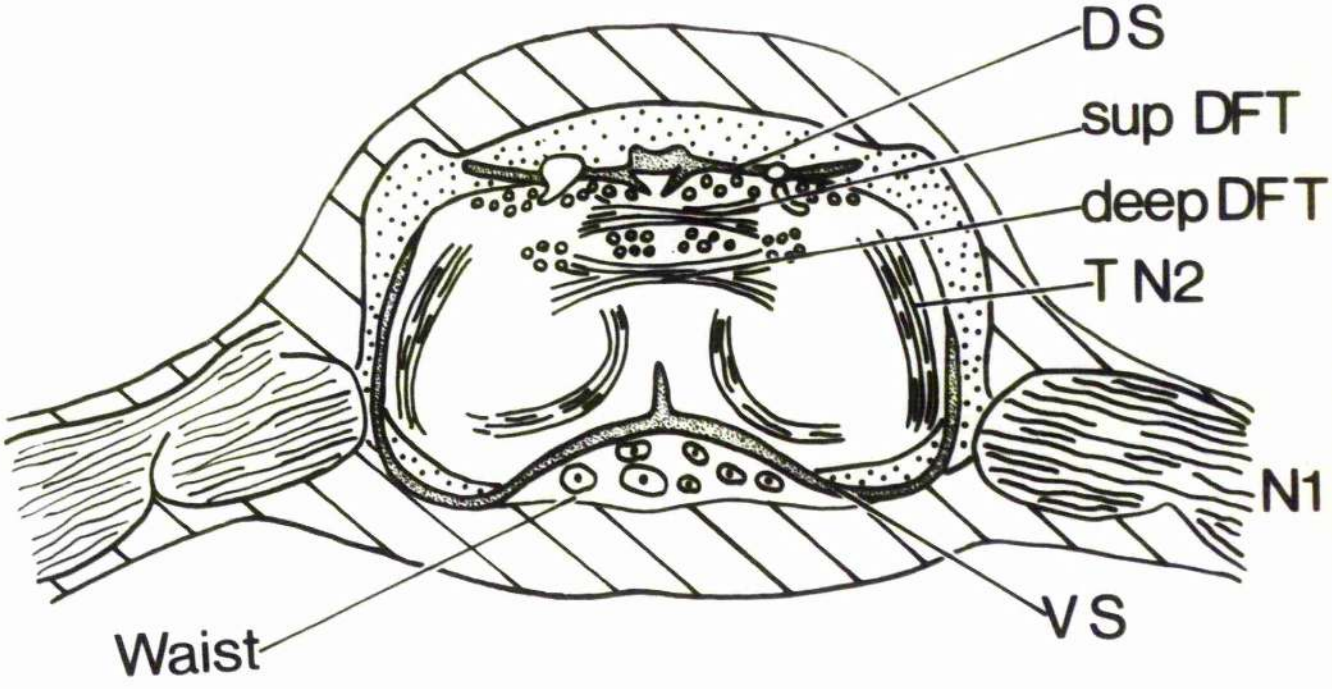
B 250 µ

Figure 2.5.4. T.S. of 6A.G. through second commissure.

Scale mark 122.



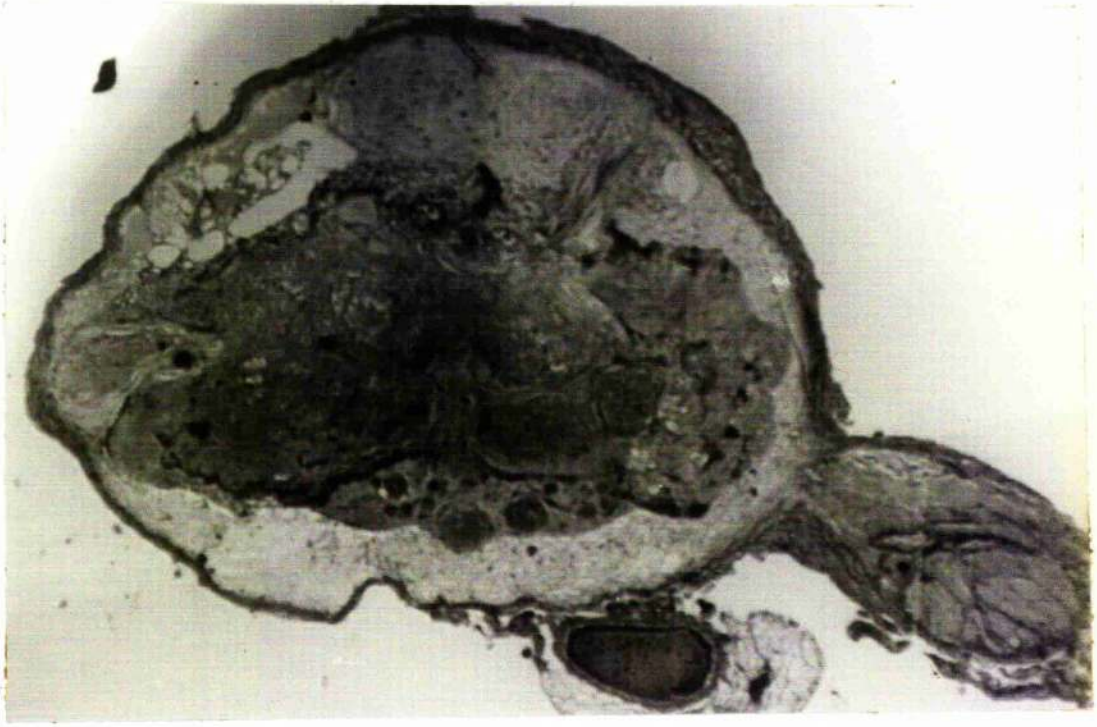
A



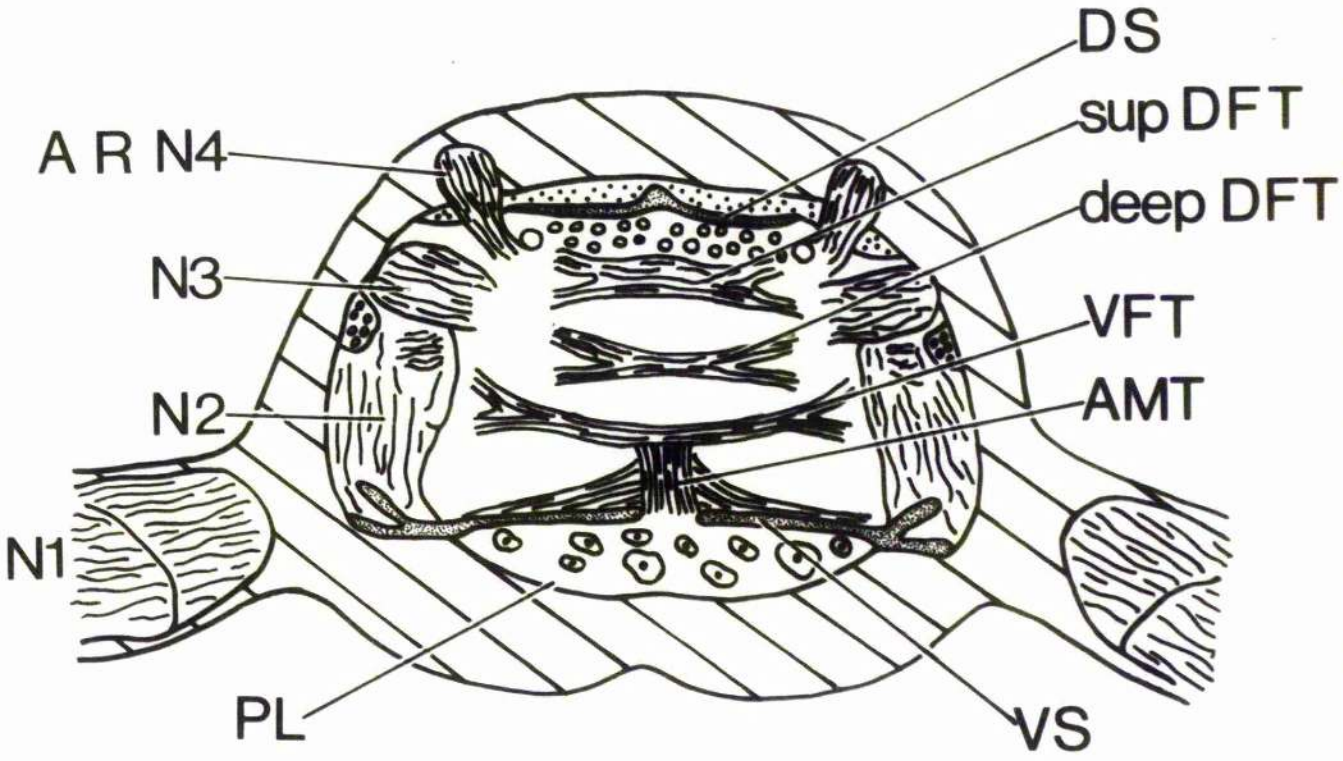
B 250 µ

Figure 2.5.5. T.S. of 6A.G. through third commissure.

Scale mark 138.



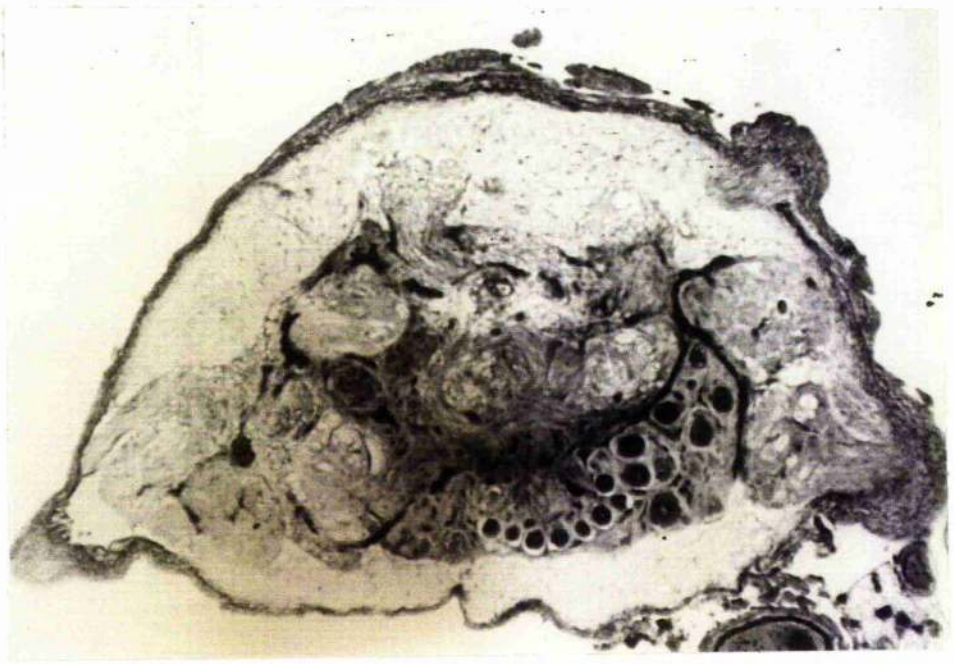
A



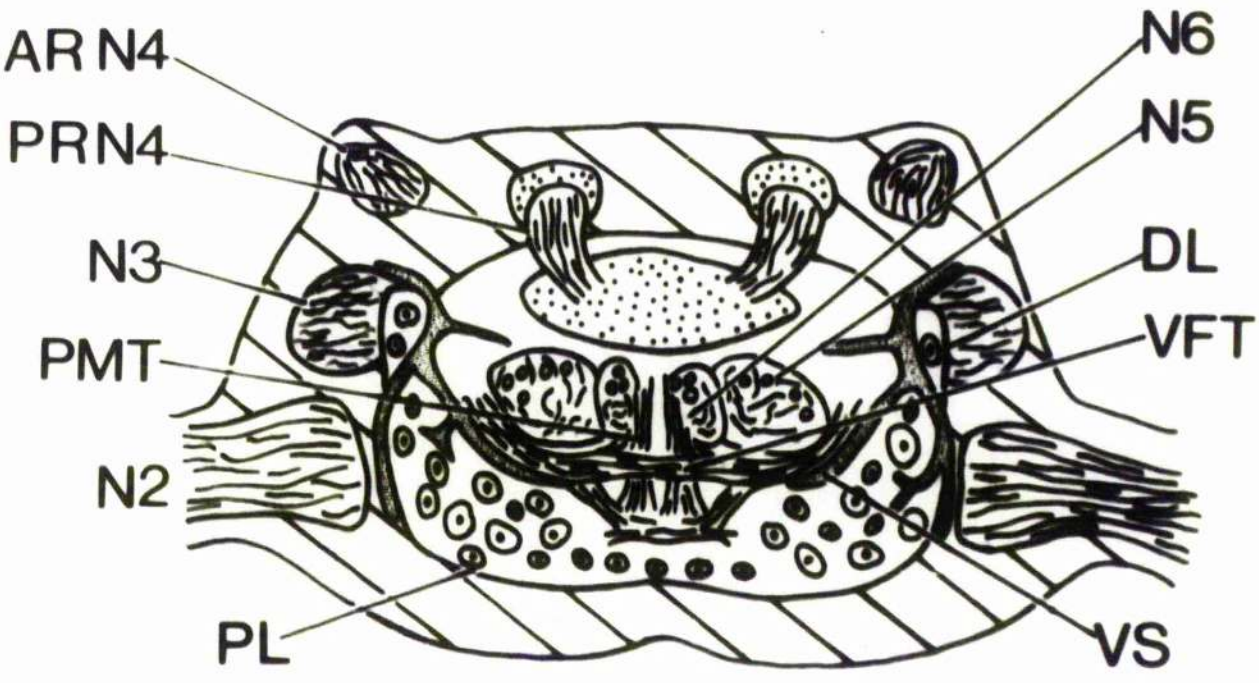
B 250µ

Figure 2.5.6. T.S. of 6A.G. through fourth commissure.

Scale mark 153.



A



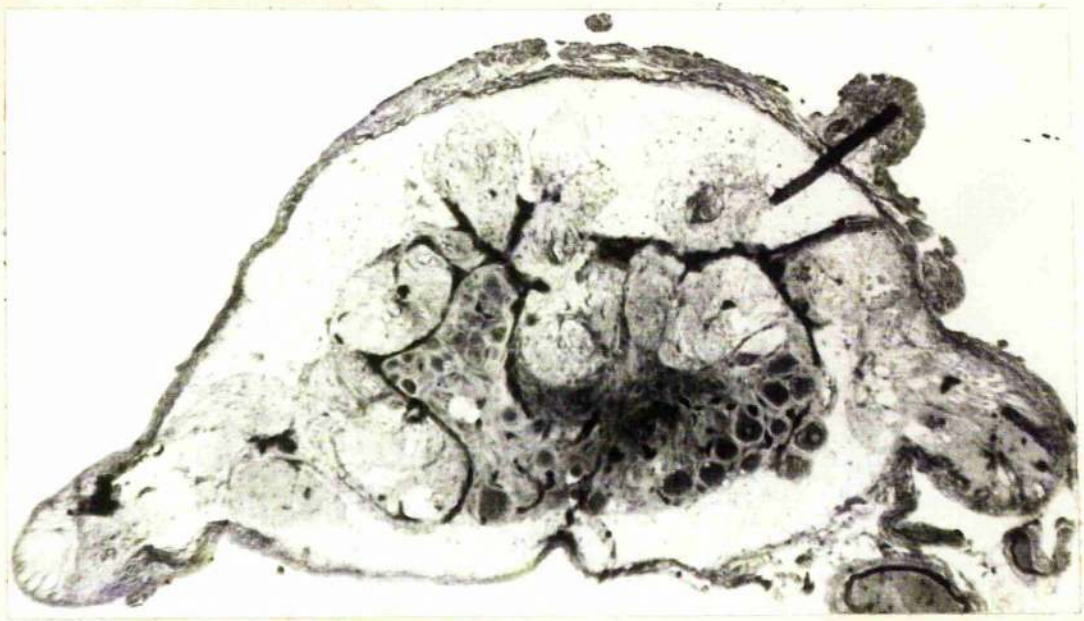
B

250 µ

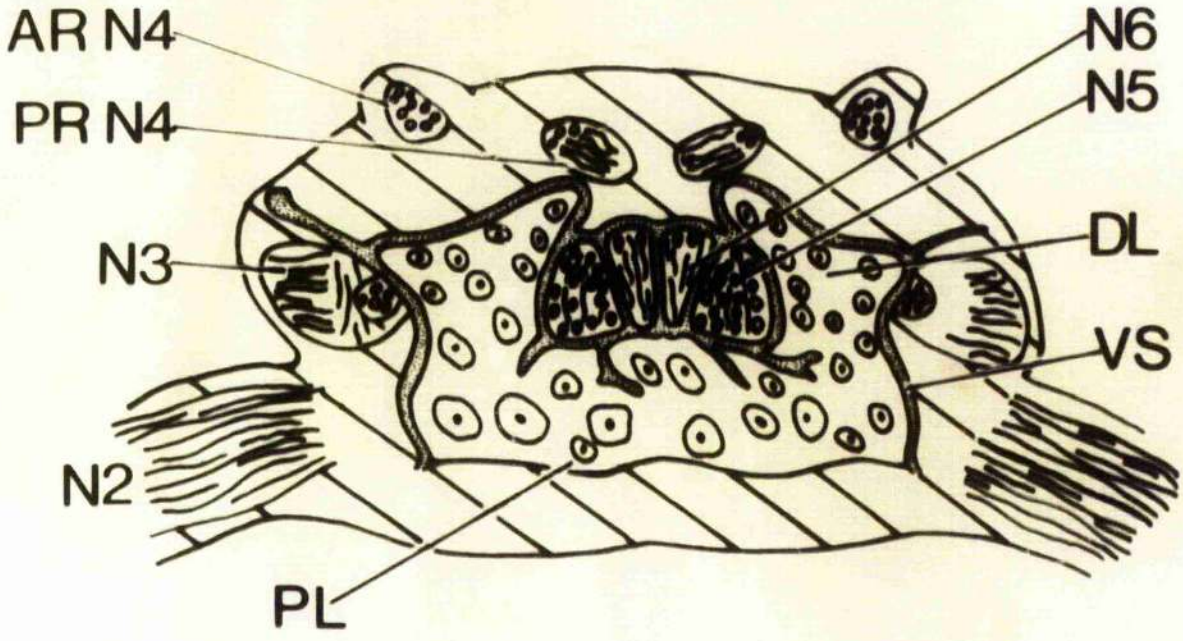
Figure 2.5.7. T.S. of 6A.G. in region of dorsal lobes of cell bodies.

Scale mark 160.

A



B

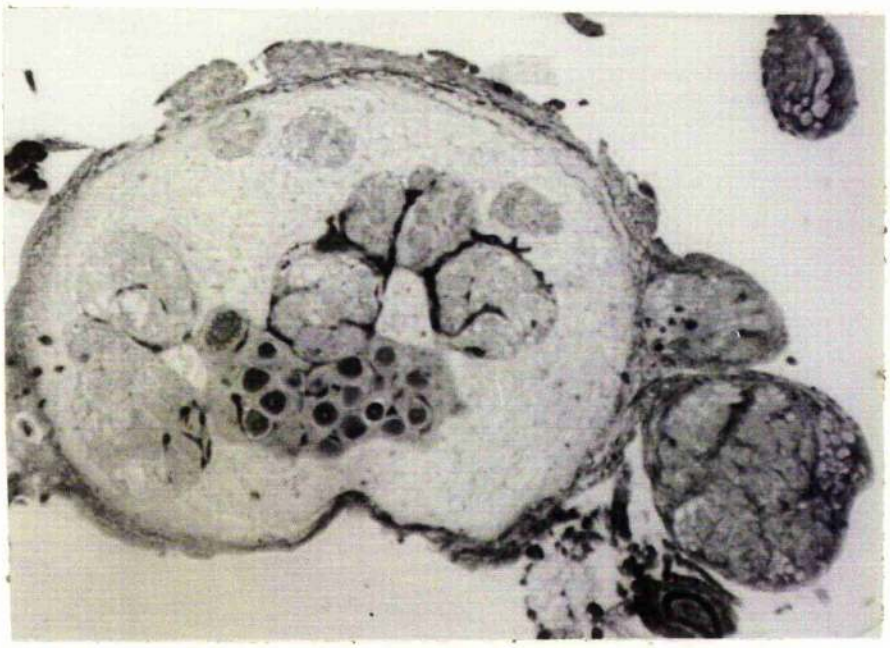


250 µ

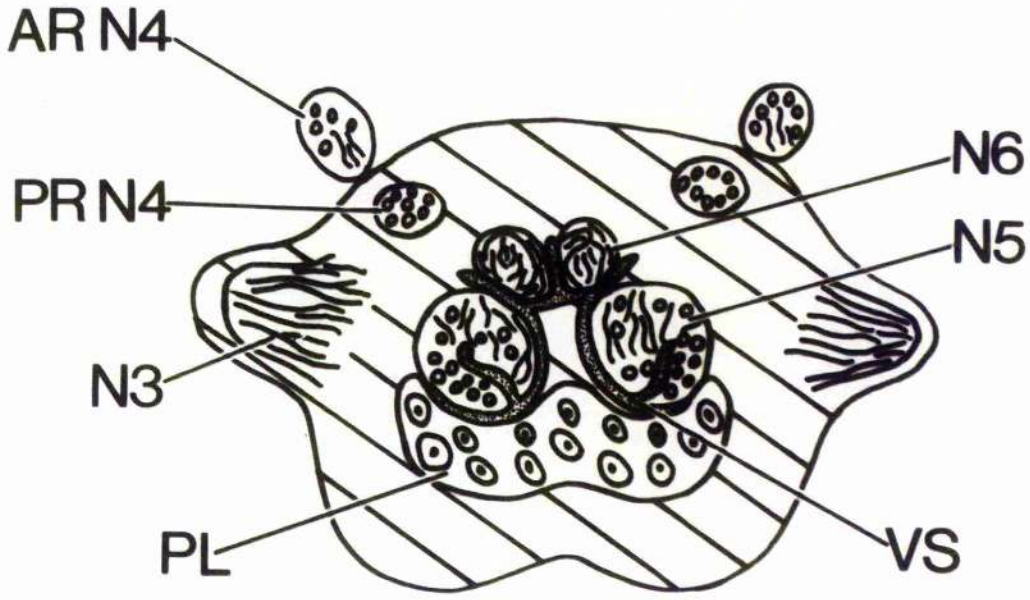
Figure 2.5.8. T.S. of 6A.G. posterior to dorsal lobes of cell bodies.

