

PROPRIOCEPTIVE NEURONS OF CHORDOTONAL ORGANS IN THE  
CRAB, *CANCER MAGISTER* DANA (DECAPODA, BRACHYURA)

BY

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ABSTRACT

The proprioceptive organs for the limbs of *Cancer magister* contain sensory endings embedded in an elastic strand to detect stretch and relaxation. The dynamic movement-sensitive neurons are of two types: those sensitive to relaxation and those sensitive to stretch of the chordotonal strand, which corresponds to flexion or extension of the joints. The cell bodies of sensory neurons associated with the planar chordotonal organs in the limbs are arranged within the elastic strand, whereas the organs that are more like a cord have the cell bodies of the dynamic movement-sensitive neurons outside the connective tissue strand. This study observed that the dynamic movement-sensitive neurons that are sensitive to relaxation have larger cell bodies than cells sensitive to stretch. No stretch-sensitive neurons were observed for the cord-shaped chordotonal organs. The static, position-sensitive neurons are distal in all the organs and have the smallest cell bodies. Mapping the functional distribution of the neurons has revealed that the various types of chordotonal organs have a similar pattern in soma location as in function. The purpose of this study is to describe this anatomical arrangement of the sensory neurons on a subset of chordotonal organs within the limb of the crab, *Cancer magister* so that species comparisons can be made, in the future.

RÉSUMÉ

Les organes propriocepteurs des pattes de *Cancer magister* contiennent des terminaisons sensorielles incluses dans une bande élastique qui détectent l'étirement et le relâchement. Les neurones sensibles au mouvement sont de deux sortes: ceux sensibles au relâchement et ceux sensibles à l'étirement de la bande chordotonale, mouvements qui correspondent à la flexion ou l'extension des articulations. Les corps cellulaires des neurones sensoriels associés aux organes chordotonaux aplatis sont arrangés à l'intérieur de la bande élastique, alors qu'au niveau des organes en forme de corde, les corps cellulaires des neurones sensibles au mouvement sont à l'extérieur de la bande élastique. Cette étude a montré que les neurones sensibles aux mouvements de relâchement présentent des corps cellulaires plus grands que les cellules sensibles à l'étirement. Aucun neurone sensible à l'étirement n'a été observé au niveau des organes chordotonaux en forme de corde. Les neurones sensibles à une position statique sont distaux dans tous les organes et présentent les corps cellulaires les plus petits. La cartographie de la distribution fonctionnelle des neurones a montré que

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les différents types d'organes chordotonaux présentent un même arrangement cellulaire et une même fonction. Le but de cette étude est de décrire l'arrangement anatomique des neurones sensoriels des organes chordotonaux à l'intérieur des pattes du crabe *Cancer magister* afin de permettre des comparaisons futures.

## INTRODUCTION

Somatesthesia, the sense of the body, is due to the activity of a variety of receptors, some of which are proprioceptors. Proprioceptors consist of position-and-movement (kinesthetic) receptors (Burgess et al., 1982). Arthropods, like vertebrates, have articulated appendages, and therefore it is not surprising that the described proprioceptors of vertebrates have their counterparts in arthropod limbs and joints. Those of Crustacea Decapoda, especially those of crabs, lobsters, and crayfish, have been examined in some detail in relation to joint receptors. Chordotonal receptors (Burke, 1954; Bush, 1965a, b), force-sensitive mechanoreceptors (Shelton & Laverack, 1970), cuticular stress detectors (Wales et al., 1971), muscle tension receptors (Macmillan & Dando, 1972; Cooper & Hartman, 1994), and touch-sensitive hairs (Norris & Hartman, 1985) have all been examined at some level of detail in the past. In spite of what at first glance appears to be a complex anatomy of the sensory endings in proprioceptors, crabs are particularly suitable experimental animals for the study of these joint organs in relation to neuronal function and anatomical arrangement, because of the size and robust structure of the chordotonal organ.

The walking legs of crabs have six joints, each of which has one or two proprioceptive chordotonal organs. These chordotonal organs consist of an elastic strand into which the sensory endings of neurons are inserted. The neurons signal the direction of the moving and static positions of the joint (Wiersma, 1959; Bush, 1965a). Using Alexandrowicz's (1967) designation, these receptors are named in accordance with which joint they are monitoring (i.e., the CP is the organ between the carpus and the propodus; the MC is the organ between the merus and the carpus; etc.). When two organs are present at the same joint, as with the carpus and the propodus, they are distinguished by labels such as CP1 and CP2 (fig. 1A).

Since Burke's work in 1954, when he first described vibration and proprioceptive responses in the PD (propodus to dactylus) organ of the crab, *Carcinus maenas* (Linnaeus, 1758), there has been an increased interest in arthropod proprioception: Alexandrowicz (1958, 1967, 1972) described the gross anatomy of the limb proprioceptive organs in the limbs of a variety of crustaceans. Whitear (1962, 1965) and others (Lowe et al., 1973; Mill & Lowe, 1973; see review by Mill, 1976) examined the fine structure of the organs related to mechanical transduction into electrical

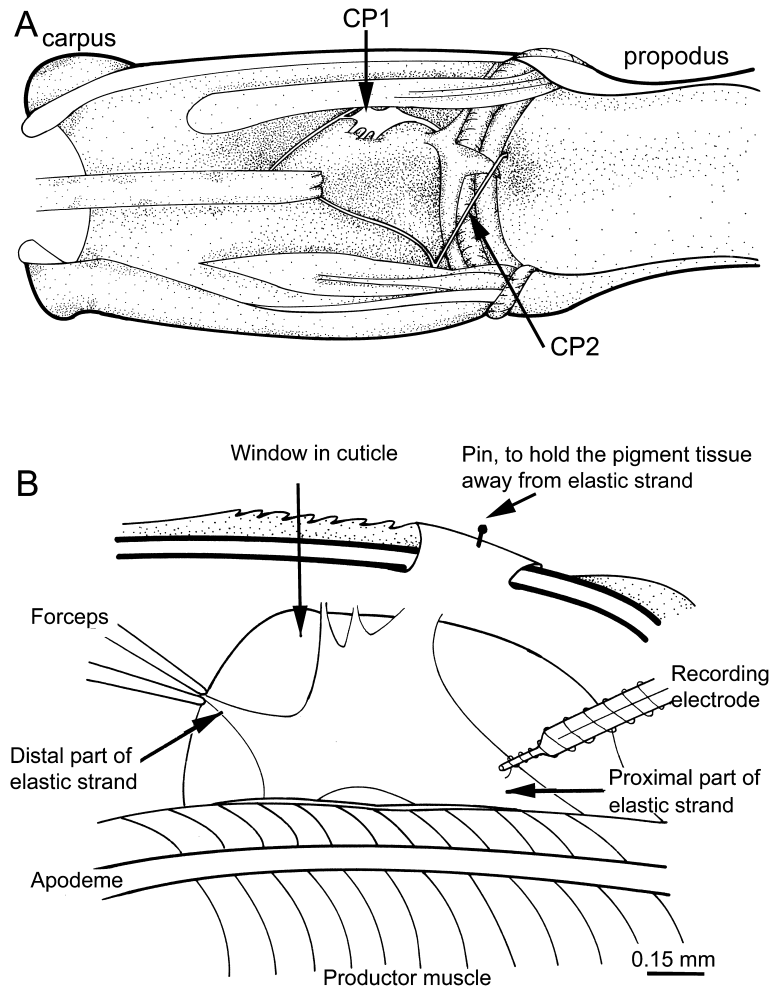


Fig. 1. Anatomy of the *Cancer magister* Dana, 1852 walking limb within the carpus segment. A, dorsal view into the segment of the right first walking leg; the main nerve bundle is cut off just after the CP2 nerve joined the leg nerve; the apodeme is shown with the muscle removed for ease in showing the location of the attachments of the chordotonal organs; B, the recording arrangement for the cells within a CP1 strand of the right first walking leg: in this view, a patch (window) of cuticle is cut out on the medial side for transmitted light; a fire-polished glass suction electrode is placed over the soma of particular sensory neurons. The view in B is with the distal part of the leg on the left, whereas in A distal is on the right side.

signals. Motor-nerve reflex response to movement of the joint, in particular, chordotonal organs was investigated over a number of years (Bush, 1962b; Evoy & Cohen, 1969; Muromoto & Shimaozawa, 1970; Spirito et al., 1972, 1973; Lindsey & Gerstein, 1979a, b; Vedel & Clarac, 1979; Lindsey & Brown, 1982; LeRay et al., 1997). Chordotonal organs were thought to be responsible for resistance reflexes,

which are reflexes that can be demonstrated by passively moving a joint in one direction while recording from antagonist motor neurons.

