

Sensory Representation of a Non-Planar Joint in the Walking Legs of the Horseshoe Crab, *Limulus polyphemus*

ROBERT A. GREGG† and DOUGLAS A. EAGLES‡

Department of Biology, Georgetown University, Washington, D.C. 20057

The patello-tibial joint in the walking leg of the horseshoe crab can move in the flexion-extension and promotion-remotion planes with the freedom of movement of a ball-and-socket joint. Its mechanical status is represented by at least seven populations of sensory neurons and these include three different types of proprioceptors; articular membrane receptors, muscle length receptors and muscle tension receptors. A population of each of these types of proprioceptor is associated with structures on the anterior and posterior sides of each leg. In this report we describe the representation of joint movement in both planes by two populations of each type of proprioceptor. In general, the articular membrane receptors were most sensitive to joint flexion; receptors on the anterior side of the leg were biased toward promotion, while those on the posterior were biased toward remotion. Muscle length receptor activity was increased by joint extension and was slightly augmented, in posterior and anterior length receptors, by promotion and remotion, respectively. Length receptor tonic activity was a linear function of muscle length for extension but a non-linear function of muscle flexion. Tension receptor activity was always highly dependent upon active muscular contraction. Tension receptors associated with posterior tibial flexor showed enhanced activity when the joint was promoted; the activity of those associated with the anterior flexor was enhanced by remotion. The relationships between tension receptor activity and muscle length, and between tension receptor activity and joint position, were also explored. While no single receptor population appears capable of representing the status of this joint in both planes, the output of all of the receptor populations studied appears to provide the CNS with unambiguous information about several parameters representing the status of this complex joint.

Supported by the Department of Biology, Georgetown University, and by a Georgetown University Summer Academic Research Grant to DAE.

† Present address: Division of Laboratories and Research, N.Y. State Department of Health, Empire State Plaza, Albany, N.Y. 12201.

‡ Person to whom reprint requests should be addressed.

INTRODUCTION

Information detailing joint position, movement, and the status of working muscle is provided to the CNS of arthropods by a number of different mechanoreceptors. Multipolar neurons sensitive to muscle length or to change in muscle length have been described in insects (Anwyl, 1972; Guthrie, 1967; Suzuki, 1979), crustaceans (Alexandrowicz, 1952; Wiersma and Pilgrim, 1961) and the Xiphosuran, *Limulus* (Eagles and Gregg, 1979). Such receptors have also been described in molluscs (Laverack, 1970; Laverack and Bailey, 1963). Information about the status of muscle is also provided by more complex sensory structures, such as the muscle receptor organs of insects (Burrows, 1975), crustaceans (Page and Sokolove, 1962) and arachnids (Bowerman, 1972a, b).

Joint status is represented by at least three anatomically distinct classes of receptor. These include (1) simple strand receptors (e.g., Alexander, 1969; Burke, 1954; O'Tanyi and Barber, 1966; Stutt and Laverack, 1979) and chordotonal organs (e.g., Burns, 1974; Bush, 1965a, b; Hartman and Austin, 1972; Orchard, 1975; Usherwood *et al.*, 1968), (2) receptors associated with specialized muscle (Barber and Hayes, 1964; Cohen, 1963; Hayes and Barber, 1967; Fountain, 1973) and (3) receptors innervating an articular membrane (e.g., Bowerman and Larimer, 1973; Hayes and Barber, 1967). For a review of arthropod mechanoreception, see Mill (1976).

