

A Survey of the Reflex Activity Elicited in Muscles of the *Limulus* Walking Leg in Response to Stimulation of Joint and Tension Afferents

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Electrical stimulation of tension afferents in the walking legs of the horseshoe crab elicited reflexes which varied in sign and strength, depending upon stimulus voltage and frequency. Electrical stimulation of articular membrane receptor afferents in an isolated leg-ganglion preparation of the horseshoe crab elicited movements at all major joints in the walking leg. Stimulation of articular membrane afferents at the trochanter-femoral joint (T-F) led to increased activity in efferents innervating tibial, patellar and trochanteral flexor muscles, giving a leg posture similar to that observed during feeding. Stimulation of articular membrane afferents at the patello-tibial (P-T) joint led to increased activity in efferents innervating tibial and patellar flexors and trochanteral extensors, giving a leg posture similar to that observed when the leg is lifted from the substratum during walking. The number of efferent units excited, and their frequency, generally increased with an increase in either the voltage or the frequency of the stimulus applied to P-T or T-F afferents. Electrical stimulation of tension afferents innervating the tibial flexor muscles elicited reflexes which varied in sign and strength, depending upon stimulus voltage and frequency. Only the tibial flexor muscles appeared to be recruited as a result of these tension-mediated reflexes, although the tibial extensors were not observed. We suggest that a major difference between reflexes mediated by tension afferents and those mediated by other classes of proprioceptive afferents may be that the projection of the former is limited to the autogenetic motor neuronal population.

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INTRODUCTION

The patello-tibial joint of the horseshoe crab walking leg moves in two planes with a freedom comparable to that of a ball-and-socket joint. The status of this joint is represented by three articular membrane receptor organs (Hayes and Barbet, 1967) and the sensory representation of the flexor muscles which operate this joint includes tension and length reception for the posterior flexor (Eagles and Gregg, 1979; Eagles, 1978) and at least tension reception for the anterior flexor (Gregg and Eagles, 1984a). In the second report of this series, the activities of tension, length and articular membrane receptors representing the status of the P-T joint in both axes were characterized in detail (Gregg and Eagles, 1984b). Here we report some of the motor reflexes elicited by stimulation of different types of afferents at the P-T joint, and of articular membrane afferents at a more proximal, planar joint, the trochanter-femoral (T-F) joint, in the horseshoe crab walking leg, and describe a major qualitative difference in the projection of tension- and articular membrane-receptor-mediated reflexes.

MATERIALS AND METHODS

The methods employed for animal care and electrophysiological recording have been described previously (Eagles and Gregg, 1979). Third and fourth legs from either side of the animal were removed by cutting through the anterior and posterior articular membrane and the apodemes which attached the leg to the prosoma. Each of the walking legs receives sensory and motor innervation from one ganglion in the ring of circumoral ganglia which comprise the brain. After cutting the connectives to adjacent circumoral ganglia, the entire leg, with its ganglion and innervation, was removed intact. The leg was pinned to a Sylgard-coated dish, anterior side uppermost, and bathed in aerated, cold (5–10°C) *Limulus* saline at pH 7.3–7.5. Excess tissue was cleared from the ganglion, and the sheath surrounding it was cut and reflected, facilitating access by the aerated saline. After exposing the ganglion, the leg was repositioned so that the posterior side was uppermost. Only preparations which exhibited spontaneous movements at all leg joints were used for

investigation. The terminology of Snodgrass (1965) was used in the naming of leg segments (see Figure 1).