Chordotonal organs of other appendages, apart from those of the limbs, have been described also. These include: the antennules of *Panulirus* (cf. Wyse & Maynard, 1967; Hartman & Austin, 1972), the swimmerets of *Homarus* (cf. Davis, 1968), and the third maxillipeds of *Homarus* (cf. Wales et al., 1970). There has been only one study of chordotonal organs where single-unit extracellular recordings were made from individual axons (or cell bodies) in order to determine the specific function of individual neurons, and to relate the location of the cell on the elastic strand. This work was done on perhaps the simplest of the chordotonal organs, the PD organ, by Hartman & Boettiger (1967). Mill & Lowe (1972), using *C. maenas*, repeated this early work and found similar results. The anatomical arrangement of CP1, CP2, MC1, and MC2 in *Cancer magister* Dana, 1852 also permits such unit analysis. The CP1 and MC1 are of particular interest, since the elastic strand forms a semi-flat sheet proximally, where obvious differential forces are exerted during joint movement to activate cells. Because their cell bodies are large (10 to 40  $\mu\text{m}$  in diameter) and located close to the organ that they monitor, the sensory neurons are easily identified and readily accessible for electrophysiological recordings. Since the site of spike initiation is distal to the soma, the action potentials are easily recorded with an extracellular focal electrode placed on the soma.

The purpose of this study was to investigate whether there is an orderly anatomical arrangement, according to function, of the chordotonal organs on the CP1, CP2, MC1, and MC2 strands of *C. magister*.

#### MATERIALS AND METHODS

Male *Cancer magister*, measuring 13-15 cm across the carapace, were obtained by trapping in the boat basin at Charleston, Oregon. Upon capture, the crabs were either shipped by Air Express to the laboratory and maintained in 303-liter aquaria containing artificial sea water (Instant Ocean) at 34 ppt and 15°C, or they were used for experimentation at the Oregon Institute of Marine Biology. Animals were fed squid periodically and used in experiments within two weeks of capture.

The *C. magister* saline (Macmillan & Dando, 1972) adversely affected the viability of the neurons and particularly the muscles. The viability problem was solved by using the animal's own serum as a saline. Whole blood was obtained by cardiac puncture from living, commercially caught *C. magister* crabs at the Point St. George Fisheries of Point Orford, Oregon. To obtain serum, the blood pooled from many crabs was cooled in an ice bath. The clot that formed was discarded,

and the resulting serum was filtered through a Whatman shark-skin filter, placed in vials, and stored at  $-80^{\circ}\text{C}$ .

The first or second walking legs were removed by slowly pinching across the merus, which caused the crab to autotomize the limb. After the joint in question was isolated from the rest of the leg, it was put into a Petri dish and bathed in cold *C. magister* serum. The serum was periodically changed during the dissection and approximately every 30 minutes during the recording sessions. All the preparations were viewed by transmitted, fiber-optic light with a green filter interposed to enhance the viewing of the neurons. Each preparation was pinned to a Petri dish lined with transparent Sylgard. During recordings, the preparation was maintained at  $12-15^{\circ}\text{C}$  by circulating ice water through the lumen of a hollowed, aluminum base to which the Petri dish was attached, using a zinc heat-sink compound. That dish in turn was fastened to a larger dish and surrounded by ice. When working with the CP chordotonal organs, muscle and exoskeleton beyond the distal half of the propodus, as well as proximal to the carpus, was trimmed away. In order to expose the CP1 chordotonal organ, the elastic strand was approached from the ventral side after cutting away the lateral half of the cuticle. A small window cut in the medial side of the carpus allowed the entry of transmitted light for better viewing. After removing the reductor tendon and muscle, the CP1 cell bodies could be seen clearly at a magnification of  $100\times$ . To expose the CP2, the medial cuticular halves of both the carpus and propodus were removed, and a window was cut laterally in the carpus. The productor tendon and muscle were also removed. For both preparations, the leg segments were pinned to the Sylgard, ventral side up.

After the main leg nerve was exposed in the preparations (CP1 or CP2), the nerve that innervates the chordotonal organ was located at the base of the elastic strand. The CP nerve bundle was teased away from the main leg nerve, and the main leg nerve was then removed. Dealing only with the CP nerve bundle, it was then possible to isolate the tension nerve, which projects toward the apodeme. The remaining CP nerve, which carries proprioceptive information, could then be split away by using fine glass needles to isolate the individual axons or subnerves for recording.

The MC1 was revealed by cutting the ventral connective tissue that articulates between the merus and the carpus. A longitudinal cut along the ventral midline was made, as well as one along the medial side, for the entire length of the merus at a level just dorsal to the medial MC condyle. The rest of the exoskeleton, except between these two cuts, was removed. The flexor tendon and muscle were removed carefully, with keen attention to not stretch the main leg nerve. The removal of the extensor tendon was accomplished by cutting the connective tissue between the tendon and its cuticular attachment. To prevent damage to the MC1 strand

and its neurons, the easiest approach was to find, before cutting the nerve bundle, where the MC1 nerve bundle branched from the main leg nerve. This ensured against pulling on the MC1 neurons when the extensor tendon and main leg nerve were removed. The best approach to expose the MC2 was to remove the dorsal half of the merus exoskeleton, flexor tendon, and flexor muscle. By reflecting the main leg nerve in the carpus region, the MC2 strand could be exposed without damage. With the nerve bundle taut, the axons were easily separated and isolated for recording.

A large diameter (10  $\mu\text{m}$  inner dia.) suction electrode, made up of a fire-polished glass electrode, was used to place directly over a cell body or to record from groups of axons. A Grass p-15 amplifier recorded the signals. As described in detail in an earlier report (Cooper & Hartman, 1999), a speaker was used to move the strand in desired wave forms and rates. The distal regions of each strand were pinched by a pair of tweezers attached to a DC speaker that served as a servomechanism. This speaker was driven by waveform inputs from a Hewlett-Packard 3300A low frequency function generator that controlled the elastic strand movements, in a manner similar to that described by Wiersma & Boettiger (1959). The servomechanism was triggered by a pulse delivered from a Grass S88 Stimulator at pre-selected intervals. The 1 mm experimental displacements were driven by triangular wave-forms with durations of 1, 2, and 4 sec. This resulted in a rate of displacement at 1 mm/sec, 0.5 mm/sec and 0.25 mm/sec, respectively.

Data on VHS tapes were retrieved for analysis on a Macintosh computer and the MacADIOS A/D converter. The data were acquired at a rate of 5.2 KHz. The spike trains were divided into a set number of bins, the number of bins depending on the rate at which the joint displacements were given. The length of each bin was either one-sixteenth or one-eighth of a second, depending on the analysis used (specified in the Results). The responses of ten trials were averaged to obtain frequency plots of the movement-sensitive cells, with bin widths of one-sixteenth or one-eighth of a second. The averaged response was then normalized to impulses-per-second and plotted as frequency versus time. When the recording period was over, the overall anatomical arrangement of each preparation was determined by methylene blue staining. Anatomical drawings were made with a camera lucida attached to a Wild dissecting microscope (model M7A). The neuronal cell bodies were discerned without staining for placement of the recording electrode, however, to see all the neurons in respect to the ones recorded, the methylene blue staining aided the anatomical mapping. Combining results from various preparations ( $\sim n = 20$ , for each chordotonal organ) and the multiple neuronal recordings in each preparation, a unified trend appeared in the anatomical arrangement.

## RESULTS

## Anatomy

The anatomical arrangement of the neurons located in the MC1 and CP1 chordotonal organs of *Cancer magister* is very similar. The number of neurons seen in the MC1 chordotonal organs was always greater than that in the CP1 organ within a given leg. Fig. 2A, B demonstrates the location of the cell bodies and the dendrites of these sensory neurons in typical MC1 and CP1 chordotonal organs. The distally located cells in both MC1 and CP1 organs are sensitive to static positions. This distal portion of the elastic strand undergoes too much movement during the displacements to allow single cell recordings. But, fortunately, the axons of these cells form two separate nerve bundles, allowing relatively easy recordings from subsets of neurons. The most medial-position nerve bundle consists of the axons from the distally located static position cells. The lateral-position nerve originated from the next most proximally located static-position cells. These two nerve bundles are still grouped as neurons with cell bodies distally on the strand. The two separate-position nerve bundles are seen most clearly in MC1 chordotonal organs. The separation of the two-position nerves allowed recordings to be made of one group independently of the other.

The neuron cell bodies, which are more proximal than the position-cells bodies in both CP1 and MC1 organs, are responsive to dynamic movements. The larger cell bodies show activity while the elastic strand is being relaxed (dynamic, relaxation-sensitive). In contrast, the smaller proximally-located cell bodies are active while the elastic strand is being stretched (dynamic, stretch-sensitive).

The MC2 and CP2 chordotonal organs are both narrow elastic strands, and the neurons in both of these organs are similarly arranged (fig. 3A, B). The neurons with large cell bodies are not situated in the elastic strands as they are in the MC1 and CP1 elastic strands. The static position cells are located in the distal part of the elastic strand, but as in the MC1 and CP1 elastic strands, the cells undergo a lot of movement during the displacements of the elastic strands. Thus, recordings of the static position cells were made from a nerve bundle, just as were the static position cells in MC1 and CP1 elastic strands. The large proximally-located cell bodies are only active while relaxing the elastic strand. No cells were found in MC2 and CP2 elastic strands that could be classified as dynamic stretch-sensitive cells.

