The anatomy and physiology of the posterior stomach nerve (p.s.n.) in some decapod crustacea

BY M. R. DANDO AND M. S. LAVERACK

Gatty Marine Laboratory and Department of Natural History, University of St Andrews

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One of the major paired nerves in the decapod stomatogastric nervous system innervates the posterior part of the gastric mill. The morphology has been completely described previously only in Pugettia producta. This nerve is called the posterior stomach nerve (p.s.n.) in this paper. It is redescribed for Homarus vulgaris, and less detailed information is given for Palinurus vulgaris and Cancer pagurus. In these three species the p.s.n. contains numerous cell bodies. The majority of the cells are in one group and the long distal processes of these cells form a large proportion of the fibres in the nerve which runs to the gastric mill. Many of these fibres terminate by ramifying in the connective tissue which invests the ossicles of the gastric mill.

The Brachyuran C. pagurus was used for most of the physiological experiments reported here. Only a small percentage of the animals exhibited spontaneous movements of the gastric mill when the carapace was removed and this activity ceased rapidly. However, a cycle of movements was usually observed in the active gastric mills and the p.s.n. contains many elements which respond to these normal movements. The p.s.n. does not appear to contain motor fibres. Intracellular recordings show that cells in the p.s.n. are proprioceptors. The distal processes of the major group of cells are morphologically dendrites, but they probably support action potentials. Repetitive electrical stimulation of a p.s.n. when it is isolated from the gastric mill evokes changes in the output from the stomatogastric ganglion. The p.s.n. sensory system could therefore function in the normal reflex control of the activity of the gastric mill. This investigation may assist in the analysis of the functions of the stomatogastric ganglion.

INTRODUCTION

The anatomy of the stomatogastric nervous system of the decapod crustacea was recently reviewed by Bullock & Horridge (1965). The general plan of a group of small ganglia and mainly bilaterally arranged nerves is uniform throughout the group. The value of this system for neurophysiological analysis has been demonstrated by Maynard's (1966, 1967) analysis of the generation of a temporally patterned output by the 30 to 35 neurons of the stomatogastric ganglion and by Larimer & Kennedy's (1966) study of an unusual multipolar sensory cell in the same ganglion.

Heath (1941), who worked on the kelp crab Pugettia producta, is the only author to have fully described the course of one of the larger paired nerves ('s' in his terminology) in the stomatogastric nervous system. These nerves terminate peripherally around the insertion of the posterior gastric muscles on each side of the gastric mill. From this point each nerve runs in an antero-lateral direction to the region above the mandible. It then descends ventrally to run back, usually fused with the mandibular nerve, into the thoracic ganglion. Staining with methylene
blue demonstrated that in *Homarus vulgaris* and *Cancer pagurus* these nerves, which we term the posterior stomach nerves (p.s.n.s.), contain numerous cell bodies. The nerves which Bullock & Horridge call the posterior stomach nerves will be called the postero-lateral nerves after Mocquard (1883).

The morphology of the cell bodies in the p.s.n.s. strongly resembles that of previously described crustacean mechanoreceptors (for example, Pabst & Kennedy 1967) and suggests that the p.s.n.s. have a sensory function. The position of the nerve endings on the posterior arch of the gastric mill suggests that if the nerves have a propriosensory function the information transmitted centrally affects the output from the stomatogastric ganglion to the muscles of the mill. The present work demonstrates the validity of these assumptions. The p.s.n. does contain neurons which respond to normal movements of the gut, and stimulation of a p.s.n. when it is isolated peripherally but connected centrally does affect the output from the stomatogastric ganglion. This paper describes the anatomy and physiology of the p.s.n. sensory system in some detail.

**Materials and methods**

*Homarus vulgaris* and *Cancer pagurus* were obtained locally. Specimens of *Palinurus vulgaris* were obtained from W. Harvey, Fish Merchant, Penzance, Cornwall. The *Homarus* used were between 20 and 22 cm long and the *Cancer* between 15 and 17 cm broad across the carapace.

The methods of Alexandrowicz & Whitear (1957) were used for the methylene blue staining. In both the lobsters and crabs the gastric mill is covered with thick white tissue. This tissue had to be removed gradually as the nerves stained to facilitate further staining. For the examination of the nerve with the electron microscope the tissue was fixed in 2% osmium tetroxide solution diluted with an equal volume of sea water. Acetone was used as the dehydrating agent and Araldite as the embedding medium. Thick sections were cut with a Porter–Blum microtome and stained with toluidine blue. Thin sections were cut with an LKB microtome, stained with lead citrate and uranyl acetate, and examined with an AEI EM 6B electron microscope.