Scale mark 175.

A



B



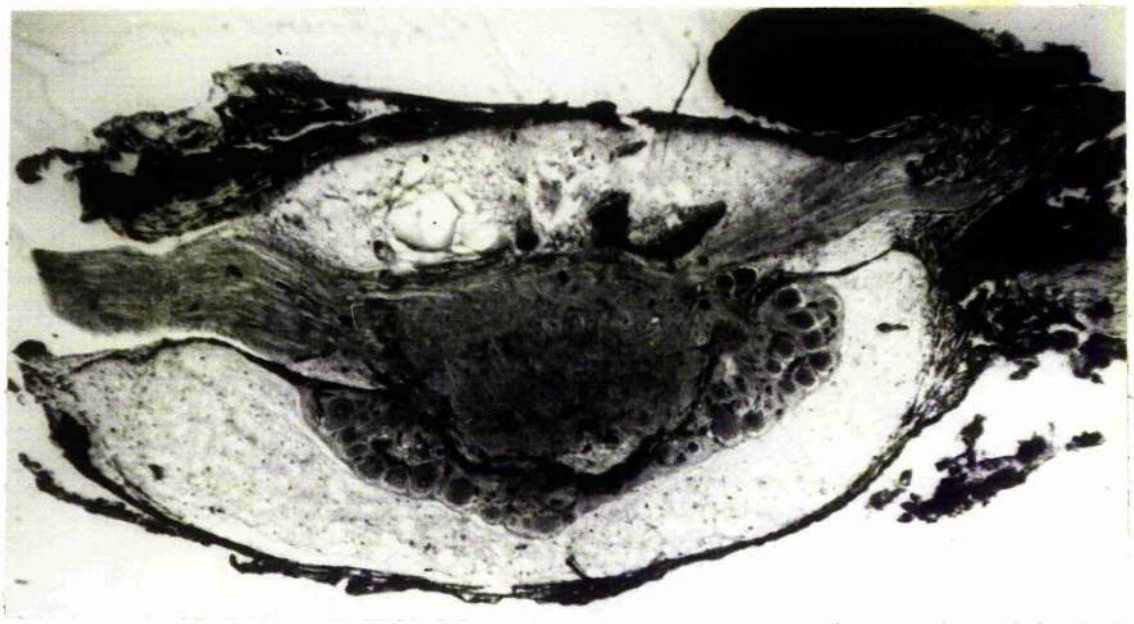
250 µ

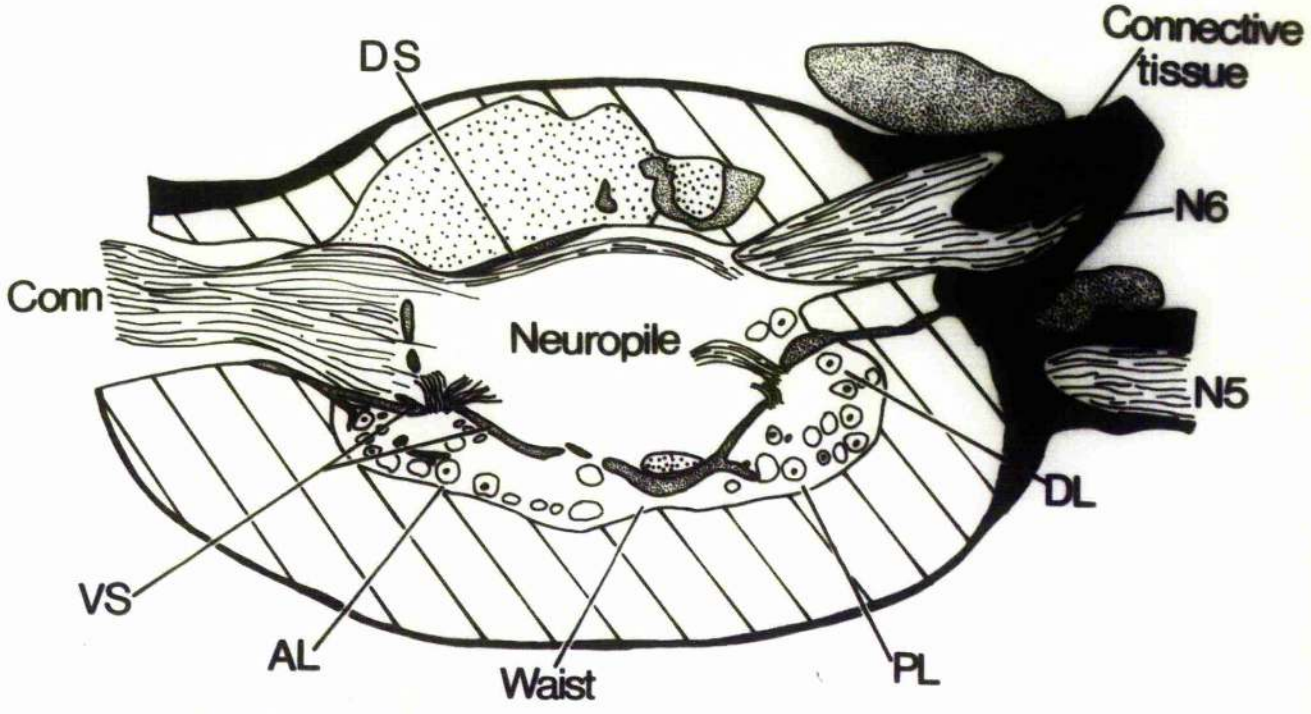
Figure 2.5.9. L.S. of 6A.G. 25μ to the left of the midline.

A. Mallory stained section.

B. Diagrammatic representation of A.

A

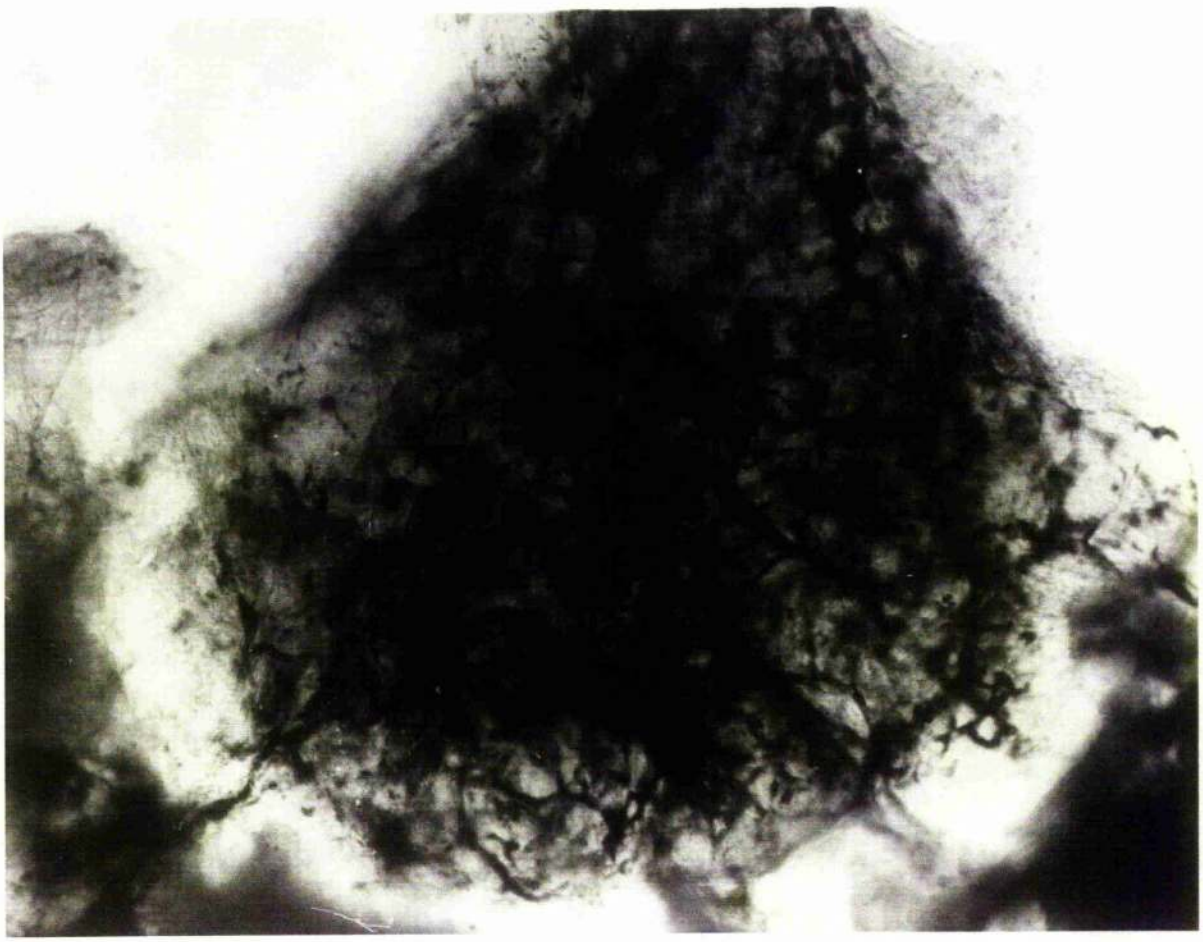




B

500 µ

Figure 2.5.10. Whole mount of indian ink injected specimen of 6A.G. from ventral aspect. This shows the network of fine capillaries ramifying through the ganglionic cortex and supplying individual neurone somata.



1mm

Section 2.6. Determination of the Region of the Sixth Abdominal Ganglion Responsible for Hindgut Control.

Introduction

It has recently been demonstrated in the lobster, Homarus americanus, that there is a high degree of constancy in the size, position and connections of any individual neurone (Otsuka, Kravitz and Potter, 1967). According to Otsuka et al, neurone somata tend to be grouped in relation to their embryonic origins. Hence the biochemical properties and the transmitters synthesized by members of the group are of the same type. Thus in Homarus americanus the somata of units with different transmitters, but supplying the same muscle, may lie in several different ganglia. In addition Kennedy, Remler and Selverston (1969) have suggested that since the soma is merely the trophic region of a given neurone, its position needs to have no relation to the geography of its processes so long as soma and processes remain connected. However, Cohen and Jacklet (1967) have shown that axons passing out through the same root of the metathoracic ganglion of the cockroach, Periplaneta americana, tend to be grouped in clusters. Further Bentley (1970), working on the locust flight system, has demonstrated that the somata of functionally similar neurones innervating the same muscle lie adjacent to one another and their fibres remain in a compact bundle throughout the neuropile. Kandel and Wachtel (1968) have suggested that the somata of neurones controlling the various organs and muscles supplied by the abdominal ganglion of the gastropod mollusc, Aplysia, are grouped according to the organs which they supply. The neurones of these groups not only have different embryonic

origins from one another, but in addition all those within a particular region show similar responses to a given transmitter.

Therefore, it can be seen that in invertebrates "the biochemical and functional properties of neurones correlate well with the topographical location of their somata" (Kandel and Kupfermann, 1970) within the ganglionic cortex. From this evidence it would seem that the somata of functionally similar cells innervating a given muscle, such as the circular muscle coat or the longitudinal muscle strands of the hindgut, might lie in adjacent positions to one another.

As a preliminary to initiating intracellular micro-electrode studies of the 6A.G. of Homarus gammarus in relation to hindgut function, I have attempted to define the general region in which the cell bodies controlling the hindgut lie. A previous knowledge of both the normal output of the 6A.G. (see Section 2.4) and its structure (see Section 2.5) were both necessary prerequisites to this work.

Materials and Methods

Two types of experiment were performed on the 6A.G.. In both series the ganglion was first exposed by removal of the ventral integument. It was then desheathed ventrally. In the first series of experiments an anterior median split was then produced using a fine scalpel. The second type of experiment involved the induction of lesions or ablations of the ventral cell bodies. Specific areas were ablated using fine forceps, or lesions were produced using a heat probe. This heat probe was constructed from fine tungsten wire (0.005 inches in diameter) through which a current was passed from a 6 volt power-supply. Best results were

obtained with the heat probe since it allowed more accurate localization of the lesion.

Prior to the experimental lesions, the V.N.C. was always crushed at the level of the 1-2 connectives in order to determine whether or not the hindgut was functioning normally. Post-lesion crushing of the anterior abdominal nerve-cord was also carried out and any alterations in hindgut notility were noted. The 6A.G./rectum complex and several abdominal ganglia were then removed from the animal and transferred to the plasticine-bottomed experimental dish described in Section 2.4. Recordings of spontaneous and evoked activity in the P.I.N.a.'s were then obtained. Finally, the ganglion alone was taken and prepared for light microscopy by the methods described in Section 2.1. The precise areas of the ganglion in which lesions had been induced were then determined and related to the motor output previously recorded from the ganglion. In this way the regions of ganglionic cortex, in which the cell bodies of neurones controlling hindgut function occurred, could be resolved.

Each of these experiments was carried out twice and in most cases three times.

Results

A. Splitting of the Ganglion

It was initially hoped that the ganglion could be split more and more posteriorly in successive preparations. However, the damage caused to the ganglionic cortex by splitting further than about the level of the first or second commissure (scale marks 105-122, with reference to Figure 2.5.1), proved too great for me to be able to detect any consistent effects on motor output. Splitting from

the posterior end forward also caused much unwanted damage. The results obtained are limited to four experiments in which the ganglion was split longitudinally in the midline from anterior to posterior. These results are summarised in Table 2.6.1.

In all cases it proved impossible to elicit the normal defaecatory response after inducing the lesions which were, in all but the first instance, to the level of the first commissure only. After creating the lesion, normal paired spontaneous activity remained in the P.I.N.a.'s. This is shown in Figure 2.6.1, which is taken from experiment 4. However, it was impossible to either modulate the activity of these units or cause burst formation by stimulation of either connective in any of the experiments. That interneurones causing tonic activity decussate in the first commissure is further borne out by the fact that the act of creating the lesion always causes hindgut motility.

In two cases (experiments 1 and 4) it was possible to elicit weak hindgut movements by crushing the V.N.C.. In these experiments normal phasic output was also found to occur on stimulation of the abdominal connectives. Thus if the interneurones causing phasic output decussate it is probable that they do not do so in the first or second commissures since phasic activity occurred in experiment 1 where both of these commissures were sectioned.

B. Extirpation of Neurone Somata

The effects of extirpation of various regions of the ganglionic cortex are summarised in Table 2.6.2 and Figure 2.6.2. Only the dorsal cell bodies were not destroyed due to major difficulties in approaching them. However, from the data obtained they do not seem to play a part in

Experiment	Commissure(s) severed	Hindgut movements elicited by crushing V.N.C.		Occurrence of hindgut movements as lesion made.	Activity in P.I.N.a.'s		
		1. Prior to lesion	2. After lesion		1. Spontaneous (paired)	2. Due to stimulation of V.N.C.	
						a. Tonic	b. Phasic
1	1st and 2nd	+	±	+	+	-	+
2	1st	+	-	+	+	-	-
3	1st	+	-	+	+	-	-
4	1st	+	±	+	+	-	+

The effect, on hindgut movements and motor activity down the P.I.N.a.'s, of splitting the 6A.G. anteriorly in the midline.

\+ indicates the presence of a particular kind of neural activity, or hindgut movements.

\- indicates the absence of the above phenomena.

± indicates the presence of very weak hindgut movements.

TABLE 2.6.1.

TABLE 2.6.2. The effect of extirpating various regions of the cortex of the 6A.G. on hindgut movements and motor output in the P.I.N.a.'s.

+	indicates the presence of hindgut movements or neural activity.
-	indicates the absence of these phenomena.
±	indicates the presence of a very weak response. This only occurs in the one case and presumably the hindgut movements are being driven by phasic units which have been shown to produce a weak response (Section 2.2.4).

TABLE 2.6.2.

Region Extirpated	Hindgut movements elicited by crushing V.N.C.		Activity in P.I.N.a.'s			Comments	See Figure
	1. Prior to lesion	2. After lesion	1. Spontaneous	2. Elicited a) Tonic	2. Elicited b) Phasic		
All anterior ventral cell bodies to level of 1st commissure.	+	+	+	+	+	Hindgut control centre not in this region.	2.6.2.A
Posterior ventral cell bodies between 1st and 4th commissures.	+	-	-	-	-	Control centre lies in this region	2.6.2.B. and 2.6.2.F.
Posterior ventral cell bodies posterior to 4th commissure	+	+	+	+	+	Control centre not in this region	2.6.2.C.
Medial posterior ventral cell bodies posterior to second commissure	+	±	-	-	+	Phasic units lie anterior to 2nd commissure	2.6.2.D.
Lateral regions of posterior ventral lobe.	+	+	+	+	+	Control centre not in these regions.	2.6.2.E.

control of hindgut movements.

Total ablation of the anterior lobe (Figure 2.6.2.A) does not affect hindgut movements nor the normal activity patterns of the motor output to the hindgut. The same is also true of lateral or posterior regions of the posterior ventral cell bodies (Figures 2.6.2.C and E). However, lesions induced in the anterior regions of the posterior ventral lobe (Figure 2.6.2.B) resulted in elimination of both hindgut movements and all motor output. This leads me to believe that any somata connected with motor neurones to the hindgut lie in the anterior central region of the posterior ventral lobe, as indicated in Figure 2.6.2.F.

I assume that no neurone somata of any significance to hindgut movements lie ventral to the dorsal lobes (i.e. between scale marks 145 and 170 with reference to Figure 2.5.1). This area was left intact in the experiment represented by Figure 2.6.2.B, but no motor output was recordable in that experiment. The same is true of the cells in the dorsal lobes which, as mentioned above, were never destroyed. When neurone somata in the medial region of the posterior lobe, posterior to the second commissure, were destroyed, as shown in Figure 2.6.2D, all activity except that of phasic units was abolished. Thus it would seem that the phasic units lie somewhere in the region of the first to second commissures (scale mark 105 to 125 with reference to Figure 2.5.1). The units which respond only tonically are thought to lie between the second and fourth commissures (i.e. between scale of numbers 125 and 155 with reference to Figure 2.5.1).

Whilst admitting that this is only a rough guide to the position of the somata controlling hindgut motility, it is useful in that a long search of the whole ganglion

with microelectrodes is now unnecessary. This search can be restricted to the region shown in Figure 2.6.2.F.

Figure 2.6.1. Spontaneous activity in the P.I.N.a.'s recorded from a split ganglion preparation. Continuous trace. This recording was made from the preparation used in experiment 4 of Table 2.6.1. Normal 'pairing' of at least 4 units takes place as is denoted by the arrows labelled 1 to 4. This figure is comparable with Figure 2.4.11 in which the ganglion was intact.

Upper beam - cut central end of right P.I.N.a.

Lower beam - cut central end of left P.I.N.a.

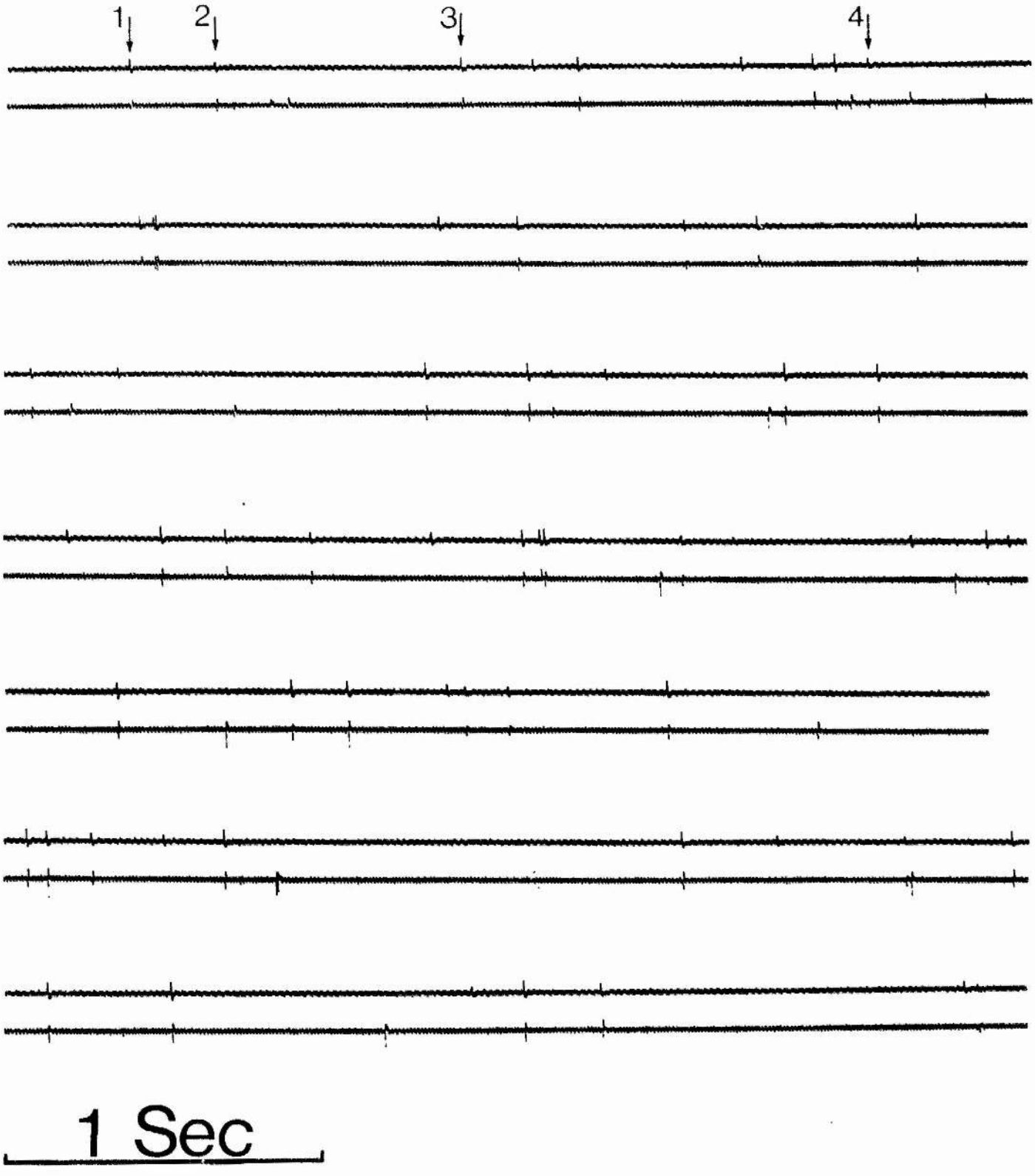

1
2
3
4
1 Sec

Figure 2.6.2. Diagrammatic representation of the regions of the 6A.G. extirpated as described in Table 2.6.2. Cross-hatching indicates the regions destroyed.

