

ASYMMETRY OF THE MOTOR SYSTEM IN THE HERMIT CRAB *PAGURUS GRANOSIMANUS* STIMPSON

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INTRODUCTION

Although much is known about the physiology of the neuromuscular junction and its role in initiating muscular contraction in crustaceans, less attention has been devoted to the central events which select and control contraction. The mechanisms which shape the discharge patterns of motoneurons are best known for the escape reflex of the crayfish abdomen, mediated by giant fibres, and for the neurogenic heart beat in decapods. The latter has provided a model (Maynard, 1955) which has proved useful in the analysis of rhythmic reflexes, and has been applied to explain the formation of repetitive-reflex patterns in a number of preparations. These systems appear to use pacemaker cells which are the sources of excitation linking together various motoneurons. Interconnexions of several pacemakers with one another and with motoneurons (which may also be interconnected one with another) are complex versions of the same general model.

The tonic slow flexor system of the hermit crab is similar in many respects to that of its symmetrical relative, the crayfish, which has recently been studied by Kennedy & Takeda (1965*a, b*) and Kennedy Evoy & Fields (1966). The functional asymmetry of the hermit crab abdomen might be expected to have imposed changes upon the nervous system, and in particular upon the central mechanisms which maintain the tonic control of the slow flexor system; these changes might lead us to a greater knowledge of these central mechanisms. The hermit crab should provide us with insight into the ways in which natural selection alters a nervous system to a different function from that for which it was originally evolved. The conservative nature of the decapod nervous system was demonstrated in a study of comparative innervation patterns in the limbs of decapods by Wiersma & Ripley (1952). Within the Reptantia there was no variation in the distribution of excitatory motoneurons to the various muscles of the pereopods but some changes had occurred in the patterns of inhibitory innervation. Bush (1963) showed that the reflex resistance to passive movement of the limbs was similar in spite of different patterns of inhibitory innervation. He suggested, however, that the more stereotyped reflex response of brachyurans and anomurans, in comparison with macrurans, might be due to a shift from polysynaptic to monosynaptic reflex control of passive movement. These two studies suggested that an investigation of the reflex control of the abdominal muscles of the hermit crab

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would show many gross similarities to homologous systems in the crayfish, but many minor alterations appropriate to the asymmetrical requirements of the hermit crab.

The abdomen of the hermit crab has a relatively restricted set of functions. A previous study (Chapple, 1966) indicated that the anatomy of the abdominal nervous system and the receptive fields of the sensory receptors in the ganglionic roots are similar to those in the crayfish. Some asymmetry does occur, although much less than might be expected from the external appearance of the abdomen. In this paper the adaptations of the motor system is described: what it does, the reflex pathways of the single pair of giant fibres, and the activities of the third roots. An account of some experiments on the basis of asymmetry in the tonic branches of the third roots, together with a possible model for the central origin of the spontaneous activity is also discussed.

METHODS

The methods are similar to those described in Chapple (1966).

Function of the abdomen

The function of the abdomen and its muscles have become altered over the course of evolution from a presumed macruran prototype; instead of being used for swimming the abdomen holds the animal in its shell. It does this by a variety of mechanisms. The role of the appendages has been investigated by Bott (1949) and Brightwell (1952), but the somatic musculature is important as well. Due to the positioning of the soft parts of the abdomen, the central and ventral superficial longitudinal muscles contract against these soft parts and increase the rigidity of the abdomen so that it becomes quite rigid in a curved position, thus holding the animal in its shell. The abdomen thus appears to act as a kind of hydrostatic skeleton.

Some experiments were performed to test this hypothesis. In four animals the uropods and last two pereopods were removed and the animals were placed in their shells. If the animals were allowed to retreat into their shells so that their abdomens were hooked around the columella of the shell they were very difficult to remove, but if they were not allowed to retreat to this point they were easily withdrawn. Normal animals at this point have the right uropod hooked around the columella and the left uropod pressed against the outside wall of the shell chamber so that they are very difficult to remove. In several experiments sections of the shell were broken off so that the abdomen could be observed when the animal had withdrawn itself. In these cases the cuticle appeared to bulge slightly around the sides of the hole. This only occurred when the animal had withdrawn itself far into the shell. The muscular protuberance on the anterior ventral part of the abdomen, the columellar muscle, is important in maintaining this pressure. If the abdomen is severed at the junction of the thorax and the abdomen, it remains rigid and the soft parts are not extruded, for the thoracic-abdominal junction is narrow and the left and right thoracic-abdominal muscles acting with the columellar muscle function as a pressure bulkhead system. The nerve cord, which runs between these muscles, has little pressure on it, but the soft parts are held in place.

The central flexor muscles have different diameters on the two sides (those on the left side are about one-third as wide as those on the right) and make different angles

with the longitudinal axis of the abdomen. These muscles originate ventrally on one segment and loop obliquely and posteriorly to insert dorsally on the intersegmental membrane of the next. The forces that the muscles of the two sides thus exert have different directions which insure that the radius of curvature is greater on the left side (where most of the soft parts are located) so that when the animal contracts both muscles the abdomen becomes rigid and curled in a counterclockwise direction appropriate for life in its shell.

The giant fibres

The giant fibres which run from the supraoesophageal ganglion to the fifth abdominal ganglion in the hermit crab have been reported by Wiersma (1961) to be homologous with the medial giants of the crayfish; the present experiments confirm this. These giants, as might be expected, have a role in reflex behaviour somewhat different from that in more symmetrical decapods.

The physiological evidence for homology with the medial giants of the crayfish rests on four criteria (Wiersma, 1952; Wiersma & Turner, 1950; Turner, 1950); (a) absence of lateral connexions between giants, (b) connexions between the two giants in the brain (c) cytoplasmic continuity of a giant through the length of the animal, and (d) ipsilateral and contralateral synapses with the third-root motor fibres. The giants of the symmetrical anomuran *Callinassa* have been shown by Turner (1950) to be homologous with the medial giants of the crayfish.

