AN ORGAN FOR PROPRIOCEPTION AND VIBRATION SENSE IN CARCINUS MAENAS

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(Received 21 August 1953)

(With Plate 6)

Little is known about the mechanism of proprioception in arthropods. A number of organs or groups of cells have been described which on anatomical grounds might serve proprioceptive function, e.g. the 'chordotonal organs' in insects (Graber, 1882) and crustaceans (Wetzel, 1933), bipolar cells in insect muscle (Hilton, 1924; Rogosina, 1928) or in crustacean joints (Tonner, 1933), the 'myochordotonal' organ in the meropodite of decapod crustaceans (Barth, 1934), the 'muscle receptor organ' and 'N-cells' of various crustaceans (Alexandrowicz, 1951, 1952a, b).

Physiological evidence has been obtained for the crustacean 'muscle receptor organ' by Wiersma, Furshpan & Florey (1953), who showed that it gives rise to a discharge of afferent impulses during stretch, as was suggested by Alexandrowicz. The existence of kinaesthetic organs in the walking limbs of crustaceans was indicated by the fact that movements of the joints can elicit impulses in the limb nerves (Barnes, 1930, 1931) and in the central nervous system (Prosser, 1935). In insects there is evidence for a proprioceptive discharge from the campaniform sensilla (Pringle, 1938a, b, 1940) and the hair sensilla at the joints (Pringle, 1938c).

The present work arose from an interest in certain large nerve fibres (up to 20 µ in diameter) in the limb nerves of Carcinus maenas which accompany the two large (30 µ) motor axons to the flexor muscle of the dactylus. These were presumably sensory fibres. In preliminary experiments the nerves were traced to the periphery and found to end on an organ which lies across the propodite-dactylus joint. The response of the organ was then examined in isolation and in situ. The experiments, described below, indicate that it is concerned with proprioception and probably 'vibration-sense'.

METHODS

All experiments were performed on preparations from the walking legs of the shore crab, C. maenas. Except where stated, the histological and physiological descriptions apply to the organ lying in the propodite-dactylus joint.

Histological. The organs and nerves were stained with methylene blue, fixed in ammonium molybdate and mounted in xylene dammar, following the technique of Alexandrowicz (1951). In all cases, the organ and nerves were exposed in the leg and the entire limb immersed in methylene-blue solution. In some cases the preparations were counter-stained with picric acid.

Physiological. The preparations were dissected under a binocular microscope magnifying 20 diameters. Crustacean Ringer of the following composition was used.
throughout: Na⁺, 513 mM.; K⁺, 12-9 mM.; Ca²⁺, 11-8 mM.; Mg²⁺, 23-6 mM.; Cl⁻, 594 mM.; HCO₃⁻, 2-6 mM.

In experiments on the isolated organ, the organ was tied at either end by a silk thread which was held by the tip of a ruling pen. The preparation was then placed in light liquid paraffin and the nerves laid on two recording electrodes, one of which was earthed. These electrodes consisted of chlorided silver wires dipping into agar-crustacean Ringer in capillary tubes and connected to the preparation by agar wicks. A cathode-follower, amplifier, oscilloscope and loudspeaker were used to pick up nerve impulses. The organ could be stretched or relaxed by altering the position of the ruling pens.

Its sensitivity to vibrations was tested as follows. A small glass rod, about 3 cm. long and 1 mm. wide, was mounted vertically on the diaphragm of a 'deaf-aid'. This was driven by a beat-frequency oscillator, the output of which could be varied over the range 0-20 a.c. V. and 0-16,000 cyc./sec. The earpiece was mounted over the organ so that the glass rod dipped into the liquid paraffin. The tip of the glass rod was adjusted to be about 1 mm. from the organ; its movement was never more than about 20 μ. Since the oscillator output was low at low frequencies and the earpiece insensitive in this range, it was usually not possible to examine the effect of sinusoidal frequencies below about 100 cyc./sec.