A. Ablation of the anterior ventral cell bodies.

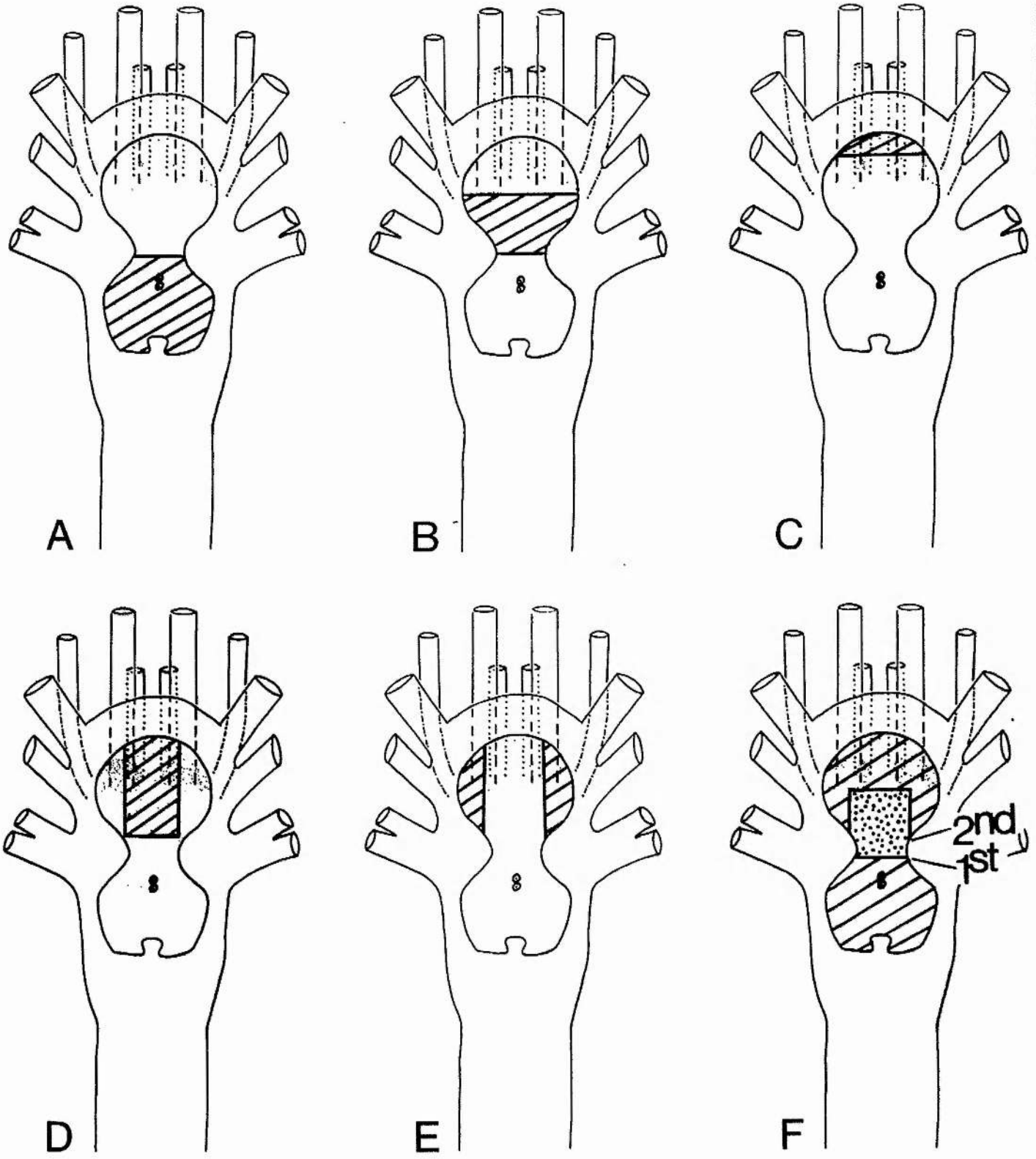
B Destruction of the anterior part of the posterior lobe to the level of the 4th commissure.

C. Ablation of the posterior lobe posterior to the dorsal lobes.

D. Ablation of the posterior lobe medially and posterior to the 2nd commissure.

E. Lateral lesions of the posterior ventral cell bodies.

F. Summary of the above results to indicate the most likely region of the ganglion in which the neurones controlling the hindgut movements should be found. Cross-hatching denotes regions of the ganglionic cortex whose somata are not connected with fibres to the hindgut. The dotted region indicates the area in which the somata of units responsible for hindgut motility are thought to occur. Phasic units apparently lie in the anterior part of this region between the first (1st) and second (2nd) commissures whilst tonic units seem to lie posterior to the second commissure.


A
B
C
D
E
F
2nd
1st

Section 2.7. Intracellular Microelectrode Studies.

Introduction

Although the neurone soma membrane of decapod crustacea is thought to be electrically inexcitable (Remler, Selverston and Kennedy, 1968) it is possible to monitor the activity in distant neurone processes, albeit in a rather attenuated form, by recording from glass microelectrodes inserted into the soma. This is especially true of motor neurones in which, unlike interneurones, the somata are thought to be joined to their branches by relatively short, thick processes (Kennedy et al, 1969). In interneurones the soma is separated from its major ramifications by a long thin process. Thus it is possible to elicit active electrical responses in crustacean motor neurones by electrical stimulation of the soma, but this is not the case with interneurones (Kennedy et al, 1969).

In previous sections (2.4 and 2.6) I determined the forms of motor output passing down the P.I.N.'s and have approximately located the region of the 6A.G. in which the somata of neurones responsible for this output occur. Earlier (Section 2.2) I demonstrated that these neurones could be activated by stimulation of interneurones which presumably originate in the brain. Hence, in the section that follows I have started probing the median anterior neurone somata of the posterior ventral lobe of the 6A.G. using microelectrodes, in the hope that I might penetrate the cell bodies of motor neurones supplying the 6A.G. Although this work is somewhat incomplete, some useful information is beginning to emerge.

Materials and Methods.

The 6A.G. was exposed and desheathed in the manner described in Section 2.6. The abdominal V.N.C., posterior to the second abdominal ganglion and with the rectum attached, was then transferred to the experimental dish and maintained as in Section 2.4. The 6A.G. was carefully freed from its connections with the hindgut and pinned out ventral surface uppermost. Recordings from the P.I.N.'s were made as in Section 2.4. The ventral somata of the 6A.G. were illuminated using a perspex light guide attached to a Prior dissection lamp. Glass microelectrodes filled with 2M.KCL and of 15-25 megohm tip resistance were used in all experiments. They were mounted on a Narashige micromanipulator and used to probe the cells of the medial anterior region of the posterior ventral lobe of the 6A.G. Shifts of membrane potential due to the electrode entering cell bodies were displayed on the oscilloscope via a Bak high impedance preamplifier with a gain of one. Once entry into a neurone soma had been achieved, positive going rectangular stimulus pulses were applied to it through the Bak bridge.

Results.

The membrane potential of the neurone somata of the posterior ventral lobe lies between 40 and 50 mV in the units so far penetrated. The types of units impaled up to now have caused either phasic or tonic activity, but never both. The approximate locations of these cells are shown in Figure 2.7.1. This demonstrates that many units concerned with hindgut motility lie within the region designated in Section 2.6. Although the phasically responding units have so far been found in the

anterior part of that region, it is too early to draw any firm conclusions as to the topography of the units described physiologically.

Impaling and stimulating either phasic or tonic units usually results in paired activity in the P.I.N.'s. In Figure 2.7.2 a phasic unit (number 1 on Figure 2.7.1), lying superficially and just to the left of the mid-line, was penetrated. Stimulation at 12Hz (Figure 2.7.2.A) produced a paired output both ipsilaterally (upper beam) and contralaterally (lower beam). Stimulation of the neurone soma at 30Hz (Figure 2.7.2.B) produced rather a different effect, reminiscent of that in Figure 2.4.7.A.2 and B.2. In this case higher frequency of stimulation caused the contralateral unit to drop out, whereas the ipsilateral unit in Figure 2.4.7 was the one to drop out. Such responses imply the presence of phasic units which laterally excite one another.

The record displayed in Figure 2.7.3 is taken from the same preparation as that shown in Figure 2.7.2. The arrow on each figure denotes spontaneously active paired units in both left and right P.I.N.a.'s. Intracellular stimulation of a single neurone soma (No. 2 on Figure 2.7.1), lying one to two cells deep on the left hand side of the anterior part of the posterior lobe, causes three units to fire tonically in the right P.I.N.a. Only one unit fires in the left P.I.N.a. and that is the very small spontaneously active unit which remains paired with the larger unit in the right P.I.N.a. even during burst formation. In several other experiments single units causing bursting activity of many neurones in both P.I.N.a.'s and P.I.N.p.'s have been detected. In the case shown in Figure 2.7.3 recording conditions in the upper trace were particularly bad but it seems

that no phasic units fire in either P.I.N.a. Thus it would appear that some major unit, driving several tonic follower neurones, had been penetrated. After cessation of tonic activity there was a period of post-stimulatory depression before the spontaneously active units commenced firing again. In Figure 2.7.4 a single unit was shown to be driven either directly or via an interneurone. The cell penetrated lay on the left hand side of the ganglion about one cell deep and in the position indicated by the number 3 on Figure 2.7.1. The output in the left P.I.N.a. was recorded and rhythmic, spontaneous activity was detectable. Stimulation of the penetrated soma, even at low frequencies, provoked tonic firing (Figure 2.7.4.A). Increasing the stimulation frequency decreased the number of pulses necessary to cause burst formation, as is shown in Figure 2.7.4.B and C. This is presumably because the extent of decay of excitatory membrane potentials between stimulus pulses is greatly reduced. High frequencies of stimulation caused this unit to 'switch off' as is demonstrated in Figure 2.7.4.D. This suggests that the unit stimulated was a driver interneurone. In Figure 2.7.4.A to C there is no indication of post-stimulatory depression.

Although this section is incomplete the initial results outlined above indicate that further study of the system, with a view to understanding the interactions of the neurones involved, would be well worthwhile. At the moment three types of units are thought to exist. These are phasic, tonic and driver units. However, if driver interneurones do exist they must be different in form from those described by Kennedy et al (1969) since stimulation of their somata evokes active membrane responses elsewhere.

Figure 2.7.1. Ventral view of 6A.G. to indicate the approximate positions of the neurone somata of units giving rise to phasic and tonic responses. Units causing a tonic response are represented by empty circles, whilst those causing phasic responses are indicated by filled circles.

1. A single superficial unit causing phasic responses in both left and right P.I.N.a.'s (and presumably the P.I.N.p.'s). Figure 2.7.2 shows the output produced by stimulation of this neurone soma.

2. A single unit causing tonic activity in both P.I.N.a.'s as demonstrated in Figure 2.7.3. It lies one to two cells deep.

3. A unit producing tonic output in the left P.I.N.a. Its output is illustrated in Figure 2.7.4. It lies about one cell deep.

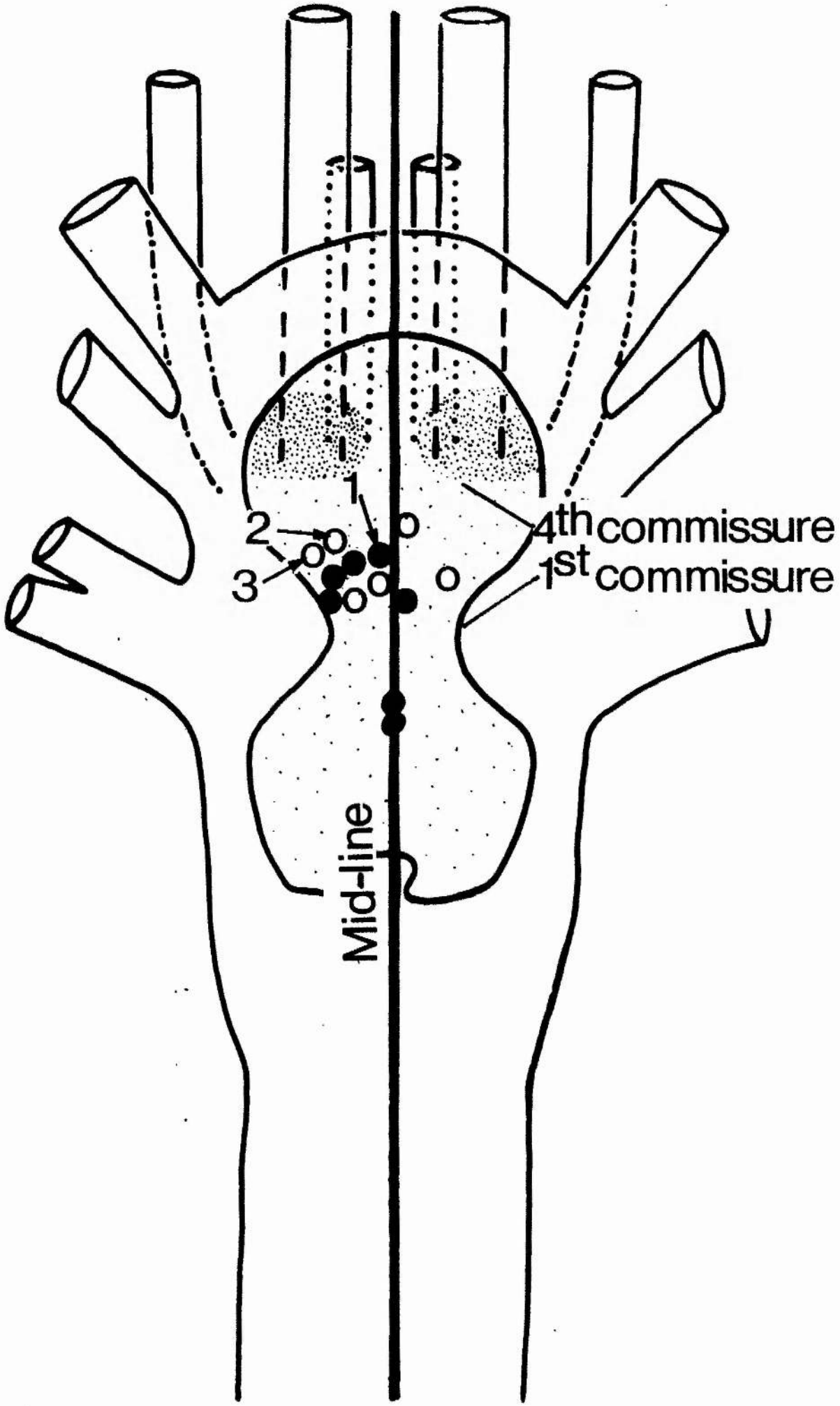

1
2
3
4th commissure
1st commissure
Mid-line

Figure 2.7.2. Phasic activity in the left and right P.I.N.a.'s produced by stimulation of a single unit lying just to the left of the mid-line in the anterior part of the posterior lobe (see Figure 2.7.1). The 6A.G. was isolated from the rectum.

Upper beam - left P.I.N.a. Lower beam - right P.I.N.a. Dots (which have been retouched) indicate stimulus pulses delivered intracellularly.

A. Stimulus pulses delivered at 12Hz cause phasic units to fire simultaneously in both left and right P.I.N.a.'s.

B. Increasing the stimulus frequency to 30Hz initially causes simultaneous phasic output in both P.I.N.a.'s, but the contralateral unit eventually slows down and then drops out. Two units, which laterally excite one another, have been postulated to explain this response. It is not thought likely that phasic output is caused by bifurcating axons (see Section 3.2.C). The arrow denotes paired spontaneously active units firing simultaneously in both P.I.N.a.'s. Their rhythmical output is unaffected by direct stimulation of the phasic unit on the left.

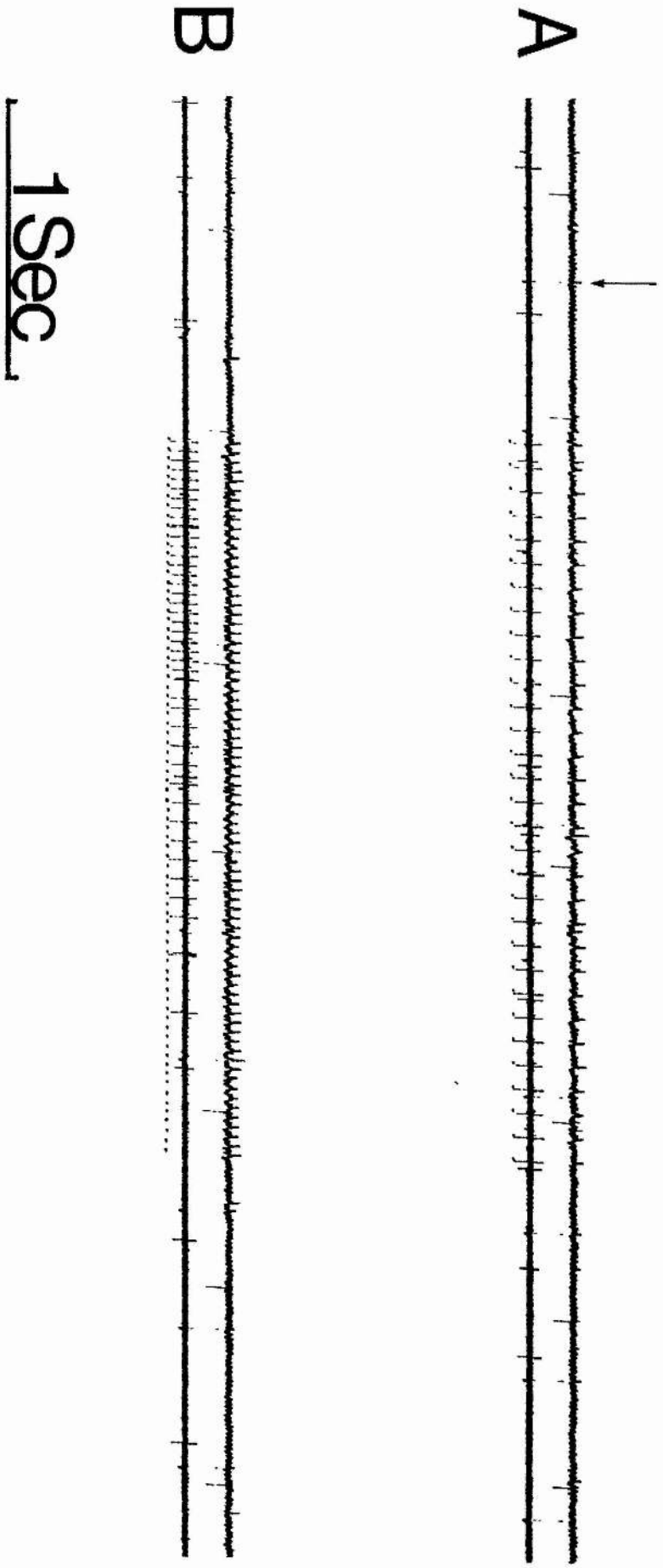

A
B
1Sec

Figure 2.7.3. Tonic activity in the left and right P.I.N.a.'s of an isolated 6A.G.

Upper beam - left P.I.N.a. Lower beam - right P.I.N.a. The arrow indicates the same pair of units as are arrowed in Figure 2.7.2. Stimulation of a single unit (number 2 on Figure 2.7.1) produces tonic activity in both P.I.N.a.'s. The arrowed units always fire simultaneously. Immediately after the cessation of the burst there is a post-stimulatory depression of spontaneous activity, but this recommences within two seconds.

