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REFLEXES MEDIATED BY NON-IMPULSIVE AFFERENT NEURONES OF THORACIC-COXAL MUSCLE RECEPTOR ORGANS IN THE CRAB, *CARCINUS MAENAS*

I. RECEPTOR POTENTIALS AND PROMOTOR MOTONEURONE RESPONSES

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SUMMARY

1. A preparation of the thoracic-coxal muscle receptor organ of the posterior leg of the shore crab, in which central synaptic efficacy of the sensori-motor reflex pathways is maintained for long periods, is described.

2. The reflex response to receptor muscle stretch commonly involves three promotor motoneurones, designated Pm1–3 in order of their recruitment.

3. Motoneurone Pm1, and less frequently Pm2 and Pm3, may be tonically active during maintained receptor length changes within the *in situ* length range of the receptor muscle.

4. The following observations suggest that the T rather than the S sensory fibre provides the afferent drive onto reflexly activated promotor motoneurones: selective section of the S or T sensory fibres; frequency 'envelopes' of individual motoneurone responses to trapezoid stretch stimuli, including features such as adaptation and velocity sensitivity of the reflex response; and the 'hysteresis' in the response to increasing followed by decreasing receptor length changes, with or without superimposed trapezoid stretch stimuli.

5. The initial reflex response to ramp stretch can be directly related to the complex 'initial component' of the T fibre receptor potential waveform. This comprises a variable spiky alpha (α) component, followed by a longer duration, more predictable beta (β) component, which depends upon stimulus parameters such as stretch velocity and the length and tension of the receptor muscle at the onset of stretch.

6. In the de-efferented receptor muscle, changes in compliance or 'tonus' resulting from receptor manipulation have a marked effect on the sensory, and hence reflex, response to stretch. As this would have profound implications for the functioning of this muscle receptor organ *in vivo*, a role for the receptor motor innervation in counteracting any such response variability seems likely.

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INTRODUCTION

Since the pioneering work of Burke (1954) on the P–D chordotonal organ of crustacean legs, studies on the morphology and physiology of crustacean proprioceptors have proliferated (for reviews, see Mill, 1976). The muscle receptor organ spanning the articulation between thorax and coxal leg segment (T–C MRO) was first described by Alexandrowicz & Whitear (1957), and investigated ultrastructurally by Whitear (1965) and physiologically by Ripley, Bush & Roberts (1968). Subsequently, the non-impulsive, decrementally conducting nature of the afferent signals (receptor potentials) in the two large diameter (40–60 μm) sensory neurones, the S (*S*trand innervating) and T (*T*endon innervating) fibres, was confirmed and investigated in terms of the parameters of stretch (Bush & Roberts, 1971) and cable properties (Roberts & Bush, 1971). It is now recognized that a variety of neurones in both invertebrates and vertebrates lack the regenerative mechanisms for the propagation of all-or-none impulses, relying instead on an analogue system of information transfer in the form of graded depolarizing or hyperpolarizing potentials (for reviews, see Pearson, 1976; Roberts & Bush, 1980).

The first demonstration that graded depolarization in such non-spiking neurones can cause the excitation of neurones one or more synapses away, came when Bush & Roberts (1968) succeeded in activating the resistance reflex to the promotor muscle by stretching the T–C receptor muscle. Subsequently, several examples of non-spiking sensory or interneurones that provide a graded presynaptic drive onto other neurones have become known (Mendelson, 1971; Shaw, 1972; Pearson & Fourtner, 1975; Paul, 1976; Shepherd, 1977; Burrows & Siegler, 1978; Dowling, 1979).

The importance of resistance reflexes is widely acknowledged. Resistance reflexes in decapod crustacean limbs mediated by chordotonal organs (Bush, 1962, 1965; Clarac, 1970; Spirito, Evoy & Barnes, 1972; Field, 1974) and by the M–C myochordotonal organ (Bush, 1965; Evoy & Cohen, 1969) are well documented. By analogy with the vertebrate myotatic reflex, passively imposed joint movements are opposed by reflex excitation of motoneurones innervating the muscles so stretched. In the context of the crustacean limb, resistance reflexes can be described as 'intrasegmental', whereas the term 'distributed reflex' (Ayers & Davis, 1977) refers to an 'intersegmental' reflex (Clarac, Vedel & Bush, 1978), this latter class of reflex recently receiving considerable attention in attempts to elucidate the complex sensory contribution to the control of the locomotory behaviour of the crustacean walking leg as a whole (Ayers & Davis, 1977, 1978; Bush, Vedel & Clarac, 1978; Clarac *et al.* 1978; Ayers & Clarac, 1978; Vedel & Clarac, 1979). The thoracic–coxal joint of the crustacean walking leg articulates in an antero-posterior plane (remotion and promotion respectively) and therefore, together with the more distal I–M and C–P joints, contributes to the antero-posterior disposition of the leg (cf. Fig. 1, Clarac *et al.* 1978). While unequivocal intersegmental reflexes emanating from the T–C MRO have not been reported, intersegmental reflex effects emanating from more distal proprioceptors onto the receptor muscle itself (Moody, 1970) and onto the remotor and particularly the promotor muscles (Clarac *et al.* 1978) have been established.