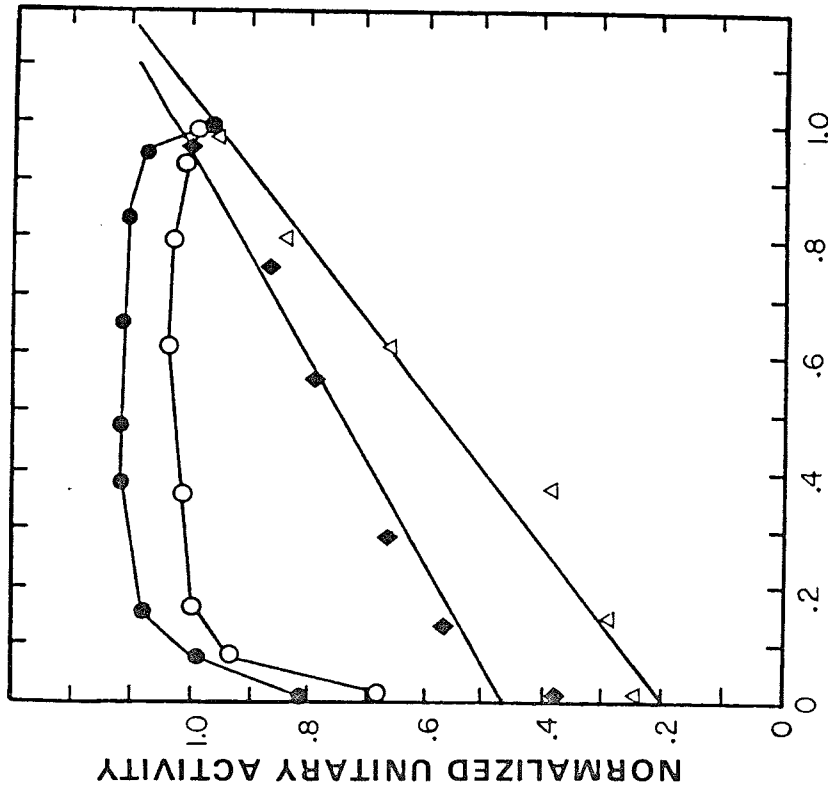
Most commonly, these receptors are associated with joints whose movement is restricted to one plane. Although arthropod mechanoreceptors have been extensively studied, the sensory representation of complex joints generally has not been investigated, with the notable exceptions of the biaxially sensitive proprioceptors at the patello-tibial joint in scorpion legs (Bowerman and Larimer, 1973), antennular receptors in *Panulirus* (Wyse and Maynard, 1965; Rossi-Durand and Vedel, 1982) and receptors associated with strands spanning two joints in the antennae of lobsters (Hartman and Austin, 1972). A major control problem at this type of joint concerns the design of a sensory array which can provide unambiguous information to the CNS about joint position and movement. This report describes the sensory representation (by all three mechanoreceptor classes) of the status of the non-planar patello-tibial (P-T) joint in the walking legs of *Limulus*.

MATERIALS AND METHODS

The methods for animal care and for obtaining electrophysiological recordings have been presented previously (Gregg and Eagles, 1984). The locations of the various nerve branches containing the different classes of mechanoreceptors are shown in Figure 1 of Gregg and Eagles (1984).

The P-T joint is freely moveable in both the promotion-remotion and the flexion-extension planes. Passive movements of the P-T joint were imposed manually by either of two methods (see Figure 1). In the first method, the P-T joint was extended and then rotated, first in a clockwise and then in a counter-clockwise direction, as seen from the tip of the claw. The tip of the tibial index was rotated at a constant radius about an axis projecting from the distal end of the patella and was held stationary at successive positions about the circumference of the circle so described. The procedure was repeated, varying the diameter of the described circle between 1 and 5 cm. As illustrated in Figure 1, the position of the P-T joint in each plane was measured relative to markings of the faces of each of the fixed reference systems. The effects of muscular activity upon the sensory system were tested by eliciting brief contractions of either of the tibial flexors. In the second method, the joint was held at a fixed angle in the flexion-extension plane and, with that angle held constant, was then moved to successive fixed positions in the promotion-remotion plane. At each trial position in the promotion-remotion plane, contractions of the anterior and posterior tibial flexor muscles were elicited (singly) by electrical stimulation of the appropriate motor nerve at constant voltage and duration in order to test the effects of muscular contraction upon sensory activity. A "grid" was completed by fixing the P-T joint at a new angle in the flexion-extension plane and repeating the motor stimulus as the joint was fixed successively at the same trial positions used previously in the promotion-remotion plane.