The size of the proprioceptive cells within a given type of chordotonal organ was the same in all the preparations. The diameter of the dynamic relaxation-sensitive cell bodies was in the range of 30-40  $\mu\text{m}$ . The dynamic stretch-sensitive cells had diameters of 10-20  $\mu\text{m}$ , whereas the static position-sensitive cells had diameters of 10-20  $\mu\text{m}$ . The number of proprioceptive cells does change with age until the adult stages are reached (Hartman & Cooper, 1994), but this was not of concern

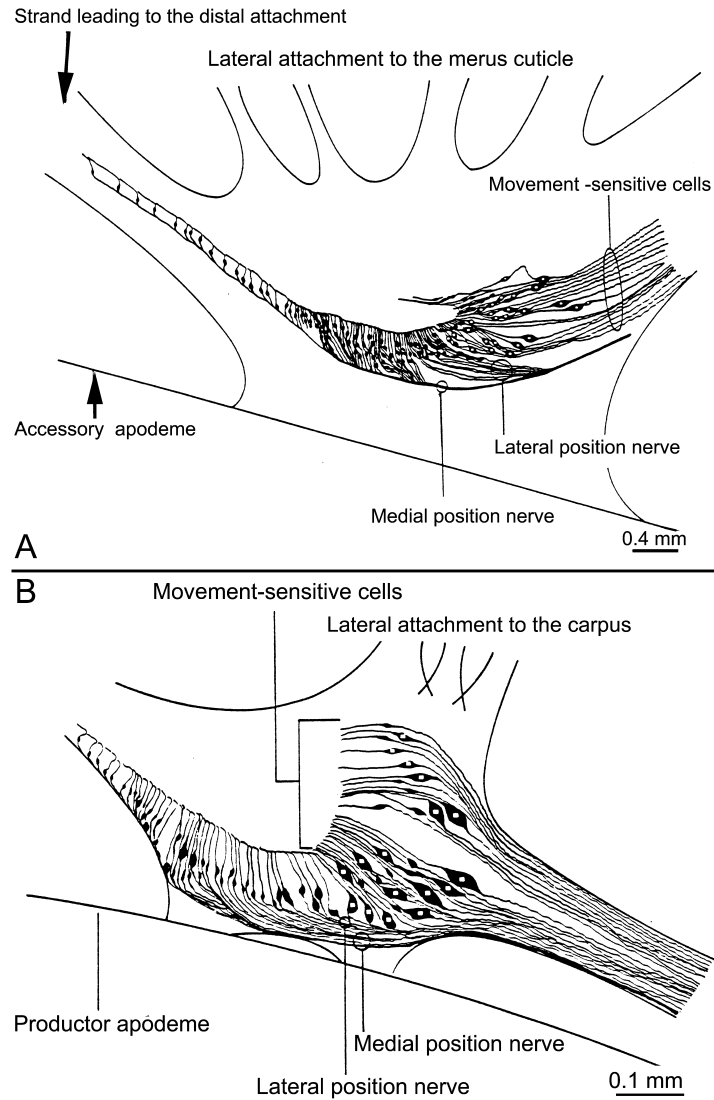


Fig. 2. The anatomical arrangement of the sensory neurons and their endings in, A, the MC1; and, B, CP1 chordotonal organs in *Cancer magister* Dana, 1852. The large somata have a clear dot in the center to delineate their function as a relaxation-sensitive subtype within the movement-sensitive neuron type; note the subnerve for the static position-sensitive neurons.

in this study, because same-size animals (13-15 cm across the carapace) were used throughout the experiments. The mean cell counts and the standard errors are as follows: CP1,  $60.2 \pm 3.2$  ( $n = 5$ ); CP2,  $29.0 \pm 1.1$  ( $n = 5$ ); MC2,  $34.8 \pm 1.5$  ( $n = 5$ ); MC1,  $124.0 \pm 3.6$  ( $n = 12$ ). A count was made also of the neurons present in the PD chordotonal organ for the same-size crabs PD,  $84.267 \pm 2.429$



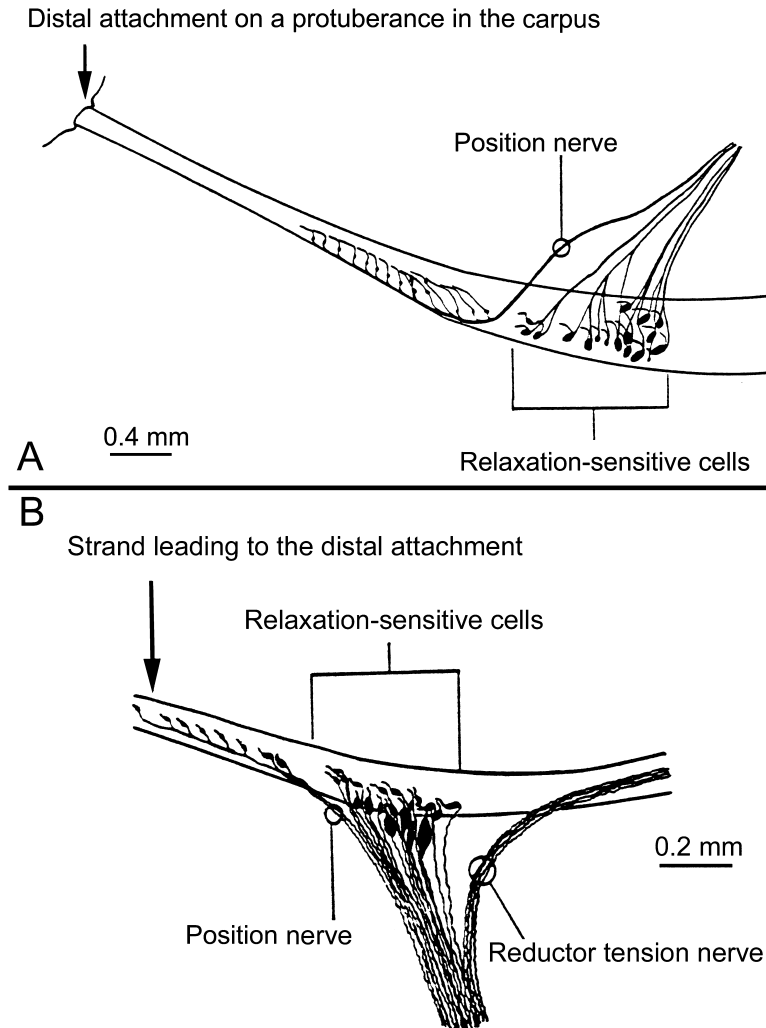


Fig. 3. The anatomical arrangement of the sensory neurons and their endings in, A, the MC2; and, B, the CP2 chordotonal organs in *Cancer magister* Dana, 1852. Note the subnerve for the static position-sensitive neurons; in B the tension nerve for the reductor muscle/apodeme is shown joining the chordotonal nerve bundle.

(n = 15). These data suggest that there is not much variation in the number of neurons between preparations for a given type of chordotonal organ, and that there is little variation between animals of the same size. This is consistent with a previous experiment that examined the number of neurons associated with the PD throughout development in the same species of crab (Hartman & Cooper, 1994).

I also used methylene blue staining to examine the PD, CB, and IM chordotonal organs in the walking legs of *C. magister*. There appears to be an arrangement

of cells similar to what was reported in other crabs. The PD, CB, and IM elastic strands are all tubular shaped, like the MC2 and CP2 elastic strands.

### Physiology of chordotonal organs

The classifying of the responses that were observed from these experiments necessitated naming three general categories of cell function. The cells in one category behaved in such a way that they were termed "static position-sensitive cells". These cells fire tonically in certain positions. They fire also during a movement, but they are not unidirectionally movement-sensitive. The cells in the second category are active only during a movement: when the movement ceases, their activity also ceases. These cells are responsive only to the unidirectional movements of either the elongating, or the relaxing elastic strand. Thus, the term relaxation-sensitive cell (RSC) or stretch-sensitive cell (ESC) was assigned to each particular cell.

### Movement-sensitive cells

These cells of the chordotonal organs (MC1, CP1, MC2, and CP2) are responsive only during the movement of the elastic strand, and most of these cells are dependent on the velocity of movement for their response. In addition, these cells are directionally sensitive, responding to stretch or relaxation of the elastic strand. The response for a particular cell to a unidirectional movement may be fractionated, firing during the onset or termination, or possibly throughout the range of a movement.