In experiments on the isolated leg, the sensory nerves, the motor nerves to the flexor muscle of the dactylus and the motor nerves to the extensor muscle of the dactylus were separately dissected out in the meropodite. The entire preparation was then lifted into light liquid paraffin and the leg held by clamping the propodite. The sensory nerves were laid on two recording electrodes as in the isolated organ preparation; the motor nerves in turn were laid on two stimulating electrodes. These were of the same type as the recording electrodes and were connected through an isolating transformer to a stimulator from which pulses could be obtained at variable voltage, duration and frequency. Movement of the dactylus was registered by attaching it to an isotonic lever moving between a light source and a twin photo-electric cell. The differential response between the two cells caused by movement of the dactylus was led off to the second beam of the oscilloscope. A thread was also attached to the dactylus so that it could be moved in either direction from outside the preparation box. In this way the response of the organ to either active or passive flexion or extension could be examined. The dactylus could also be clamped in order to observe the effects of isometric contraction.

Conduction velocity in the nerves to the organ was determined by placing them (with the organ removed) in light liquid paraffin, stimulating maximally and recording at three or four points.

RESULTS

A. Anatomy and histology

The presence of a group of nerve cells in the organ spanning the propodite-dactylus joint was observed in unstained preparations, but their arrangement and connexions were shown only after staining with methylene blue. The staining also revealed
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similar groups of cells in all three joints between ischiopodite and propodite, in each case on the flexor side.

The position and structure of the propodite-dactylus organ are sketched in Text-fig. 1. It consists of nerve cells and distal processes, embedded in a strand of connective tissue which runs across the joint. The strand is loosely attached to the

apodeme of the flexor muscle, about 5 mm. proximal to the joint; the other end is firmly attached to a small protuberance on the caudal inner surface of the dactylus, about 1 mm. distal to the joint. Some connective tissue fibres run to the hypodermis in the immediate vicinity of the protuberance, and a few small threads leave the organ along its length.

The organ is about 6 mm. long when the joint is extended. When the joint is flexed the organ is stretched by about 1 mm. due to the movement of the apodeme (see Text-fig. 1a, b). It is about 100 μ in diameter in the centre, widening at either end. The proximal processes of the nerves form a bundle which is free for about 2 mm. before reaching the main bundle, and is characteristically crinkled, presumably

Text-fig. 1. Semi-diagrammatic sketches drawn to scale to show the position and structure of the organ. (a) Propodite-dactylus joint in extended position. (b) Joint in the flexed position. Note the elongation of the organ and the movement of the nerve (n). (c) Enlarged view of organ. h = hypodermis; N = main nerve bundle; n = nerve bundle to organ; a = organ; p = distal attachment to protuberance; e = apodeme of extensor muscle; f = apodeme of flexor muscle; c = small connective tissue attachment.
Pl. 6 shows photographs of a preparation stained with methylene blue. The large cells clustered at the proximal end of the organ are ovoid and up to about 40 μ in diameter. The cells extend along the organ becoming smaller and more spindle-shaped and eventually impossible to distinguish from the 'beading' of the nerve fibres characteristic of methylene-blue staining. At least forty cells have been counted in one preparation and the number is probably greater because of uncertainties in staining. The lengths of the distal processes could not be accurately measured; some can be followed clearly for up to 200 μ. Stained elements extend to the distal third of the organ.

The meropodite-carpopodite organ lies across the joint and resembles the propodite-dactylus organ in its attachments and general appearance but is smaller in size.

The only other species examined was *Portunus depurator* in which the propodite-dactylus organ is present and resembles that of *Carcinus*.

The groups of bipolar cells described by Tonner (1933) are similar to those described here, but apparently not associated with a muscle. The organ seems related to the 'myochordotonal' organ of Barth (1934) in its general structure, large number of cells and association with a muscle. The points of difference are that the 'myochordotonal' organ does not cross a joint, has smaller cells (up to 10 μ in diameter) and makes contact with the muscle only through a membrane in which the cells lie.

### B. The discharge of the isolated organ

A resting discharge which might continue for several hours was recorded from the nerves and this discharge could be modified in a consistent manner by certain mechanical stimuli. If the nerves were severed or crushed near the organ, both resting discharge and response to sensory stimulation ceased. Crushing the organ from the distal end progressively reduced the discharge. The discharge was also reduced or abolished by a drop of 0.2% procaine placed on the organ. It may be inferred that the discharge originates in the organ and is sensory in nature.