Five animals were placed with the ventral side down but the abdomen was left intact and unrestrained. The carapace and soft parts of the thorax were removed so that the thoracic-abdominal connectives were accessible; stimulating electrodes were mounted on either side. As the stimulus intensity was gradually increased, a point was reached at which the right flexors contracted with a twitch and the right uropod retracted. Stimulation of the left side produced flexion of the left central muscles and retraction of the left uropods. Thus there appeared to be a functional reflex separation of left and right giants.

In fifteen animals the animal was dissected ventrally and stimulating electrodes were placed on the left and right connectives between the third and fourth ganglia, while recording electrodes were placed on left and right thoracic-abdominal connectives. (In four animals the positions of recording and stimulating electrodes were reversed, but this was technically more difficult and there was no difference experimentally between the two sites of stimulation and recording.) In response to a single stimulus (Fig. 1) a short latency response was followed by a muscle potential (from the ipsilateral muscle) and twitching of the ipsilateral uropod. The conduction velocity of the giants ranged from 5 to 7 m./sec. depending upon the size of the animal, and impulses followed repetitive stimulation to a frequency of 200/sec. Only ipsilateral activation could be observed when precautions were taken to eliminate current spread from the stimulating electrodes. Cutting one connective between stimulating and recording electrodes entirely eliminated a response from that giant.

The medial giants in the crayfish can normally be activated by mechanical stimulation of the eyestalks and antennae while the laterals are excited by stimulation of the telson and uropods. In the hermit crab the giants could be activated by stimulation of the head but not of the telson or uropods. Since connexion between the two medial

giants occur in the brain of both crayfish and *Callinassa*, it was necessary to see whether the same was true of the hermit crab. The animal was dissected dorsally, and the connectives were cut just anterior to the first abdominal ganglion and separated. Stimulating electrodes were placed on one connective and recording electrodes upon the other. If there were connexions in the supraoesophageal ganglion (or at any other point) stimulation of one connective should have elicited activity in the contralateral giant. This experiment was performed on five animals with negative results; ipsilateral commissural stimulation showed the giant fibres to be active; contralateral but not ipsilateral stimulation of the optic nerve was also effective in evoking a spike. Thus it appears that the two giants are uncoupled in the supraoesophageal ganglion, but that contralateral synaptic activation occurs there as in the crayfish.

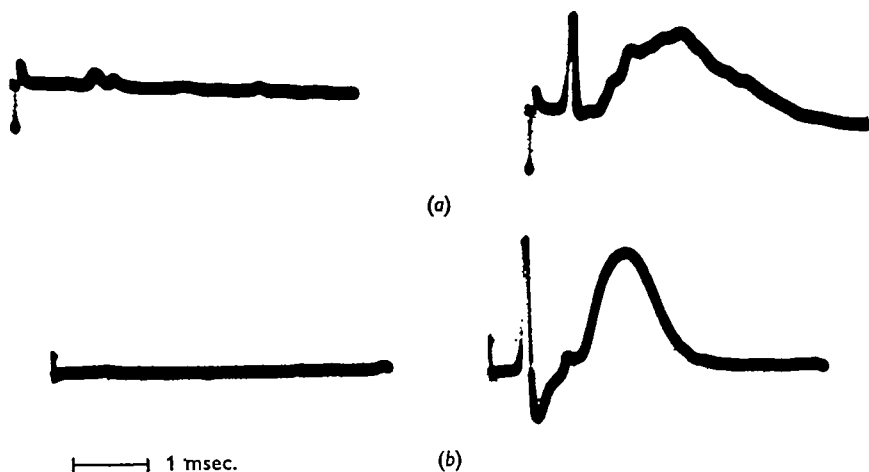


Fig. 1. Giant fibres and the muscle potentials associated with them. (a) Left connectives between third and fourth ganglia stimulated below threshold for the giant (left) and above threshold (right). Recording electrodes on the left connectives between thoracic and the first abdominal ganglia. (b) Right connectives between third and fourth ganglia stimulated; recording electrodes on right thoracic-abdominal connectives. Below threshold (left) and above threshold (right).

Serial sections of the connectives cut transversely at $10\ \mu$ failed to show septa such as those which occur in the lateral giant fibres of the crayfish. The giants were traced to the posterior part of the supraoesophageal ganglion where there are a number of large cell bodies, but it was not possible to determine which somata belonged to the giants.

Several attempts were made to record from the motor fibres of the central flexor branch of the third root, but this is technically difficult in *Pagurus granosimanus* and was only successful on two occasions. Ipsilateral but not contralateral driving of two units was observed. In contrast to the anterior branch of the third root, the posterior branch is much more accessible; simultaneous recording from left and right posterior branches (Fig. 2) showed only ipsilateral activation.

Motor functions of the ganglionic roots

In the crayfish the first roots contain motor fibres which control movement of the pleopods, the second roots have fibres which innervate the dorsal extensor musculature and control the bias of the muscle receptor organs, while the third roots, strictly

motor, innervate the central flexors and the ventral superficial flexors. In the hermit crab all three pairs of ganglionic roots contain motor fibres as in the crayfish, but in only two of them, the first and the third roots, is there any asymmetry in either the number or discharge frequency of their units on the two sides.