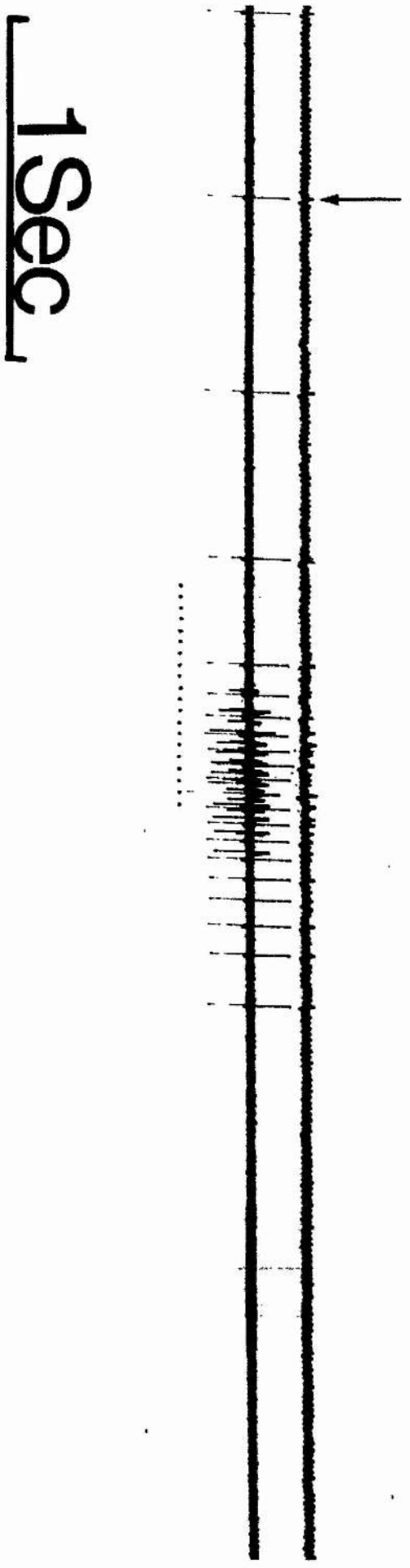
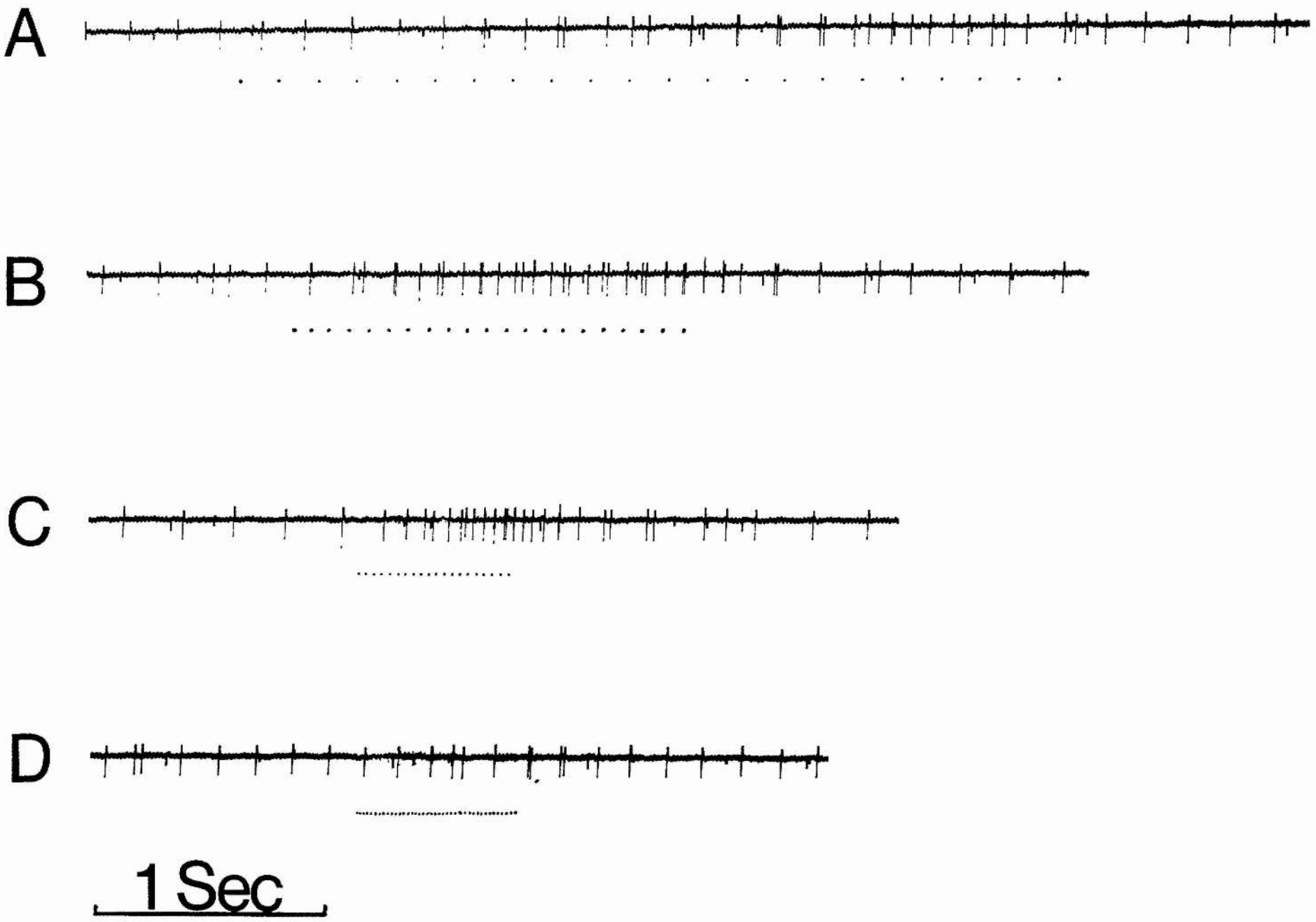

1Sec

Figure 2.7.4. A single tonic unit in the left P.I.N.a. of an isolated 6A.G. driven at a variety of frequencies and at constant stimulus amplitude. The frequencies used were:- A - 6Hz; B - 12Hz; C - 30Hz; D - 60Hz.

In A 17 stimuli were delivered before tonic activity was elicited, whilst in B and C only 7 or 8 stimuli were necessary. Presumably the excitatory potentials summating in the stimulated unit (which is number 3 of Figure 2.7.1) must have an initial slowly decaying phase, so that an increase in stimulus frequency from 12 to 30Hz makes little difference to the number of stimulus pulses necessary to elicit the response.

In D a stimulus train delivered at 60Hz produces no tonic response. The reason for this is unknown but it is possible that a driver cell was penetrated and the efficacy of its synapse breaks down at these higher stimulus frequencies. This correlates with the loss of tonic output above 50Hz demonstrated in Figure 2.4.12.


A
B
C
D
1 Sec

PART 3. DISCUSSION

Section 3.1. Anatomical Considerations.

A. The Hindgut.

As mentioned in the Introduction the hindgut of several macrurous decapods has previously been described. The relative lengths of the hindgut in relation to the midgut are very obviously different from one species to another. According to Alexandrowicz (1909) and Miller (1910) the rectum of the crawfish, Palinurus vulgaris and the crayfish, Astacus fluviatilis and Cambarus, is very elongated whilst the midgut is very short. In some marine types, such as Nephrops norvegicus (Yonge, 1924) and Homarus gammarus (Miller, 1910 and present observations), the hindgut is much shorter than the midgut.

In most macrurans the arrangement of longitudinal and circular muscles in the rectum is constant, but in Palinurus the number of longitudinal muscle strands is twelve as compared with the more usual six. In those species, such as Homarus, with a short hindgut, the circular muscle coat of the anterior region is rather better developed than that nearer the anus (see Figures 2.1.5 and 2.1.6) and the lumen is much wider anteriorly. In those types with a long rectum the posterior rectal region is highly muscular at the place corresponding to the anterior hindgut of types such as Nephrops and Homarus. In Homarus the muscular portion of the hindgut has now been shown to be the main faecal expulsion region (see Figure 2.2.2).

Cattaneo (1888), Guiyesse (1907) and Alexandrowicz (1909) have shown that the longitudinal muscles eventually

insert into the proctodaeal cuticle by what Cattaneo terms small tendons. The fibres inserting themselves into the chitin apparently have a tuft-like appearance according to Alexandrowicz. In all these types the proctodaeal cuticle is transversely ridged and according to Janisch (1923) the longitudinal muscles are divisible into strands some of which insert onto the anterior faces of the ridges and run caudad whilst others insert onto the posterior and inner faces of the ridges and run rostrally. He suggests that those muscles running forward act as levators, whilst those running posteriorly act as retractors on the ridges. The retractor muscles seem rather better developed and presumably depress the ridges during longitudinal muscle contractions. Levation of the transverse ridges may well assist the hindgut in gripping the faeces during the peristaltic part of the defaecatory response (see Section 2.2).

The five radial muscle groups lying around the anus (see Figure 2.1.1) have not been adequately described in previous publications although both Janisch and Miller acknowledge the presence of a dorsal and a ventral group which seem to correspond to R3 and R4 of Figure 2.1.1 respectively. The radial muscles arise from the proctodaeal cuticle as do the longitudinal muscles. It therefore seems probable that the embryological origins of these muscles and perhaps their properties might be very similar (see Section 3.2.A).

The arched muscles which are a continuation of the circular muscles are present around the anus of Homarus, Cambarus and Astacus, but according to Yonge they are absent in Nephrops. They may act as an anal sphincter muscle although this is most unlikely since they do not surround the anus.

B. The Posterior Intestinal Nerves.

According to all the major authors (Krohn, 1834; Lemoine, 1868; Police, 1908; Alexandrowicz, 1909; Miller, 1910; Janisch, 1923; Yonge, 1924 and Orlov, 1926) the P.I.N.'s of Astacus, Homarus, Palinurus, Cambarus and Nephrops arise from a single median root which then divides to send a pair of branches toward the anterior end of the hindgut and a single median branch into the region of the anus. Krohn, who worked on Homarus and Astacus, states that the root of the P.I.N.'s may sometimes be paired. In Homarus gammarus I have found that the P.I.N.'s usually arise from a paired root and each nerve then bifurcates to send ipsilateral branches to both the anterior hindgut and to the anal region (see Figure 2.1.1). It has already been noted (Section 2.1) that these nerves are very variable in their finer details and the P.I.N.p.'s may occasionally arise from the anal nerves. The presence of a single median nerve suggests that the neurones supplying the hindgut may arise from a single median region of neuropile whilst paired nerve roots are suggestive of a bilaterally symmetrical neuropile. The central positioning of neurones controlling hindgut function is discussed below (Section 3.1.C).

C. The Ganglionic Roots.

As has been demonstrated above (Section 2.2) the anal nerves have no motor function with respect to the hindgut and the so called anal dilator and anal compressor muscles, which were named by Schmidt (1915), are concerned with telson flexion. The anal nerves have been shown to carry afferent information from the telson and to give off branches in the region of the anus (Sections 2.1 and 2.3).

They also carry efferent information to the anal dilator and anal compressor muscles. The side branches passing towards the anus are assumed to carry afferent information from the soft cuticle around the anus on the basis of the evidence presented in Section 2.3. Thus the term 'anal' nerve (Keim, 1915) is a misnomer. Perhaps they should be renamed the 'caudal' or 'terminal' nerves when one considers their position. It might even be best to refer to them as 'root 5 of the 6A.G.'.

Larimer and Kennedy (1969) have shown that the anal dilator and compressor muscles of the crayfish, Procambarus clarkii, may incidentally change the shape of the anal orifice since they insert distally onto the soft cuticle around the anus, but their more important action is to flex the tail fan by their action on the telson. Both muscles are innervated by the 6A.G. but receive an entirely separate innervation. The anal dilator and compressor muscles respectively are suggested (Larimer and Kennedy) to behave like the serial homologues of the tonic superficial flexor and the phasic anterior oblique muscles of more anterior abdominal segments. They go on to suggest that the anal nerves of the crayfish are equivalent to the third roots of more anterior ganglia. However, the anal nerves of Homarus have also been shown to contain afferent fibres from soft cuticle receptors and this suggests an homology with the first or second roots of the more anterior ganglia. It is difficult to form a precise theory of the homologies of the roots of the 6A.G. with those of more anterior abdominal ganglia.

The first root of the 6A.G. supplies the swimmerets of the fifth segment and is thus homologous with the first root of a 'normal' ganglion. The uropod nerve, which is the second root of the 6A.G., supplies the anterior

oblique muscles, in common with the second roots of other abdominal ganglia. It also supplies many muscles of doubtful homology, which supply the uropods. It is possible that some of these muscles are equivalent to certain of the axial muscles of more anterior segments. I have, therefore, assumed the second root of the 6A.G. to be equivalent to the second root of other abdominal ganglia.

The third roots of abdominal ganglia 1 to 5, of Homarus, arise on the connectives posterior to each ganglion. It supplies the anterior and posterior oblique muscles as well as the superficial and deep flexors. The ventral telson nerve (3rd root) of the 6A.G. again supplies the anterior oblique muscles as well as the anal compressors and anal dilators. It also supplies many of the muscles associated with the uropods. As mentioned above the anal dilators and anal compressors are thought to be equivalent to the tonic superficial flexors and the phasic deep flexors (Larimer and Kennedy). Thus the third root of the 6A.G. is equivalent to the third root of a normal abdominal ganglion, but the additional nerve supply to the uropods suggests that it also has an affinity with a normal first root. My assumption is that the third root of the 6A.G. is equivalent to a fusion of the third root of a normal abdominal ganglion and the first root of a seventh ganglion.

The dorsal telson nerve (4th root) of the 6A.G. supplies both the anterior and posterior telson flexor muscles and the anal compressor muscle. The posterior and anterior telson flexors are both phasic muscles which are, according to Larimer and Kennedy, equivalent to the transverse and posterior oblique muscles of other segments respectively. The transverse muscles are

normally supplied by the second root of an anterior ganglion, whilst the posterior oblique muscles are usually supplied by the third root. In addition, the anal compressors are equivalent to the deep flexors which are normally supplied by the third root. Thus the fourth root of the ganglion is apparently compounded from normal second and third roots.

As mentioned above, the anal nerve may also be compounded from first or second and third roots which leads me to suspect that:-

a) normal second and third root function is unevenly divided between the 4th and 5th roots of the 6A.G., which would then be assumed to be a fusion of two ganglia,

or b) the 4th and 5th roots of the 6A.G. are equivalent to the fused roots of two separate ganglia which have become telescoped into one another.

Thus the 6A.G. would be composed of three separate ganglia, the first of which would be equivalent to a normal anterior abdominal ganglion as would be the second (with the proviso that its appendages would be the uropods rather than the swimmerets). The third ganglion would then be terminal and associated with the telson and proctodaeum. As such it would probably be lacking appendages so that the anal nerve would be equivalent to fused 2nd and 3rd roots.

The 6th root of the 6A.G. is the P.I.N. This is not homologous with any root in the other abdominal ganglia and would probably be associated with a terminal ganglion.

The affinities of the roots of the 6A.G. are summarised in Table 3.1.

TABLE 3.1.

Root of 6A.G.	Muscles supplied.	Homologue of root in abdominal ganglia 1 to 5.	Ganglion with which roots are associated.
N1 - anterior nerves.	Swimmeret muscles and pleura.	1st	6th
N2 - uropod nerves.	Anterior oblique muscles and muscles to uropods.	2nd	6th
N3 - ventral telson nerves.	Anterior oblique muscles. Muscles to uropods. Anal compressor) Anal dilator) muscles.	3rd + 1st	7th
N4 - dorsal telson nerves.	Anterior and posterior telson flexor muscles. Anal compressor muscle.	2nd + 3rd	7th
N5 - anal nerves.	Anal compressor and dilator.	2nd + 3rd	Terminal.
N6 - P.I.N.'s.	Muscles of hindgut.	No homologue.	Terminal.

D. The Sixth Abdominal Ganglion.

As shown in Section 2.5 the 6A.G. of Homarus is a rather complex structure though somewhat less so than any of the thoracic ganglia.

The function of the layer of vacuolated cells, lying external to the nervous elements of the ganglion, is unknown. It is possible that it may serve as a shock-absorber to prevent undue activation of central neurones by pressure during violent abdominal movements such as are involved in the tail flick. Such tissue has not so far been described in other ganglia of the lobster V.N.C. The non-vascular space is another feature of the 6A.G. whose function is unknown. It does not seem to be in continuity with the blood system, although it is difficult to be certain of this point.

The vascular system of the 6A.G. is highly complex. The delicate tracery of capillaries surrounding the neurone somata leaves one in little doubt as to their trophic function. Many cells in the abdominal ganglia of Homarus have been shown to be neurosecretory in character (Johansson and Schreiner, 1965; Schreiner, Staaland and Johansson, 1969). The presence of the massive ventral sinus lying dorsal to the ventral cell bodies and even wrapping over them, leads me to wonder whether the axonal processes of the neurosecretory cells ever reach the neuropile. It is quite conceivable that they could discharge their contents into the ventral sinus if their dendrites were activated in the neuropile.

Like other ganglia of the V.N.C. the 6A.G. is very obviously a bilaterally symmetrical structure and the two halves are linked by commissures. Kendig (1967) has shown that there are three major commissural groups in the third abdominal ganglion of Procambarus clarkii, whilst Horridge

(1965) indicates that there are only two in the first and second abdominal ganglia of Astacus. In Homarus gammarus there are four major commissural groups lying across the 6A.G. which indicates that the ganglion was formed by fusion of at least two ganglia during evolution. The structure of the cortex bears out this fact, there being two major ventral lobes and a pair of symmetrical dorsal lobes of cell bodies (not three dorsal lobes as Johansson and Schreiner (1965) state).

As was mentioned above (Section 3.1.C) the 6A.G. may have been the product of fusion of three ganglia. Support for such a theorem may be gained when one considers the ganglion more closely, even though the number of commissural groups is still quite low. Fusion of ganglia is presumably the result of either reduction or shortening of body segments in decapod crustacea. This would cause fusion of neuropiles and presumably the loss of some duplicated units. However, those units remaining could be contained in a lesser volume of neuropile than previously, due to shortening of dendritic and axonal processes, especially in the case of units involved in interganglionic communication. The cortex might be relatively larger in relation to the neuropile in such fused ganglia and would then tend to enfold the neuropile. In the case of the 6A.G. the anterior and posterior ventral lobes may have arisen from the cortices of the sixth and seventh abdominal ganglia respectively. The dorsal lobes which wrap over the posterior surface of the 6A.G. would then be the neurone somata of the terminal ganglion of the ventral chain. The positions of the cell bodies of the neurones controlling hindgut movements (see Sections 2.6 and 2.7) would seem to belie this proposition, but presumably there would be changes of position of nerve cells if ganglia were to be

fused in this drastic manner. The close apposition of the origins of the anal nerves and the P.I.N.'s (see Figure 2.5.6) adds further weight to the argument that both arose from the terminal abdominal ganglion.

If the above hypothesis proved to be correct, what would be the function of the dorsal cell bodies? Since the anal nerves supply the anal compressor and anal dilator muscles,it is assumed that the dorsal cortical lobes contain the motor neurone somata of the units innervating these muscles. The somata of the nerve fibres terminating in the multitudinous sensory structures of the telson have never been detected. It is possible that these somata might lie in the dorsal lobes of the 6A.G. Alexandrowicz and Whitear (1957) provide evidence that sensory cell bodies may occur in the central nervous system. The receptor cell described above (Section 2.3) and those indicated by Pabst and Kennedy (1967) all lie within major nerve roots, thus demonstrating that the somata of receptor neurones can be quite variable in position. Such positional characteristics imply that the dendrites must be capable of active discharge and Pabst and Kennedy have shown this to be the case. Active discharge within dendrites was first shown to occur by Mellon and Kennedy (1964) and was later demonstrated by Mendelson (1966).

Section 3.2. Physiology.

A. Hindgut Motility.

As was indicated above (Section 2.2) the hindgut of Homarus can produce powerful peristaltic waves when driven by stimulation of the V.N.C. In this way the defaecatory response, outlined in Figure 2.2.2, may be caused. That this response is under direct central control is not in

doubt since peristaltic waves have been shown to be induced by bursts of activity in the P.I.N.'s (Section 2.4). The neurones responsible for control of hindgut movements lie in the 6A.G. (see Sections 2.6 and 2.7), but these represent only a final motor pathway to the hindgut.

Miller's work (1910) demonstrated that neurogenic mechanisms controlled the hindgut, but a comparison of his work with my own (Table 2.2.1), indicates certain discrepancies which I have attempted to explain below:-

a) Miller found that stimulation or severance of the anal nerves produced a single anal opening. With this I disagree. Miller's diagram of the 6A.G./rectum complex in Homarus shows the anal nerves to give off major branches to the posterior rectum as well as to the anus. As I have shown elsewhere (Section 2.1), the P.I.N.p.'s and the anal nerves of Homarus are occasionally fused and I suspect that Miller carried out his experiments only on animals in which this fusion had taken place. Severance of the combined anal nerves and P.I.N.p.'s proximal to their ultimate division could well produce a single anal opening. However, it is less easy to see why direct stimulation of such combined nerves for several seconds would fail to produce the anal rhythm. It is probable that Miller did not earth his preparation, although he gives little in the way of experimental detail and it is not possible to confirm this supposition. However, if current spread occurred, stimulation 'distal' to the division of the nerves may have been sufficient to induce a single anal opening. Support for such a suggestion is found in Miller's statement that the extent of anal opening was more marked on the stimulated side.

b). I found Miller's assertion that direct stimulation of the hindgut would produce co-ordinated hindgut

and anal movements, to be insubstantiated. Perhaps this discrepancy was again due to current spread down the rectum.

Although the neurogenic basis of the defaecatory response has been conclusively demonstrated (Sections 2.2 and 2.4), myogenically originating hindgut movements also seem to occur (Figure 2.2.10). Such movements could be produced by pacemaker cells of a myenteric plexus, if this exists. Therefore the possibilities of neurogenic or myogenic control of hindgut movements are discussed below.

1. Neurogenic Hindgut Movements.

It is apparent from the experiments in which various branches of the P.I.N.'s were severed that the hindgut is divisible into regions (Figures 2.2.3 to 2.2.6): an anterior region controlled by the P.I.N.a.'s and a posterior region, including the extrinsic anal muscles, controlled by the P.I.N.p.'s. Stimulation of the V.N.C. after splitting the hindgut into separate anterior and posterior regions, revealed that the basic co-ordination of the defaecatory response was undisturbed by such treatment. At least a single cycle of the defaecatory response can be caused by a short burst of activity in the P.I.N.'s (Figure 2.4.16.B). In fresh preparations such a burst of activity will evoke complex hindgut movements and an anal rhythm as well as the defaecatory response (Figures 2.4.17 and 2.4.18). Thus it seems that bursting activity in the P.I.N.'s will co-ordinate the initial response of the hindgut, but once the response is initiated other factors come to bear. The factors underlying the anal rhythm (Figure 2.2.7.E) may well be similar to those governing the spontaneous contractions of the hindgut (Figure 2.2.10), except that the anal rhythm must first be set going by

activity in the P.I.N.p.'s.

2. Myogenic Hindgut Movements.

The spontaneous rhythmicity of the hindgut displayed in Figure 2.2.10 is thought to be due to the presence of numerous independent oscillators within the longitudinal and circular muscles of the hindgut, whether the 6A.G. is present or absent. These movements are of much less amplitude than the movements involved in the defaecatory response (see Section 2.2). Various authors (Alexandrowicz, 1909; Ebara, 1969) have observed the independent contractions of individual longitudinal muscle ridges of the hindgut in other macrurous decapods. Figure 2.2.10 demonstrates that the same situation obtains in Homarus. Ebara, working on the crayfish Procambarus clarkii, concludes that each longitudinal muscle strip of the hindgut is governed by a pacemaker, which causes rhythmic contractions of the longitudinal muscle. The frequency of beat (about 10 per minute) was rather greater than that in Homarus, which rarely exceeded a frequency of 1 per minute. In Procambarus the site of the pacemaker in isolated longitudinal muscle strips was fairly constant and lay anteriorly. Destruction of a pacemaker site resulted in a more posterior pacemaker driving the rhythm. Successive obliteration of pacemaker sites caused a steady diminution in the contraction rate until contractions of the muscle strip finally ceased. Measurement of the pacemaker potentials driving these muscles showed them to depolarize very slowly, i.e. anything from 60-190mV per second during the 'rapid depolarization' phase. The amplitude of the action potential at the pacemaker site (about 20mV) was lower than elsewhere in the muscle strip.