In this and the second paper in the series (Cannone & Bush, 1980*a*) an attempt is made to adequately describe the resistance reflex to the promotor muscle. A detailed description of the stimulus parameters which evoke reflex excitation of motoneurones is essential in view of the marked differences in response of the S and T sensory fibres to receptor stretch. These differences are attributable to the in-parallel and in-series arrangement of the S and T fibres respectively with the receptor muscle, the S fibre showing more closely the characteristics of a displacement detector, while the T fibre responds primarily to a tension change, and is therefore subject to receptor efferent modulation (Bush & Roberts, 1971; Bush & Godden, 1974; Bush, Godden & Cannone, 1975; Bush, 1976, 1977; Cannone & Bush, 1980*b*, *c*). Relating reflex activity in individual motoneurones to receptor muscle length, and to stretch-evoked receptor potentials recorded simultaneously in the sensory fibres, establishes the contribution of these two sensory fibres to the resistance reflex (this paper). Injecting depolarizing and hyperpolarizing constant current pulses into the non-impulsive sensory fibres enables a more quantitative analysis of input–output relationships, and a more rigorous determination of numbers and discharge characteristics of motoneurones reflexly recruited by either the S or T fibres (Cannone & Bush, 1980*a*). Aspects of this study have been reported elsewhere (Bush & Cannone, 1973; Bush, 1976, 1977; Cannone & Bush, 1977). With the resistance reflexes described, attempts to integrate the T–C MRO into the functional proprioceptive repertoire of the crustacean limb should be facilitated.

METHODS

Male shore crabs (*Carcinus maenas*) of 50–70 mm carapace width were used for most of these experiments. They were supplied by the Marine Biological Laboratory, Plymouth, and kept in aquarium tanks of recirculating aerated, filtered, artificial sea water at temperatures of 10–15 °C. Of 54 reflexly active preparations used, the majority involved the T–C MRO of the right posterior walking leg (5th pereiopod) for reasons of accessibility, and because the sensory fibres are longer in this leg than in the more anterior legs.

Preparation

Synaptic efficacy in the dissected preparations could be maintained for many hours (20 in one preparation tested) by using the basic technique (Bush & Roberts, 1968) of cannulation of the sternal artery and perfusion of the thoracic ganglion with crab saline (Roberts & Bush, 1971). Before perfusion, the haemolymph in the intact animal was transfused by cutting a window in the carapace, opening the pericardial cavity, and immersing the animal in refrigerated saline until no further haemolymph emerged. This procedure reduces the risk of coagulation in the network of fine blood vessels ramifying through the thoracic ganglion (Sandeman, 1967). The legs, abdomen, carapace, mouthparts, viscera and gills were then removed under cold, running saline. The remaining ventral thorax was pinned under cold crab saline onto the transparent sylgard base of a perspex dish, and viewed under the dissecting microscope with

transmitted and obliquely incident light. The sternal artery was cannulated and perfused with refrigerated and oxygenated saline at a controllable rate of about 1·5–2·5 ml/min throughout the experiment. Perfusion rates greater than this sometimes produced adverse effects on the reflex sensitivity of the preparation (Fig. 4), due possibly to the excessive intra-ganglionic pressures which ensue. It was found necessary to retain as long a length of sternal artery as possible, since there are at least two blood vessels which leave the sternal artery dorsal to the thoracic ganglion, often at a considerable distance from it. Injection of pontamine sky blue into the perfusion cannula revealed that these vessels branch extensively within the sheath of the dorsal surface of the ganglion, thereby contributing significantly to the blood supply of the ganglion in addition to the vessels previously described (Sandeman, 1967). In addition, the continuous circulation of a coolant fluid through a heat exchanger in the preparation dish enabled the temperature of the saline bathing the preparation to be maintained within the range of 12–14 °C.