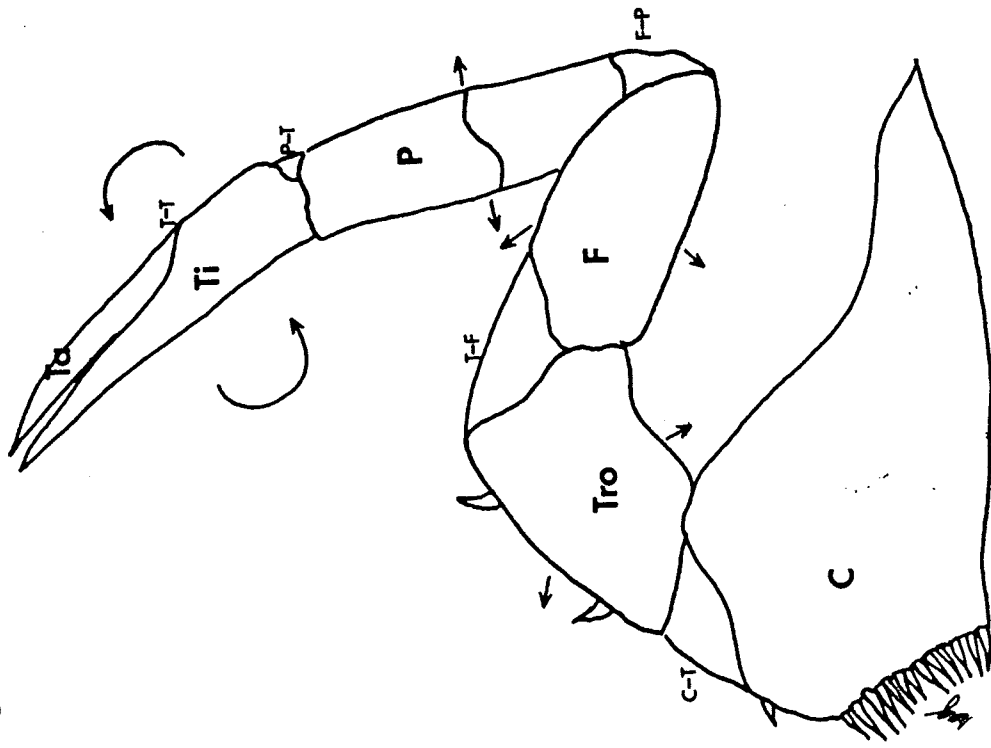


FIGURE 1 The exterior of the walking leg segments of *Limulus* as seen from a posterior view. The arrows indicate the directions in which each segment may move relative to the next proximal segment. Abbreviations: leg segments (bold letters), Ta = tarsus, Ti = tibia, P = patella, F = femur, Tro = trochanter, C = coxa; leg joints (finer letters), C-T = coxal-trochanter, T-F = trochanter-femur, F-P = femoro-patellar, P-T = patello-tibial and T-T = tibio-tarsal joint. Note the freedom of rotation at the P-T joint.