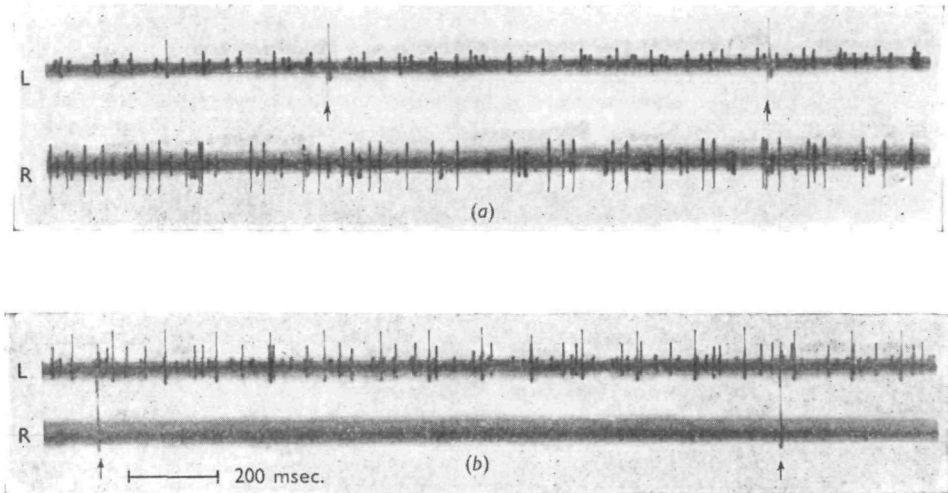


Fig. 2. Recording from both left and right third-root posterior branches while stimulating left thoracic-abdominal connectives (a) and right thoracic-abdominal connectives (b). Gain reduced on left side in (a) and right side in (b).

Efferent discharges recorded from left and right first roots of the third ganglion cut distal to the electrodes (Fig. 3) showed six active fibres on the left side and three on the right side. Stimulation of the left first roots elicited anterior-posterior movement of the pleopod of the segment and closure of the exopodite joint.

The second roots showed no asymmetry in their efferent discharge; furthermore, stimulation of the second roots of either side produced no apparent effect on the dorsal region of their segment (there is no dorsal musculature in *P. granosimanus*), so the motor fibres may have some other function, perhaps control of the short muscles running between the sclerites of the pleuron. Due to the presence of extensive connective tissue in this region, contractions of these muscles could easily have been missed.

The third roots of the hermit crab are exclusively motor, as in the crayfish. In the crayfish the branch to the central flexors has no spontaneous activity and acts as a fast twitch system, while the other branch has a number of spontaneously active units which innervate the tonic flexor system (Kennedy & Takeda, 1965*a, b*). In the hermit crab the anterior branch to the central flexors has no spontaneously active units, while the posterior branch to the superficial ventral muscles, as in the crayfish, has a number of tonically firing fibres. Examination of the ventral superficial musculature reveals no asymmetry in the lateral distribution of the muscle fibres resembling that in the central muscles. When the left and right third-root branches to these muscles were cut distal to the electrodes and recorded from simultaneously, units of the left side had a higher

average frequency (Fig. 4) and reflex activation of a burst by displacing the sensory hairs on the telson indicated that during this discharge the asymmetry persisted.

It seemed possible that the observed asymmetry might be due to different reflex inputs to motor fibres on the two sides. To investigate this, the left and right third-root posterior branches of the third ganglion were recorded from and a prod was used to elicit reflex activity of the fibres by touching various parts of the body. The reflex discharge of Fig. 4 was most easily elicited by stimulation of the sensory hairs on the telson, but movement of the uropods or the epidermis evoked it as well. In contrast it was evoked by mechanical stimulation of the antennae or eyestalks only occasionally. Electrical stimulation of the connectives between the fourth and fifth ganglia, or of the thorax and the abdomen, produced the discharge provided that the stimulus frequency was above 10/sec.

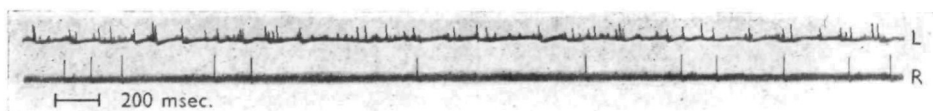


Fig. 3. Motor discharge from the left and right first roots of the third ganglion.
Nerves cut distal to the electrodes

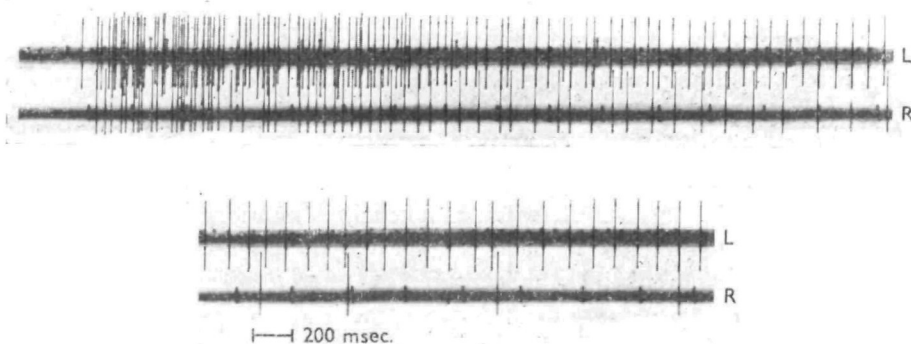


Fig. 4. Motor discharge of the left and right posterior branches of the third roots of the third ganglion. Nerves cut distal to the electrode; sensory hairs on the telson stimulated mechanically.

Stimulation of the sensory hairs on the telson indicated that an asymmetry existed opposite to that existing in the tonic firing of the motor fibres. A glass needle mounted on a loudspeaker was used to stimulate the sensory hairs, since it provided a deflection relatively constant in amplitude and direction. Under these conditions a deflexion of the tactile hairs produced a massive discharge that had a latency of about 55 msec. By varying the position of the glass needle sensory hairs on the left and right sides of the telson were stimulated. In all the animals examined deflexion of the sensory hairs of the left side of the telson produced a lower frequency of discharge in both roots of the third ganglion than did deflexion of the sensory hairs of the right side at the same stimulus intensity. Since the needle could be moved from left to right side repeatedly in a single preparation without altering this effect, the asymmetry of threshold does

not appear to have been caused by a fortuitous arrangement of the needle. Electrical stimulation of the left and right telson roots gave the same results as mechanical stimulation, establishing that the asymmetry is not due to different mechanical coupling conditions between receptor neurons and epidermis on the two sides. Above a threshold intensity, frequency rather than intensity determined the response frequency of the motoneurons. Tactile hairs normally produce a high-frequency burst of impulses in response to mechanical stimulation, and thus the characteristics of the receptors appear to be matched to the central characteristics of the reflex. The asymmetry in the intensity of the response appears to be due neither to differences in receptors on the two sides nor to the number of fibres activated; the ratio of frequencies as between the left and right roots was unaffected by this asymmetry. Whatever is responsible for the asymmetry of spontaneous activity and reflex response in the motoneurons cannot be responsible for the imbalance of telson input, since the two effects are asymmetric in opposite directions.