On the basis of this evidence it seems likely that individual striated muscle fibres of the longitudinal muscle bundle are capable of producing spontaneous activity similar to that occurring in the myogenic hearts of molluscs and vertebrates (Prosser and Brown, 1962). However, the pacemaker strips have not been proved to be completely free of ganglion cells. Prosser et al (1965) assert that the electrical activity of the crustacean abdominal intestine is nervously conducted. Their findings are not in conflict with Ebara's results since the terminals of the motor nerves ramifying over the intestine would naturally be stimulated either by the drugs or stimulus pulses which they applied. It is unnecessary to assume that these procedures activated a myenteric plexus mediating the responses of the hindgut. I would support the view, expressed by Campbell and Burnstock (1968), that the major co-ordinating activities of the arthropod gut occur either in peripheral ganglia (e.g. the stomatogastric ganglion) or within the central nervous system. They make the valid point that "the arthropodan plexus is more reminiscent of a somatic system than of the enteric system in other phyla". The plexus is rather superficial and appears to be made up largely of efferent fibres arising outside the gut and efferent fibres arising from the neurosensory cells of the gut wall. The presence of ganglion cells within the gut wall has not been demonstrated. In the vertebrate gut co-ordination is mainly carried out via axosomatic synapses which do not occur in the arthropod gut. However, axosomatic synapses do not occur in the arthropod central nervous system either and would not be expected to occur in the gut. Campbell and Burnstock concede that axoaxonic or axodendritic synapses might be responsible for the intrinsic nervous control of

intestinal motility. Such synapses are as yet undemonstrated and could only occur between sensory and motor units in the absence of interneuronal elements. Even though Alexandrowicz (1909) reports the sensory and motor elements to be in contact, Orlov (1926) denies this state of affairs.

The general conclusion is that the hindgut of Homarus is under direct central control from the 6A.G. However, the motor pathways from the 6A.G. to the hindgut are dominated by interneurones (see Section 3.2.C) descending from the brain. Much spontaneous activity occurs in the P.I.N.'s and this may very often be rhythmic (see Figures 2.7.2 and 2.7.3), but in the absence of commands from higher centres burst formation never occurs. The outputs of the stomatogastric ganglion (Morris and Maynard, 1970) and the cardiac ganglion (Maynard, 1960) are likewise governed by regulatory neurones from the central nervous system. These ganglia are more capable of independent action than the group of neurones in the 6A.G. which supply the hindgut. They are more truly autonomous in relation to the central nervous system, but all endogenously active systems must ultimately be controlled by some means so that the organism can withstand alterations of environmental conditions (whether internal or external).

The presence of a continuous barrage of spontaneous activity passing to the hindgut down the P.I.N.'s (see Figure 2.4.11) is likely to have some effect on the muscles of the hindgut. Schreiner et al (1969) observed that, in specimens of Homarus vulgaris (= gammarus) from which the 6A.G. had been extirpated, the hindgut was greatly dilated as compared with that of control animals. The spontaneous efferent barrage, if it has a function, may thus maintain the balance of tone between longitudinal and circular muscles of the rectum.

The radial muscles are of interest in that they rarely respond spontaneously, although the anal rhythm may continue for long periods (57 sec. in one of Miller's experiments) after cessation of stimulation of the P.I.N.'s. As mentioned in Section 3.1.A the radial muscles may have similar origins to those of the longitudinal muscles. Their rhythmic properties may thus be a development of mechanisms similar to those producing spontaneous rhythmic hindgut contractions (see Figure 2.2.10). Figure 2.1.1 illustrates that the radial muscles are directly innervated by the P.I.N.p.'s in much the same way as the somatic muscles. I have never observed the radial muscles to be polyneuronally innervated, nor have I observed any development of a plexus upon them. Experiments on immobilised radial muscles indicate that their rhythmicity is an inherent phenomenon (see Figure 2.2.9) and not due to simple mechanical factors such as rebound of the soft cuticle around the anal lips, nor to the participation of the arched muscles around the anus. Mechanical factors might enhance either the frequency or amplitude of the anal rhythm in the intact animal. The basic cause of such rhythmic behaviour is thought to be due to an endogenous oscillatory mechanism within the radial muscles, activated by stimulus pulses or motor output and causing rhythmic shifts of membrane potential. The first maximal radial muscle contraction is assumed to be connected with activation of the oscillator, since graded responses of less than maximum amplitude are directly related to the number of stimulus pulses delivered to the P.I.N.p.'s at a given frequency (see Figures 2.2.7 and 2.2.8). Spontaneously active oscillators occur in the longitudinal strands and in the circular muscle coat of the rectum (Figure 2.2.10). Perhaps the radial muscles represent an

evolved form of such an oscillator gradually brought under central nervous control.

The sarcomere lengths of the various hindgut muscles are summarised in Table 2.1.1. All lie in the upper part of the 4-8μ range. Hoyle (1967) suggests that such a sarcomere length would imply passively or gradedly responding fibres. This fits with their known properties of slow contraction and with the slow rise times and low amplitudes of the membrane responses recorded by Ebara (1969).

B. Function of Receptors.

The receptors occurring on the soft cuticle around the anal lips are reminiscent of the cutaneous mechanoreceptors described by Pabst and Kennedy (1967). The somata of these cells are located in the proximal region of the first and second ganglionic roots of each abdominal ganglion of the crayfish, Procambarus clarkii. They innervate the hypodermis of soft cuticle associated with the superficial flexor muscles and the bases of the appendages. Individual cells may innervate widely separated areas of hypodermis and their average dendritic conduction velocity is about 0.6-0.8 metres/sec., though that of their most proximal thickened portion may be as much as 1.5 metres/sec. They produce reflex suppression of motor discharge to the postural flexor muscles and the swimmerets. Many units are involved - up to six per ganglionic root - and they may pass centrally either ipsilaterally or contralaterally. They converge onto 21 single multisegmental interneurones most of which suppress motor discharge to the flexor muscles.

The neurones I have described in the anal nerves (see Section 2.3) also respond to deformation of the hypodermis

of the ventral soft cuticle. I envisage them to be the last in the abdominal series of soft cuticle receptors. They do not appear to reflexly modulate the motor discharge to the hindgut. Comparison of Figure 2.4.16.B, in which the nerves to the hindgut were all intact, with Figure 2.4.13, in which an isolated portion of the abdominal ganglionic chain was studied, indicates that the form of the afferent bursting discharge down the P.I.N.'s is fixed. This apparent immutability of the discharge might not be so apparent if whole animal preparations were used. The axons of the anal receptors may converge onto the multisegmental interneurones described by Pabst and Kennedy and hence suppress motor discharge to the postural flexor muscles. Put more simply, they may merely be non-specific mechanoreceptors which respond to anal movements only because of their position and in a most incidental way. The soft cuticle receptors in the region of the anus may well be the anatomical manifestation of the physiologically described phaso-tonic proprioceptors responding to telsonal flexion in Procambarus clarkii (Barth, 1964). Soft cuticle receptors of more anterior segments respond to swimmeret movements and it is likely that soft cuticle receptors near the anus respond to movements of the uropods. On the basis of the argument laid out in Section 3.1.C soft cuticle receptors should be found in all the somatic roots of the 6A.G., since all have homologies with the first or second roots of more anterior ganglia. The presence of sensory fibres in the P.I.N.'s is by no means certain, but it would be possible for receptor cells to innervate the soft cuticle of the proctodaeum.

I have taken the view that the anal receptors correspond to the receptors which Wiersma and Hughes (1961) describe as discharging onto ipsilateral and contralateral

interneurones during pulling out of the anal valve in Procambarus. The proximal terminations of these interneurones is as yet unknown. They must run at least as far as the third abdominal ganglion since Wiersma and Hughes recorded from the 3-4 connectives and it is possible that they may terminate in the brain. Thus the soft cuticle receptors may well subserve a multiplicity of functions due to their apparent lack of specificity.

Although it has not been possible to demonstrate the presence of receptors innervating the hindgut physiologically, their presence has been demonstrated on several occasions (Alexandrowicz, 1909; Janisch, 1923; Orlov, 1926). It is just possible that the fine dendritic processes of these cells innervate the proctodaeal cuticle in much the same way as the cells described above innervate the ventral soft cuticle of the abdomen. Weight is added to this supposition by the fact that the proctodaeal cuticle is an invagination of the ventral soft cuticle which forms the anal lips. In addition, the longitudinal muscle strands are associated with the proctodaeal cuticle as has been demonstrated by Cattaneo (1888) and Janisch (1923). Cuticular deformation must thus be a concomitant of hindgut movements. However, even if such receptors do occur their presence or absence does not affect the motor discharge down the P.I.N.'s, at the level of the 6A.G. (Figures 2.4.16.B and 2.4.13). These receptors, if present, may pool their input onto a common interneurone terminal, possibly in association with the input from the anal lips. The receptor discharge is not thought to be caused by the presence of faeces in the hindgut (see below) although it would probably occur in response to defaecatory movements. The interneurone(s) responding to this afferent information could easily reflect a patterned discharge

anteriorly. The rhythmic contractions of the intestinal muscles might well cause a constant receptor barrage to impinge on anterior going interneurones. Such an input into the brain would maintain the excitatory state of neurones causing hindgut movements. Any additional input, from whatever source, might then be sufficient to activate these interneurones and thus the motor network supplying the hindgut.

In addition to the receptors described above, Orlov (1926) has indicated the presence of pyloric sensory cells (see Introduction) whose dendrites innervate the hindgut after passing posteriorly from the pyloric region of the stomach. The axons of these cells enter the commissural ganglion. Three types of receptors are thus thought to innervate the hindgut:- ventral soft cuticle receptors, proctodaeal cuticle receptors and pyloric sensory cells. None of these modulate the motor activity to the hindgut at the level of the 6A.G. All may be centrally represented in the tritocerebral region of the brain.

From evidence presented by Herrick (1895) it would seem that lobsters tend to forage for food at night and are comparatively inactive during the day. This is the opposite of the situation in the gastropod mollusc, Aplysia californica, which forages during the daytime and is quiescent at night. Strumwasser (1967) has demonstrated the presence of a parabolic burster neurone, in the parieto-visceral ganglion. This neurone acts as an endogenous oscillator. In the absence of all synaptic input it emits spontaneous bursts followed by periods of silence. In isolated parieto-visceral ganglion preparations from animals exposed to several cycles of photoperiod (12 hours light followed by 12 hours darkness)

the parabolic burster shows a large peak of impulse rate around the projected dawn. Such neurones may occur in many other phyla, and are probably responsible for the persistent diurnal rhythmicity of Astacus, Orconectes, Procambarus and Cambarus (Brown, 1961), when these types are kept in constant darkness. The role of such endogenously active neurones would then be to cause arousal in previously quiescent animals. If they exist in lobsters they may initiate foraging and feeding among other activities. Receptors of the stomach and mouthparts (Dando and Laverack, 1969; Laverack and Dando, 1968) may then impose their excitation on the groups of neurones in the brain which control the hindgut. Summation of e.p.s.p.'s would then activate the interneurones causing the defaecatory response.

In view of Morris and Maynard's (1970) investigations, in which the activity of the stomatogastric system was suspected to be modulated from higher centres, and my findings that the hindgut is controlled from the brain, it now seems very likely that the presence of a major centre co-ordinating all intestinal movements will be detected, probably in the tritocerebrum. This suggests that the processes of feeding and defaecation are entirely automatic, and dependent upon one another, but I have observed that lobsters which have been starved for many weeks still defaecate. Perhaps the defaecatory response is driven by some cerebral oscillatory mechanism whose activity is only modulated in time by feeding. The absence of a receptor discharge (see Section 2.3) during artificial dilatation of the rectum suggests that faeces entering the rectum do not cause a reflexive output from the 6A.G. to promote the defaecatory response. In fact it is thought, from observations on many lobsters, that faeces are always

present in the rectum and midgut and are bound together in a mucous string. Defaecation would then simply drag this string further through the midgut, which is only weakly contractile (Yonge, 1924), and push its posterior end out through the anus. Lobsters maintained in aquaria may often have short strings of faeces hanging from the anus (personal observation) and these may remain there for several hours.

According to Horridge (1968) "the normal pattern of a sequence of motor impulses need not depend on feedback from the periphery" especially in invertebrates. Normal patterning (though often at reduced frequencies) in the absence of afferents has been demonstrated. Pasztor (1969) found that rhythmic motor output would continue to issue from the isolated sub-oesophageal ganglion to the scaphognathite of several macrurous decapods even though there is normally a rich sensory input from that organ. Similarly, rhythmic beating of the maxillipeds of many brachyurans has been shown to be due to the activity of a central oscillator (Burrows and Willows, 1969) and according to Davis (1969) the cyclic motor patterns controlling swimmeret beating in the lobster, Homarus americanus, are produced by purely central nervous mechanisms. The intrasegmental reflexes of the swimmeret system merely serve to amplify the cyclic motor patterns. Finally, Wilson (1961) has demonstrated that normal locust flight movements can carry on at half-normal frequency in the absence of a very rich sensory input. All that is required to produce the flight pattern is the non-phasic stimulus of air blowing on the sensory setae of the head.

The defaecatory response of the lobster is also thought to be a centrally derived phenomenon. The fixity of the form of the bursting discharge (see Figure 2.4.13) and its

immutability in the absence of sensory input (see above) bears out this assertion.

C. Neural Mechanisms Underlying Hindgut Movements.

The approximate positions of the somata of neurones initiating hindgut movements have been determined (Figures 2.6.2.F and 2.7.1). They do not occur in the dorsal cortical lobes, as stated by Horridge (1965), but in the posterior ventral lobe. Although intracellular studies of these neurones have only been initiated, something of their interactions and structure can be surmised from the results obtained (Sections 2.4, 2.6 and 2.7).

1. Command Interneurones.

It has been indicated (Section 2.2) that stimulation of either oesophageal connective anterior or posterior to the commissural ganglion could activate the mechanism within the 6A.G., controlling hindgut movements. The two types of efferent activity recordable from the P.I.N.'s by stimulation of abdominal connectives at different stimulus amplitudes show that at least two command interneurones, presumably originating in the brain, control neurogenically originating hindgut movements. As mentioned above (Section 3.2.B) the foregut is controlled from the tritocerebrum which includes the commissural ganglia, and it is thought likely that hindgut function is controlled from the same region. The command units eliciting tonic activity in the hindgut have been shown to decussate in the first commissure of the 6A.G. (Section 2.6). It seems unlikely that the interneuronal units causing phasic activity decussate in the first or second

commissures when one considers Table 2.6.1, but the results presented in Figure 2.4.7.A.2 and B.2 suggest that either these command neurones or the phasic units themselves decussate in commissures three or four. It seems likely that the interneurones must decussate, since direct driving of a phasic unit at high frequencies results in the contralateral response dropping out (Figure 2.7.2.B) and not as in Figure 2.4.7 where the ipsilateral response drops out first. In addition, the increase in conduction time between stimulus pulse and phasic response when ipsilateral interneurones are stimulated instead of contralateral interneurones (Figure 2.4.3), suggests that excitation of motor units or their driver units takes place contralaterally and that this activity is then reflected back to the ipsilateral side of the ganglion. Thus the profiles of these interneurones might be envisaged as in Figure 3.1, although more than one class of phasic interneurone (I1) is now thought to exist.

2. Neural Networks* in the 6A.G.

Two types of efferent activity to the hindgut have been described (Section 2.4). These are phasic activity and tonic activity. The two types of output are basically activated by the two different kinds of interneurones envisaged in Figure 3.1. The interneurones (I1) driving phasic units can generally be stimulated at lower stimulus amplitudes than those driving tonic units (I2). However,

* I have used the term network as a convenient term to refer to neuronal models. I do not imply any similarity to the diffuse neuronal systems such as are found in coelenterates.

Figure 3.1. Decussation of command interneurones activating the defaecatory mechanisms within the 6A.G.

I1 - interneurone(s) activating phasic motor units.

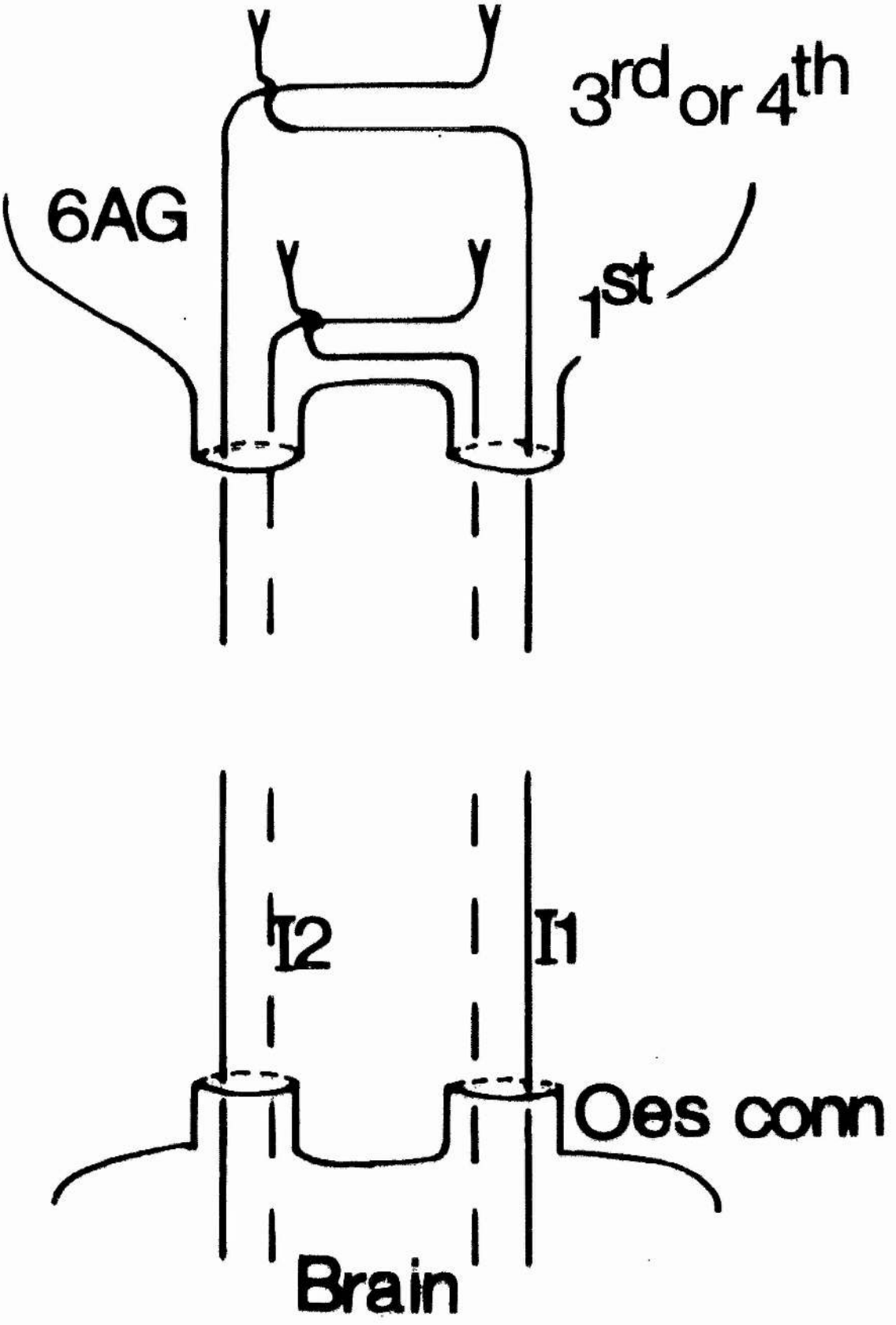
I2 - interneurone activating tonic motor units.

Brain - tritocerebral region of the brain.

Oes. conn. - oesophageal connectives.

1st - first commissure.

3rd or 4th - third or fourth commissure.


3rd or 4th
6AG
1st
I2
I1
Oes conn
Brain

units responding in a phasic manner to I1 may respond tonically to I2 (Figure 2.4.3). That phasic and tonic motor neurones generally exist as separate classes is demonstrated in the records of Section 2.7 where intracellular stimulation was utilised. In some cases phasic units were shown to be driven by intracellular depolarisation (Figure 2.7.2) whilst in others penetration of a single neurone soma caused three units to respond tonically (Figure 2.7.3). This suggests that tonic units must either be closely connected with one another so that by lateral excitation they produce a grouped discharge (see Figure 3.2.A) or they may in fact be a type of phasic unit activated by a tonically discharging driver interneurone (see Figure 3.2.B). Such tonic driver cells might also elicit the apparent tonic response of otherwise phasic units (see Figure 2.4.3). In this case I1 could be envisaged as acting directly on a phasic unit whilst I2 would act on both classes of motor unit through a tonic driver neurone (Figure 3.3). Inhibition of I1 during firing of the tonic driver unit may also occur, although such a circuit is not thought to be vital. A branch of the driver unit could well inhibit I1 as shown in Figure 3.3.

a. Phasic Network.