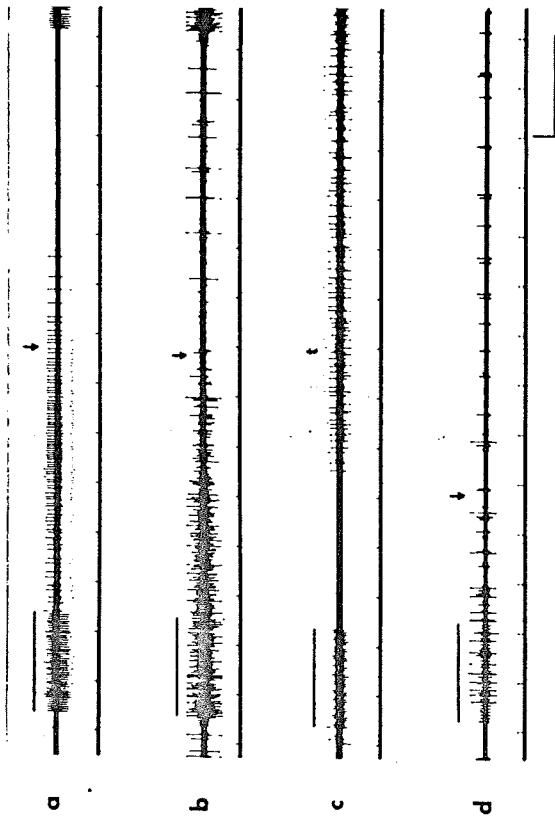


FIGURE 8 The effects of suprathreshold stimulation of P-T joint proprioceptor afferents (PTP) on the activities of units within the motor nerves innervating several leg muscles. The upper trace of each record shows the activity elicited by electrical stimulation of the PTP in units within efferent nerves to: (a) the tibial flexor, at 2 V and 50 Hz; (b) the patellar flexor, at 2 V and 50 Hz; (c) the trochanter flexor (depressor), at 2 V and 50 Hz; (d) the trochanter extensor (levator), at 1 V and 30 Hz. Each stimulus was a 1 second train of pulses 10 msec in duration. Bar denotes application of the stimulus; arrow marks the onset of muscular relaxation, determined visually. The "r" represents onset of muscular contraction in record (c), also determined visually. Lower trace; time mark (2 Hz). Calibration: vertical = 50 μ V (in a, c and d), 25 μ V in (b); horizontal = 1 second.

elicited contraction in the tibial, patellar and trochanteral flexors and, as shown in Figure 11(b, c), some efferents fired only as the muscular contraction ended. For the patellar and trochanteral flexors, this efferent activity was terminated by an additional stimulus to the TFP afferents (Figure 11b, c). The efferent nerve branch to the tibial flexor appeared to include multiple efferents, and it seems possible that one of these was inhibitory since its frequency clearly increased toward the end of the second contraction and possibly of the first, as well (Figure 11a). The low frequency spike activity observed following the arrow (indicating muscular relaxation) in Figure 8b also is consistent with the suggestion that the patellar flexor may receive peripheral inhibitory innervation.

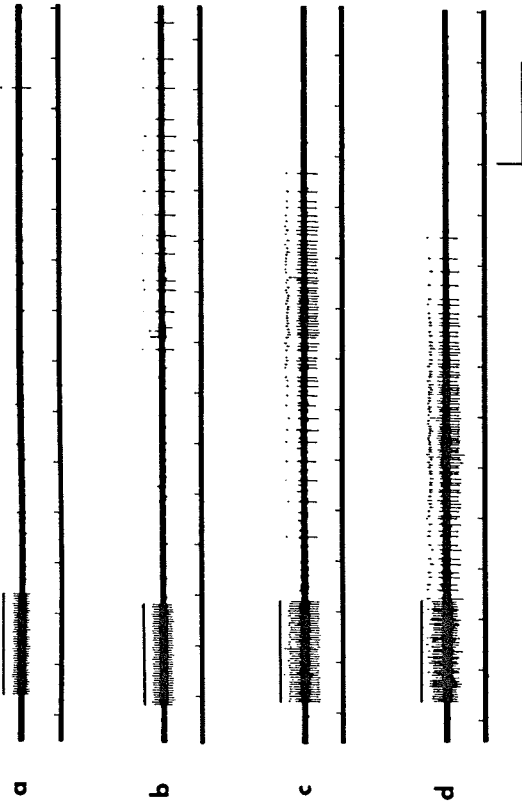


FIGURE 9 The effects of varying the strength of the stimulus delivered to the P-T joint proprioceptor afferents (PTP) on the activity of units within posterior tibial flexor efferents (upper trace). The stimulus delivered to the PTP (bar above upper trace) was a 1 second train of pulses, 10 msec in duration at 40 Hz and (a) 0.25 V; (b) 0.40 V; (c) 0.80 V; (d) 2 V. Lower trace; time mark (2 Hz). Calibration: vertical = 50 μ V; horizontal = 1 second.

DISCUSSION

The most general observation from these studies is the recognition of the marked difference in projection of the reflexes elicited by the stimulation of tension and articular membrane receptor afferents. Tension-mediated (PTU) reflex actions appeared confined to the homonymous muscle, the posterior tibial flexor, and its synergist, the anterior tibial flexor. Although activity in the tibial extensors was not monitored, there was no reflex effect upon the patellar flexor or upon either the extensor or flexor of the trochanter. By contrast, stimulation of the afferents from receptors associated with the articular membranes of either the patello-tibial or trochanteral-femoral joints elicited reflex effects upon both tibial flexors, the patellar flexor and both the extensor and flexor of the trochanter.