For the electrophysiological experiments two preparations were used. After induced autotomy of the chelae and walking legs *Cancer* provided a minimally dissected preparation by simple removal of the carapace and epidermis over the gastric mill. Although the p.s.n.s. were always near the exposed surface it was usually necessary to isolate the nerves from the surrounding tissues. In order that the p.s.n.s. could be located easily and a reasonable length of the nerves exposed, the large specimens were used. This produced difficulties when the chelae were removed because it was not always possible to make the animal autotomize these limbs. If these limbs were removed surgically the blood losses were stemmed with tissue paper. After dissection the animal was transferred as quickly as possible to an experimental bath containing aerated saline (Pantin 1962). The saline temperature was kept between 7 and 12 °C by a flow of water around the experimental bath. The anterior aorta was left intact in all experiments.
For experiments with *Homarus* the thorax was isolated and the digestive gland removed from it. The thorax and stomach were thoroughly washed with sea water at this stage. Then the thorax was split longitudinally from the ventral surface. On one side the whole urocardiac tooth of the gastric mill was retained. This half thorax, which contained the majority of the gastric mill, was laid on its outer side and the ventral part of the stomach was removed. The apodeme of the posterior adductor muscle was then cut and the p.s.n. was separated from neighbouring tissues thus exposing a long length from the edge of the mandible to the posterior adductor muscle of the mandible. Finally the ventral part of the body wall was cut away and the apodeme of the posterior adductor muscle pinned aside. This procedure enabled recording from the region of the p.s.n. proximal to the main group of cells. Recording from the distal portion of the nerve was accomplished after the entire posterior adductor muscle was removed. Cool (10 to 15 °C) filtered sea water was used as the bathing fluid.

Extracellular recording and stimulation were carried out with fine wire electrodes on which the nerve was lifted into liquid paraffin. Glass microelectrodes filled with 3M KCl and having a resistance of 20 to 30 MΩ were used for the intracellular recordings. Conventional methods of amplification and display were used.

**Anatomy**

*Homarus vulgaris*

The p.s.ns., which in *Homarus* are branches of the outer mandibular nerves, are symmetrically arranged on each side of the animal. Each p.s.n. proceeds ventro-laterally along the mandible and then turns dorsally to run up the side wall of the thorax just anterior to the cervical groove. The nerve then enters the ventral surface of the posterior adductor muscle of the mandible, and after passing through this muscle, runs onto the gut by way of the posterior external gastric muscle. The nomenclature used is that of Keim (1915) and Schmidt (1915).

Figure 1 shows the nerves which occur in the region of the mandible on the right side of the animal. The outer mandibular nerve leaves the circumoesophageal connective as a single trunk in the majority of animals and divides into two near the ventral head muscle. Both branches pass under the ventral head muscle and run out towards the lateral adductor muscles. Just after the first division of the main nerve the posterior branch divides again giving rise to a nerve which courses ventrally under the major abductor muscle onto the inner surface of the mandible. This is the posterior stomach nerve. The nerve runs along the posterior edge of the mandible and then onto the side wall of the thorax. It gives off small branches to sense organs on the surface and borders of the mandible. Many of the fibres in these branches are recurrent and run parallel to the p.s.n. for a short distance. A few cell bodies, usually with processes innervating the epidermis, are often present in the nerve trunk in this region. Most of these cells are bipolars but some tripolar cells may be seen.

Figure 2 shows the direction taken by the left p.s.n. on the lateral wall of the thorax. The p.s.n. runs in a straight line usually just anterior to the bow of the
Figure 1. The origin of the right p.s.n. from the outer mandibular nerve in *Homarus vulgaris*. The dorsal part of the animal has been removed and the interior of the mandible is seen from above. The anterior of the animal is at the top of the diagram. The outer mandibular nerve (omn) leaves the connective (oc) as a single trunk. Just after the first division of the nerve the posterior branch divides again giving rise to the posterior stomach nerve (psn) which drops down onto the mandible (mand) under the major adductor muscle (ma.abd). The divisions of the outer mandibular nerve usually occur near the ventral head muscle (vhm). The other two branches of the outer mandibular nerve run out towards the lateral adductor (l.add) muscles. The posterior stomach nerve crosses the edge of the mandible behind the minor adductor muscle of the mandible (mi.abd) and runs out towards the thoracic base of the posterior adductor muscle of the mandible, the mandibular apodeme (apd) of this muscle is shown here.

Figure 2. The course of the left p.s.n. on the lateral wall of the thorax of *Homarus vulgaris*. The anterior of the animal is to the right, and the top of the diagram is dorsal. The apodeme of the posterior adductor muscle of the mandible has been cut and pulled dorsally. The posterior stomach nerve (psn) crosses the mandible (mand) between the minor adductor muscle of the mandible (mi.abd) and the adductor muscle of the coxopodite of the 1st maxilla (c.abd). It crosses the thorax in front of the bow of the cervical groove (cg). The main group of cell bodies (cbs) occurs just before the nerve enters the posterior adductor muscle (p.add) and about level with the thoracic base of the anterior dorso-ventral muscle (dvm). The other muscles bounding this region are the attractor muscle of the epimeral plate (at.ep) and the remotor muscle of the antenna (r.ant).
cervical groove. The nerve is usually about 250 μm in diameter in this region. The position of the individual cell bodies and the distribution of the minor branches of the nerve which innervate the epidermis vary widely even between the two sides of the same animal. The main group of cells, however, always occurs immediately before the nerve enters the adductor muscle, and the distal processes of most of these cells run to the gastric mill. It was difficult to obtain good methylene blue staining of all the cells in any one preparation. Nevertheless, in many preparations 60 cells were clearly visible and up to 80 were seen in some preparations. This compares with a total of about 105 to 125 fibres in the nerve just distal to the group of cells (see later, figure 4B). No other cell bodies are present between the major group and the ending of the nerve on the gastric mill but a few cells are always present between the major collection and the mandible. The cell bodies are of two types. The majority are simple bipolars of about 60 x 40 μm (figure 3A, plate 30). The others are varieties of bipolars which tend towards a monopolar type (figure 3B), and are of the same size as the simple bipolars. No anatomical connexions were observed between the cells in the group.