In these experiments, the displacements of the elastic strand were made at three different rates (1 Hz, 0.5 Hz, and 0.25 Hz), with approximately 1 mm movement of the distal part of the strand. The analysed responses from each chordotonal organ showed that all the directionally-sensitive cells were velocity-sensitive. Fig. 4 shows an integrated record of a CP1 cell that is sensitive to relaxation of the elastic strand as well as to the rate of relaxation. During the relaxation movement at a given rate, the cell's response was relatively constant. At the 1 Hz (1 sec) displacement, the frequency of the activity was approximately 65 Hz; and for the 0.5 Hz (2 sec) and 0.25 Hz (4 sec) displacements, the activity had a frequency of 55 Hz and 40 Hz, respectively. A control run of 1 Hz was again performed after the series of 1 Hz, 0.5 Hz, and 0.25 Hz displacements. The control run was always consistent with the initial 1 Hz trial. The activity was maintained until the direction of movement was changed. A more typical response for the RSCs is seen in fig. 5. This unit is velocity-dependent but does not maintain a set frequency during the movement, as did the cell depicted in fig. 4. When the movements of the elastic strand were produced in a decreasing order (1 Hz, 0.5 Hz, 0.25 Hz), the activity of

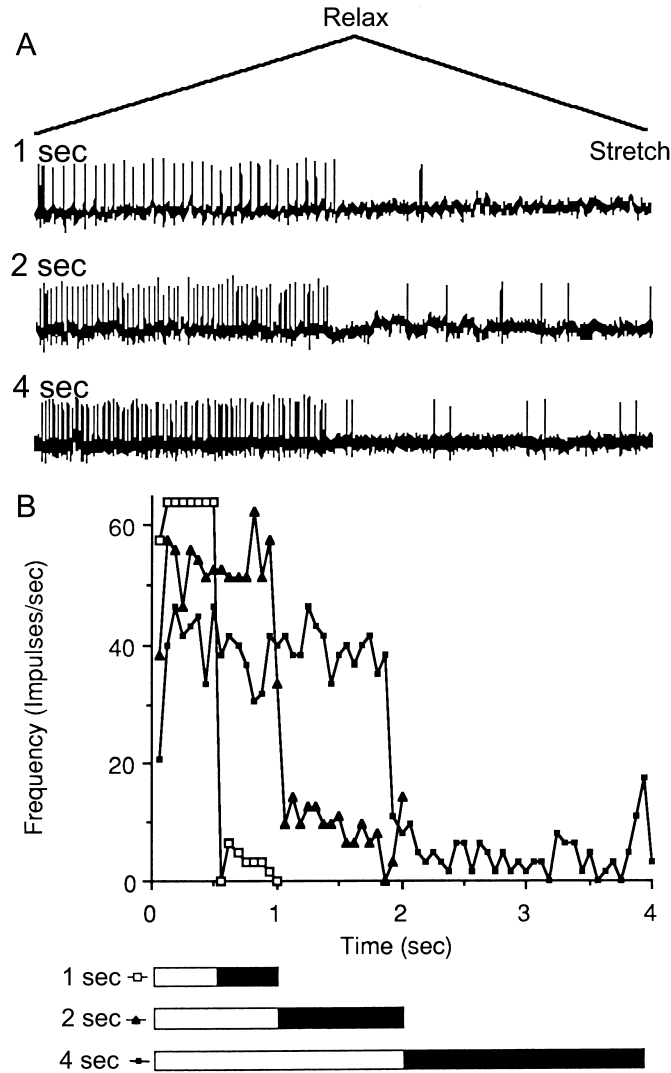


Fig. 4. A representative response recorded from a single neuron on a CP1 organ that is sensitive to relaxation of the strand. A, The total movement of the strand is about 1 mm over 1 sec, 2 sec, or 4 sec time frame from a stretched to relaxed and back to a stretched position; note the time scale differences; to index the firing frequency the displacement was repeated 10 consecutive times and an average activity profile was obtained; B, the number of spikes was converted to the impulses per second or frequency for each sixteenth-of-second bin; the horizontal bars represent each displacement period with the open box showing the time from stretched to relaxed and the solid box represents the time frame from relaxed to stretch for each of the different displacement rates.

the cell being recorded usually decreased in firing frequency. Some cells decreased in firing frequency more rapidly than other cells.

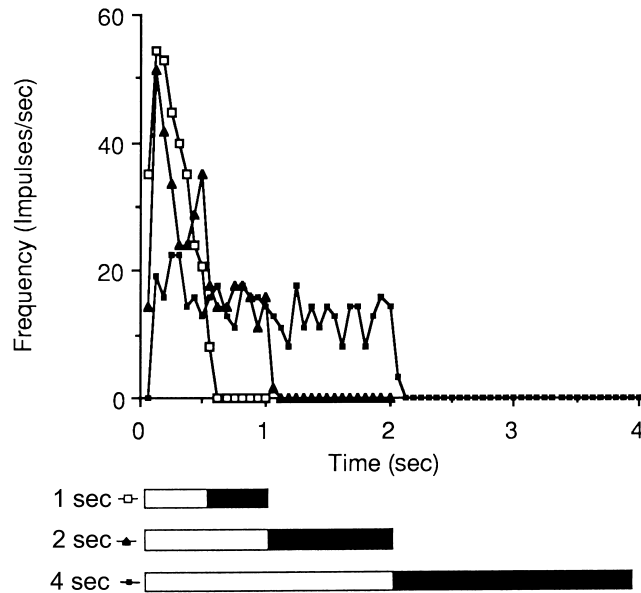


Fig. 5. Responses from a dynamic relaxation-sensitive neuron on an MC1 strand that shows adaptation for the rapid movements (1 sec and 2 sec) but little adaptation for the slow movement (4 sec); the firing frequency is also reduced as the rate of movement is decreased; the displacement was repeated 10 consecutive times and the average number of spikes was converted to impulses per second for each sixteenth-of-second bin.

The cells' responses that have been depicted so far correspond to relaxation of the elastic strand. While these neurons are the more common movement-sensitive cell type in the chordotonal organs examined, there are also cells that responded only to stretch of the elastic strand. As with the RSCs from MC1 and CP1 chordotonal organs, they are unidirectional in response. As may be seen in fig. 6, the relaxation-sensitive activity stops upon initiation of the strand stretching, and ESCs come in at the onset of stretch and maintain their activity throughout the phase. Since the recordings are extra-cellular, activity from neighboring cells is seen in most of the recordings.

In addition to a cell being relaxation- or stretch-sensitive, some cells show also range fractionation of unidirectional movements. That is, some cells fire only during the early part of relaxation, while other cells may fire later. Fig. 7A shows how a late RSC behaves while another low activity cell, as shown in fig. 7B, is sensitive only during early relaxation. Fig. 8A shows an stretch-sensitive cell that fires throughout its stretch. Fig. 8B, however, shows the activity of a cell that is sensitive only to late stretch. The activity in fig. 8C was unique in that this was the only cell found to behave in this way. The quiescent period during the mid-range of stretch was seen also during the 0.5 Hz and 0.25 Hz displacements. One might speculate that some distortion occurred to the elastic strand when a certain

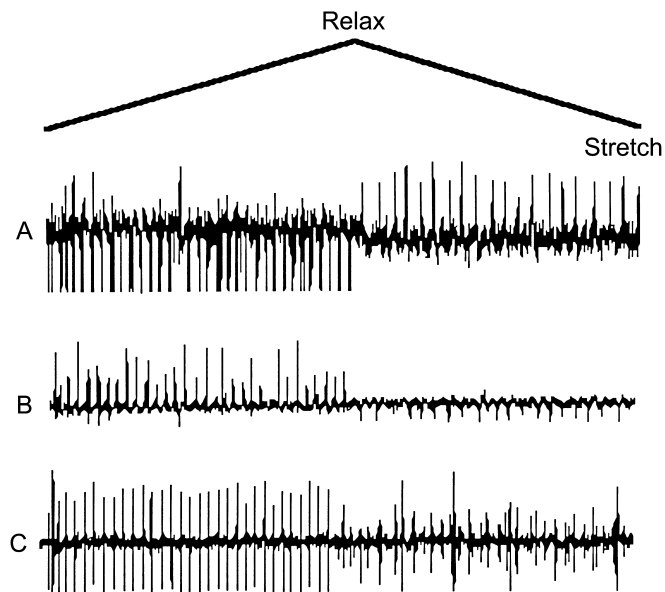


Fig. 6. Unidirectional dynamic, movement-sensitive cells that are responsive to either, A, relaxing; or, B, stretching of the MC1; or, C, the CP1 chordotonal strands. The extracellular recordings, in all three cases, over the soma of the predominantly relaxation-sensitive cell, is also picking up the neighboring stretch-sensitive cell or its axon, which is in close proximity; in cases A and B, the stretch sensitive responses are in the opposite direction of the field potential for the relaxation sensitive cell; in C, there is a bidirectional field potential for the stretch sensitive cell; all three large responses were recorded from a 1 second displacement of 1 mm in distance, all these responses were consistent for the observed 10 trials.

position was reached, but why would the activity then return past the position when the movement was unidirectional?