Intraganglionic reflexes

The preceding experiments thus raised the question of the locus of asymmetry of the motor fibres. By severing the connectives posterior to the third roots and anterior to the ganglion itself the ganglion could be treated as an isolated reflex unit and could be examined for evidence of self-contained asymmetry. In the first set of experiments the first and second roots of the third ganglion were left intact while the connectives were cut as described above. Mechanical stimulation with a glass needle or with watchmaker's forceps was used to explore the reflex pathways. The results were as follows:

(1) Stimulation of the body wall or articular receptors in the pleural sclerite produced a short-latency rapidly adapting discharge. By cutting the first roots and leaving the second roots intact (or vice versa) it was established that both roots were involved in this reflex.

(2) Anterior-posterior stretch of the ventral body wall of the next posterior segment produced tonic inhibition of spontaneous activity. This reflex was shown to be mediated by second-root input.

(3) Stretch of the body wall produced tonic increases in spontaneous activity mediated by first-root afferents. Electrical stimulation of the first and second roots was used to confirm the results of root-section experiments; in addition to confirming the three reflex categories above, electrical stimulation of either second or first root produced a greater response in the left root than in the right. Furthermore, the frequency of spontaneous activity was found to be consistently higher in the left root, establishing that some of the 'weighting' of the left side is accomplished within the ganglion itself.

In both cases there appeared to be two 'reflex groups' of fibres within the two third roots of a single ganglion. One group of fibres consisted of three units: (1) a large normally inactive fibre, (2) a medium spontaneously active tonic fibre, and (3) a much smaller spontaneously active unit. All were excited extraganglionically by telson stimulation and uropod retraction. The other two units (4) one medium-sized and normally inactive, and (5) one smaller and spontaneously active (at about the same frequency as the smallest of the first group) were inhibited during passive or

active uropod retraction and excited during extension. Such correlations are quite difficult to determine since the fibres in the two groups are similar in size and during the burst they are almost impossible to distinguish from one another. It was, however, possible to differentiate between the medium-sized and small-sized units in two preparations. The differences between the two groups was not evident in the intra-ganglionic reflexes.

Isolation and spontaneous activity

The observation that spontaneous activity persisted upon isolation of the third ganglion, and that the left root normally exhibited a higher level of spontaneous activity, just as in the intact preparation, was another indication that the asymmetry was intrinsic to the ganglion itself. Caudal isolation, accomplished by cutting the connectives between the third roots of the third ganglion and their entrance into the fourth ganglion, had a much greater effect upon the spontaneous activity than did rostral isolation. Rostral isolation left spontaneous activity in both roots indistinguishable from that of the normal state, but caudal isolation diminished the irregularity of the spontaneous activity. Spontaneous activity after total isolation resembled that observed after caudal isolation. In one such experiment the two tonic medium-amplitude fibres of the left and right third roots (presumably homologues) were recorded during 'steady state' conditions for 20 sec and a small portion was selected for further analysis. In this portion the mean frequency of the left side unit was 11.7/sec. with a standard deviation of 1.1/sec.; the unit of the right side had a mean frequency of 7.1/sec. and a standard deviation of 0.6/sec. The interval histogram most closely approximated a normal distribution.

In addition total isolation revealed, in favourable preparations, a cyclic alternation of the two reflex groups of fibres described. Initially (Fig. 5) three units are spontaneously active (nos. 2, 3, and 5) in the isolated ganglion. At the beginning of a 'burst' they are joined by a fourth (4), one of the three (3) becomes more active and the other two are inhibited for about a second while the fourth fibre reaches a peak of fifteen to twenty impulses per second before shutting off abruptly. The initial three fibres return to their previous frequencies. No asymmetry is apparent in the discharge of the normally silent fourth unit, and the discharge frequencies of the small fibres on the two sides may only differ by a few spikes per second; but the medium tonic fibres (2) show marked asymmetry of spontaneous activity; the remarkable correlation in the change of frequency between the two sides is difficult to explain by assuming that branches of the same fibre go out via the two roots, since individual impulses in the two sides are not precisely phase-correlated, and antidromic stimulation (see below) of one third root has no effect upon the activity of the other. The corresponding units appear as closely correlated by non-identical fibres.

The interval between these variations in spontaneous activity is extremely variable (from 3 sec. to a minute or more) but the duration of discharge of the fourth fibre and of the inhibition of two of the other three is quite regular. These 'bursts' are very similar to the behaviour of the units during uropod extension, although the frequency is lower and there are fewer units active during the 'pre-extension' period. It is clear that this reflex behaviour is not involved in uropod retraction or extension but is a correlated reflex, since uropod retraction can be evoked by touching the tactile hairs on the telson after isolating the fifth ganglion from the rest of the nervous system.

Antidromic stimulation

The close coupling of the discharge in the units of the two sides and the asymmetry within the ganglion made it important to determine whether the source of excitation is located in the motoneurons themselves or is presynaptically situated. One method is to determine whether antidromic stimulation of the neurons in question re-sets the rhythm of the spontaneous activity (Hagiwara & Watanabe, 1956; Wall, 1959; Kennedy & Preston, 1963).