The variation in the sign of the tension-mediated reflexes noted in

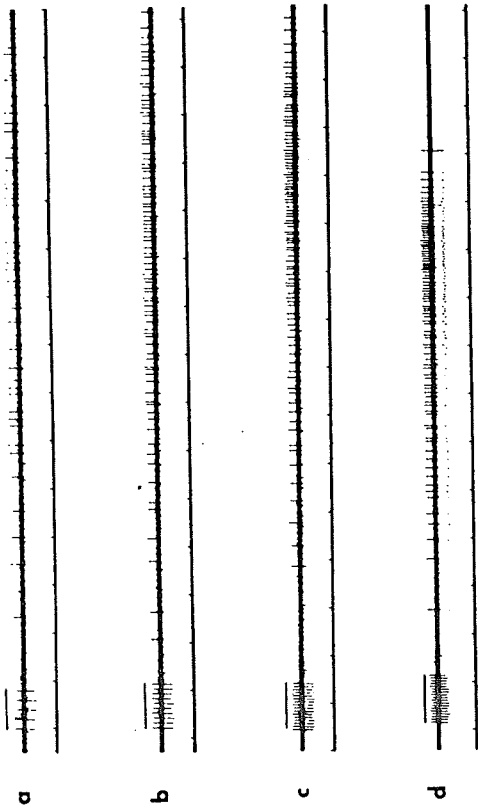


FIGURE 10 The effects of varying the frequency of the electrical stimulus to the P-T joint proprioceptor afferents (PTP) on the activities of units within the tibial flexor efferents (upper trace). The stimulus delivered (bar) was a 500 msec train of pulses 10 msec in duration at 1.0 V and: (a) 10 Hz; (b) 30 Hz; (c) 50 Hz; (d) 50 Hz. Lower trace; time mark (2 Hz). Calibration: vertical = 50 μ V (in a, b and c); 35 μ V in (d); horizontal = 500 msec.

the present study has been a common observation in studies of arthropods. The earliest studies of such reflexes in crustaceans showed that the sign of the reflex effect upon the homonymous muscle was dependent upon the intensity of the stimulus to the afferent nerve (Clarac and Dando, 1973; Dando and Macmillan,

TABLE I

Nerve	Muscle	Activity change	Joint	Action
PTU	tib. flexor	+	P-T	flexion/none
	tib. flexor	+	P-T	flexion
	pat. flexor	+	F-P	flexion
PTP	tro. flexor	+	Tr-F	flexion
	tro. extensor	-	Tr-F	flexion
	tib. flexor	+	P-T	flexion
	pat. flexor	+	F-P	flexion
	tro. flexor	-	Tr-F	flexion
	tro. extensor	-	Tr-F	extension

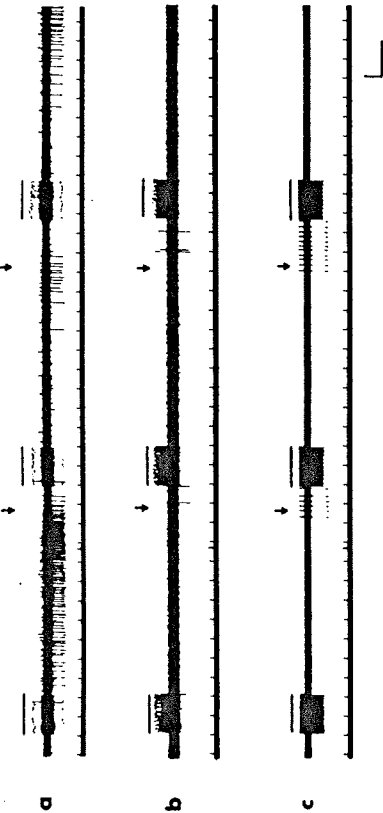


FIGURE 11 The effects of suprathereshold stimulation of the T-F joint proprioceptor afferents (TFP) on the activity of units in subdivisions of the motor nerves innervating the: (a) tibial flexor, (b) patellar flexor and (c) trochanteral flexor muscles (upper trace). Following the stimulus, each muscle contracted. Onset of muscular relaxation, determined visually, is marked by arrows. The stimulus delivered to the TFP (bar) was a 1 second train of pulses of 10 msec duration at 0.60 V and 50 Hz. Lower trace; time mark (2 Hz). Calibration: vertical = 50 μ V (in a and c), 25 μ V in (b); horizontal = 1 second.