A large number of nerve fibres ramify in the thoracic epidermis surrounding the p.s.n., particularly near the base of the anterior dorso-ventral muscle. Many of the fibres run into the region from other nerves, but the p.s.n. also sends fibres to this area between the mandible and the adductor muscle. Sometimes the fibres are given off in groups which form minor branches of the nerve, and occasionally processes lead directly from a cell body in the p.s.n. out into the epidermis. The fibres innervating the epidermis often split up with characteristic multiple bifurcations which spread the distal processes from one cell over a wide area. Correlated with the branching of the main p.s.n. is a decrease in the number of fibres in it (as shown by electron microscopy) from about 160 to 180 on the thoracic edge of the mandible to about 105 to 125 immediately distal to the main group of cells (figures 4A, B, plate 30). The fibres in the nerve form a fairly homogeneous collection with regard to diameter and none are larger than 8 μm. Near the main group of cell bodies the proximal processes are nearly all alike in structure and similar to previous descriptions of crustacean nerve fibres (for example, that of the crab leg nerve, Horridge & Chapman 1964). One striking point of difference shown in the cross-sections of the p.s.n. is the roundness of the fibres (figure 5, plate 31). There is no great difference between the nerve processes proximal and distal to the major group of cells.

The part of the p.s.n. which runs to the gastric mill is often divided as it enters the adductor muscle but these separate trunks join up within the muscle and the total diameter of the nerve decreases. Few small side branches leave the main trunks after they enter the posterior adductor muscle. The mandibular nerve and a large blood vessel run in the same gap in the muscle as the p.s.n., but the p.s.n. is most superficially positioned. As the p.s.n. reaches the base of the gastric muscle it is often crossed by another nerve which supplies the body wall epidermis in this region. On the posterior external gastric muscle the p.s.n. spreads out and many of the fibres divide by multiple bifurcations as the nerve approaches the gastric mill.

The exact location of the endings of the p.s.n. on the gastric mill was difficult to
ascertain. This difficulty was caused by the presence of an anastomosis between the p.s.n. and several other nerves near the gastric mill. The other nerves forming the junction are the lateral ventricular nerve, and the three nerves that run respectively over the cardio-lateral muscle, round the posterior gastric muscles, and onto the pyloric region of the gut. The lateral ventricular nerve contributes many fibres to the last two nerves mentioned. These fibres are easily traced because of their large size. Figure 6 is a diagram of this region though the precise anatomy is variable between different preparations. Often there are anastomoses, involving small numbers of fibres, between the p.s.n. and branches of the nerve running around the gastric muscles before the major junction. The p.s.n. can contribute fibres to all other nerves partaking in the anastomosis and branches of single fibres do pass into different nerves. Despite this complexity one significant feature is often seen in preparations in which the p.s.n. divides before the major anastomosis. In such preparations some branches of the p.s.n. do run to the gastric mill without encountering any other nerves (that is to say, bypassing the junction). The fibres in these branches ramify in the connective tissue which occurs in the regions between the posterior gastric muscles and the dorsal edges of the exopyloric and zygocardiac ossicles. (This connective tissue fills all the spaces between the ossicles of the mill and is easily distinguished from the thick white tissue which overlays the gastric mill.) Nerve fibres also run onto the top of the mill between the insertion of the posterior gastric muscles and the propyloric ossicle, and laterally along the dorsal edge of the zygocardiac ossicle. In all preparations there were nerve fibres in these regions and often some could be traced back to the p.s.n. Though some fibres run deeply into the connective tissue none were traced directly onto the ossicles.

**Palinurus vulgaris**

In this species the p.s.n. follows a similar course to that described for *Homarus*. The main group of cell bodies is usually nearer the mandible than in *Homarus*, and its position is often obscured by a blood vessel. The cell bodies are large (80 × 40 μm) and are generally pear-shaped with the blunt end facing centrally. There is also a small proportion of cells tending towards the monopolar type. The p.s.n. is

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**DESCRIPTION OF PLATE 30**

**Figure 3.** Nerve cells in the p.s.n. of *Homarus vulgaris* stained with methylene blue and fixed in ammonium molybdate. A. A collection of bipolar cells from the main group of cells of a preparation. The deeper-lying cells are only lightly stained but this is not due to them having thick sheaths as in the cells of *Cancer* (figures 8 and 9, plate 31). B. A cell which is almost of a monopolar form. In both A and B the peripheral processes are at the top of the picture.