Only very small potentials or none at all were recorded from the organ itself; no prolonged depolarization of the type found near the nerve terminations of a muscle spindle (Katz, 1950a, b) was seen. It is probable that the nerve within the organ is sufficiently long for the action potential to have reached its normal shape at the point of nerve exit. Any local electrical events inside the organ are presumably short-circuited and, therefore, not recordable.

The resting discharge varies with the length of the organ (see Text-fig. 2). When the organ is allowed to shorten as far as it can, the discharge becomes greater than it is at the lengths normally attained in the leg. In the physiological range of lengths, the rate of discharge in some fibres is dependent, in others apparently independent of length.

Judging from the size of the spikes the resting discharge appears to occur in small
An organ for proprioception and vibration sense in Carcinus maenas fibres. When the organ is suddenly stretched or quickly allowed to relax, there is a burst of large spikes more or less coincident with the movement. These impulses travel evidently in large fibres which are normally at rest. Rapid stretch is usually more effective in eliciting this discharge than is rapid relaxation.

Vibratory stimuli

The sensitivity of the isolated organ to vibration was very high. The organ responded to a tap on the bench or even to heavy footsteps several yards away. The freedom of movement of the organ in the isolated condition was much greater than in the leg, yet it would respond to a vibration which caused no movement visible to the naked eye.

Many axons, but especially the large fibres which respond to sudden stretch or relaxation, can be caused to discharge during vibration. When the organ is stimulated with the ‘vibrator’ (see Methods) the type of record shown in Text-fig. 3 is obtained. There was no indication that any unit was tuned to a particular frequency within the band employed. The higher the frequency the higher was the intensity of stimulus required to produce the same response. With the present apparatus no response could be obtained above 1000 cyc./sec.

The rate of firing in any one unit during a vibratory stimulus was irregular and did not follow the frequency of the stimulus. The maximum mean rate of firing observed in a single unit over a period of 200 msec or more was 100 impulses/sec. The minimum interval seen between two impulses was 4 msec. (temperature
The response to continuous vibration showed little or no adaptation (see Text-fig. 3).

In one experiment, the meropodite-carpodite organ and nerve were mounted in liquid paraffin. Resting discharge and response to a brief vibration were similar to those of the propodite-dactylus organ.

![Text-fig. 3](image)

**Text-fig. 3.** Response of the isolated organ to vibrational stimulus at 350 cycles/sec. Signal on lower beam indicates the duration of the stimulus. The large spikes have been retouched.

**C. The discharge of the organ in situ**

A recording could be made from the nerve bundle to the organ by exposing it in the meropodite (see Methods). This bundle is easily recognized by its large fibres and is fairly discrete. Its identity was always confirmed after the experiment by dissecting it up to the organ. In some cases, a few nerve fibres from other bundles were present, but in these experiments the results differed in no way from those in which only the bundle supplying the organ was used.

The resting discharge occurred in small fibres and was similar to that in the isolated preparation, although it disappeared sooner, possibly due to asphyxia and accumulation of metabolites from contracting muscle. The discharge varied with the position of the joint, certain units discharging at different rates according to the position, others behaving in an irregular way. The units which respond to positional changes adapt slowly if at all, the result being somewhat obscured by the gradual deterioration mentioned above.