For comparative purposes, an index of directional preference (IDP) was assigned to each receptor. The IDP was defined as that vector which bisected the angle between half peak spike frequencies, plus or minus the value of the angle between this vector and either of the half-peak frequency angles. For example, if the peak frequency achieved by the receptor during rotation of the P-T joint



INDEX OF MUSCLE STRETCH

FIGURE 10 The correlation between muscle length and tension receptor activity for anterior (ATU) and posterior (PTU) tension receptors. Closed figure-ATU responses measured as the anterior flexor was stretched while the tibia was deflected toward the anterior (diamonds, $r' = 0.940$) or toward the posterior (circles). Open figure-PTU responses measured as the posterior flexor was stretched while the tibia was deflected toward the anterior (circles) or toward the posterior (triangles, $r' = 0.998$). Muscle length is expressed as the Index of Muscle Stretch, and tension receptor sensory activity is normalized and averaged from the responses of 8 anterior and 6 posterior tension units, respectively.

displayed in Table I, individual units exhibited linear relationships when compared to joint position along the flexion-extension plane, but only when the joint was deflected either medially (median $r' = 0.974$) or toward the opposite side (e.g., anterior, for posterior units) of the leg (median $r' = 0.975$). Deflections along the same side (e.g., posterior, for posterior units) of the leg as the receptor population under study evoked a nonlinear and generally retarded response (median $r' = 0.775$).

DISCUSSION

The sensory representation of the status of the non-planar P-T joint of horseshoe crab walking legs includes information from receptors monitoring joint position and movement, muscle length, and forces either acting upon or generated within the musculature. The complexity of representing this already complex system is increased by the fact that the next proximal (F-P) joint affects the status of the tibial flexor muscles, since one of the three heads of each of the flexors originates in the femur. Our results describe sensory information provided by each type of mechanoreceptor and suggest that inputs from more than one receptor population are required to represent the posture of the limb in space.

The sensory maps of the P-T joint proprioceptor activity during

TABLE I

The linear coefficients r' , calculated from the activity of tension units at fixed P-T joint positions along the flexion-extension axis as the tibia was deflected laterally toward either the side of the leg on which the receptor population under study was located (same), toward the side opposite (opposite), or along the midline (medial)

Unit #	same side r'	medial r'	opposite side r'
1	0.999	0.880	0.760
2	0.904	1.000	0.630
3	0.986	0.956	0.913
4	0.989	0.992	0.775
5	0.940	0.974	0.866
6	0.815	0.965	0.701
7	0.975	0.997	0.957
median	0.975	0.974	0.775

associated with the anterior flexors) as it was extended, the spike frequency at any given angle in the flexion-extension plane was lower than that observed when the tibia was displaced toward the opposite side of the patella. These findings suggest that the dendritic fields of the length receptors are maximally distorted by P-T joint extension toward the side of the leg opposite that in which the afferents are located. The precise relationships between length receptor dendrites and muscle fibers or apodemes are unknown. Parsons (1980) has suggested that the dendrites of crustacean tension receptors are stimulated by the bending of the apodemes in which they are embedded. Perhaps these *Limulus* length receptors are excited similarly. In any case, the combined bilateral inputs from the length receptors provide symmetrical information about flexor muscle length and complement the bilateral inputs from proprioceptors signalling joint position.

Forces generated within or acting upon the flexors are represented by the activity of the tension receptors both the posterior (Eagles, 1978) and anterior (Gregg and Eagles, 1984) flexors. Except when the tibia was displaced toward the side of the leg on which the receptors are located (Gregg and Eagles, 1984), tension receptor activity was linearly proportional to total muscle tension. Furthermore, the discharge rate was independent of the direction of tibial movement (flexion or extension), provided that a given level of tension was attained (i.e., there was no observable hysteresis). Again, it seems reasonable to suggest that the observations of Parsons (1980, 1982), suggesting that the apodeme must bend for crustacean tension receptors to be stimulated, may also apply to those of *Limulus*. Alternatively, or perhaps in addition, the tension receptors may innervate one side of the tendon or muscle and be asymmetrically excited when the tibia is displaced to opposite sides of the midline of the patella. This would also account for the observed dependency of tension receptor activity upon the side of the patella toward which the tibia is displaced during measurements of tension receptor activity. Presently, the precise anatomical relationships between the dendrites of any of these receptors and the tissues they innervate are unknown. The array of sensory receptors innervating the musculature in the patella and articular membranes at each joint provides the CNS with exquisitely detailed information which appears sufficient to define any posture and loading of the