**Figure 4.** Transverse sections of the p.s.n. of *Homarus vulgaris*. These nerves were fixed in osmium tetroxide and stained in toluidine blue. It is sometimes difficult to decide if small objects are fibres, but electron micrographs show that there are very few small unsheathed fibres in the p.s.n. The figures quoted in the text are taken from counts of five preparations. A. Section of a p.s.n. on the thoracic edge of the mandible. There are about 170 processes in this section. B. Section of the same nerve as in A immediately distal to the position of the main group of cells. There are about 120 processes in this section.
For legend see facing page.

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commonly divided into a number of trunks distal to the main group of cells. The nerve runs external to the posterior adductor muscle of the mandible and through a gap in the anterior dorso-ventral muscle. We did not continue working with this species because yellow tissue (see Alexandrowicz & Whitear 1957) obscured the central end of the p.s.n., and the gastric mill is much weaker than that of Homarus.

Cancer pagurus

The course of the two p.s.ns. on the dorsal surface of Cancer (figure 7) is similar to that described for Pugettia by Heath. At the base of the posterior external gastric muscles each nerve runs laterally for a short distance and lies alongside a small muscle which is an extension of the internal abductor muscle of the mandible. This small extension is not described by Pearson (1908) in his monograph on Cancer. The p.s.n. often divides in order to pass round this muscle. Most of the cell bodies are in a group which is near this muscle or between it and the gut. (In a few preparations the group of cells was at some distance central to the internal abductor.) From the small muscle the p.s.n. passes forward and becomes associated with the epidermis before reaching the cuticular insertion of the external abductor muscle of the mandible. A few cell bodies are often found along this region of the nerve between the two muscles. The nerve runs centrally along the external abductor muscle and then into the thoracic ganglia with the mandibular nerve. A group of 10 to 15 cell bodies occurs in the common root of the p.s.n. and the mandibular nerve. Some of the distal processes from these cells innervate the tissue overlaying the mandible but many appear to run in the p.s.n. There are usually 20 to 25 cells which stain in the main group of cells.

Cross-sections of the nerve show that it has a much thicker sheath than in Homarus and that many of the cell bodies also have thick individual sheaths (figure 8, plate 31). All the p.s.n. cells are bipolars with dimensions of between $30 \times 20 \mu m$ and $50 \times 30 \mu m$. Good staining with methylene blue was difficult to achieve. Many of the cells appear to have a large pale surround (figure 9, plate 31).

Description of Plate 31

Figure 5. An electron micrograph of typical fibres in the p.s.n. of Homarus vulgaris. This section is of processes just distal to the main group of cells from the same nerve as is shown in figure 4, plate 30. $d =$ nerve fibre, probably the peripheral process of a cell in the p.s.n.; $i =$ inner sheath of nerve fibre; $m =$ mitochondrion; $s =$ outer sheath of nerve fibre; $g =$ sheath cell nucleus.

Figure 8. Transverse section of part of the p.s.n. of Cancer pagurus. This nerve was fixed in osmium tetroxide and stained with toluidine blue. $is =$ individual sheath of a cell body; $cb =$ cell body; $ns =$ sheath of p.s.n. The large peripheral processes ($p$) of some cells are at the top of the picture.

Figure 9. Nerve cells in the p.s.n. of Cancer pagurus. These cells were stained with methylene blue and fixed in ammonium molybdate. The large diameter peripheral processes of the cells run towards the top of the picture. These cells show the clear halo around the stained part of the neuron. This effect is almost certainly caused by the failure of the large individual sheaths of the cells (figure 8) to stain.
The sheaths probably explain the capricious results of the methylene blue staining. The cells with the thick sheaths are of the same sizes as the other cells in the nerve which stain normally. However, sequential transverse sections of the nerve confirm an impression gained from methylene blue staining, that the initial parts of the peripheral processes of many cells are of greater diameter than the central processes.

**Figure 6.** The peripheral termination of the right p.s.n. on the posterior of the gastric mill of *Homarus vulgaris*. The anterior of the animal is to the right. The lateral ventricular nerve (lvn) has been cut and pulled clear of the thick white tissue which still overlays the dorsal surface of the gut. This tissue has been removed from the base of the posterior external gastric muscle (pegm). The posterior stomach nerve (psn) runs along the ventral surface of the posterior external gastric muscle and gives off a small branch to the connective tissue which invests the region between the posterior gastric muscles (pigm = posterior internal gastric muscle) and zygocardiac ossicle (zo). The posterior stomach nerve then anastomoses with the lateral ventricular nerve. From this anastomosis a nerve (cln) runs over the cardiolateral muscle (clm). The thick white tissue has been removed from this muscle down to the infero-lateral ossicle (ilo). Other nerves from the anastomosis run over the pyloric region of the gut (prn) and around the gastric muscles (pgm).

In the region of the nerve between the two mandibular muscles (i.e. central to the usual position of the cell bodies) there are usually 40 to 45 fibres. These fibres are similar in structure to those described for *Homarus*. The counts of fibres and cell bodies in the nerve are again fairly close. As they approach the gut many of the fibres in the p.s.n. divide into smaller processes, again with the typical bifurcations.