The effects of passive movements and vibration are shown in Text-fig. 4 and of active movements in Text-fig. 5. With the exception of the effects due to a prolonged stimulus to the motor nerves there are no obvious differences between them. The active movements were produced by applying a series of stimuli to the motor nerves, the frequency being varied according to the speed of movement required. The duration of stimulation was \( \frac{1}{4} \) sec. for a brief contraction. For a prolonged contraction, stimulation was maintained for 4 sec. or more. The possibility that electrical stimulation of the motor nerves might accidentally stimulate the sensory nerves was ruled out: (i) by crushing the sensory nerves between the recording electrodes and the organ, thereby abolishing all discharge; (ii) by the fact that if the movement was delayed the sensory discharge was likewise delayed and coincided with it; (iii) because the difference between isotonic and isometric contraction of the flexor muscle (see below) can only be explained by active movement.
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The results are summarized in Table 1. The phasic discharge takes place primarily in the large fibres and depends on the rate and extent of movement. The faster the movement the greater the frequency of discharge (see Text-fig. 4b, c). The farther the joint is moved the greater the number of impulses at a given rate (see Text-fig. 4c). It is possible to reduce the discharge considerably, and perhaps avoid it altogether, by moving the joint very slowly. Flexion is more effective than extension in eliciting a discharge.
When the leg relaxes, after brief muscular contraction, there is usually a second discharge similar to that during passive stretch and relaxation (see Text-fig. 5 a, c). The effect of a maintained contraction, however, differs from that due to a passive change of position. With prolonged contraction there is a continuous sensory discharge, often in large fibres, in spite of the fact that visible movement has ceased (Text-fig. 5 b, d). The explanation may be that the contraction is really an incompletely fused tetanus, the irregularities of movement being too small to be recorded but sufficient to stimulate the receptors.

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<th>Static and phasic responses from the organ</th>
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<th>Position (flexed)</th>
<th>Large fibres</th>
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<tr>
<td>Position (extended)</td>
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<td>Movement (active or passive flexion)</td>
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<td>'Isometric' contraction (extensor muscle)</td>
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Isometric contraction of the flexor muscle produced a similar though less intense sensory discharge, in spite of the fact that no external movement was allowed (the dactylus being clamped) (Text-fig. 5 e). This result is not unexpected since contraction will move the apodeme slightly. Isometric contraction of the extensor muscle produced no discharge, which is probably explained by the fact that this muscle has no direct connexion with the organ.

A brief vibrational stimulus—a tap on the preparation box—produced a discharge in both large and small fibres (see Text-fig. 4 d), the intensity of the discharge increasing with the strength of the stimulus. The sensitivity was much lower than with the isolated preparation, but the vibration was still effective without causing any recordable movement of the joint. In view of this, it is not surprising that an apparently smooth tetanus gave rise to continued discharges in the vibration receptive axons.

Conduction velocity in the nerves

The fastest speed of conduction measured was about 3.5 m./sec. at 21.5° C. There are perhaps a dozen fibres with velocities above 1 m./sec. and many whose velocities could not be measured. Because of the large number of nerve fibres in the bundle, the conduction of individual impulses may be regarded as taking place in a medium of low resistance. The fastest fibres are thus comparable to the motor axons whose conduction velocities in sea water range from 3.1 to 5.5 m./sec. at 21° C. (Hodgkin, 1939).
Text-fig. 5. Response of the organ to contraction of the flexor and extensor muscles of the dactylus produced by stimulation of the motor nerves. The beginning and end (where visible on the original photograph) of the series of stimulus artifacts are indicated by white bars. The second beam records movement of the dactylus but, because of an adjustment to the lever between (b) and (e), extensor of the joint in (d) and (e) is shown in the same direction as flexion in (a) and (b). Recording from the nerve in the meropodite. All records from the same experiment. Large spikes retouched. (a) Active flexion (brief contraction). (b) Active flexion (maintained contraction; end of stimulus off the photograph). (c) Active extension (brief contraction). (d) Active extension (maintained contraction). (e) Brief 'isometric' contraction of flexor muscle.
The organ can signal the rate and extent of a movement of the joint. Whether it can signal the direction of the movement or the resting position of the joint is not clear from the experiments, although there are some indications that this might be done by a variation in the pattern of the discharge in different fibres. Judging from a single experiment, these conclusions can probably be applied to the meropodite-carpopodite organ.

The fact that movements are signalled by large fibres whose conduction velocity approaches that of the motor axons is probably important in eliciting rapid reflex action.