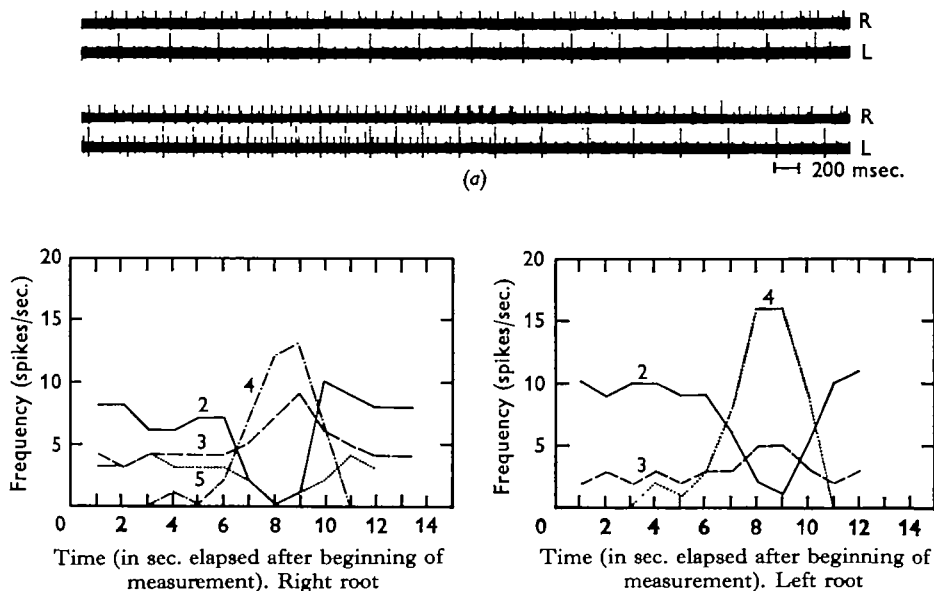
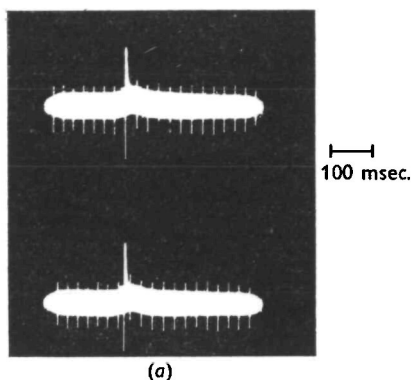


Fig. 5. Spontaneous burst discharges in left and right posterior branches of the third roots of the isolated third ganglion. (a) Oscilloscope record of steady-state frequency and burst. (b) Plots of frequency vs. cumulative time of each fibre of the two roots.

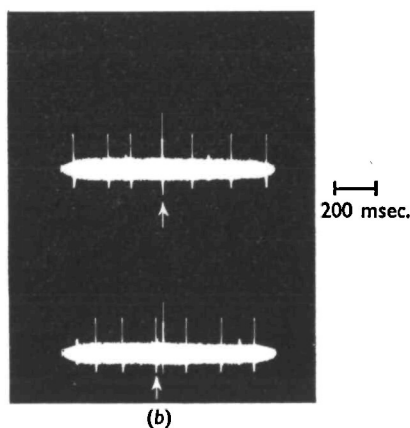
The left third root was mounted with the recording electrodes proximal to the connectives and the stimulating electrodes $1\frac{1}{2}$ mm. along the nerve. At stimulus intensities greater than 10 V. the stimulus artifact blocked the pre-amplifier, but below this intensity four fibres could be driven at very short latencies, showing that at least four of the five fibres were antidromically activated. Under these conditions there was no shift in phase of the spontaneous activity (Fig. 6a), as would be expected if the spike was invading a pacemaker zone, nor was there any other sort of alteration in activity. Re-setting spontaneous activity has been possible in some pacemaker interneurons in the crayfish cord (Preston & Kennedy, 1962); thus antidromic invasion of the pacemaker site must occur in these units. Takeda & Kennedy (1964) have also shown that antidromic invasion of motoneurons in the crayfish third root proceeds to a point near the soma. The failure to re-set in the present case thus makes it likely that the motoneurons themselves are not the source of spontaneous activity.

A second kind of experiment was performed to test the possibility that the correlation between discharge in the two third roots was achieved by collaterals of the motoneurons of one root synapsing upon those of the other. The left third root was

mounted on stimulating electrodes and activity in the right third root was recorded. Left-root stimulation had no discernible effect upon the spontaneous activity of the opposite root (Fig. 6*b*). It seems likely that some common pre-synaptic source of excitation provides the coupling observed in the behaviour of corresponding fibres in the two roots.



(a)



(b)

Fig. 6

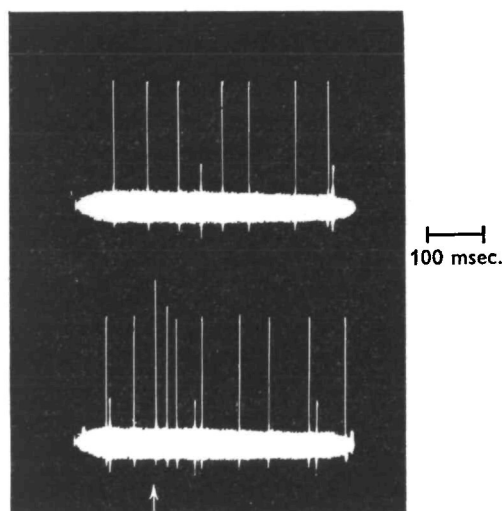


Fig. 7

Fig. 6. Antidromic stimulation of posterior branches of the third roots of the third ganglion. (a) Recording and stimulating electrodes on right third root. (b) Recording from right third root while stimulating the left one.

Fig. 7. Synaptic lack of re-set of third root discharge. Stimulation of the right second root of the third ganglion while recording from the left third-root posterior branch of the same ganglion. Stimulus intensity of top trace 0 Volts (control); bottom trace 5 V.