The p.s.n. on each side runs on to the gut near the base of the posterior external gastric muscles. Each nerve usually anastomoses with the lateral ventricular nerve on the same side of the gut and then joins the other p.s.n. just behind the propyloric ossicle. Branches of the posterior stomach nerves ramify in the connective tissue which invests the ossicles of the mill but the innervation appears to be concentrated more around the propyloric ossicle than in *Homarus*.

In *Carcinus maenas* there is a p.s.n. which runs on to the gastric mill in a similar manner to that described for *Cancer pagurus*. This nerve also contains a group of cells in the same position as in *Cancer*. *Galathea strigosa* also possesses a nerve running on to the posterior part of the gut in the same position as in *Cancer*. It was
difficult to identify cell bodies in Galathea because in the small number of specimens examined the nerve joined the epidermis much closer to the gut than in Cancer and made vital staining difficult.

Figure 7. The arrangement of the stomatogastric nervous system on the dorsal surface of the gastric mill of Cancer pagurus. The thick white tissue has been removed from the dorsal surface of the gastric mill and the p.s.ns. cleared of obscuring viscera up to the point where they descend ventrally. On each side of the mill the posterior stomach nerve (psn) contains a group of cell bodies when it is near the internal abductor muscle (i.abd). The p.s.ns. then run onto the gut near the insertion of the posterior gastric muscles. For clarity the usual anastomosis with the lateral ventricular nerves (lvn) is omitted. The p.s.ns. join up behind the proplyloric ossicle and fibres from one side probably often cross to the other. The region in front of the proplyloric ossicle is heavily innervated. The connective tissue here overlays the reflected urocardiac ossicle (see figure 10). Anteriorly there are four nerves arising from the region of the stomatogastric ganglion. The largest nerve is probably analogous to the dorsal ventricular nerve (d.vn) of Homarus minus the innervation of the cardio-pyloric muscles (cpm) which here are innervated by a separate short ventricular nerve (svn). This is often a branch of the d.vn. close to the ganglion. The dorsal ventricular nerve divides into two lateral ventricular nerves. The paired outer lateral nerves (oln) are probably analogous to the median ventricular nerves of Homarus as they appear to innervate the same muscles. There are variable anastomoses between these four stomatogastric nerves. The stomatogastric ganglion lies on the anterior surface of the cardiac stomach and it is not shown in this diagram. agm, anterior gastric muscles; pgm, posterior gastric muscles.

Physiology

Cancer pagurus

This species was used for most of the work as it was available in large numbers and because the nervous system on the dorsal surface of the mill is more accessible that that of Homarus. Figure 7 shows the normal resting position of the gastric mill and the anatomy of the nerves running posteriorly from the stomatogastric ganglion in Cancer. This diagram should be compared with Maynard’s (1966) figure for Homarus. In Cancer the p.s.ns. can be isolated and physiological activity recorded with little difficulty. The nerves in Homarus are overlain and obscured by the posterior external gastric muscles. The disadvantage of using Cancer is that the stomatogastric ganglion usually lies on the anterior surface of the gut almost at right angles to the plane of the nerves shown in figure 7. The relevant ossicles of the gastric mill of Cancer are illustrated in figure 10. The anterior gastric muscles insert on the medial part of the pterocardiac ossicles. The posterior gastric muscles insert
on the pyloric and exopyloric ossicles, and the cardiopyloric muscles span the gap between the anterior and posterior arches of the mill. In open preparations the movements of the major dorsal ossicles can be followed during normal and induced activity.

Figure 10. The dorsal ossicles of the gastric mill of *Cancer pagurus* as seen from the inside of the mill. The anterior arch consists of the median mesocardiac ossicle (mc) and on each side a pterocardiac ossicle (pt). The distinction between these and the median urocardiac ossicle (uc) which bears the medial tooth (mt) is emphasized in this drawing. Seen from this aspect the medial tooth would lie above the propyloric ossicle (pr.p) in life. Here the propyloric ossicle is shown in a more posterior position and displaced more than 90° from its usual alinement. The apex of this ossicle connects with the urocardiac ossicle, and the base with the posterior arch. The medial tooth is reflected backwards at rest. The medial pyloric ossicle (p), two paired exopyloric ossicles (ep), and the two zygocardiac ossicles (zc) make up the posterior arch. The zygocardiac ossicles connect with the outer ends of the two pterocardiac ossicles in life. The zygocardiac ossicles each bear a lateral tooth (lt). In life these teeth would be rotated in towards the medial tooth and would lie above it if seen from this aspect.