1973) and that variable reflex effects were evoked in the antagonist (Clarac and Dando, 1973). In the locust, Theophilidis and Burns (1979) showed that tension-mediated responses of the homonymous muscle could be either excitatory or inhibitory and that the activity of the antagonistic extensor muscle was always of the opposite sign. Recently, Macmillan and Laverack (1982) showed that the sign of tension-mediated reflexes in *Cancer* depended upon whether the tension afference occurred during motor excitation from central nervous inputs or from other sensory inputs. In *Carcinus*, Parsons (1982) found that tension-mediated reflexes to the limb flexor muscles were always positive, but of variable duration. In the present study, variation was observed in the magnitude of the reflex action which followed the delivery of identical stimuli to the tension afferent nerve. Such reflexes were always more pronounced when the leg was undergoing spontaneous flexion, suggesting that tension reception may elicit "assistance" reflexes. These are similar to those induced, for example, by crustacean N-cell multipolar stretch receptors (Suzuki, 1979) or as described in the antenna of the rock lobster (Vedel and Rossi, 1978).

It is interesting to note that, for all of the studies cited, the only muscles whose activities were described were the homonymous muscle and, in some cases, its antagonist. Since it is unclear whether the activity of other muscles was monitored, the inference that there was no other muscular activity is unwarranted. In *Limulus*, however, tension-mediated reflexes appear to involve the homonymous flexor and its synergist (the extensors were not observed), but none of the other muscles whose activity was observed. We suggest that a projection limited to the autogenetic motor neuronal population may be a general characteristic of tension-mediated reflexes in arthropods. The same generalization may also apply to vertebrate tension reflexes (see review by Binder *et al.*, 1982).

The articular membrane proprioceptors described here were first described by Hayes and Barber (1967). The receptors identified as the TFP in this study correspond to those of the T-F organ of Hayes and Barber (1967) and our PTP's correspond to the afferents in their TT2 and TT3 sensory nerves since, in the present study, stimuli were applied to the sensory nerve proximal to the TT2-TT3 bifurcation. As shown in the second paper of this series (Gregg and Eagles, 1984b), P-T proprioceptors fired in response to P-T flexion; TT2 afferents were maximally excited by flexion biased toward the anterior and TT3 afferents were maximally excited by flexion biased toward the posterior side of the leg. Electrical stimulation of PTP afferents evoked reflex contractions in anterior and posterior tibial flexors, the patellar flexor and the trochanteral extensor. This intersegmental and intrasegmental reflex activation of the leg musculature led to a posture which approximated the position of the leg when it was retracted into the umbrella of the prosoma, or was elevated from the substratum during the promotion phase of forward walking. Note that the intrasegmental reflex was positive; the afferents were excited by P-T joint flexion and stimulation of the PTP afferents led to flexor activation.

Barber (1960) showed that the afferents innervating the medial side of the articular membrane spanning the T-F joint (TFP) included receptors excited by joint flexion and others excited by T-F extension. Despite the heterogeneity of the receptor population, variations in the voltage of the stimulus applied to the afferent nerve elicited stable reflex effects with variations in magnitude but not of sign. Stimulation of the T-F proprioceptive afferents elicited reflex