Another piece of supporting evidence for this view is provided by the effect of certain types of presynaptic input to the spontaneously active units. As mentioned in the section on intraganglionic reflexes, stimulation of the skin produces a short-latency rapidly adapting discharge which is modulated through the first and second roots. By stimulating the second roots of either side (Fig. 7) it was shown that there was no effect upon the phase of the spontaneous activity; a short burst of impulses is evoked in the medium-sized motor axon, but this burst had no effect upon the spontaneous activity. Although the latter was obscured, the interval between the last

spontaneous spike and the first one after the burst was a simple multiple of the normal interval between spontaneously active spikes. Hence there seems to be no interaction between this input and the spontaneous activity, suggesting that this input impinges directly on the motoneuron while the more tonic inputs (to be described below) act on the presynaptic source of excitation, possibly a pacemaker. Wiersma (1952) observed a similar failure of re-setting in the spontaneous activity of second-root motoneurons by giant-fibre stimulation in the crayfish; and the converse case, in which presynaptic input does re-set the spontaneous activity, has been more recently observed in crayfish interneurons (Kennedy & Preston, 1963).

Tonic effects

The dichotomy between the short-latency rapidly adapting responses and the tonic increases and decreases in spontaneous activity is quite marked. A single stimulus of 0.1 msec. duration delivered to the second roots produces a burst of four or five spikes in the medium tonic unit in the third, but the same stimulus to the telson roots has little effect regardless of its intensity. On the other hand, a train of four or five stimuli delivered at 100/sec. to the telson roots produces the same effect as deflexion of the tactile hairs on the telson. The medium fibres fire at 50–60/sec., the large fibre at 40/sec., and the smaller ones at lower frequencies. This activity appears to be 'turned on' by a burst of sensory impulses; when the units are firing at their peak frequency further stimulation does not affect the frequency. During the course of experiments on telson-root stimulation reported above, it was observed that the intensity of the tonic discharge was some function of the frequency and duration of stimulation. If the duration of stimulation was longer than 100 msec. there was no additional increase in the frequency of the burst, but the length of the burst increased with increases in the duration of the stimulation. These relationships cannot be more precisely specified since two or three fibres firing simultaneously at over 50/sec. produce an extracellular record that is exceedingly difficult to analyse.

Finally, if the tonic drive were derived from a single source of excitation the ratios of the frequencies of homologous fibres on the two sides would be expected to be relatively constant. In one experiment stimulation of the body wall of the left third segment, with only the thoracic-abdominal connectives cut, produced a tonic burst discharge. The frequencies of the several units were plotted during stimulation, and after its cessation while frequency was returning to a 'steady-state' value. Under these conditions a constant proportionality emerged (Fig. 8) which is consistent with the model of a single pacemaker providing excitation for units on both sides. In contrast to this, high-frequency stimulation of one of the second roots produced, in addition to the phasic rapidly adapting burst, a tonic inhibitory effect during which the proportionality was not preserved at all. Different preparations showed this departure from the constant ratio in different ways, and it has been difficult to get consistent results on this point.

Picrotoxin

In the cardiac ganglion of the lobster stimulation of the inhibitory nerve produces effects in both pacemaker and follower cells (Hagiwara, 1961) and it seemed plausible that a similar inhibitory process might be altering the constant ratio (referred to above) between fibres on the two sides. Much evidence now implicates γ -amino butyric acid

(GABA) as the transmitter substance mediating peripheral and central inhibition in decapod crustacea (for summary see Grundfest, 1964; Hichar, 1960) and it is suggested that picrotoxin blocks inhibitory synapses. In view of the antagonistic action of the two it seemed possible to determine to what extent the spontaneous activity, its asymmetry, and departures from a constant proportionality were dependent upon inhibition.

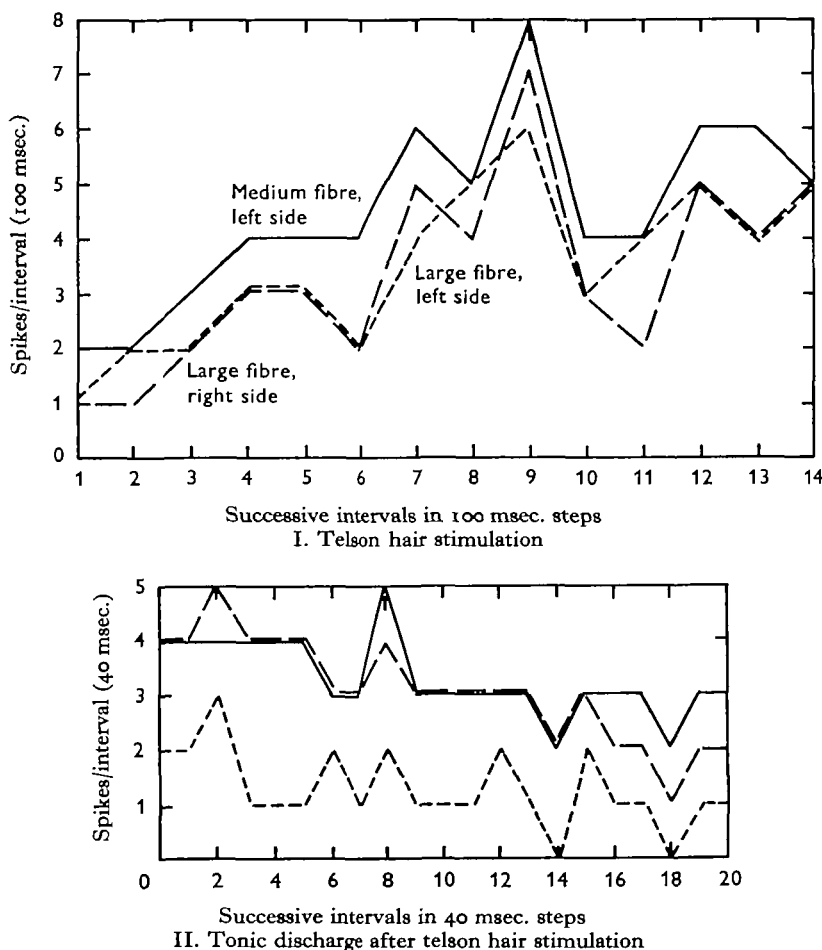


Fig. 8. Correlation in the frequency of fibres of the left and right posterior branches of the third root of the third ganglion. Medium fibre of the left side presumably homologous with the large fibre of the right. Short stimulation of the sensory hairs on the telson (a) followed by gradual decrease in the frequency of the fibres of the two sides (b).