Velocity-sensitive responses have been mentioned already, but their behavior was not fully examined. Some cells (fig. 9) showed a decrease in firing frequency when the rate of displacement decreased. Other velocity-sensitive cells showed not only a decrease in firing frequency, but also an alteration in onset and termination of activity. Fig. 9 shows how a cell's activity changes from firing throughout relaxation to firing during early relaxation only, as the rate of displacing the elastic strand changes from 1 Hz to 0.25 Hz. The movement-sensitive cells show no background activity when the joint is at rest.

#### Static position-sensitive

Cells that respond in a tonic fashion to a static position are termed "static position-sensitive cells". These cells will also fire during a dynamic movement, but they are not unidirectional movement-sensitive.

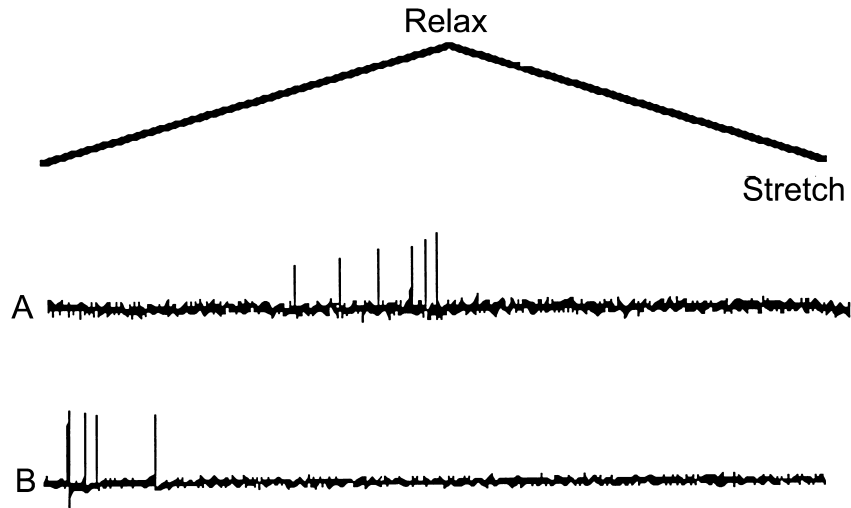


Fig. 7. Responses from two different types of relaxation-sensitive cells. A, the responses are from an MC1 organ that only responds to the late phase of relaxation; other neurons in the same strand respond to earlier phases of relaxation; B, where a cell on a CP1 organ only responded to the early phase of relaxation but not the later phases; in both cases the displacement was at 1 Hz (1 sec for a full period).

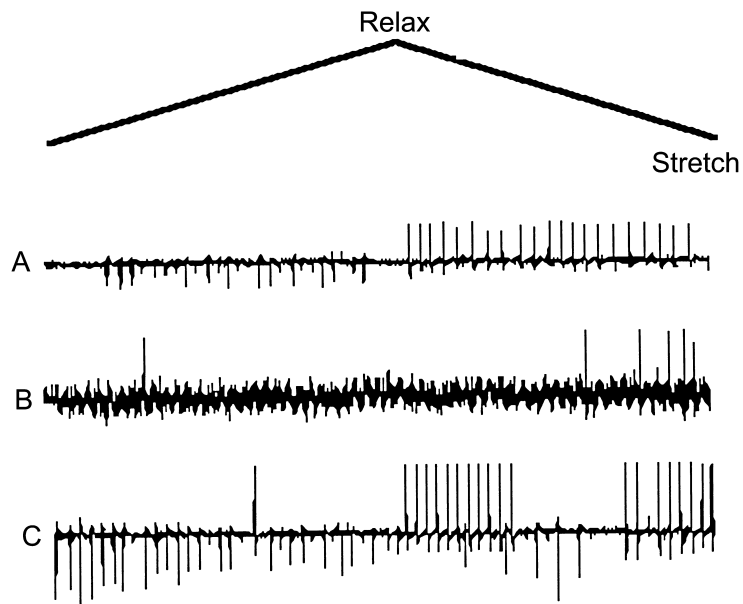


Fig. 8. Responses from three different types of stretch-sensitive cells. A, the responses are from a CP1 organ that responded throughout stretching, while another neuron (B) in the same strand responded only to the later phase of stretch; C, a stretch-sensitive cell on an MC1 organ showed a very unique response to stretch that was reproducible with a gap in its responsiveness to a set stretching phase of the strand; the displacement was at 1 Hz in all cases.

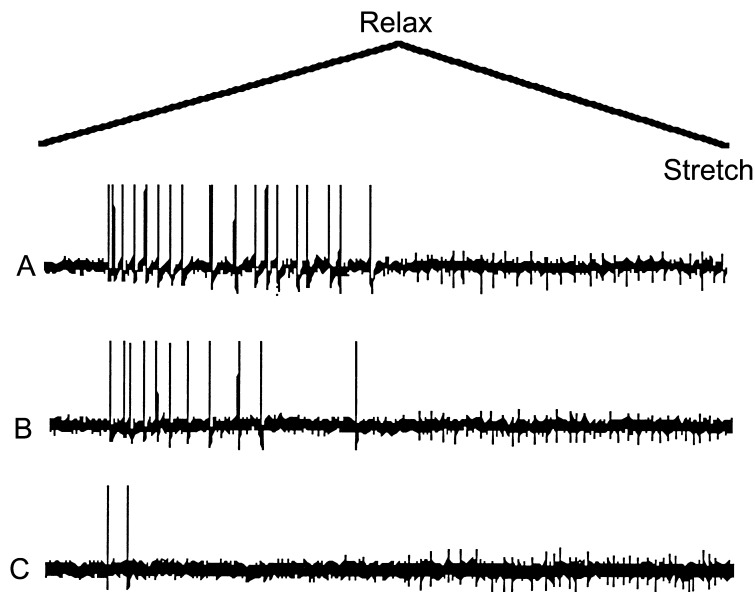


Fig. 9. A relaxation-sensitive neuron on an MC1 organ that shows phase and displacement rate sensitivity. This particular cell has a large soma and tended to favor the early phase of relaxation at all three displacement rates; a stretch sensitive response from a neighboring neuron can be seen barely above the noise level that maintains its firing frequency at all three rates, while the relaxation sensitive neuron does not; A, 1 sec; B, 2 sec; and C, 4 sec displacement rates are shown.

Since the position-sensitive cells are located distally along the elastic strand, they move too much during the displacement, which prevents individual recording. So, recordings were made from groups of the position-cell axons at a more proximal location along the elastic strand. A typical response from these cells in an MC1 chordotonal organ, at five different strand positions, is shown in fig. 10. The frequency plot indicates that the cells increased their activity when the elastic strand reached a more relaxed position, since the recordings are mass recordings. The same held true in other preparations of MC2 chordotonal organs, one of which presented eight different strand positions (fig. 11A). To show that these static position-sensitive cells have tonic activity at a given position, they were analysed at five different strand positions for four seconds each. To obtain frequency plots, the activity of all the spikes in the recordings were used. This was discerned by a window discriminator set above the baseline trace. The frequency plot indicates that the cells do indeed have a tonic activity, and that the firing frequency increases as the elastic strand reaches more relaxed positions (fig. 11B). These types of responses were seen consistently in all the whole-position nerve recordings in the four different chordotonal organs (CP1, CP2, MC1, and MC2).

As already mentioned, the static position cells show a range fractionation, with some cells becoming more active at particular degrees of relaxation. In the CP1 and

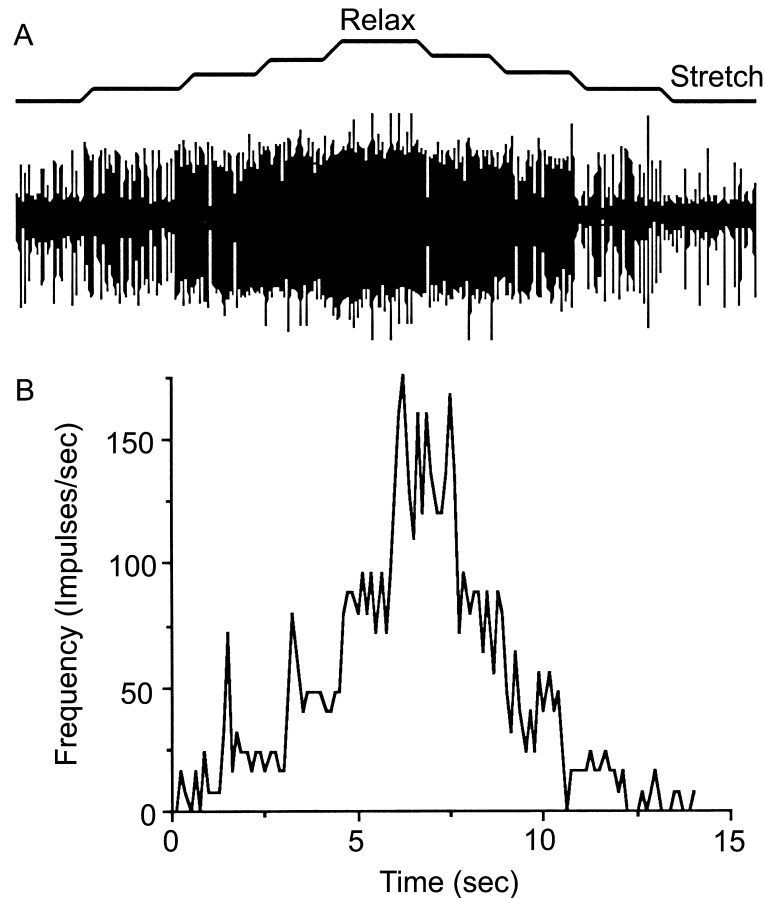


Fig. 10. Responses during hold-move-hold displacements from the static position-sensitive bundle that is composed of the axons from the cells with small somata. A, these neurons make up the unique cells that are shown as a distal string along the strand; B, the frequency of activity for each 1/8 sec; the entire trace shown in A is processed in the frequency plot (B, 14 sec).