We were not able to make a detailed study of the natural movements of the gut since in most preparations the gastric mill did not move spontaneously. In those preparations in which the mill did move after the carapace was opened the movements did not last longer than a few minutes. Starvation of the animals or starvation followed by feeding just before the operation, had little effect on the number of animals in which the mill moved spontaneously after dissection. The movements of the active mills consist of a synchronous motion of the mesocardiac ossicle backwards and a smaller forward motion of the propyloric ossicle. In mills 1.5 cm long from the mesocardiac to the propyloric ossicle, the mesocardiac ossicle was often observed to move backwards by 0.4 to 0.5 cm. These movements were followed by a slower concurrent return of the ossicles to their resting positions. The time taken for the complete cycle of movements to occur varied from 2 to 5 s. It was occasionally possible to observe one of the ossicles moving without the other also moving, but normally the two movements were coordinated. The movement of the mesocardiac ossicle necessarily imposes a movement on the central parts of the pterocardiac ossicles, and of the urocardiac ossicle. The movements of the propyloric ossicles probably require simultaneous movements of all the other ossicles in the posterior arch of the mill. These normal movements must involve backward and
forward motions of the medial tooth and probably movements of the lateral teeth. In a small number of preparations the mesocardiac ossicle also moved forward from the resting position taken up between the normal cycle of movements. This movement appeared to be caused by contraction of the anterior gastric muscles and not just relaxation of the cardio-pyloric muscles.

In about half of the preparations examined movements of the gastric mill could be evoked when the d.v.n. (dorsal ventricular nerve) and s.v.n. (short ventricular nerve), nerves which run over the dorsal surface of the gastric mill from the stomatogastric ganglion, were isolated from the ganglion and stimulated by a burst of electrical shocks. The resultant cycle of movement was the same as that observed in minimally dissected crabs and of the same order of magnitude. A forward motion of the mesocardiac ossicle between the cycles was never evoked by stimulation but sometimes the whole mill moved backwards. Equivalent stimulation of the peripheral part of the p.s.n., when it was isolated from the c.n.s., evoked no noticeable movement of the gut. This indicates that the p.s.n. probably lacks motor fibres. Concurrent stimulation of the d.v.n. and s.v.n. nerves, and the peripheral part of the p.s.n. did not interfere with the movements of the mill. This indicates that the p.s.n. does not carry inhibitory fibres.

Spontaneous electrical activity was recorded in the p.s.n. when there was no visible movement of the stomach. This activity was recordable distal and proximal to the major group of cells. It is a fairly regular discharge. If the nerve was cut central to the usual position of these cells the activity did not noticeably decrease in that part of the nerve distal to the cut. In the central stump of the nerve few units were evident after the injury discharge.

Normal movements of the gastric mill caused by stimulation of the d.v.n. and s.v.n. nerves evoked changes in the activity of units in the p.s.n. (figure 11). The units recorded all had a phasic and a tonic component. Larger movements of the

![Figure 11](http://rspb.royalsocietypublishing.org/)

**Figure 11.** Cyclic movements of the gastric mill of *Cancer pagurus* evoked by stimulation of the nerves from the stomatogastric ganglion which run posteriorly over the dorsal surface of the mill; and the response of a unit in the left p.s.n. recorded at a position central to the main group of cells. In all records the top line shows when a stimulus is delivered. The second line is a record of the movements of the left pterocardiac ossicle midway along its length monitored with a mechano-electrical transducer (RCA 5734). The bottom line is a record of a unit in the p.s.n. A. The response to a stimulus of 10 V. B. The response to a stimulus of 15 V. The time base is the same as in A. The transducer rod on the pterocardiac ossicle moved back 2 mm at this stimulus intensity. C. The response to a stimulus of 10 V with the speed of film increased to twice that in A. D. The fourth and fifth responses in a series of equal stimulations of 10 V with the same time base as in A.
min produced an increased response from the units. Similar units were readily recorded in the p.s.n. following simple mechanical movements of the mesocardiac ossicle which simulated normal movements (figure 12). A range of resting frequencies and responses to a similar stimulus were encountered but no pure phasic or tonic receptors were found. The units are sensitive to the rate (figure 13) and to the magnitude of displacement. Smaller responses could be obtained from these units by forward movements of the mesocardiac ossicle. It is possible that the extent of movement necessary to evoke these responses is outside the normal physiological range of movements. Unfortunately it was difficult to stimulate all of the nerves which innervate the anterior gastric muscles in order to check the response. The single units recorded in the p.s.n.s. could be stimulated by probing widely spaced (even contralateral) parts of the posterior of the gastric mill. Great care was necessary in these experiments to prevent the p.s.n. from being stretched by movements of the mill and perhaps thereby producing false results.

The electrical activity in the nerves running over the gastric mill from the
stomatogastric ganglion often occurred in regular patterned bursts (figure 14). This patterning remained if the peripheral part of the nerve was cut away from the gut. The nerves most thoroughly studied were the paired outer lateral nerves in the group which run back over the mill (figure 7). These nerves are almost certainly equivalent to the median ventricular nerves of *Homarus* as described by Maynard.

**Figure 14.** Examples of the patterned output in nerves originating in the stomatogastric ganglion. A. Output in the d.v.n. This record was filmed at half of the film speed of record B. It demonstrates the regularity of the output over a period of time, which was obtained in good preparations. B. Top line is a record of the output in the d.v.n. Bottom line is the output in the left o.l.n. of the same preparation recorded simultaneously. The time mark applies to this trace. C. The output in the right and left l.v.n.s of a preparation, recorded at twice the film speed of B. Most preparations had recognizably similar units in these nerves but many were much more irregular. The cycle frequency also varied in different regular preparations.