The third ganglion was isolated rostrally and caudally, and recording electrodes placed upon both roots. Picrotoxin dissolved in sea water was added to the preparation so that the final concentration was 5.5×10^{-5} M. Before addition of the picrotoxin the discharge was asymmetric and cyclic bursts occurred. Fig. 9 shows the activity before and after the addition of picrotoxin. In certain parts of the record, before the drug was introduced, the correlation between the two sides departed from the ratio to be expected if one pacemaker was driving homologous motoneurons on the two sides.

Three minutes after the addition of the drug the activity of both sides and the number of units increased markedly (all five units were now firing) and the variations in spontaneous activity (the bursts) were still present, suggesting that these were not dependent upon central inhibition. The asymmetry between the two sides was now maintained in a constant ratio, without the departure from the ratio which were noted before the addition of the drug. Five minutes after the addition of the drug the spontaneous activity of both sides was seven to eight times higher in frequency than before, the bursts varied in duration and intensity, and the correlation between the two sides persisted. Units which were reflexly inhibited before addition of the drug now fired without interruption and their frequency increased and decreased with that of the other motoneurons.

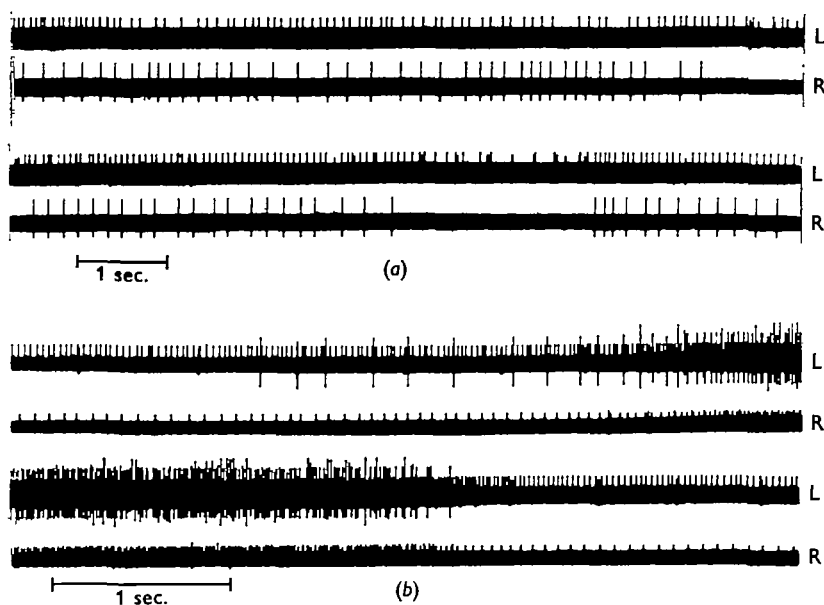


Fig. 9. Burst discharge of left and right posterior branches of third-root motoneurons of the third ganglion (a) prior to the addition of picrotoxin; (b) 3 min. after the addition of 5.5×10^{-6} M of picrotoxin in sea water.

The effect of picrotoxin suggests several conclusions, despite the lack of certainty about its precise central role: (1) The ratio of frequencies of the two sides is maintained despite a very much higher level of central excitation—presumably resulting from a central interference with inhibitory junctions. (2) The lack of correlation between the frequencies of the two sides which occurs in the normal record disappears and therefore may be due to inhibition. (3) The long-term 'bursts' are maintained and become more frequent and regular, suggesting that they are an intrinsic attribute of the excitatory process. (4) Units which are reflexly inhibited in the normal case now fire all the time, suggesting that the 'switching off' of some units by excitation in others is achieved by inhibitory cross-connexions, but not at the final motoneuron level. (5) All of the units are excited during bursts but the frequency ratio is preserved between the two sides, suggesting that a central oscillator may be responsible for these bursts.

DISCUSSION

The motor system of the hermit-crab abdomen appears to have changed much more drastically during the course of its presumed evolution from the symmetrical macruran system than has the sensory system. The homologues of the medial giant fibres have become uncoupled so that they do not necessarily fire together. The first pair of ganglionic roots have different numbers of motor fibres, related to the absence of pleopods on the right side. The central muscles are structurally asymmetrical, although their motor neurons do not appear to be. Conversely, there seems to be no evidence of asymmetry in the superficial ventral muscles, yet the tonic fibres of the posterior branch of the third roots are not only spontaneously active at different frequencies, but maintain this asymmetry during their reflex activity and after isolation of the ganglion.

In both crayfish and hermit crab there is a division of the third roots into two branches which appear to have similar functions in the two animals, similar numbers of fibres, similar reflex patterns of activation with input from telson and uropods predominating, reciprocal coupling of efferent fibres in the same root, and some form of intrinsic ganglionic pacemaker which maintains the excitability of the spontaneously active motor fibres.

The asymmetry of spontaneous activity and reflex excitation indicates that the primary area of adaptation has been in the 'micro-structure' of the reflex arc rather than by some gross alteration in the muscles or the number of neurons innervating them. The first-root case, in which the number of motor fibres has been reduced on the right side, provides an interesting contrast to the third roots. In the former, reduction in the number of motor fibres is probably related to the absence of peripheral structures (the right pleopods). In the third root, in contrast, the alteration has been achieved centrally. The locus of asymmetry may be within a pacemaker, at its terminal endings on the motoneuron (by a reduction in the amount of transmitter produced by each spike), or possibly within the motoneurons themselves. The observed asymmetry of phasic reflex bursts in the two roots suggests that since it is common to both tonic and phasic effects the most parsimonious explanation might be that the asymmetry is post-synaptic. A common pacemaker, according to this scheme, would have a similar presynaptic input to the homologous motoneurons but the threshold of the motoneurons on the left side would be lower.