MC1 preparations, there were always medial and lateral position nerves. Fig. 11C shows the activity from the medial-position axon bundle of an MC1 preparation.

An isolated position nerve is harder to obtain in CP2 and MC2 preparations, because the axons from neighboring cells join the position nerve more distally than in the CP1 and MC1 elastic strands. Responses from position-sensitive cells and from dynamic relaxation-sensitive cells of the CP2 elastic strand are very similar to those in the MC2 organ. The bursting activity from the dynamic cell is seen only during the movements. Also, the dynamic cells produce larger amplitude spikes than those of the static position cells. This is due to the smaller size of the static position cells.



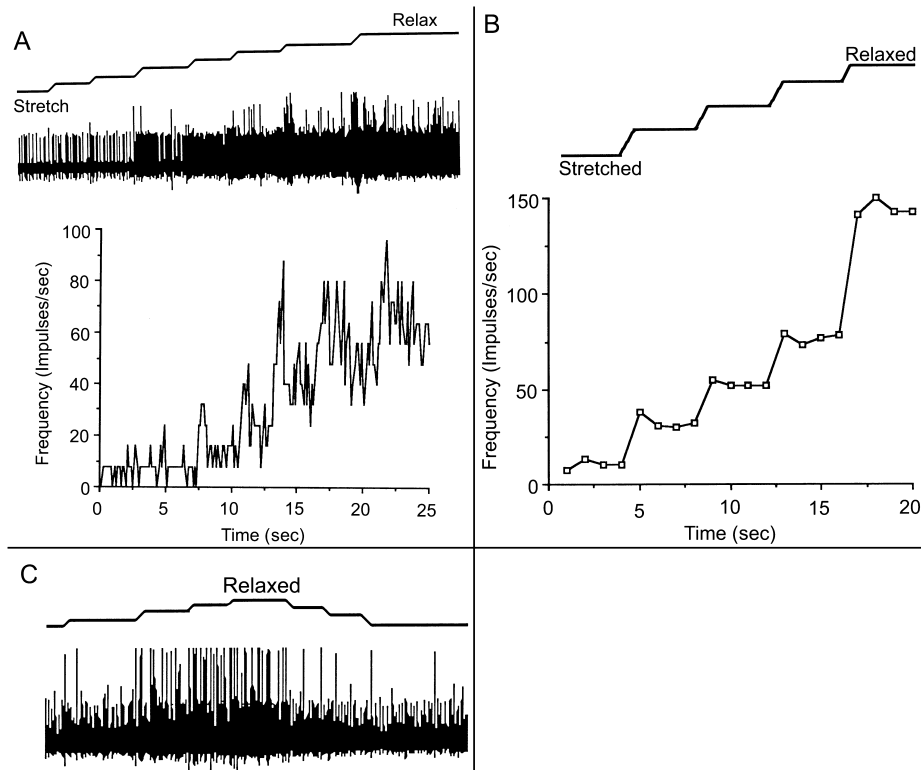


Fig. 11. A, responses from the bundle of axons that comprise axons along the distal aspect of the strand for a CP2 organ over a range of hold-move-hold displacements; the activity plot for the nerve recordings shows the greatest activity during relaxation of the strand; B, a similar hold-move-hold displacement and activity profile for the distal nerve bundle of an MC2 organ; C, as shown for the other organs, the distal neurons in the MC1 organ show a similar pattern of activity for the hold-move-hold displacement; the frequency plots represent the time frame shown in the raw traces.

### Dynamic versus static

Dynamic directionally-sensitive cells fire only during movement and become quiescent during the opposing directional movement. This is seen clearly in the recording of a relaxation-sensitive cell during a 1 Hz movement and during the move-hold conditions (fig. 12A). Notice that in both cases (fig. 12A, B) there is no activity during stretch of the elastic strand.

Static position-sensitive cells fire throughout a 1 Hz displacement, and they do not respond to one direction of the movement (fig. 12C). The frequency of firing changes considerably during movement. Under the move-hold procedure, the cell maintains a tonic state of firing at each position (fig. 12C, D). The movement-sensitive cells would not be active if the elastic strand were held in a static position.

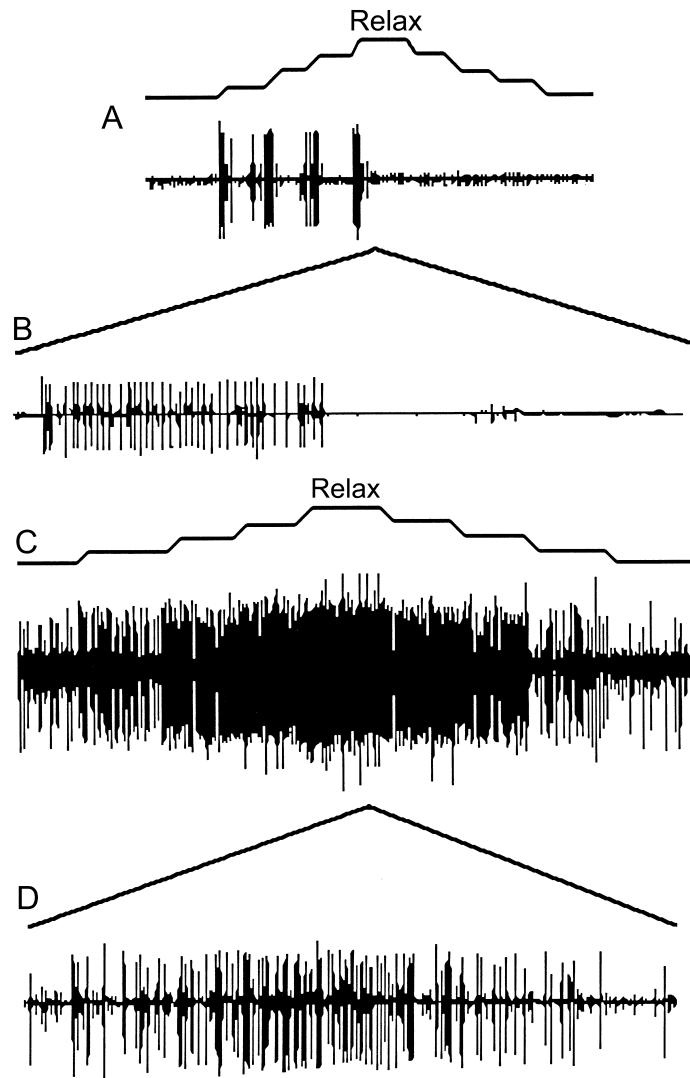


Fig. 12. Activity of dynamic-sensitive and static position-sensitive neurons to ramp and hold-move-hold displacements. A, a relaxation-sensitive neuron on an MC2 organ exposed to the hold-move-hold displacement only presented activity during the movements and not the static positions throughout the entire range of displacement; B, the same cell monitored in A responded only during the relaxation phase of a ramp displacement; C, the static position-sensitive neurons in an MC1 organ increased activity as the strand was held in more relaxed static positions; and D, the firing frequency was highest during ramp displacement for the most relaxed range during the relaxation or stretching of the strand; one second for stretch-relaxation-stretch in all examples.

## DISCUSSION

## Structure-function relationship

The sensory cell mapping of the chordotonal organs (MC1, MC2, CP1, and CP2) in the walking legs of *Cancer magister* revealed that neuronal function is related to the location of the single sensory ending on the elastic strand, and to the size of its soma. Recordings within chordotonal organs enabled the quantitative characterization of the proprioceptive response. Cells located more distally showed a tonic response whereas those more proximal are movement sensitive and accommodate quickly in a static position.