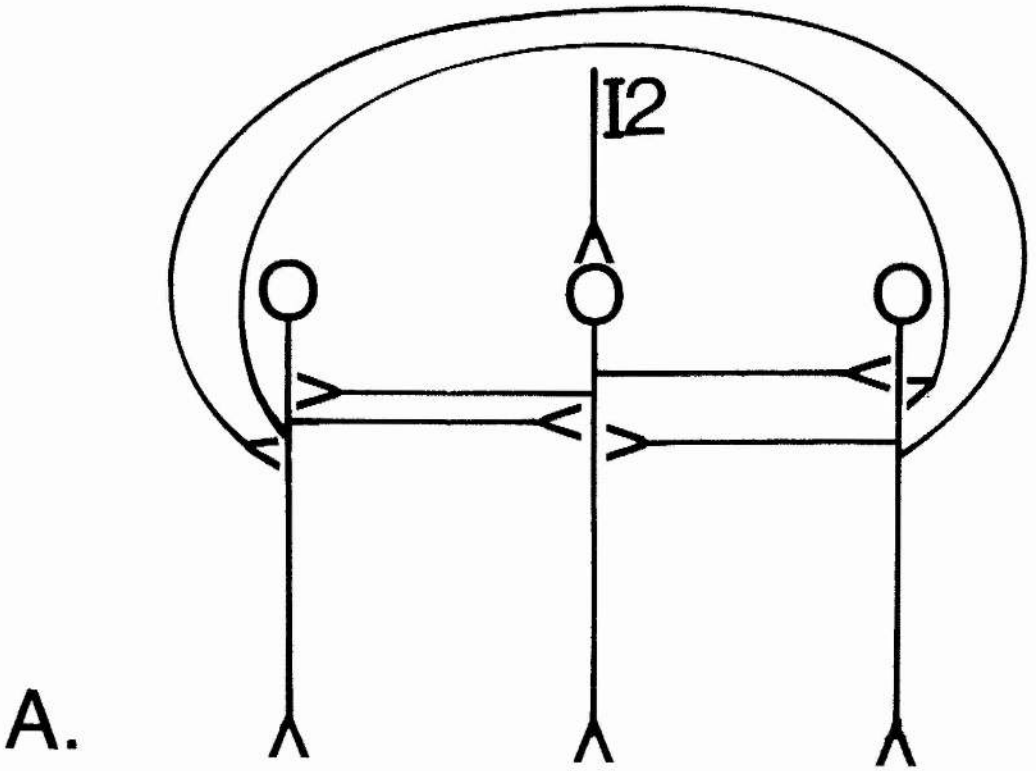
The results obtained, whilst studying phasic units, indicate that the same units tend to respond regardless of which abdominal connective is stimulated (Figures 2.4.3.A and B and 2.4.8). There is some discrepancy as to the way in which the units might be linked to one another. The way in which ipsilateral units are shown to drop out during stimulation of the connectives (Figure 2.4.7), compared with contralateral units dropping out

Figure 3.2. Possible connections of tonic units to produce a grouped discharge.

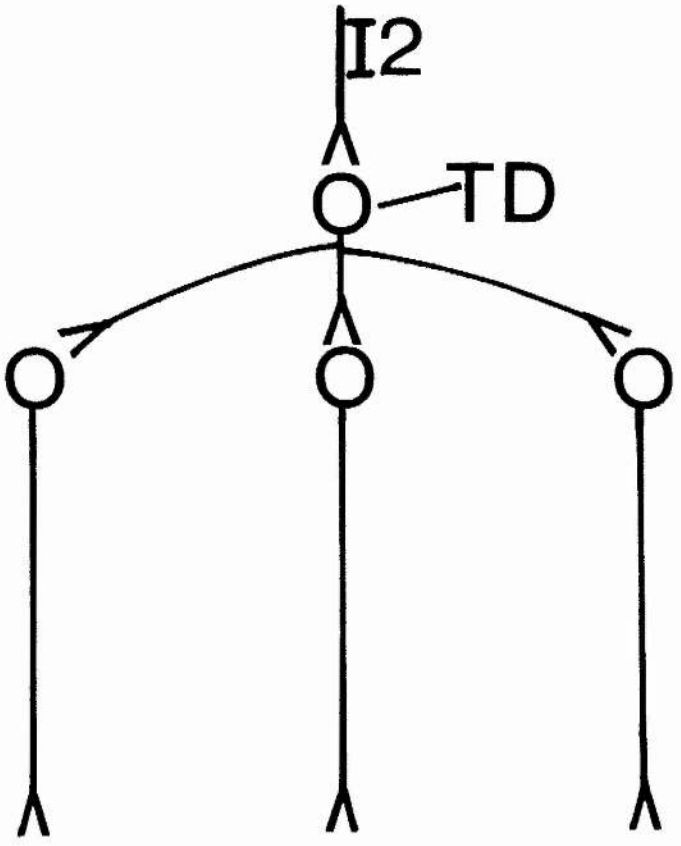
A. All units laterally excite one another so that stimulation of one produces a discharge from all others. The response of all motor units is tonic.

B. A phasic input from I2 fires a driver cell which may respond tonically. It would then cause an apparent tonic response in phasic motor neurones.

T.D. tonic driver cell.



A.

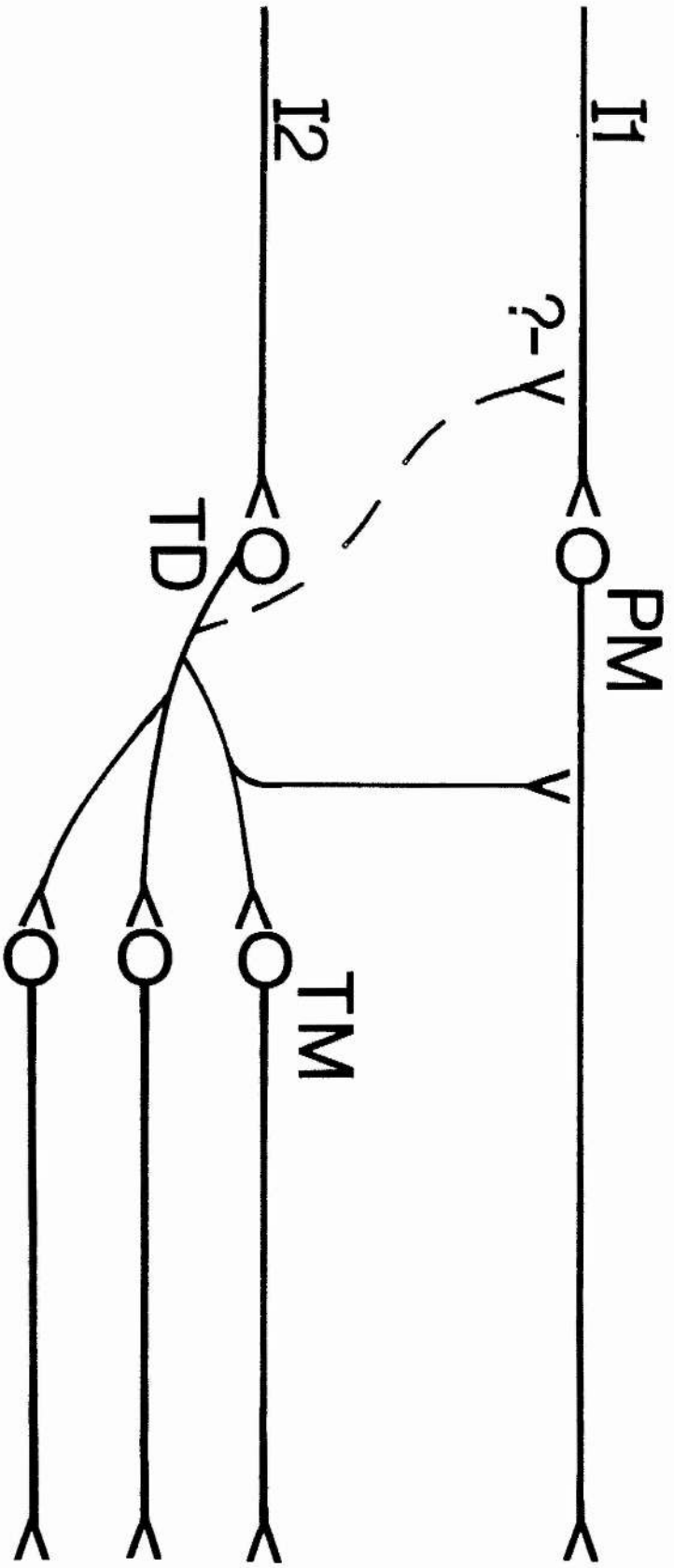


B.

Figure 3.3. Excitation of both phasic and tonic motor units.

I1 may drive phasic motor units (P.M.) directly whilst I2 drives tonic motor units (T.M.) through a tonic driver neurone (T.D.). This driver may also excite the phasic motor unit.

?- Possible inhibition of I1 by the dashed branch of the tonic driver unit.


I1
I2
2-γ
TD
PM
γ
TM

when a neurone soma is directly stimulated (Figure 2.7.2.B), suggests that:-

a) the interneurones producing phasic output decussate (probably in the third or fourth commissure) and

b) phasic units are linked to one another through synapses. Such linkages might be through lateral side branches of the motor cells themselves (Figure 3.4.A). There are also three other possibilities concerning the connections of the I1's:-

i) The I1's may in fact synapse at their decussation (Figure 3.4.B).

ii) They may bifurcate so that each I1 supplies both phasic units (Figure 3.4.C).

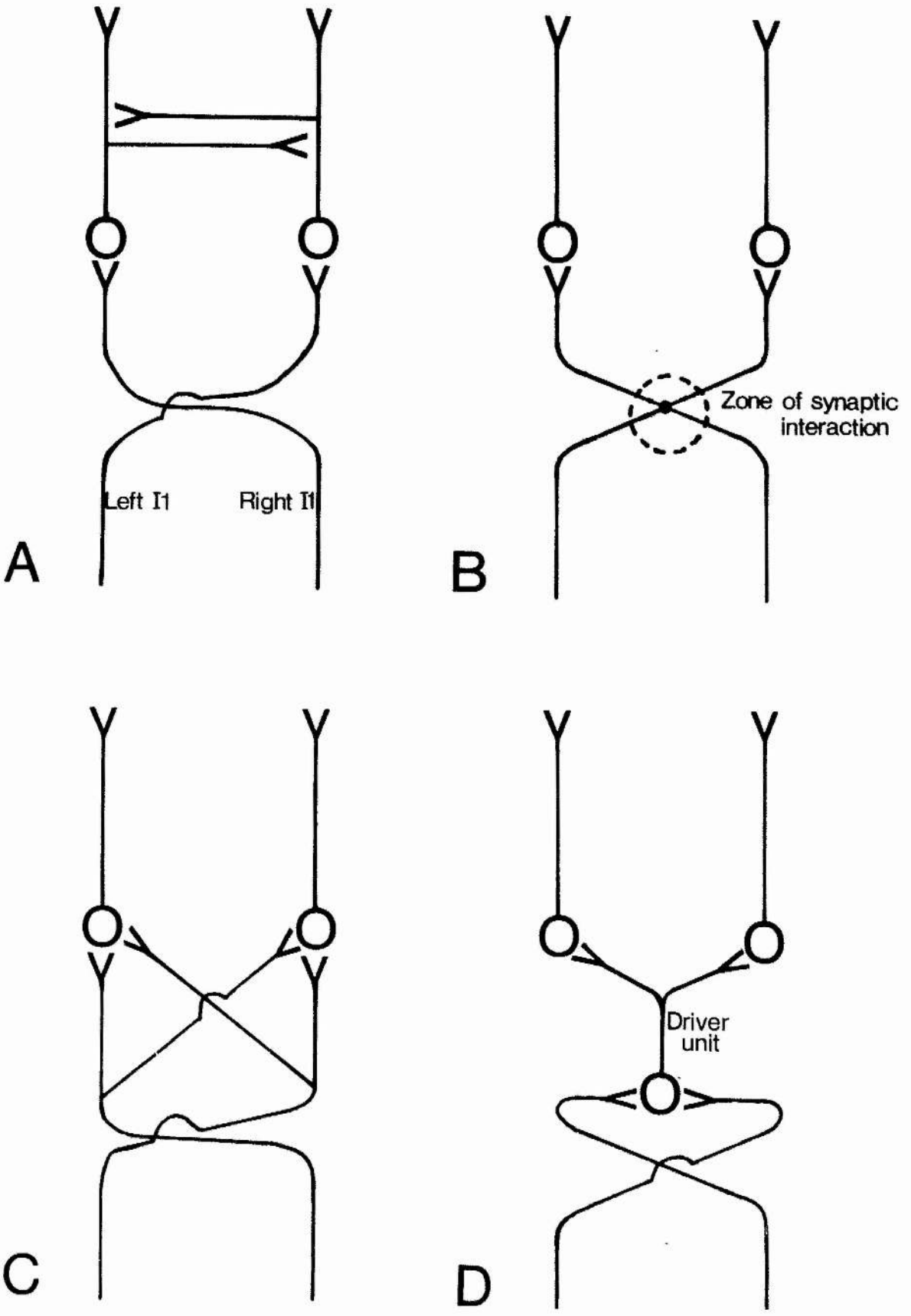
iii) They may both synapse onto the same driver unit (Figure 3.4.D).

Other variations are, of course, possible. In Figures 3.4.A, B and C there would be a variable delay in the response of a given unit to stimulation of contralateral or ipsilateral I1. Stimulation of the contralateral I1 would always produce the most rapid response. In Figure 2.4.3.A and B the same phasic units in the right P.I.N.p. fire regardless of whether the right or left connective is stimulated. However, there is a slightly greater delay (5 m.sec.) between the stimulus pulse and the response when the ipsilateral rather than the contralateral connective is stimulated. The results displayed here (and in Figure 2.7.2) suggest that these units are laterally linked and that the efficacy of the synapse on the side branch is less than that between I1 and the motor axon. Thus the network represented in Figure 3.4.A is the one most likely to fulfill these properties.

In other cases a different situation arises (Figure

Figure 3.4. Possible mechanisms underlying pairing of phasic units.

A. Motor cells cross excite one another.
B. I1's synapse at the point of decussation.
C. I1's bifurcate to supply both motor units.
D. Both I1's synapse onto the same driver unit which then bifurcates to supply both motor units.


Left I1
Right I1
A
Zone of synaptic interaction
B
C
Driver unit
D

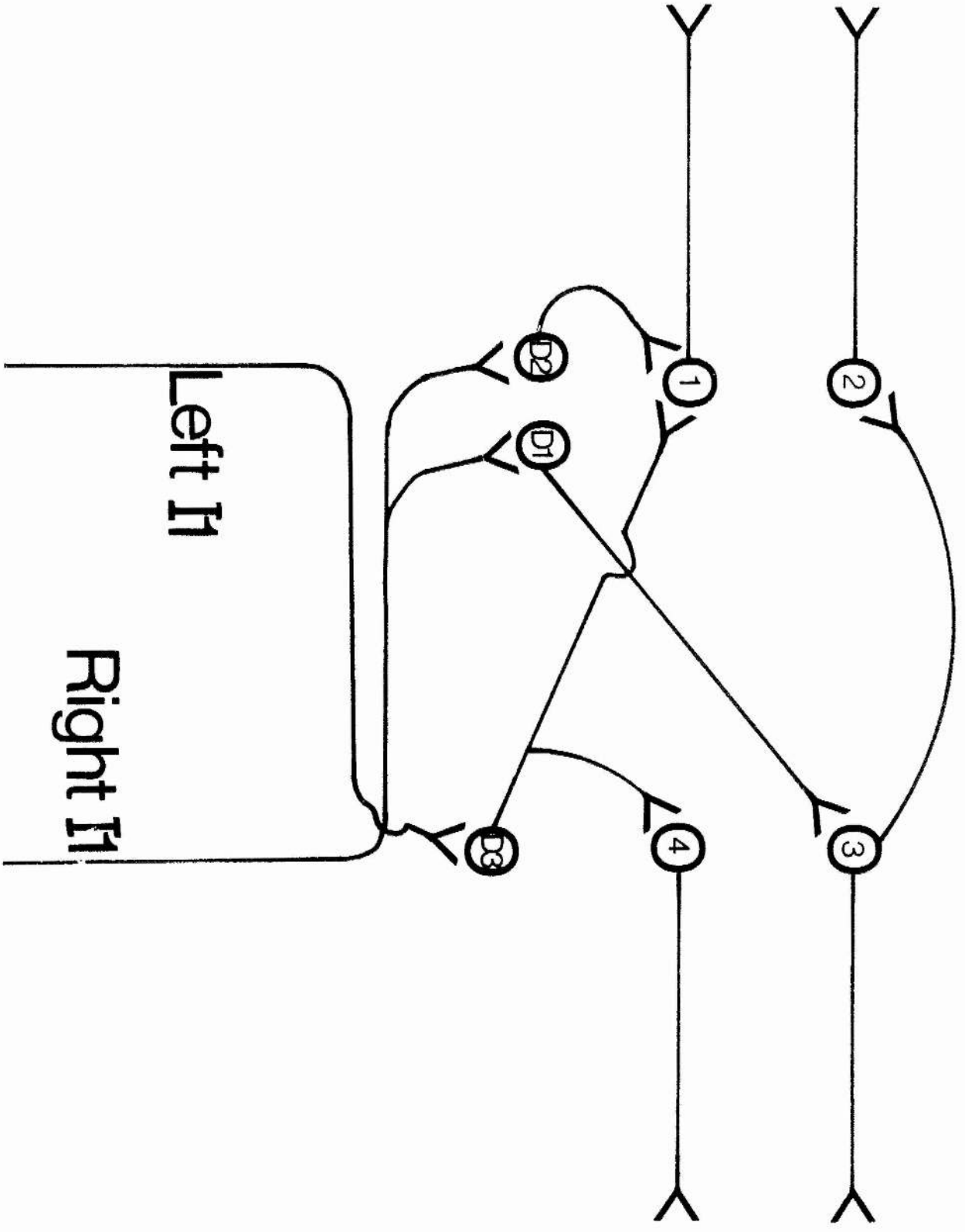
2.4.8). The same units discharge in both P.I.N.a.'s when either connective is stimulated and in this case there is no detectable change in the delay time between stimulus and pulse, nor is there any variation in the delay between the pulses in the upper or the lower beam. What is more, at high stimulus frequencies both ipsilateral and contralateral units drop out simultaneously, regardless of which connective is stimulated. Only the model represented in Figure 3.4.D would explain this phenomenon. The simultaneous adaptation of both pulses would presumably be due to loss of synaptic efficacy between I1 and the median driver unit.

The only way to resolve such a conflict seems to be to suggest that in these experiments two classes of interneurone were active. That this is a fair proposition is borne out by Wolfe (personal communication) who states that from one to five interneurones of the second and third abdominal connectives affect the gut in the crayfish, Procambarus clarkii.

Further levels of complexity in the phasic network are indicated elsewhere (Figures 2.4.6 and 2.4.7). Differences in output from the P.I.N.a.'s are detectable according to which connective is stimulated (Figure 2.4.6). Stimulation of the right 4-5 connective initiates a response by two contralateral units (1 and 2) firing as a doublet and one ipsilateral unit (3) (Figures 2.4.6.A and 2.4.7.A). On stimulation of the left 4-5 connective unit 2 drops out leaving only unit 1 (both of these are now ipsilateral) and unit 3 (which is now contralateral) is replaced by unit 4 (see Figures 2.4.6.B and 2.4.7.B.1). A possible network to account for such phenomena is indicated in Figure 3.5. In this network units 1, 2 and 3 are coupled by two driver cells (D1 and D2) which are activ-

Figure 3.5. Possible neural connections to account for the outputs of Figures 2.4.6 and 2.4.7.A and B.1.

The right I1 stimulates two driver cells, coupling units 1, 2 and 3 at 10Hz. At 30Hz units 2 and 3 drop out simultaneously, which suggests breakdown of a single synapse. These units may therefore be linked by a unilateral driver (D1) 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 (D2). Units 1 and 4 may be connected by a bifurcating driver cell (D3).

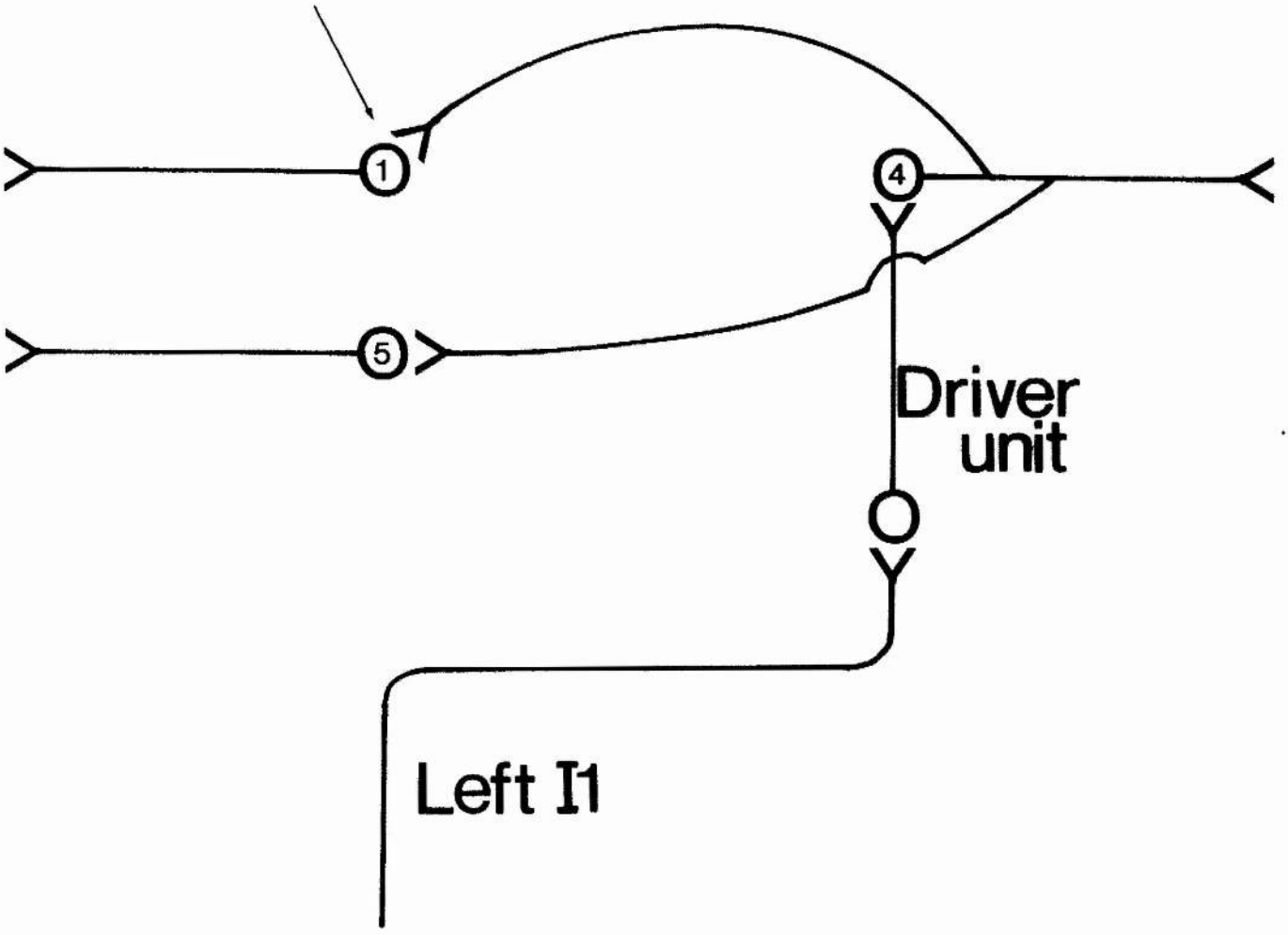

Left H
Right H
D2
D1
D3
1
2
3
4

ated by the right I1. At low frequencies units 1, 2 and 3 fire in synchrony. Units 1 and 4 may be activated by a bifurcating driver cell (D3) since they are closely coupled at low frequencies. At slightly higher frequencies of stimulation units 2 and 3 fail simultaneously and it is thought that stimulation of one or the other, rather than both units, would be more likely to produce this phenomenon than if two synapses had to fail simultaneously. The excited unit (3) might then be envisaged as activating its contralateral homologue (2). Of course 2 might be the unit initially activated. A further aspect of higher frequency stimulation is that units 1 and 2 gradually separate and come to fire on alternate stimulus pulses. In the first part of Figure 2.4.7.A.2 unit 1 gradually slows with respect to unit 2. This may be due to some decrease in synaptic efficiency of D2. Unit 1 then fires erratically on several stimulus pulses and misses altogether on some. Missing of stimulus pulses may account for the abrupt shift of phase of unit 1 with respect to 2 in the latter part of the trace when units 1 and 2 fire alternately. Presumably the synaptic efficacy of D2 was restored at this time. In Figure 2.4.7.A.3, after an uncertain start, units 1 and 2 again fire alternately.