The promotor (leg protractor) muscle with its innervation intact was exposed from the dorsal aspect by removal of all overlying muscle bundles and endoskeletal structures. In the majority of preparations, all leg nerves, except that under study, were transected near the ganglion in order to (*a*) isolate the periphery from motor activity which could produce disruptive movement of the preparations, and (*b*) isolate the thoracic ganglion from the periphery in order to eliminate the possibility of intersegmental reflex interactions. Care was taken to preserve the underlying leg arteries intact, thereby ensuring adequate perfusion pressure (indicated by slight swelling of the sternal artery at the flow rates used). The motor nerve to the receptor muscle was also cut, leaving the T–C MRO in the open-loop, de-efferented condition. The receptor muscle was dissected free from the in-parallel promotor muscle fibres, retaining a small piece of tendon attached to its distal end for insertion into the nylon chuck of a puller device.

Mechanical stimulation

The puller assembly comprised a 3 ohm loudspeaker with a stainless steel probe attached to the coil former. A flexible stainless steel pin fixed onto the anode shaft of a RCA 5734 transducer valve provided a linear output signal over a displacement range of 1·5 mm. The assembly was mounted on a micromanipulator fitted with a dial gauge calibrated in 10^{-2} mm, to enable accurate positioning and monitoring of the rest length of the receptor muscle. The puller was operated by a linear, double ramp-function (trapezoid) generator, with variable and independent control of stretch and release velocities and 'plateau' durations. Servo-control of the puller enabled the linear response characteristics of the ramp to be retained with displacements of up to 1·5 mm and with rise and fall times as short as 10 ms. This displacement represents *ca.* 13% of the 11–12 mm fully extended *in situ* length of the receptor muscle (or approximately 37% of its total effective length change of about 4 mm over the full T–C joint angle) in crabs of *ca.* 60 mm carapace widths.

Recording techniques

Intracellular recordings of receptor potentials and excitatory muscle junction potentials were made with 3 M KCl filled glass micropipettes. Extracellular recordings

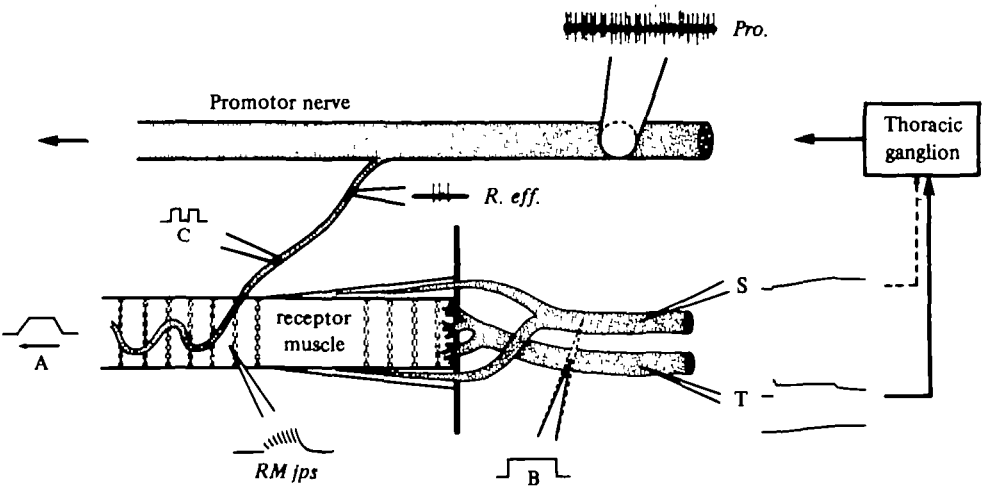


Fig. 1. Diagrammatic representation of the thoracic–coxal muscle receptor preparation showing the three stimulus routes leading to reflex excitation. (A) trapezoid stretch stimulus applied to the isolated distal end of the receptor muscle; (B) constant current pulses injected into the in-series T and in-parallel S sensory neurones using intracellular glass micropipettes; and (C) repetitive stimulation of the motor nerve to the receptor muscle using a suction electrode. Recordings can be made of T and S fibre receptor potentials, receptor muscle and promotor muscle junction potentials (intracellular), and promotor and receptor motor nerve action potentials (extracellular, suction electrodes). Responses of S and T fibres to a ramp-function stretch are shown leading to reflex excitation of promotor neurones. *RM jps*, receptor muscle junction potentials; *R. eff.*, receptor efferent impulses; *Pro.*, promotor impulses.