The MC2 and CP2 chordotonal organs contain few position-cells when compared to their partners, the MC1 and CP1 organs. However, the responses from static position cells were like those of the MC1 and CP1 static position cells. That is, activity in the nerve increased as more relaxed, static positions were imposed. In all the position-nerve recordings (MC1, MC2, CP1, and CP2), recruitment of additional larger units occurred as the elastic strand was held in more relaxed positions. Also, unlike movement cells, these neurons showed hysteresis to changes in direction. Depending on the direction of movement, relaxation-to-stretch versus stretch-to-relaxation, the static position cells might show an initial difference in activity at the same given point during a displacement. If the strand is held at a given position for a second or more, regardless of which direction the strand was moved to obtain that position, the firing rate will be tonic and representative to that position. Wiersma (1959), while recording from whole nerve bundles of CP1 and CP2, reported activity to maintained positions. Bush (1965a) indicated that the MC1 and CP1 nerves have tonic activity, the responses being most active when the elastic strand was held in relaxed positions. I did not record responses from single cells that would fit Wiersma & Boettiger's (1959) definition for intermediate cells. As mentioned earlier, they classified cells as an intermediate type based on recordings from groups of axons. Activity recorded from a group of axons in a CP2 preparation displays the type of response that Wiersma & Boettiger (1959) would have termed an "intermediate type" of response. The activity shown in fig. 12A arose from position axons and movement axons. In other CP2 preparations, when recordings were taken from just the position nerve, no bursting of activity was seen during the movement phase of the move-hold displacements. The response that Wiersma & Boettiger (1959) obtained was probably from a mixed nerve of position- and movement-sensitive neurons.

In all the preparations, the more proximal cells were found to be exclusively movement-sensitive. The neurons having cell bodies measuring 30-40  $\mu\text{m}$  in diameter were found to be sensitive to relaxation of the elastic strand. Less

numerous, smaller (10-20  $\mu\text{m}$  in cell body diameter), co-mingled neurons in the MC1 and CP1 chordotonal organs proved to be responsive to elastic strand stretch. However, this latter category of neurons (stretch-sensitive cells) was not found on the MC2 and CP2 chordotonal organs. This is indicated, because during the ramp displacements no cells showed sensitivity to the stretch movements.

Most encountered RSCs and ESCs fired throughout the range of imposed movements. RSCs that fired a burst only at the initiation of strand relaxation were found occasionally (6 cells out of 98 cells), but no ESCs of this description were seen. Twelve out of 36 ESCs showed sensitivity to the later phase of stretch. Not one of the ESCs fired only during the early phase of stretch. Since Wiersma & Boettiger (1959) recorded from very few isolated cells, it was hard for them to gain an appreciation of how the majority of the cells respond to dynamic movements. From whole-nerve recordings, as they were in the past (Wiersma & Boettiger, 1959; Bush, 1965a, b), it is almost impossible to determine whether one cell demonstrates unidirectional fractionation (i.e., early or late responsiveness).

All the movement-sensitive cells from all of the four chordotonal organs showed sensitivity to the rate of displacing the elastic strand in which they were embedded. In all cases, the response frequency decreased with decreasing rates of displacement while holding the amount of displacement constant. Some cells can maintain a constant firing frequency throughout a unidirectional movement at a set rate. In most cases, the response frequency changes at a fixed rate during a unidirectional movement. In some cases, the number of spikes for the range of displacement increased at reduced displacement rates, but the frequency of activity always decreased at the slower displacement rates. This study is the first, to date, of movement-sensitive cells decreasing their firing frequency and shifting their activity toward late or early phases of a movement as the rate of displacement decreases. Consistency in responses to repetitive trails of displacements is addressed in an earlier study (Cooper & Hartman, 1999).

Since all the movement-sensitive cells show sensitivity to the velocity of displacement, they should show acceleration sensitivity also. For example, if there were a relaxation-sensitive cell that could maintain a constant firing frequency for a fixed rate of displacement, and if a sine wave relaxation displacement were given, the firing frequency of the cell would change during the movement, because the velocity changes at different points along the sine wave. Recall that most of the movement-sensitive cells cannot maintain a set firing frequency during a triangular displacement of a given rate, so one cannot analyse, with accuracy, whether a cell is sensitive to velocity of acceleration when a sine wave displacement is given. So possibly these cells could qualify as being acceleration sensitive.

## Comparisons to other preparations

Wiersma (1959) recorded from CP1 and CP2 nerve bundles in the walking legs of *Carcinus maenas* and found that these nerve bundles were more active during relaxation than for stretch of the elastic strands. In the majority of the cases, he reported that neither CP1 nor CP2 had any activity during a stretch movement of the elastic strand. While I have not examined the chordotonal organs in *C. maenas*, I suspect that he failed to record either the ESC responses or the additional RSCs and position-sensitive cells in the CP1 organ: he used sea water as a preparation bathing medium. It is my experience that even the most carefully prepared crab saline, let alone filtered sea water, yields far less neural activity than crab serum. The ESCs (which are small), and the very small position units are probably killed by sea water. Wiersma (1959) recorded little stretch-sensitive activity from the CP2 nerve bundles, which is in agreement with my results for *Cancer magister*. He also recorded from MC1 and MC2 nerve bundles while moving the merus-carpus joint. He noted that the “MC2 nerves typically, but not exclusively, signal joint flexion (stretching the MC2 elastic strand), whereas MC1 has a preponderance of extensor movement (relaxation of MC1 elastic strand) and position fibres”. His results with MC1, but not with MC2, are in accordance with the results I obtained in *C. magister*. These differences might have been due not only to bathing medium but also to species differences.

Bush (1965a) also made recordings from whole chordotonal nerve bundles of CP1, CP2, MC1, and MC2 elastic strands in *C. maenas*. His results from whole-nerve recordings were similar to mine. In contrast to Wiersma (1959), he did not record any movement-sensitive responses from the MC2 nerve during the stretching of the strand. He did show, for two isolated relaxation-sensitive nerve fibers, that the responses varied with the rate of relaxing the elastic strand, which is normal adaptation.

Once one knows the physiological significance of the single neurons within the CP1, CP2, MC1, and MC2 chordotonal organs, one is inclined to ask: why are there two chordotonal organs in both the carpus-propodus and merus-carpus joints, when there is only one chordotonal organ present in the propodus-dactylus joint? As already mentioned, the MC1 and CP1 organs are similarly shaped (planer-2D) and the MC2 and CP2 organs are both rod-shaped, like the PD organ. There are differences in the way the analogous chordotonal organs are arranged in their respective joints. In the carpus-propodus (CP) joint, the CP1 is proximally attached to the ventral edge of the productor apodeme and distally attached to the distal-ventral cuticle within the carpus and on the same side (medial) as the productor apodeme. The CP2 organ is proximally attached to the ventral surface of the reductor apodeme, but distally it spans the CP joint and crosses from the lateral to

the medial side of the leg to attach to the proximal ventral cuticle in the propodus. Due to the arrangement of the chordotonal organs, contraction of the producer muscle results in the production of the CP joint. This would cause stretch of the CP1 elastic strand and relaxation of the CP2 elastic strand. The sensory response to this movement would be that ESCs and some static position cells in the CP1 organ would become active, whereas, in the CP2 organ, the RSC's and static position cells would be stimulated. An opposing response would be seen if the reductor muscle were to contract. That is, reduction of the CP joint would cause the CP2 elastic strand to become elongated, and, at the same time, the CP1 elastic strand to undergo relaxation. Since the CP2 elastic strand does not contain ESCs, only the static position cells would be active during this movement, whereas in the CP1 organ, both RSCs and static position cells would be stimulated. One might predict that the central nervous system is able to sort out this redundant sensory information, and would, therefore, know exactly what angle the joint is at during a movement. But, why does the crab need two chordotonal organs sensing joint information when the CP1 organ is capable of sensing all the degrees of movement and position within the joint? Redundancy of sensory information might be the answer. There is only one chordotonal organ in the propodus-dactylus (PD) joint, which undergoes an even greater degree of movement during normal locomotion than the CP joint. There are neurons on the PD organ capable of detecting all movements (RSCs and ESCs) and positions of the elastic strand. The number of neurons in the PD chordotonal organ is approximately the same as in the CP2 and MC2 chordotonal organs. Since the PD joint functions well with one chordotonal organ, one cannot rationalize satisfactorily why the CP and MC joints have two chordotonal organs.

The same questions should be asked about the MC1 and MC2 chordotonal organs. Although the MC joint does undergo large movements and there is a different anatomical arrangement of the two chordotonal organs in this joint as compared to their analogous pairs in the CP joint, there is still no need for two chordotonal organs. The MC1 elastic strand is proximally attached to the accessory apodeme and distally to the ventral edge of the merus cuticle. The MC2 is proximally attached to the distal surface of the flexor apodeme, which is joined by the accessory apodeme. Distally, the MC2 elastic strand spans the MC joint to attach to a cuticular protuberance located proximally and ventrally in the carpus. When the MC joint is flexed, both the MC1 and MC2 elastic strands are elongated. In this situation, both chordotonal organs are sending the same information to the CNS, with the exception that the MC2 organ does not contain any ESCs. With extension of the MC joint, there would be complete redundancy of sensory information. There could be differences in activity from single cells in the MC1 and MC2 chordotonal organs that I am unable to distinguish when the

strand is displaced, but it appears to me that the MC1 organ is able to sense all the proprioceptive information needed in the MC joint.