In Figure 2.4.7.B.2 and 3 additional complications arise. At higher frequencies of stimulation unit 1 fires rather spasmodically and a further ipsilateral unit (5) is shown to be activated. It is thought that this small unit always fires in correlation with unit 1 during stimulation of the left connective, but its presence is probably obscured by unit 1. A possible network to include unit 5 is set down in Figure 3.6. At low frequencies unit 5 is not normally discernible due to its simultaneity of

Figure 3.6. Possible neural connections to account for the output in Figure 2.4.7.B.2 and 3.

Units 1, 4 and 5 are coupled by a single unilateral driver cell which synapses onto unit 4. Unit 4 then laterally excites units 1 and 5. As the preparation fatigues the synapse onto unit 1 (arrow) quickly becomes exhausted even at low frequencies. Units 4 and 5 go on firing in synchrony after 1 drops out.


1
4
5
Driver unit
Left I1

firing with 1. A reasonable way for such simultaneity to be achieved, as well as retaining the coupling with 4, would be for 4 to be driven either directly from I1 or from a unilateral driver unit. Unit 4 itself might then excite both 1 and 5 to fire simultaneously. Thus failure of a single synapse (that between 1 and 4) would be sufficient to cause unit 1 to fail. Later (Figure 2.4.7.B.3), unit 1 failed at low frequency leaving unit 5. The preparation was fatiguing at this point in time.

It is improbable that the branching axons shown in Figure 2.1.3 correspond with phasic units since the site of spike initiation of such a bifurcating neurone would probably be single and would lie in the unbranched part of the axon. If this were not the case the distance, over which electrotonic current spread would need to occur to reach separate spike initiating zones in the individual branches, would be quite large (about 2 mm. in the preparation from which Figure 2.1.3 was taken). However, Ripley, Bush and Roberts (1968) have shown current to spread electronically over surprisingly large distances (up to 10 mms. in Carcinus and Potamon).

Thus a complex network underlies the activity of phasic neurones. These are thought to lie in bilaterally symmetrical groups, which laterally excite one another. The groups themselves might be excited by the two classes of interneurones indicated in Figure 3.4.A and D. The mechanisms represented here are acknowledged to be highly speculative and many more experiments will need to be carried out to determine the precise interconnections of the various units.

b. Tonic Network.

The results obtained in Figure 2.7.3 show that a single unit is capable of driving several other units, in a tonic manner, in both the contralateral and ipsilateral P.I.N.'s. This would suggest that the unit penetrated was either a driver unit or that all units in the tonic network mutually excite one another to produce cascading activity in the P.I.N.'s (as in Figure 3.2.A). Elsewhere (Figure 2.4.4) both ipsilateral and contralateral I2 are shown to produce the same tonic output in the right P.I.N.a.'s and P.I.N.p.'s. One more stimulus pulse is required to elicit the response when the ipsilateral I2 is stimulated rather than the contralateral I2. This may be due in part to the greater delay imposed by decussation of the ipsilateral I2. However, stimulation of both I2's considerably decreases the latency of the onset of the response. In the absence of detailed studies of the latencies between almost simultaneous bursts in the P.I.N.'s, it is not possible to make well-founded predictions of the form or position of the neurones mediating bursting activity. However, the change in delay mentioned above (Figures 2.4.4.A and B) suggests that the neurones involved in a burst may lie in two lateral groups which cross excite one another in some way. On the basis of Figure 2.4.4.C I would suggest that both I2's converge onto driver units, which cannot be immediately discharged by only one I2. I also suggest that these units cross excite one another. However, if both I2's were stimulated simultaneously (as in Figure 2.4.4.C), then this input may be sufficient to shift the membrane potential of the driver cells (D) to threshold by summation of p.s.p.'s. The drivers, which may or may not be tonic, would then drive one or more units of their

ipsilateral group. Assuming these groups to be laterally exciting, a tonic, cascading group discharge would then be produced and might perhaps be self-generating over a short period. Figure 3.7 is a diagrammatic representation of this postulated network. A network of this type has been demonstrated in the brain of the nudibranch mollusc Tritonia, by Willows and Hoyle (1969).

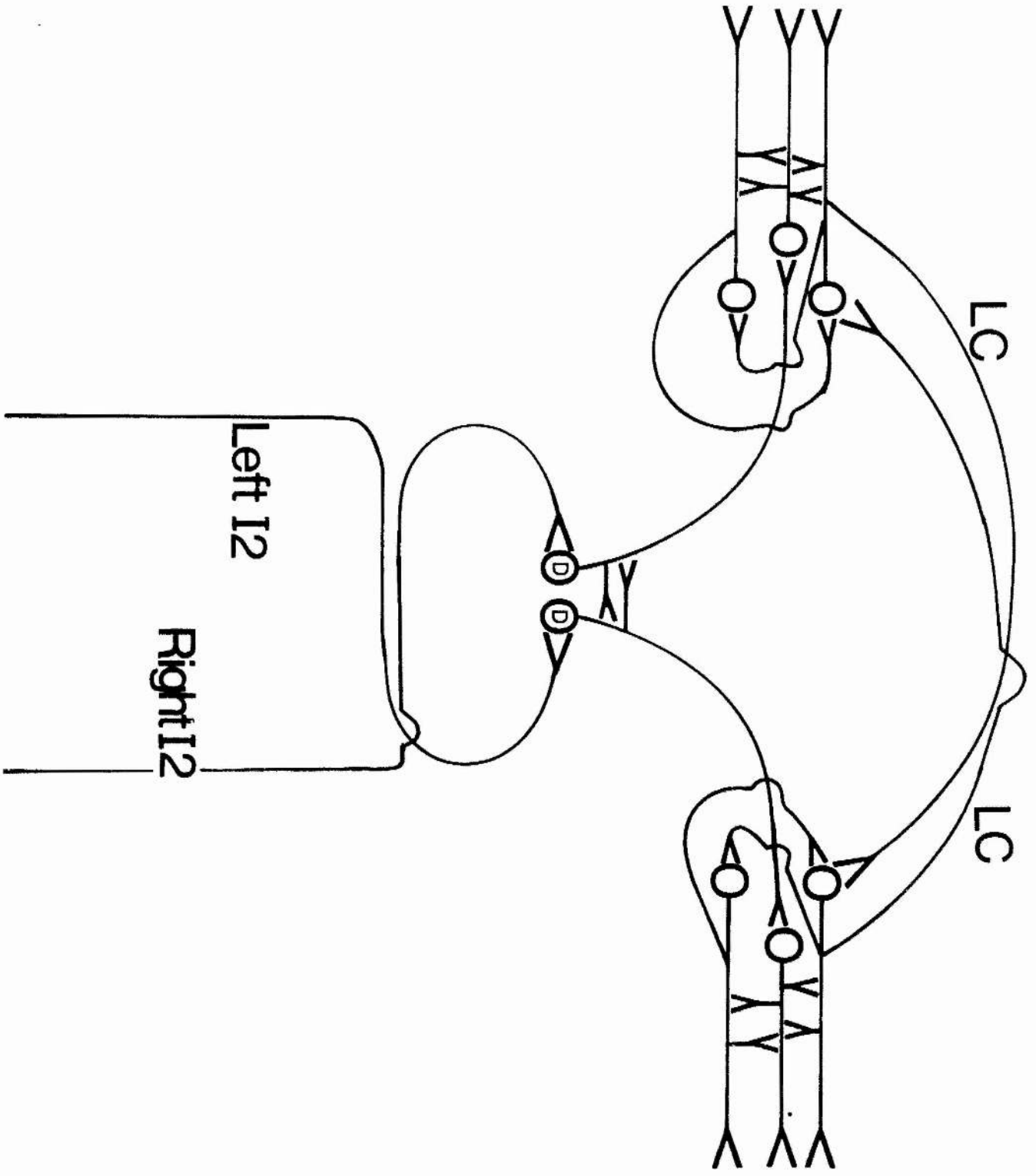
Synaptic delay(s) between the drivers might cause the delay shown in Figure 2.4.4. Further assurance of the sirultaneity of discharge could be attained by cross excitation of each of the lateral groups. In this way a fixed motor pattern could be produced, especially if some of the neurones of a lateral group were asymmetrical or inhibiting in their connections. The fixity of the tonic discharge pattern (see also Section 3.2.B) is demonstrated in Figure 2.4.16.B and D. Increasing the duration of stimulus delivered to the left 4-5 connective does not alter the form of the subsequent burst of activity in the right P.I.N.a.

The simultaneous appearance of both tonic and phasic activity in all branches of the P.I.N.'s is of great interest. It is postulated that these responses may be partly mediated by multibranched axons (Figures 2.1.2 and 2.1.3) passing down the anterior and posterior branches of both ipsilateral and contralateral P.I.N.'s. Further evidence of the presence of multibranched axons with branches to both right and left P.I.N.'s is provided in Figure 2.3.1. Stimulation of the left P.I.N.a. elicits a response by three units in the right P.I.N.a. On the basis of the conduction velocities of these spikes, which is not less than 2 - 3.5 metres/sec., even for the slowest axon, I think it is unlikely that sensory fibres would conduct impulses centrally to

Figure 3.7. Possible neural connections to account for the outputs shown in Figures 2.4.4 and 2.7.3.

Both I2's synapse onto driver units (D) which laterally excite one another. Each driver unit then excites an ipsilateral group of neurones whose individual units are assumed to cross stimulate one another. In addition these major neuronal groups are thought to laterally excite one another via a lateral coupling (L.C.) across the ganglion.

Such a series of connections would ensure the almost simultaenous delivery to the hindgut, of bursts of motor activity down both right and left P.I.N.'s.


LC
Left I2
Right I2
LC

initiate the response. Pabst and Kennedy have shown (see Section 3.2.B) that the maximum conduction velocity attained in the soft cuticle receptors they describe is only 1.5 metres/sec. Thus, at least three multibranched units are likely to occur on the basis of this evidence andeach lateral group might contain such units (see Figure 3.8). However, if multibranched axons lay in two lateral groups and innervated both P.I.N.'s, then the nature of the tonic response could vary, due to delay in firing of some neurones, depending on which I2 was stimulated. Clearly this does not happen and the form of the tonic response would seem to be immutable (Figures 2.4.3, 2.4.4, 2.4.12 and 2.4.13). Thus it seems that there is a single network of tonic units (as in Figure 3.9) rather than two lateral networks. Presumably such a network could either be directly driven by convergence of both I2's, or the I2's might converge onto a single driver interneurone. It is unlikely that these interneurones are in contact distally (Figure 2.4.4), although they may be activated simultaneously in the brain.

The possibility of there being a single median network of tonic units driving the hindgut conflicts with some earlier results obtained from arthropods. According to Kennedy et al (1969) 'partner' neurones in bilaterally symmetrical ganglia correspond in position to one another quite precisely and Cohen and Jacklet (1967) have found that many neurones of the cockroach metathoracic ganglion are arranged in bilaterally symmetrical pairs. These pairs are often quite widely separated. In his study on the mesothoracic ganglion of the locust, Schistocerca gregaria, Bentley (1970) indicated that motor units passing to the flight muscles usually had a symmetrically placed homologue and were

Figure 3.8. Convergence of input onto a tonic multibranched motor neurone. Such units would send branches down both P.I.N.a.'s and P.I.N.p.'s.

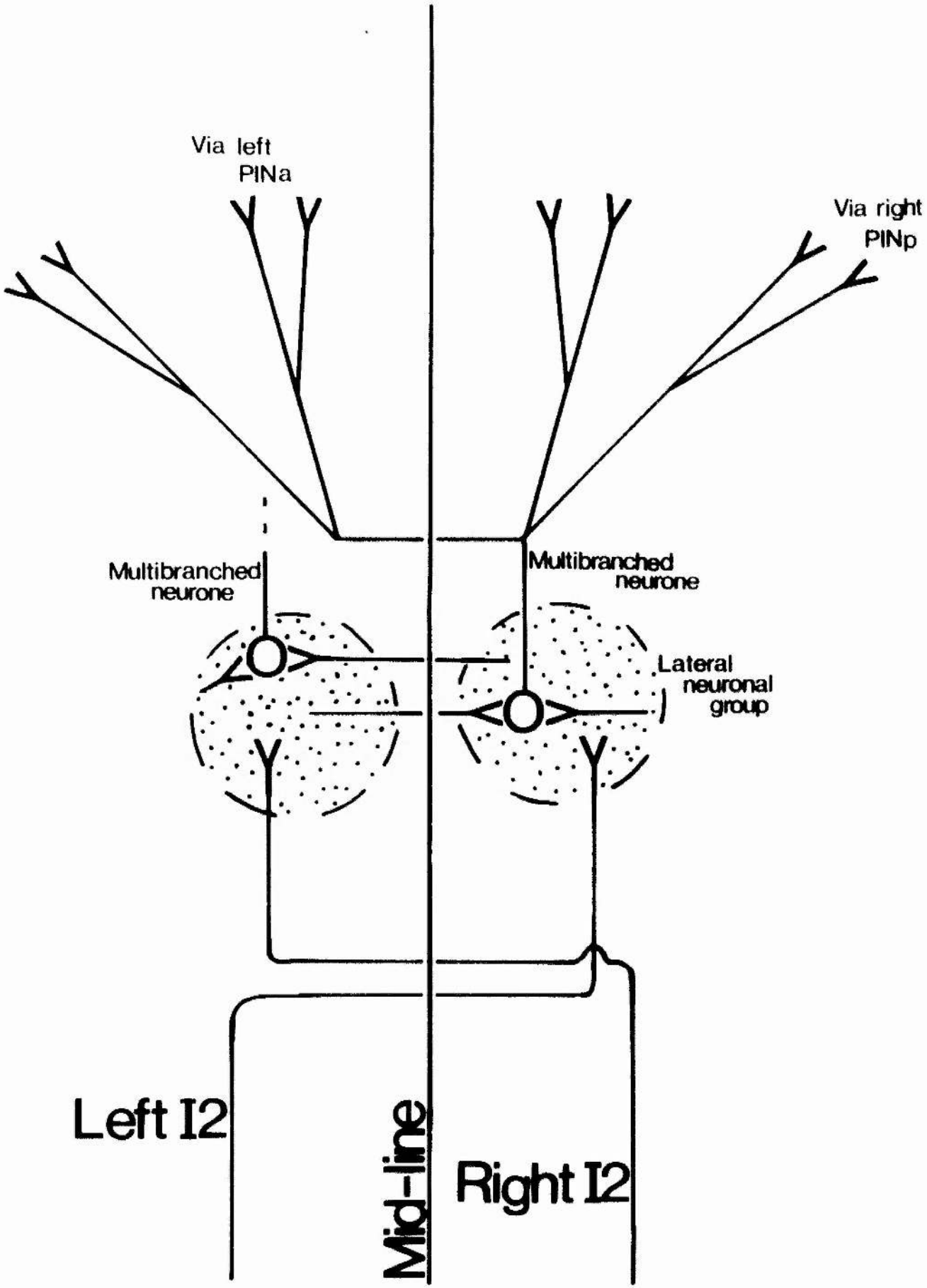
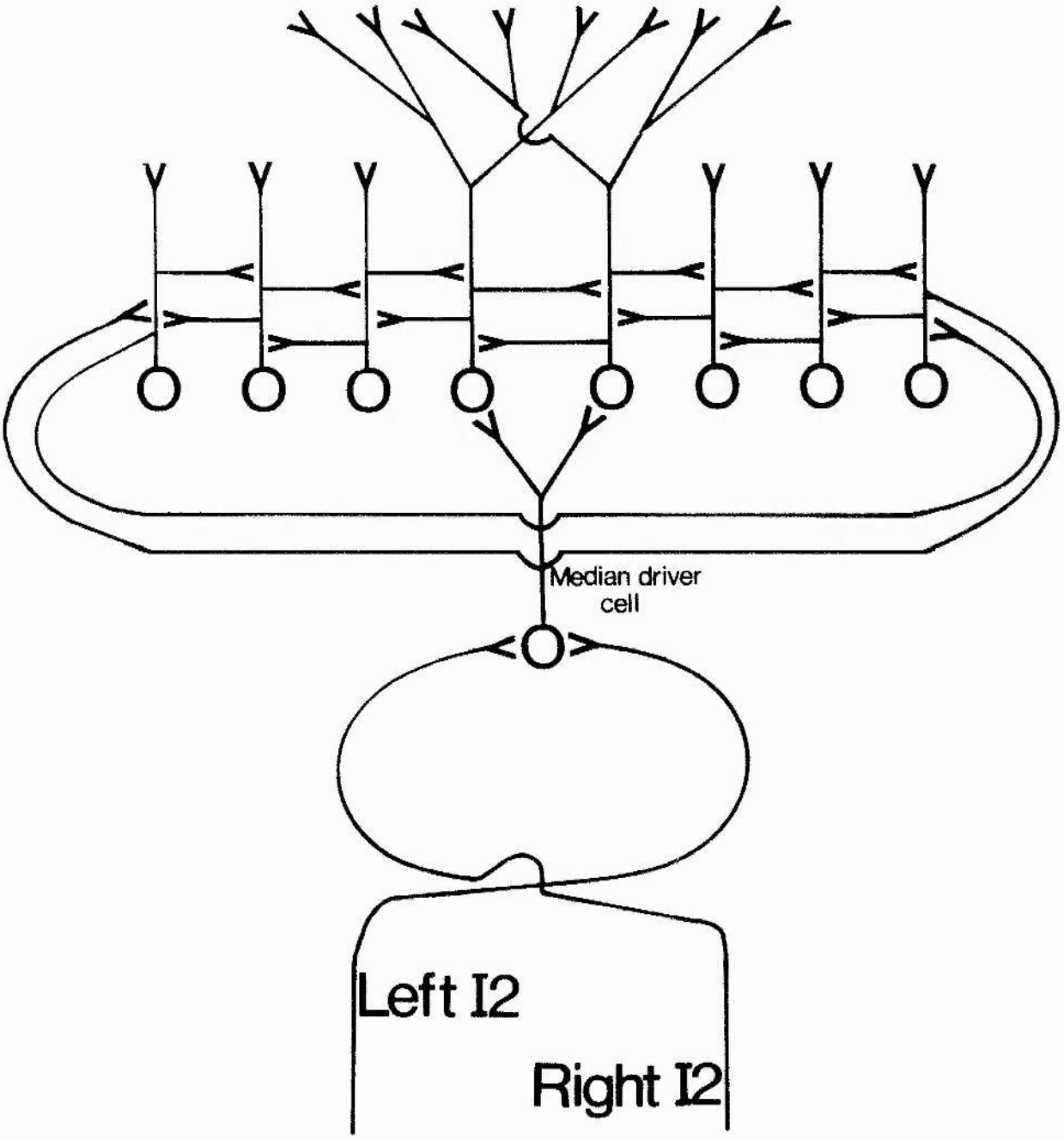

Via left
PINa
Via right
PINp
Multibranched
neurone
Multibranched
neurone
Lateral
neuronal
group
Left I2
Mid-line
Right I2

Figure 3.9. Convergence of the left and right I2 onto a median driver cell driving a network of tonic interneurones, some of which are multibranched.


Median driver
cell
Left I2
Right I2

ipsilateral with respect to the muscle which they innervated. In addition these units were shown to be weakly coupled to their contralateral homologues. Coupling of lateral giant fibres occurs in each crayfish abdominal ganglion (Kennedy et al, 1969), so that stimulation of either fibre will lead to activation of both symmetrical somata of certain flexor motor neurones of the third root.

Thus the general situation in arthropods is that the ganglia are symmetrically arranged 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 is medianal in position and contains a group of non-symmetrical interacting cells which produce synchronized 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 is also median in its position and lies within the anterior aorta on the antero-dorsal 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. 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 bi-axonic 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 my proposition that the hindgut is partly controlled by a median group of tonic 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.

3. The Functions of Phasic and Tonic Units.

In Figure 2.4.16 the phasic and tonic units are shown to produce rather different responses. Phasic units will often produce weaker rhythmical contractions of the hindgut, although these movements are of much greater amplitude than the spontaneous hindgut contractions shown in Figure 2.2.10. The major point of difference in the response of the hindgut is that tonic units produce a massive peristaltic movement which considerably elongates the hindgut, whilst this elongation is not so marked with phasic units. It is thought that the phasic units may only innervate the longitudinal muscles. The weaker peristaltic contractions caused by phasic units 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 tonic units is usually of large amplitude and a neurally evoked circular muscle contraction is thought to be involved so that tonic units apparently innervate both longitudinal and circular

muscles. The function of the phasic units may be, in part, to 'prime' the longitudinal muscles and thus facilitate the initial defaecatory response. The primary phase of hindgut movements represented in Figure 2.2.1 was probably induced by tonic units and the subsequent rhythmic contractions may have depended solely on a phasic discharge.