rotation of the joint illustrate the behavior of two populations of articular membrane proprioceptors. The population associated with the anterior articular membrane of the P-T joint (TT2 of Hayes and Barber, 1967) was maximally excited when the joint was both flexed and deflected anteriorly; that associated with posterior regions of the articular membrane (TT3 of Hayes and Barber, 1967) showed maximal activity when the joint was flexed and deflected posteriorly, in a manner similar to that described at the P-T joint of scorpion walking legs (Bowerman and Larimer, 1973). The experimental procedures used in these studies selected for slowly adapting joint proprioceptors. Characteristically, none of these joint proprioceptors showed any sign of adaptation to static stimuli, even over periods of time exceeding ten minutes. A similar lack of adaptation appears to be characteristic of the position neurons of the J2-J3 chordotonal organ in the antennae of *Palinurus* (Rossi-Durand and Vedel, 1982). The properties of a third population of P-T articular membrane receptors, first described by Stuart (1953) and identified as TT1 by Hayes and Barber (1967), were not studied.

The length of the tibial flexor muscles is a function of the positions of the two outermost joints in the walking legs. Flexor length is represented by the length receptors which innervate the insertion regions of each muscle (Eagles and Gregg, 1979). Although the term *length receptors* defines the general quality of these sensory neurons, the receptors responded with a phasic burst, the frequency of which was also dependent upon the rate of change of muscle length. Thus, at least part of the length receptor discharge was movement sensitive. Positive changes in stretch (increasing muscle length past the resting lengths) accelerated ongoing activity, whereas negative changes depressed background activity. A tonic, but slowly adapting, discharge at a maintained position followed the phasic burst. The tonic activity of these receptors was found to be linearly related to the stretch within the muscle but this relationship held only for positive changes in muscle stretch; negative changes (as during flexion of either joint) resulted in a nonlinear and attenuated discharge rate. Length receptor spike frequency was also dependent upon whether the tibia was deflected toward either the anterior or the posterior side of the patella as it was flexed or extended. When the tibia was held toward the side of the patella on which the receptors were located (i.e., anterior side for receptors

appendage which would be confronted under normal physiological conditions.

Acknowledgement

The authors wish to express their sincere appreciation to Dr. Gordon A. Wyse and to Dr. H. Bernard Hartman for their helpful suggestions on earlier drafts of this manuscript.

References

- Alexander, C. G. (1969). Structure and properties of mechanoreceptors in the pereopods of *Ligia oceanica* Linn. (Crustacea, Isopoda). *Comp. Biochem. Physiol.* **29**, 1197-1205.
- Alexandrowicz, J. S. (1952). Receptor elements in the thoracic muscles of *Homarus vulgaris* and *Palaemonetes vulgaris*. *Q. Jl. Microsc. Soc.* **93**, 315-348.
- Anwyl, R. (1972). The structure and properties of an abdominal stretch receptor in *Rhodnius prolixus*. *J. Insect Physiol.* **18**, 2143-2153.
- Barber, S. B. and Hayes, W. F. (1964). A tendon receptor organ in *Limulus*. *Comp. Biochem. Physiol.* **11**, 193-198.
- Bowerman, R. F. (1972a). A muscle receptor organ in the scorpion postabdomen. I. The sensory system. *J. comp. Physiol.* **81**, 133-146.
- Bowerman, R. F. (1972b). A muscle receptor organ in the scorpion postabdomen. II. Reflexes evoked by MRO stretch and release. *J. comp. Physiol.* **81**, 147-157.
- Bowerman, R. F. and Larimer, J. (1973). Structure and physiology of the patella-tibial joint in the scorpion pedipalps. *Comp. Biochem. Physiol.* **46A**, 139-151.
- Burke, W. (1954). An organ for proprioception and vibration sense in *Carcinus maenas*. *J. exp. Biol.* **31**, 127-139.
- Burns, M. D. (1974). Structure and physiology of the locust femoral chordotonal organ. *J. Insect Physiol.* **20**, 1319-1339.
- Burrows, M. (1975). Monosynaptic connections between wing stretch receptors and flight motoneurons of the locust. *J. exp. Biol.* **62**, 189-219.
- Bush, B. M. H. (1965a). Proprioception by chordotonal organs in the Mero-Carpopodite and Carpo-Propodite joints of *Carcinus maenas* legs. *Comp. Biochem. Physiol.* **14**, 185-199.
- Bush, B. M. H. (1965b). Leg reflexes from chordotonal organs in the crab, *Carcinus maenas*. *Comp. Biochem. Physiol.* **15**, 567-587.
- Cohen, M. J. (1963). The crustacean myochoordotonal organ as a proprioceptive system. *Comp. Biochem. Physiol.* **8**, 223-243.
- Eagles, D. A. (1979). Tension receptors associated with muscles in the walking legs of the horseshoe crab, *Limulus polyphemus*. *Mar. Behav. Physiol.* **5**, 215-230.
- Eagles, D. A. and Gregg, R. A. (1979). Receptors sensitive to muscle length in the horseshoe crab. *Mar. Behav. Physiol.* **6**, 211-223.
- Fountain, R. L. (1973). Motor control of accessory muscles in the tendon receptor organ in *Limulus polyphemus*. *Comp. Biochem. Physiol.* **44A**, 511-517.
- Gregg, R. A. and Eagles, D. A. (1984). The relationship between sensory activity and muscle tension in the anterior flexor muscle of the horseshoe crab walking leg. *Mar. Behav. Physiol.* **10**, 283-301.