The arrangement of the organ seems to provide better shielding against extraneous stimuli and give a more accurate 'muscle-sense' than, for example, the campaniform sensilla or the hair sensilla in the joints of insects. Unlike the 'muscle receptor organ' in crustaceans (Alexandrowicz, 1951) and the vertebrate muscle spindle, the present crab-limb organ lies in series with the main muscle bundle; contraction of this muscle therefore stretches the crab-limb organ while it relieves the tension on the other two. The crab-limb organ also differs from the others in that it responds to sudden relaxation as well as sudden stretch. This suggests that the movement receptors undergo only transient deformation such as might be produced by friction or viscous drag during the movement.

It is more difficult to decide whether the crab uses the organ to perceive vibrations. Many tissues which cannot be regarded as specific 'vibration-receptors' respond incidentally to a vibrational stimulus (e.g. the teeth, Pfaffmann, 1939; the skin, Adrian, Cattell & Hoagland, 1931, Newman, Doupe & Wilkins, 1939). Any receptor which is excited by mechanical deformation will probably respond to vibration of sufficient intensity; the question is whether normally the tissue experiences vibrations of greater than threshold intensity. The stimuli used on the isolated leg of the crab were small, and it is reasonable to suggest that the crab can perceive vibrations due to nearby moving objects. It might seem unusual that the same fibres should convey two different types of mechanical sensation. However, there is good reason to believe that in vertebrates the same end-organs (stretch receptors in the muscle) are responsible both for deep vibratory sensibility and for appreciation of changes in posture (Echlin & Fessard, 1938; cf. also Newman et al. 1939). The suggestion of Echlin & Fessard (1938) that the sensations can be resolved centrally because of the different pattern of the two types of discharge seems plausible.

The present experiments may also explain the isolated-leg 'reflexes' described by Barnes (1931). Barnes found, for instance, that passive flexion of the dactylus of the isolated limb of Eupanopeus produced a maintained active flexion. Supposing that the nervous anatomy is similar to that in Carcinus, the initial flexion will produce a discharge of impulses in nerves which lie in close proximity to the motor nerves to the flexor muscle. 'Cross-excitation' of these motor nerves might take place near the cut ends.
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SUMMARY

1. An organ lying across the propodite-dactylus joint of the walking leg of *Carcinus maenas* has been described; this organ is supplied by a nerve bundle which contains some large fibres (up to 20 μ in diameter) and which accompanies the motor nerves to the flexor of the dactylus. A similar organ lies in the meropodite-carpodopodite joint.

2. The organ is embedded in a strand of elastic tissue which is in a stretched condition at all positions of the joint, the stretch being greater the more the joint is flexed.

3. The organ with its afferent nerve has been isolated and shown to contain sensory receptors. It reacts to vibration and to sudden changes in length with a burst of impulses in both large and small fibres. There is a resting discharge in small fibres which varies with length.

4. Experiments on the organ in situ show that it can serve to signal the rate and extent of movement in the propodite-dactylus joint. In addition, it may be used as a vibration-receptor. It is probable that some of its nerve fibres can signal the position of the joint and the direction of movement.

Some preliminary experiments were done with Dr Xenia Machne to whom I am greatly indebted. I am extremely grateful to Dr J. S. Alexandrowicz for showing me his methylene-blue technique and for much helpful advice. My thanks are due to Prof. B. Katz for constant encouragement and advice, to Mr J. Armstrong for the photographs of the organ, to Miss A. Paintin and Mr K. Copeland for technical assistance, and to Mr J. L. Parkinson for constant help.

REFERENCES


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EXPLANATION OF PLATE 6

Propodite-dactylus organ and nerve supply. Photo-micrographs made from a preparation stained with methylene blue, fixed with ammonium molybdate and mounted in xylene dammar. 

(a) Low-power photograph showing almost the entire organ lying across the centre, the nerve supply (two branches) joining at the left. The proximal attachment of the organ is seen faintly on the left, the distal attachment is outside the picture to the right. The cells and nerve processes can be seen extending along the upper part of the organ. Other material in the photograph is flexor muscle and hypodermis. The organ has been damaged slightly in the distal region and has been allowed to shorten by about a third in fixation. 

(b) High-power photograph of the nerve-organ junction in the same preparation. Distal part of the organ on the right.
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