The proposed mechanism of spontaneous activity, a pacemaker cell which drives follower neurons, is analogous to the case of the lobster cardiac ganglion (Maynard, 1955; Hagiwara & Bullock, 1957). The demonstration of this mechanism in the present case rests upon whether or not the failure of antidromic stimulation to re-set the tonic discharge of the motoneurons is due to a presynaptic locus of excitation or to the location of a pacemaker zone in the motoneuron on a branch which is unaffected by the antidromic stimulus. The follower cells of the lobster cardiac ganglion (Hagiwara & Bullock, 1957) can be re-set by antidromic stimuli as can many crustacean interneurons (Preston & Kennedy, 1962). There are cases, however, in which interneurons in the crayfish connectives are not re-set (Kennedy *et al.* 1966), but whether this is due to a presynaptic origin of spontaneous activity or to failure of the antidromic stimulus to invade a branch of the axon is not clear. Recent studies on

crustacean motoneurons (Takeda & Kennedy, 1965) showed that antidromic impulses normally invade the neuron soma in the smaller cells running to the fast flexor muscles. Since the cell bodies of the slow flexor motoneurons are still smaller it seems likely that antidromic impulses normally invade the main axon up to and usually including the soma. In the present experiments neither phasic presynaptic nor antidromic inputs re-set the rhythm, although the phasic input was clearly interpolating a spike in the

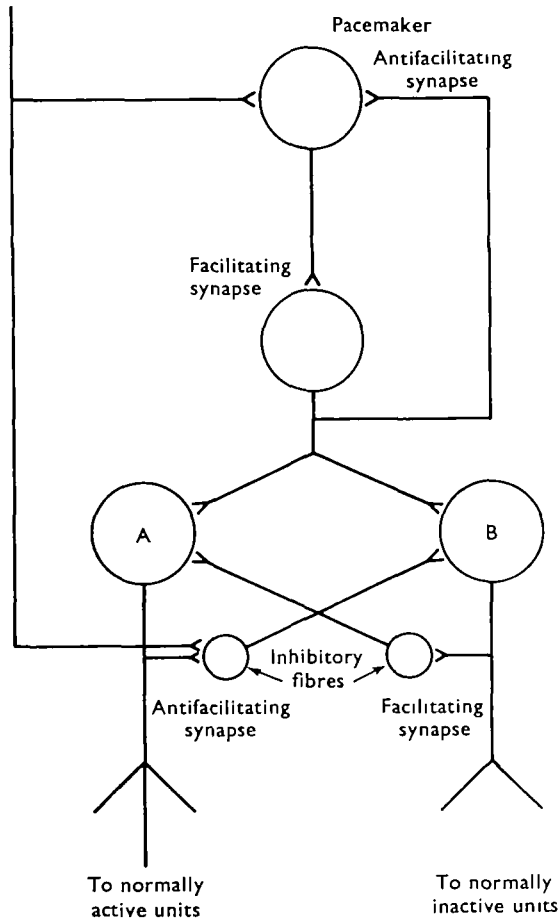


Fig. 10. Hypothetical model of the mechanism of burst formation in the third roots of the third ganglion. For detailed description see text.

tonic discharge. The close correlation of tonic discharge frequencies on the two sides together with the maintenance of asymmetry suggests a common source of excitation rather than pacemaker sites upon branches of each motoneuron which interact in some fashion but are not influenced by activity in the main axon.

Several other points rest upon the central action of picrotoxin and the tacit assumption (now made explicit) that GABA is the only central inhibitor. If this is true, the alternating activation of units with opposite reflex functions cannot be explained purely by inhibitory interconnexions since the burst which apparently triggers this alternation is not abolished by picrotoxin.

A possible model for the burst in the hermit-crab system is shown in Fig. 10. We assume that the pacemaker is a tonically firing unit with a normally distributed frequency histogram. The regular pacemaker fires a follower interneuron which in turn fires interneurons that activate either the normally active units (A) or the normally inactive ones (B)—by analogy with Hughes's (1953) study of the effects of direct current on the insect ganglion. These two interneurons have inhibitory connexions with each other; below a certain frequency A inhibits B. As A's frequency increases so its effect upon B decreases while B increasingly inhibits the other. This would produce alternations of reflex units in response to changing frequencies of the pacemaker. To produce the reflex discharge of A, B is overridden by increasing the inhibition upon it as well as firing the pacemaker. A similar mechanism would produce reflex discharge of B. The 'bursts' are produced by excitatory feedback from the interneuron following the pacemaker to the pacemaker itself. The synapse between pacemaker and this interneuron is assumed to be facilitating, thus producing a reflex discharge as the frequency of the pacemaker increases. The synapse from follower interneuron back to pacemaker is de-facilitating so that at increasing frequencies its effect will be diminished. The 'bursts' are the result of slight increases in the pacemaker frequency causing a discharge which is then cut short by the decrease of positive feedback to the pacemaker. Normally feedback connexions between ganglia would prevent such instability from arising.

SUMMARY

1. The decalcification of the abdomen and the asymmetry of the central flexors is a 'hydrostatic' mechanism by which the abdomen is expanded to fit the shell tightly.
2. The single pair of giant fibres is homologous to the medial giants of the crayfish except that no contralateral activation of the flexor muscles was observed.
3. Two kinds of asymmetry in the motor system were observed; (a) loss of motor fibres in the first ganglionic roots of the right side; (b) greater frequency of 'spontaneous activity' in the slow flexor branch of the third roots on the left side.
4. Sensory stimulation evoked different sets of fibres in these third root branches; one set fired spontaneously even after total ganglionic isolation, the other, when reflexly activated, reciprocally inhibited the first group.
5. Antidromic and synaptic inputs failed to re-set the spontaneous activity; two kinds of reflex activation, phasic and tonic suggest two different sites for inputs, one to the motoneurons, the other to a pre-synaptic pacemaker.
6. Alternations of the two reflex groups occurred in the totally isolated ganglion; addition of picrotoxin to the saline abolished reciprocal inhibition but variations in the frequency of spontaneous activity persisted.
7. A possible mechanism for this system is proposed.

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