- Guthrie, D. M. (1967). Multipolar stretch receptors and the insect leg reflex. *J. Insect Physiol.* **13**, 1637-1644.
- Hartman, H. B. and Austin, W. D. (1972). Proprioceptor organs in the antennae of Decapoda Crustacea. *J. comp. Physiol.* **81**, 187-202.
- Hayes, W. F. and Barber, S. B. (1967). Proprioceptor distribution and properties in *Limulus* walking legs. *J. exp. Zool.* **165**, 195-210.
- Laverack, M. S. (1970). Responses of a receptor associated with the buccal mass of *Aplysia dactylomela*. *Comp. Biochem. Physiol.* **33**, 471-473.
- Laverack, M. S. and Bailey, D. F. (1963). Movement receptors in *Buccinum undatum*. *Comp. Biochem. Physiol.* **8**, 289-298.
- Mill, P. J., ed. (1976). *Structure and Function of Proprioceptors in the Invertebrates*. John Wiley and Sons, New York.
- Orchard, I. (1975). The structure and properties of an abdominal chordotonal organ in *Carassius morosus* and *Blaberus discoidalis*. *J. Insect Physiol.* **21**, 1491-1499.
- O'Tanyi, T. J. and Barber, S. B. (1966). Stretch receptors in *Limulus* limbs. *Am. Zool.* **6**, 519-520.
- Page, C. H. and Sokolove, P. G. (1972). Crayfish muscle receptor organ: role in regulation of postural flexion. *Science*. **175**, 647-650.
- Parsons, D. W. (1980). The morphology and ultrastructure of tension receptors in the walking legs of the crab, *Carcinus maenas*. *Cell. Tissue Res.* **211**, 139-149.
- Parsons, D. W. (1982). Responses and central interactions of tension receptors in the leg flexor of *Carcinus*. *Comp. Biochem. Physiol.* **72A**, 391-399.
- Rossi-Durand, C. and Vedel, J. P. (1982). Antennal proprioception in the rock lobster *Palaemonetes vulgaris*: anatomy and physiology of a biarticular chordotonal organ. *J. comp. Physiol.* **145**, 505-516.
- Stuart, R. W. (1963). Proprioceptive mechanisms in *Limulus polyphemus*. Honors B.A. Thesis, Williams College, Massachusetts.
- Stutt, I. and Laverack, M. S. (1979). Proprioceptors in the uropods of *Ligia oceanica* (L.). *Mar. Behav. Physiol.* **6**, 269-276.
- Suzuki, Y. (1979). Abdominal abductor muscle in crayfish; physiology properties and neural control. III. Tonic system. *Comp. Biochem. Physiol.* **64A**, 483-492.
- Usherwood, P. N. R., Runion, H. I. and Campbell, J. I. (1968). Structure and physiology of a chordotonal organ in the locust leg. *J. exp. Biol.* **48**, 305-323.
- Wersma, C. A. G. and Pilgrim, R. L. C. (1961). Thoracic stretch receptors in crayfish and rock lobster. *Comp. Biochem. Physiol.* **2**, 51-64.
- Wyse, G. A. and Maynard, D. M. (1965). Joint receptors in the antennule of *Palaemonetes argus* Latreille. *J. exp. Biol.* **42**, 521-535.