As mentioned in Section 3.1.A the origins of the radial and longitudinal muscles may be similar. It is interesting to note that both can be driven rhythmically (see Figures 2.2.1 and 2.4.16.C) by phasic 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. Eloffson et al show noradrenaline to be distributed among both longitudinal and circular muscle groups. Thus it is possible that the phasic units may utilise a different transmitter (acetylcholine or related compounds) from tonic units which may utilise noradrenaline or related substances. The response of both radial and longitudinal muscles to phasic units may reflect similarities in their pharmacological properties.

The nature of the responses of the hindgut in life are unknown, but some aspects of its control have been studied here. Its function in relation to the rest of the intestine might provide a useful future study.

Section 3.3. Conclusions.

From the evidence presented in the preceding sections it is concluded that the hindgut of the lobster, Homarus gammarus (L.), is under direct central control from the 6A.G. with respect to the defaecatory response. Central patterning initiates the response which may then be carried on by the highly exciteable hindgut muscles. The hindgut is divisible into anterior and posterior sections controlled by the P.I.N.a.'s and the P.I.N.p.'s respectively. The innervation of the hindgut resembles that of the somatic muscles. No myenteric plexus capable of independent co-ordinating activities is thought to exist. The hindgut is capable of unco-ordinated low amplitude excursions which are myogenically initiated by pacemakers within the muscles. The muscles may exhibit a graded or passive response on stimulation.

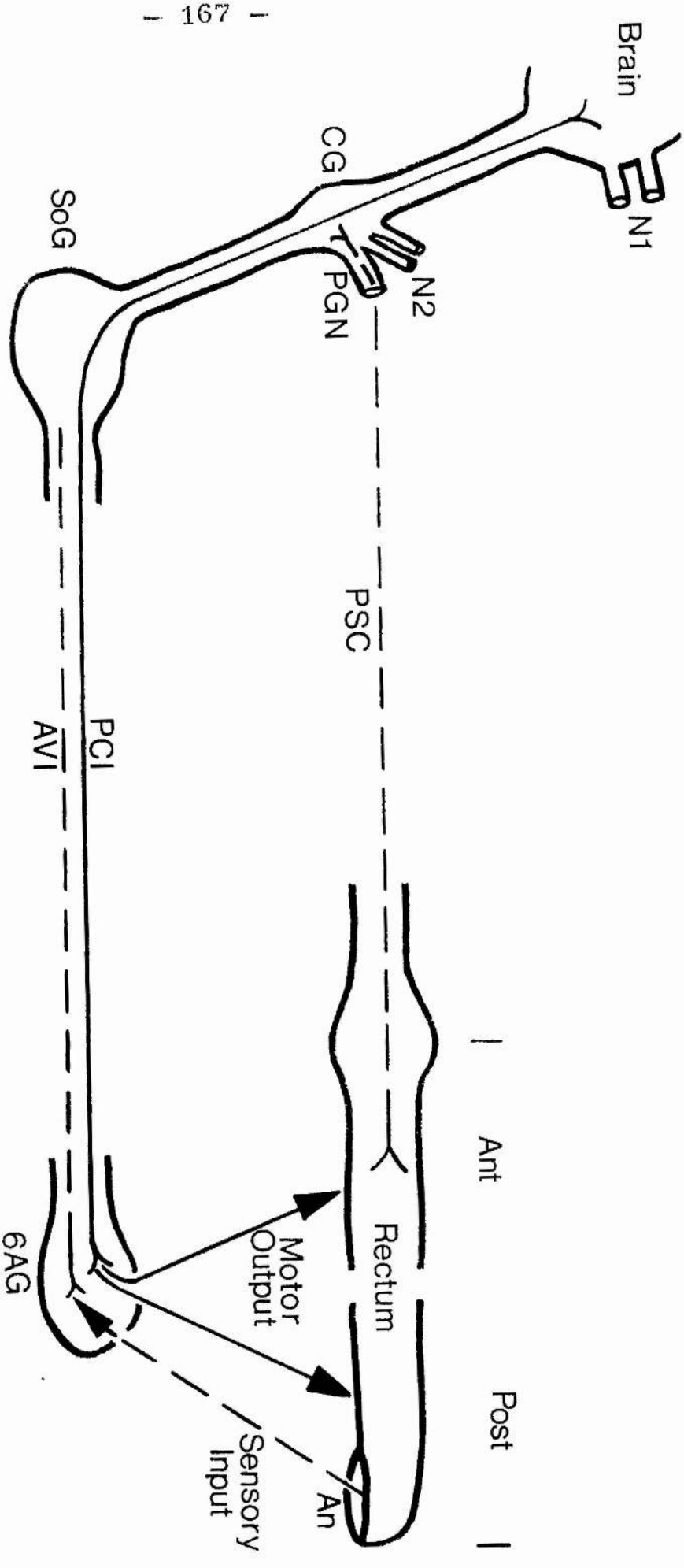
Anal movements are monitored by sensory cells responding to deformation of the soft cuticle of the anal lips. Their input does not modulate motor discharge and their central connections are unknown. They may synapse onto interneurones which are responsive to pulling out of the anal valve (Wiersma and Hughes, 1961). Some of these points are summarised in Figure 3.10.

Phasic and tonic units control hindgut motility. The phasic units are thought to lie in two lateral networks, whilst the tonic units lie medianly. Phasic units interact with one another by lateral excitation. Many tonic units may be multibranched and send axonal branches to all the P.I.N.'s. Tonic units alone are capable of driving the powerful defaecatory response. Phasic units are thought to 'prime' the muscles of the hindgut, but they can also drive the longitudinal and radial muscles rhythmically. The different types of

Figure 3.10. The innervation of the hindgut in relation to the central nervous system of Homarus gammarus.

The bipolar sensory cells lying on the hindgut are not shown. The cell bodies of Orlov's (1926) pyloric sensory cells lie on the pylorus and their axons pass to the commissural ganglion.

Brain - tritocerebral region of the brain.
6A.G. - sixth abdominal ganglion.
C.G. - commissural ganglion.
So.G. - suboesophageal ganglion.
P.G.N. - posterior gastric nerve.
N1 - nerves to oesophageal and stomato-gastric ganglia.
N2 - nerves to oesophageal ganglion.
Ant. - anterior region of the hindgut.
Post. - posterior region of the hindgut including the extrinsic anal musculature. The hindgut is broken to indicate the independence of anterior and posterior regions.
An. - anus.
Motor output - motor output down the P.I.N.'s.
Sensory input - sensory input from the receptors of the anal lip.
A.V.I. - interneurones responding to pulling out of the anal valve (Hughes and Wiersma, 1960). Their proximal terminations are unknown.
P.C.I. - presumed command interneurones probably originating in the brain.
P.S.C. - pyloric sensory cells of Orlov.
Dashed lines - primary sensory fibres and the interneurones onto which they discharge.
Solid lines - motor fibres and command fibres.


Brain
N1
CG
SoG
N2
PGN
PSC
PCI
AVI
6AG
Motor
Output
Rectum
An
Sensory
Input
Ant
Post

units may utilise different transmitters. These networks only represent a final motor pathway and are ultimately controlled by several interneurones originating in the brain, presumably in the tritocerebral region (see Figure 3.10).

The somata of the units controlling the defaecatory response have been localised. They lie in the anterior region of the posterior cortical lobe of the 6A.G.

The 6A.G. may have been derived from three fused ganglia - the sixth, seventh and terminal abdominal ganglia.

Several lines of evidence are presented to support the view that the longitudinal muscles of the hindgut and the extrinsic radial muscles of the anus may be derived from the same embryological origins.

Section 3.4. Comparison of the Cockroach and Lobster 6A.G./Rectum Complex.

A number of recent publications (Belton and Brown, 1969; Brown and Nagai, 1969; Nagai and Brown, 1969; Nagai, 1970) report the results of work carried out on the proctodaeal muscles of the cockroach, Periplaneta americana L. The hindgut of the cockroach is very similar to that of Homarus except that the six symmetrically placed longitudinal muscle strips lie external to the circular muscles and only stretch over the anterior two thirds of the rectum. In the posterior part they are replaced by six symmetrical dilator muscles which are distally attached to the body wall in a manner reminiscent of the five radial muscle groups of the lobster.

The nerves supplying the cockroach hindgut arise in the sixth abdominal ganglion. The hindgut is supplied

bilaterally by the proctodaeal nerves (a branch of nerve XI, the cercal nerve). These nerves are divisible into anterior and posterior branches as in Homarus (see Figure 2.1.1). The anterior branches supply the longitudinal muscle straps, the ventral dilators, the circular muscles of the anterior rectum and all the muscles of the midgut, whilst the posterior branches run to the dorsal and lateral dilators and the circular muscles of the posterior rectum. Thus the hindgut of Periplaneta may be almost as completely divided into two regions as that of Homarus.

Stimulation of the 5-6 connectives of the cockroach elicits motor activity in the proctodaeal nerves and this activity can be knocked out by ganglionic blocking agents. Thus the motor fibres are thought, by Brown and Nagai (1969), to be centrally controlled. The somata of motor neurones to the hindgut have been shown to lie in groups of four to six cell bodies, on either side of the midline, in the posterior region of the sixth abdominal ganglion. This is very similar to the position of the motor neurone somata in Homarus.

Afferent activity has been recorded from sensory cells lying below the circular muscle layer. Such cells may be similar to those described by Alexandrowicz (1909) in decapod crustacea. Ganglion cells have never been observed on the cockroach hindgut although its surface is interlaced with a periproctodaeal net which is thought to be made up of modified muscle fibres rather than nerve fibres.

Two types of motor output exist, one of which is rapidly conducted (0.5 metres/sec.), whilst the other is much slower (0.2 metres/sec.). Both conduction velocities are much less than those indicated in Figure 2.3.1, for Homarus. Only the fast fibres evoke p.s.p.'s in muscles

of the hindgut. The function of the slow fibres is unknown.

The longitudinal muscle fibres of the cockroach proctodaeum are innervated multiterminally and polyneuronally. Muscle action potentials are of the graded type (as is suspected in Homarus) and could be triggered either by summation of centrally generated p.s.p.'s or by a smoothly fluctuating muscle membrane potential of critical amplitude. The longitudinal muscles of the cockroach proctodaeum appear to be myogenic, but under the control of the central nervous system. I have reached precisely this conclusion for the hindgut musculature of Homarus. In the cockroach rhythmic muscle action potentials can also be evoked by stretch of the proctodaeal muscles. According to Nagai (1970) these properties are similar to those of vertebrate smooth visceral muscles, as well as the papillary muscles of the mammalian heart. Their pacemaker sites are of variable location.

As can be seen from the review of recent literature set out above, the control mechanisms for defaecation are apparently very similar in cockroaches and lobsters. In both cases the hindgut is centrally controlled and the rhythmicity of the visceral muscles is thought to be myogenic in origin.

Section 3.5. Suggestions for Future Work.

Future investigations on the 6A.G./rectum complex must involve several lines of investigation if the neural mechanisms underlying hindgut function are to be

completely understood. The most pressing problem is, in my opinion, the need to understand the relationships of the various motor units involved in the control of the hindgut. Both extracellular and intracellular recording techniques will be necessary for such a project. Associated techniques, such as the microinjection of dyes would give the necessary anatomical details with which physiological information could, hopefully, be correlated. Stretton and Kravitz (1968) have shown that the dye Procion Yellow M4RS is of very great use in this context. Bentley (1970) and Remler et al (1968) have used this dye to construct a ganglionic map of the motor neurones of the locust flight system and to locate the neurone somata of the lateral giant fibres of the crayfish, respectively. Other methods to locate the position of neurone somata and the paths of fibres could also be tried. Various techniques involving neurone degeneration have been described. Cohen and Jacklet (1967) demonstrated the presence of a dense ring of ribonucleic acid in the perinuclear cytoplasm around the neurone soma after sectioning of the peripheral branches, whilst the Nauta stains for degenerating nerve fibres have been applied to arthropod material (Kennedy et al 1969).

It has been suggested (see Section 3.2.C) that phasic and tonic units may synthesize different transmitters. 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).

Another important aspect which must be given a great deal of consideration is the ultimate hindgut control mechanism which lies in the brain. The origins of

command interneurones emanating therefrom must be studied as a matter of urgency and the relationship of the defaecatory response to foregut and mouthpart movements should be clarified.

Anatomical and physiological investigations of the muscles of the hindgut should also be carried out in order to work out the causes of their rhythmic contractility. Electron microscopy of the muscles might shed some light on any anatomical specialisations or similarities to other muscles.

An electron microscopical investigation of the P.I.N.'s, in the search for fine sensory fibres passing into the 6A.G., would be of interest, as would a similar investigation of the proctodaeal cuticle to determine the presence or absence of sensory endings. Perhaps a search of the hindgut for axoaxonic or axodendritic synapses would also be of value.

Finally behavioural observations on the time of defaecation and its relation to feeding and body posture also need to be carried out.

It is my view that a specific relationship exists between hindgut and foregut function in Homarus gammarus (L.). I think that this relationship will eventually be revealed by an electrophysiological study of the tritocerebral region of the brain.

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THE OCCURRENCE OF AN ANAL PROPRIOCEPTOR IN THE DECAPOD CRUSTACEA HOMARUS GAMMARUS (L.) (SYN. H.VULGARIS M.ED.) AND NEPHROPS NORVEGICUS (LEACH)

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It has been known since 1910 that the peristaltic movements of the hindgut and the opening of the anus of Cambarus and Homarus can be evoked by stimulation of the nerve cord[1]. Anal opening precedes the arrival of a posteriorly directed wave of contraction passing along the hindgut[2]. The innervation of this area arises from the sixth abdominal ganglion and both motor and sensory systems are represented in the nerves reaching the rectum[3,4,5]. The details are not, however, well known, nor are they all in agreement; Alexandrowicz[4], for example, suggested that a plexus connects sensory and motor systems on the hindgut, but this idea was subsequently refuted by Orlov[5].

In recent years much information has accrued about the details of the sensory system of decapods, particularly the distribution and structure of internal mechanoreceptors. In the abdomen such receptors are typified by the well known MRO system[6], endings in the ventral soft cuticle[7], and a proprioceptor in the telson[8], but no definitive information exists as to the receptor population of the hindgut region.

We are at present investigating the mechanisms of nervous control of the rectal and anal region of the gut of Homarus and Nephrops and we report here on the occurrence of a mechanoreceptor that responds to distortion of the soft cuticle that forms the border of the anus.

In Homarus and Nephrops the hindgut is supplied by the paired posterior intestinal nerves, first described by Krohn[9] and Lemoine[10], and, apparently, named by Alexandrowicz[4]. These nerves originate from the dorsal posterior side of the sixth abdominal ganglion at a position close to two groups of

nerve cell bodies situated in the dorsal side of the ganglion as described by Retzius[11] and confirmed by us. These posterior intestinal nerves divide typically into anterior and posterior branches which can be shown to control hindgut peristalsis and anal opening[2]. The posterior branches of the nerves run towards the anus and innervate the various radial muscles which, on contraction, dilate the anus. There is no anal sphincter muscle (Fig. 1).

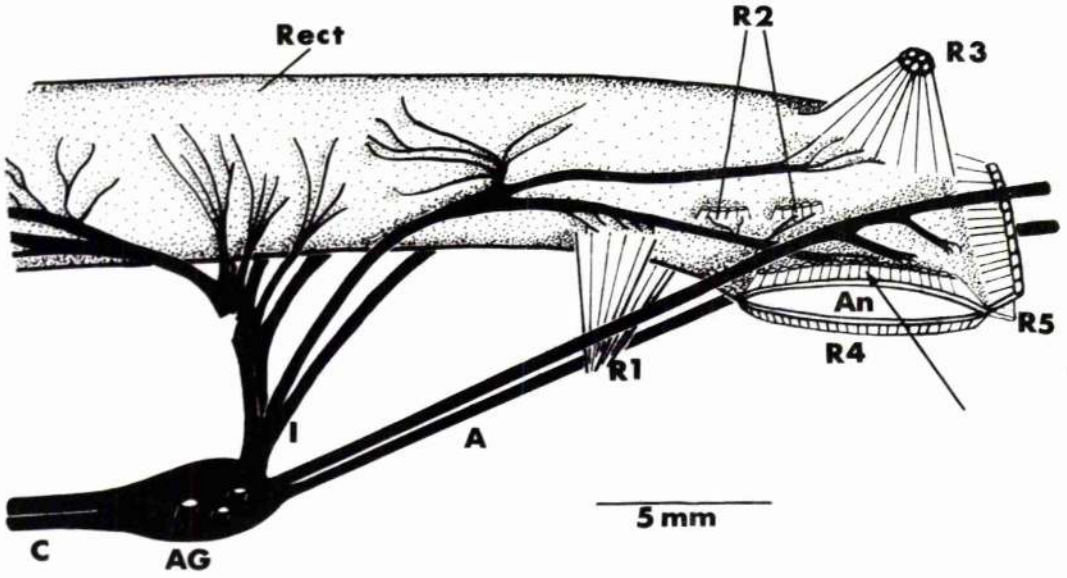


FIG. 1

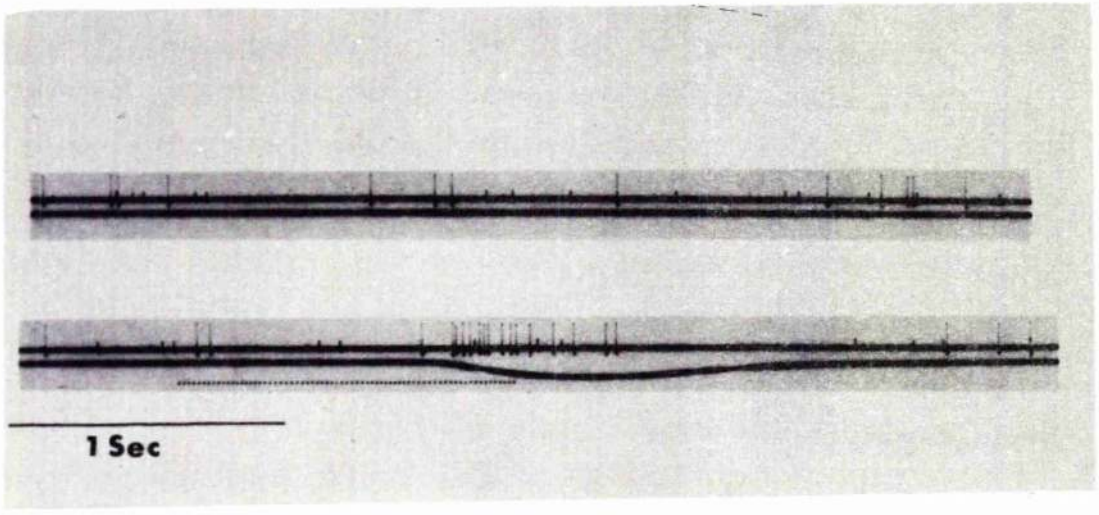
Isolated sixth abdominal ganglion/rectum complex in Homarus gammarus. The ganglion has been deflected ventrally in the interests of clarity. A, anal nerves; A.G., 6th abdominal ganglion; An, anus; C, connectives; I, posterior intestinal nerves; R_1, paired anteroventral radial muscles; R_2, paired lateral radial muscles; R_3, paired dorsal radial muscles; R_4, perianal radial muscles; R_5, posterior oblique radial muscles; rect., rectum.

The arrow denotes position of anal receptor.

The system shows several minor variations in Nephrops, e.g. the fusion of the R_1 and R_4 muscle groups and occasional fusion of the posterior intestinal nerves with the anal nerves (a condition sometimes also found in Homarus). Note that previously only muscles R_3 and R_4 have been described[1], and the suggested terminology used here applies only to position and not to any implied function.

Also originating from the sixth abdominal ganglion is a single pair of anal nerves[1,12]. In Homarus gammarus the anal nerves, in the main, carry afferent information from the sensory structures of the telson, but also give off small branches that terminate in the region of the anus. Methylene blue staining of the area indicates that in at least one ventrally going branch on each side of the anus there is one large bipolar sensory cell. The sense cell body lies deep alongside the anus and the dendrite passes posteroventrally to lie on a portion of hypodermis below the soft cuticle of the anus. The dendrite then loops to run anteriorly and finally ramifies in the hypodermis on the same side of the anus (Fig. 1). The form and position of this receptor is reminiscent of those described by Pabst and Kennedy[7] and perhaps represents the last in the abdominal series of such receptors.

The anus can be opened and closed by stimulation of the posterior branches of the posterior intestinal nerve and cutting of the anal nerves does not affect this response. It appears, therefore, that the anal nerves carry almost entirely sensory information. Teasing of the bundles to small groups enables electrophysiological recording of activity in the anal nerves from receptors that discharge during anal opening (Fig. 2), during which time the surrounding hypodermis is deformed. Removal of the overlying cuticle does not interfere with this response, but cutting the underlying hypodermis abolishes it. Part of this activity is believed to represent the response of the receptor cell described here, but in some preparations more than one unit is active and anal closure is also monitored. The location of these supernumerary sensory units is not yet known.



Response of soft cuticle receptor of anal lip during anal opening. Upper beam - Activity of receptor in teased left anal nerve. Lower beam - Record of the movements of the left anal lip recorded by means of an RCA 5734 transducer (downward deflection denotes anal opening).

A. Spontaneous activity of receptor with anus in closed position. Mean frequency of output is approximately 3Hz.

B. Activity of receptor during anal opening elicited by simultaneous stimulation of both posterior intestinal nerves with 3mS pulses at 50Hz for approximately 1.5sec. (dotted line indicates stimulus pulses).

Barth[8] described a receptor located within the telson of Procambarus clarkii as a result of physiological analysis, but gave no anatomical details. It is possible that the anal receptor represents a unit similar to the telson receptor which may be located on soft cuticle elsewhere in this region. Work is in progress to confirm or deny this proposition.

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