contractions of the two tibial flexors and of the flexors of the patella and trochanter. Thus, the reflexes elicited by stimulation of PTP and TFP afferents were of the same sign at the more distal joints (P-T and F-P) and of opposite sign at the C-T joint. The reflex posture resulting from TFP stimulation resembled that demonstrated by the leg when the tibia and tarsus were brought to the mouth during feeding. Some caution, however, must be applied in evaluating these reflex effects. Since an electrical stimulus was used, the relative contribution of flexion-sensitive and extension-sensitive afferents in eliciting these reflexes are unknown. While there can be no basis for relating the spontaneous and reflex-mediated behaviors in terms of control mechanisms, it is evident that both of these proprioceptive reflexes resulted in co-ordinated leg actions involving movement at several joints simultaneously. Such inter-segmental reflexes have been demonstrated repeatedly in the walking legs of insects (Field and Burrows, 1982; Field and Rind, 1981; Burrows and Horridge, 1974) and in the antennae (Vedel, 1980) and walking legs (Clarac *et al.*, 1978) of crustaceans. While Clarac *et al.* (1978) noted that intersegmental reflex actions upon more distal joints tended to be stronger than those upon more proximal joints in the rock lobster leg, there appeared to be little systematic difference in the polarity of the reflex projection in *Limulus* legs. Consistent with the observation of Clarac *et al.* (1978), however, that the joints which participated in intersegmental reflexes tended to be those which moved in the same plane as the joint at which the afference was generated, we find that stimulation of either T-F or P-T proprioceptive afferents led to flexion at the P-T joint. The relevance of this observation to that of Clarac *et al.* (1978) is that, while the proprioceptors at the T-F joint represent movement in the flexion-extension plane, those of the P-T joint represent components of movement in both of the planes (flexion-extension and promotion-remotion) in which this joint is free to move (Gregg and Eagles, 1983b), yet the reflex action consists of only a flexion.

In all of the reflex studies reported here the leg was free to move only at the joint under study. Studies of reflexes, especially those having intersegmental actions, require that choices be made among the classes and amounts of uncontrolled or indirectly controlled afference operating in the system under study. In these studies, immobilizing the leg shifted the total afferent input away from joint

proprioceptors and toward known (Gregg and Eagles, 1983a; Eagles, 1978), and any unknown, tension receptors. While components of the reflexes observed might have been secondary to afference from such receptors, the observation that tension-mediated reflexes appear to be restricted to the autogenetic motor population suggests that secondary reflex components were minimal. Deafferentation was not performed since reduced sensory input might have altered the central excitatory state and so influenced the presence (Cruse and Schmitz, 1983; Vedel, 1980) or altered the sign (Vedel, 1982; DiCaprio and Clarac, 1981; Bassler, 1976) of evoked reflexes. Pasztor and Clarac (1983) have suggested that the waving behavior shown by the third maxillipeds and peripods of decapod Crustacea represents a motor program expressed by a sub-population of motor neurons when proprioceptive feedback is reduced to low levels. Several observations suggest that the nature of reflex efferent output is influenced by fine details of the afferent input. In the lobster, rapid movements were sometimes required to evoke assistance reflexes, and Ayers and Davis (1978) noted that leg reflexes were tuned to the velocity of walking. Field and Burrows (1982) have shown that fast and slow leg motor neurons innervating leg muscles in the locust were preferentially excited by fast and slow leg movements, respectively, and have suggested that these reflex actions have roles in locomotion as well as in posture.

Attempts to define the roles of peripheral inputs in postural or locomotory systems would seem to require a knowledge of the projection of each of the classes of sensory input and of the various forms of reflexes resulting from such inputs. In recent years, reflexes have been shown to (a) have inter-segmental components (e.g., Burrows and Horridge, 1974), in some cases organized with respect to the plane of movement (Clarac *et al.*, 1978), (b) reverse sign (e.g., Bassler, 1976), (c) be dependent upon the central excitatory state (e.g., Vedel, 1982), (d) include positive feedback (e.g., Davis, 1969), (e) include such specificity that the identity of participating efferents was dependent upon, for example, the velocity of the imposed movement (e.g., Field and Burrows, 1982; Ayers and Davis, 1978), and (f) include inter-limb influences (e.g., Cruse and Epstein, 1982; Cruse and Schmitz, 1983), all in a variety of arthropods. The present report extends these findings by showing that joint proprioceptors elicit intersegmental reflexes in *Limulus*, a

representative of another group of arthropods, the merostomate chelicerates, and by showing a marked difference in the projection of reflexes from articular membrane proprioceptive afferents and from tension afferents. Further studies will be necessary to determine whether the relative projections of tension- and joint receptor-mediated reflexes observed in *Limulus* apply generally in arthropod neuro-muscular systems.

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