As for crustaceans, the chordotonal organs in insects show similar patterns in range-fractions and structure-function relationships. The chordotonal organs in insects have been shown to be important in reflex motor control (Kondoh et al., 1995; Newland & Kondoh, 1997; Hess & Büschges, 1999) and show a relationship between the morphology, receptive fields and CNS projections (Burrows & Newland, 1993).

#### Developmental aspects

Knowing the cell body location of certain types of neurons allows one to count the types of cells present within a chordotonal organ at any age of the animal. A developmental study has been done of the neuronal types on the PD organ in this same species of crab (Hartman & Cooper, 1994). It was shown that the dynamic sensitive neurons increase in number more rapidly throughout early development (juvenile 5 instar to adult) than the static position sensitive neurons, which remain relatively constant in number from the juvenile 5 instar stage. A continued increase in neuronal numbers for the PD organ was observed in lobsters throughout development and adulthood (Cooper & Govind, 1991). This continual increase might arise in lobsters, as distinct from crabs, because lobsters do not appear to have a terminal molt or adult size. An asymmetry in the number of sensory neurons associated with the PD organ was also observed between the cutter and crusher claws of the American lobster, *Homarus americanus* H. Milne Edwards, 1837 (cf. Cooper & Govind, 1991). The crusher claw maintained a larger number of neurons from juvenile to late adult stages.

#### Other chordotonal organs

Apart from the MC1, MC2, CP1, and CP2 organs, there are three other chordotonal organs in the walking legs: PD, IM, and CB organs. Burke's (1954) as well as Wiersma & Boettiger's (1959) research focused on determining the different types of receptor endings that were present, and on whether the PD organ could analyse both directions of movement and the degree of flexion and extension. Wiersma & Boettiger (1959) recorded response from stretching as well as relaxing the PD strand. One statement they made that does not hold true for MC1, MC2, CP1, or CP2 movement-sensitive cells in *Cancer magister*, is: "The most sensitive unidirectional movement fibers are almost completely independent of position and velocity". In addition, they traced responses to certain cell bodies and found that movement fibers had larger cell bodies than position fibers, and

were more proximally located on the PD elastic strand. A similar arrangement is seen also in the MC1, MC2, CP1, and CP2 chordotonal organs that I have studied.

Hartman & Boettiger (1967) looked at the detailed functional arrangement of the sensory cells in the PD organ of *Cancer irroratus* Say, 1817. They found that certain movement cells showed position sensitivity. Those at the distal end of the strand are sensitive to the open-arc region, while those at the proximal end are more sensitive to the closed-arc region. Mill & Lowe (1972) analysed a variety of decapods crustaceans to determine the types of sensory units present in the PD organs. Their results on the functional arrangement of the sensory cells were similar to those of Hartman & Boettiger (1967).

Anatomically, the arrangement of the cells in the CP2 and MC2 elastic strands of *C. magister* looks like the arrangement seen in the PD elastic strand. The dynamic movement-sensitive cells' responses in MC2 and CP2 did not reveal any functional arrangement like that reported for the PD cells. The reason for this difference could be that at the PD joint there is only one chordotonal organ present and it needs to discriminate all directions of joint movement. The MC and CP joints each have 2 chordotonal organs. Since the MC1 and CP1 chordotonal organs have both RSCs and ESCs, it would appear that the MC2 and CP2 chordotonal organs do not need to have complete redundancy of sensory information.

Regarding the other chordotonal organs, the CB, PD, and IM, there appears to be a similar arrangement of cells (pers. obs. by methylene blue staining). I speculate that the neurons in the PD, CB, and IM chordotonal organs would show similar anatomical and physiological function as the neurons do in the MC1, MC2, CP1, and CP2 chordotonal organs. Bush (1965a) worked with the CB elastic strand in *Carcinus maenas* and found cells to be responsive to unidirectional movements of stretching or relaxing the elastic strand. Static-position responses were also recorded in the CB organ. Regarding the IM organ, Clarac (1968) recorded from whole nerves and found the responses to be related to joint movements.

### Hemolymph

The physiological recordings in the past from chordotonal organs, in which sea water was used as a saline, while yielding general qualitative ideas about individual neurons categories, can be faulted (Wiersma, 1959; Wiersma & Boettiger, 1959). Even those employing carefully-concocted crab saline solutions with various buffers may be suspect. The use of crabs' serum as a bathing medium in the present study identified many more active cells capable of responding for many hours. Wiersma's (1959) failure to find stretch-sensitive cells in the MC1 and CP1 can be attributed to this issue. Wiersma & Boettiger (1959) and Wiersma (1959) did record cells sensitive to static positions while using seawater as a bathing medium,



but their preparations did not last long. Wiersma (1959) would have been able to record position-sensitive activity because he recorded from whole nerves. And there are many position neurons, in relation to ESCs, so even if half of the position neurons died there would still be plenty of activity present to record. However, a different species of crab was used in Wiersma's studies. So it is possible that an entirely different structure-function relationship is present.

Peetz & Winter (1980) noted alterations of activity in muscle receptor cells (muscle receptor organ, MRO) in the crayfish, *Astacus leptodactylus* Eschscholtz, 1823, due to hemolymph. They conducted a quantitative study on the effects of hemolymph and carefully prepared a saline solution. The major finding of their study was that hemolymph maintained the membrane potential and increased the discharge frequency in some cells. They postulated that there is an active substance present in the hemolymph. Previous studies had also shown that neuromodulators influence the MRO activity (Cooper et al., 2003), so similar compounds may have effects on other proprioceptive neurons.

#### Reflexes

Proprioceptive reflex responses have been researched in some detail for crustaceans. Bush (1962a, b, 1965b) studied the peripheral reflex inhibition in the claw of the crab, *Carcinus maenas*. These studies gave rise to the idea that central neural mechanisms could control the proprioceptive reflexes from the periphery. He noted that peripheral inhibition in the pereopods of decapod crustaceans was demonstrated physiologically as early as 1887 by Biedermann and later by Hoffman (1914), Wiersma (1933), and Pantin (1936). Most of the results from this early work are analogous to the results of Sherrington (1913), who discovered the vertebrate system of reciprocal central inhibition of antagonist muscles. Later research also addressed reflex control in crustaceans (Muramoto & Shimozawa, 1970; Barns et al., 1972; Field, 1974; Bush et al., 1978; Lindsey & Gerstein, 1979a, b; Lindsey & Brown, 1982; Bush & Head, 1985). These studies did not account for the effects tension receptors have on the joint reflexes in association with the chordotonal organs. Tension receptors are responsive to rapid movements of a joint (Cooper & Hartman, 1994).

#### Ultrastructure

The first electron microscopy of the proprioceptive endings in crustacean chordotonal organs was performed by Whitear (1960, 1962, 1965). She discovered that all the scolopidia of the crab leg chordotonal organs have paired sensory cells. Mill & Lowe (1971, 1973) did a careful examination of the receptor strand and movement-sensitive cells in the PD organ of *Cancer pagurus* Linnaeus, 1758. They

suggested that stretching the elastic strand will pull on the scolopales and cause a stretching of the dendrites in some scolopales. The explanation for relaxation-sensitive neurons is not as clear. They postulate that during the stretch period the physical properties of the elastic strand are not the same as during the relaxation of the strand. This alteration of physical properties in the elastic strand over the same range of movement does not seem feasible. Although this explains how the stretch-sensitive cells might be activated, it does not completely account for how the mechanical transduction of the relaxation-sensitive cells occurs during relaxation (see review of transduction in insects: Kernan, 2007). It is known that action potentials are initiated in the sensory endings within the scolopidium (Mendelson, 1963; Hartman & Boettiger 1967, 1968), and, therefore, the transduction process is located in the endings.

#### Future studies

The results of this study will be helpful for understanding how the crab's CNS is kept apprised of proprioceptive information from its appendages. Questions that remain to be answered are: (1) What is the anatomical arrangement, in the thoracic ganglion, of the axons from the chordotonal organs? (2) Since the chordotonal organs have been shown to elicit reflex responses in the muscles they monitor, are the CNS reflex arcs monosynaptic or polysynaptic? (3) How does the proprioceptive information from one joint affect the muscles in other joints in the same leg or in other legs? (4) What substances in the hemolymph are responsible for maintaining the viability of the chordotonal neurons? (5) What is the ultra-structural arrangement within the scolopale and the scolopales' arrangement in the elastic strand that determines the neurons' specificity? Such future studies may prove valuable in the study of reflex arcs in coordinated locomotion and in kinesthetic computer modeling.

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