(1966). Recordings were usually made from these trunks after they had crossed the anterior gastric muscle. There is a good deal of variation in the anatomy of the nerves on the dorsal surface of the mill, but this region of the outer lateral nerves could always be located with little difficulty. In many preparations the recordings from the nerve demonstrated the presence of two units which fired in a rhythmic manner (figure 14B). The small unit tended to cease discharging quickly, leaving the large unit alone. In about 65% of the experiments performed repetitive electrical stimulation of either one of the peripherally isolated p.s.n.s. was followed by changes in the activity of the units in the outer lateral nerve. No great difference was noted between stimulation of the p.s.n. on the ipsi- or the contralateral side of the gut to the nerve under study, though the majority of the experiments were performed with the stimulation applied to the contralateral p.s.n.

Figure 15 illustrates an example in which stimulation of the contralateral p.s.n. produced an inhibition of the firing of the large unit in the outer lateral nerve followed by an increase in activity when the stimulus ceased. In the series shown in figure 16 the inhibition and rebound become progressively greater. This unit is probably equivalent to the *m* unit described by Maynard (1967). A different result sometimes obtained was a direct increase in the activity of the large unit upon p.s.n. stimulation. In a few experiments stimulation of the p.s.n. initially caused an inhibition of the unit followed by the poststimulatory increase in activity and later in the experiment only caused a direct increase in activity. This increase may have been due to the progressive deterioration of the preparation. Occasionally, however, the patterned activity of the two units returned during the stimulation experiments.
(figure 15). These experiments may therefore indicate a lability of the junctional events in the interpolated stomatogastric ganglia. The experiments were controlled by stimulations applied to the viscera and the gastric mill to ensure that there was not some general effect on the animal produced by the shock of the stimulus. Such controls and experimental stimulations could be repeatedly alternated with no change in the response to the experimental stimulus, and no response to the shocks applied elsewhere.

**Figure 15.** The response of two units in the left outer lateral nerve of *Cancer pagurus* to repetitive electrical stimulation of the cut central end of the right p.s.n. *A.* Normal output in the nerve. Filmed at half the speed of B to E. *B.* Output in the peripherally-isolated nerve when the small unit ceases firing. *C.* Two repetitive stimulations in a short series are indicated by the second line. Note inhibition and rebound. *D.* Record of activity after the series of stimulations. This output lasted for several minutes after which another series of stimuli were given. The ‘normal’ activity then returned to the nerve. *E.* The effect of repetitive stimulation of the p.s.n. on this activity. Time mark is for *B* to *E*.

**Homarus vulgaris**

Spontaneous electrical activity was recorded in the p.s.n. when there were no visible gut movements. This spike activity was recordable both distal and proximal to the major group of cell bodies. Transections of the nerve central to the group of cells left the same level of activity in the distal section but very little in the central
Sectioning the nerve distal to the cell bodies left the same level of activity in the terminal portion. This activity was recordable in the part of the p.s.n. on the posterior external gastric muscle very close to the gastric mill.

Movements of the ossicles of the mill which mimicked normal activity observed in minimally dissected animals, evoked changes in the activity of units in the p.s.n. in a similar manner to that described for Cancer. Movements of the pyloric region alone of the gut had little effect on the activity of the p.s.n., whereas movements of the teeth of the mill from their normal alinement (as might be produced by a large amount of food being in the stomach) evoked responses in units on the p.s.n. Intracellular recordings from the cells in the main group confirmed that the cells are proprioceptors responding to normal movements of the gastric mill (figure 17).

**Figure 17.** Intracellular recordings from a cell in the p.s.n. of Homarus showing the response to forward movements of the urocardiac ossicle. A. The response to a movement of 3 mm. Time mark for A to C is 1 s. B. The response to a similar movement filmed at twice the speed of A and C. This movement was slightly smaller than in A but started from in front of the resting position. C. The response to the fourth and fifth in a series of equal stimulations. D. Records of spikes filmed at higher speed to show the form of the impulse. Calibration 80 mV and 50 ms.

**Discussion**

The mode of action of the gastric mill in the decapod crustacea has been the subject of a long debate (e.g. Mocquard 1883; Pearson 1908; Patwardhan 1935; Reddy 1935). The argument has centred on the relative importance of the anterior and posterior gastric muscles in the movements of the mill. At the present time it is only possible for us to draw tentative conclusions on this subject because of the difficulty of making open Cancer preparations function over any length of time. Previous descriptions of the movements have mostly followed Mocquard’s (1883) observations on some crabs with transparent cuticles. He states that the major feature is a forward motion of the mesocardiac ossicle from the resting position caused by contraction of the anterior gastric muscles. This may be the major movement in Homarus but it was not often seen in Cancer. Mocquard himself was careful to state that his observations may not have covered all the possible movements of the mill. In our experiments it was difficult to judge the effect of the swelling of the cardiac stomach, which usually occurs when the carapace is removed, on the activity of the gastric muscles. The important point is that the cycle of movements...
which is described does occur in most active preparations and thus constitutes a part of the normal activity of the mill.

Repetitive stimulation of the nerves which originate from the stomatogastric ganglion and run posteriorly over the dorsal surface of the mill evokes movements which are very similar to the normal cycle of movements. The response in the p.s.n. indicates that the mechanoreceptors in the nerve function during normal movements of the gastric mill. The lack of response to movements of the pyloric region of the stomach shows that the p.s.n. does not directly monitor the passage of food from the cardiac to the pyloric stomach.

The small amount of residual activity in the section of the p.s.n. central to a cut which leaves the main group of cells on the distal side, indicates that the p.s.n. contains few motor fibres unless the neurons have failed centrally. The failure of stimulation of the section of the p.s.n. distal to a cut, to elicit movements of the gut also shows that the p.s.n. has no motor neurons unless the neuromuscular junctions have failed. These observations were made on the dorsal surface of the gastric mill and it is possible, but unlikely, that small movements of other parts of the mill were not seen. We conclude that the p.s.n. carries no motor fibres innervating the gut. The possibility that the nerve carries inhibitory fibres has also been eliminated because concurrent stimulation of the p.s.n. and the motor nerves from the stomatogastric ganglion had no effect on the movements. It is not likely that the p.s.n. contains chemoreceptors or innervates glandular tissue because the fibres do not end in the gut cuticle and there are no glands to be seen in this region.

The anatomy of the p.s.n. suggests that the cells in it are sensory and this is confirmed by the intracellular recordings from the cells in the main group in Homarus. The cell body and fibre counts indicate that the peripheral processes from the cells in the main group make up at least a large percentage of the fibres in the p.s.n. which run to the gastric mill. This, combined with the similarity of the electrical activity which can be recorded central and distal to the main group of cells, indicates that many processes which are morphologically dendrites carry propagated action potentials as has been found in other decapod mechanoreceptors (Mellon & Kennedy 1964; Mendelson 1966; Hartman & Boettiger 1967).

The sensory neurons in the p.s.n. are clearly type II receptors (Pringle, 1961) having branched dendrites which end with no obvious terminal specialization, but it is not possible to be sure that some of the dendrites do not end on muscles. The units observed in the p.s.n. have all been varieties of the intermediate phasitonic type such as the unusual sensory neuron in the stomatogastric ganglion of the crayfish reported by Larimer & Kennedy (1966), and the telson receptor of Barth (1964). Distortions of the connective tissue caused by movements of the ossicles must result in the generation of action potentials very close to the gut. The fact that the fibres branch widely on the gut might be expected to impose difficulties for the c.n.s. in the interpretation of the sensory information. This topic was investigated by Pabst & Kennedy (1967) for the crayfish cutaneous mechanoreceptors which have many similar features.

The fact that repetitive stimulation of the p.s.n. alters the output in the nerves from the stomatogastric ganglion should allow a more precise determination of the
function of the information in the c.n.s. Experiments to determine the function of the p.s.n. sensory system are being carried out on *Homarus* because Maynard (1966) has already analysed some of the output from the stomatogastric ganglion, and the part of the stomatogastric nervous system between the commissural and oesophageal ganglia is well known and approachable. There is also a good deal of pertinent information on neuronal pathways in *Homarus* (Allen, 1894) and *Astacus* (Orlov 1926–29).

An important point of comparison with Maynard’s work on *Homarus* concerns the inhibition of the m unit which he found was associated with increased l and a unit activity as the stimulus to the stomatogastric nerve was increased (his figure 30). We found that although the effects of p.s.n. stimulation on the units in the d.v.n. was variable often the l unit activity increased. Stimulation of the p.s.n. in *Cancer* is definitely associated with inhibition of the m unit. This confirms that the p.s.n. sensory system is a normal input to the stomatogastric ganglia.

We suggest that the gastric mill is normally held in a given (resting) position which is under nervous control. This position may then be altered by information arising elsewhere in the nervous system. The associated sensory systems could operate in the following way. Information from mandibular and oesophageal chemo- and mechanoreceptors (Dando & Laverack 1968) could affect the stomatogastric ganglion (and the cells of through-going fibres) in much the same way as Maynard has demonstrated by stimulation of the stomatogastric nerve. The p.s.n. sensory system and the k cells (Orlov 1927; Larimer & Kennedy 1966) if they had not been activated by distortions caused by food entry, would be activated by the movements caused by altered output from the ganglion. The p.s.n. input would then feed back into the stomatogastric system and perhaps have progressively increasing effects (figure 16). Inhibition of gastric mill movements could be affected by the pyloric sensory cells (Orlov 1926) sending information about the passage of food to the midgut back to the commissural ganglion via the postero-lateral nerves. (Pyloric sensory cells, although difficult to find, do occur in *Homarus.* ) Bethe (see Bullock & Horridge) showed that a brainless crab will continue to feed beyond its capacity so presumably an inhibitory pathway from the brain to the stomatogastric system could also operate. As the stomatogastric ganglion lies in the anterior aorta it is also well-positioned to receive excitatory stimuli from chemicals in the blood. The motor cells in the ganglion may also of course be directly sensitive to movements of the mill. Further work on these sensory systems of the foregut of the decapod crustacea is in progress and will be reported later.

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