both *en passant* and from the cut ends of motor axons, were obtained, using flame-drawn polyethylene suction electrodes. A micromanipulated glass hook was used to support the sensory fibres close to the point of penetration. In many preparations, impulse activity in the promotor motoneurones was monitored during sensory fibre impalement to provide an indication of damage, if any, caused to the sensory fibre membrane. Preparations in which penetration alone precipitated a maintained motoneurone discharge were usually discarded. Two Transidyne MPS-6 high impedance d.c. preamplifiers with capacitance neutralization were used for the intracellular recordings, and two Grass P511 a.c. preamplifiers for the extracellular recordings, these all being displayed simultaneously with the stimulus monitor on a six channel Tektronix 565 oscilloscope. Permanent records were obtained on Ilford NL6 recording paper using a Grass C4 Kymograph camera in either the single frame or continuous modes. In some experiments, an instantaneous frequency meter used in conjunction with an amplitude discriminator (Matthews & Searle, 1972) provided a display of the response frequency envelope of a selected motoneurone, to facilitate comparison with the concomitant receptor potentials.

RESULTS

In this and succeeding papers the T–C MRO sensori-reflex system is described. The system can be activated via the three stimulus routes shown diagrammatically in Fig. 1, each leading to both negative and positive (Bush & Cannone, 1974; Cannone

Table 1. *Impulse amplitudes of the three commonly activated motoneurones related to reflex activation thresholds*

(The figures represent numbers of encounters in 28 experiments yielding unambiguous data in this respect. The nomenclature Pm1, Pm2 and Pm3 is adopted on the basis of activation sequence rather than on impulse amplitude (see text).)

	Reflex activation thresholds		
	Low	Intermediate	High
Small amplitude impulses (Pm1)	27	1	0
Large amplitude impulses (Pm2)	0	21	7
Intermediate amplitude impulses (Pm3)	1	6	21

& Bush, 1980*b*) reflex feedback. Firstly, activation is by receptor stretch (this paper); secondly, by injection of constant current pulses into the T and S sensory fibres (Cannone & Bush, 1980*a*), and thirdly, by repetitive stimulation of the receptor efferent nerve which causes contraction of receptor muscle fibres (Cannone & Bush, 1980*c*).

A ramp-function stretch applied to the isolated distal end of the receptor muscle (RM) most commonly elicits reflex firing in three promotor motoneurones (MNs). The nomenclature Pm1, Pm2 and Pm3 is adopted on the basis of increasing activation thresholds rather than on impulse amplitudes. These 'activation thresholds' are best defined in terms of the absolute membrane potentials of the afferent fibre(s) mediating the reflex, as described later in this paper and considered in detail in our second paper of this series (Cannone & Bush, 1980*a*). For present purposes, however, they can be defined in terms of the relative RM length in a given preparation at which each motoneurone becomes (or ceases to be) tonically active (Figs 2, 3), which in turn determines the 'steady-state' membrane potentials of the afferent fibres (see Fig. 10). Thus in the preparation of Fig. 2, for example, only the small unit, Pm1, fires tonically following stretch to 7·5 mm (B), whereas Pm2, which as in this preparation is typically the largest of the three units, remains tonically active after stretch to 8·5 mm (C). The intermediate-sized unit, Pm3, becomes active only in response to relatively large afferent fibre depolarizations, such as occur (see later) at large RM lengths in 'lively' preparations (Fig. 3), or more commonly during the *dynamic* phases of stretching, either at constant velocity (B, C) or sinusoidally (A, D).

Tabulation of those preparations for which it was possible to distinguish between these three MNs on the basis of impulse amplitude *and* relative activation thresholds (Table 1), indicates an imperfect correlation between impulse amplitude – and therefore presumably fibre diameter – and activation threshold. High velocity stretches imposed at extended RM lengths often recruit additional promotor MNs to the reflex. These higher threshold MNs are more readily observed following constant current pulses injected into the sensory fibres (Cannone & Bush, 1980*a*).

During the 'stretch' or positive dynamic phase of a trapezoid stimulus, impulse frequencies are considerably greater than during the 'hold' or static phase, imparting a marked phasic component to the resistance reflex. This phasic component is much less evident, or may even be totally occluded, when the trapezoid stimulus is superimposed upon a high frequency, small amplitude, sinusoidal oscillation of the RM

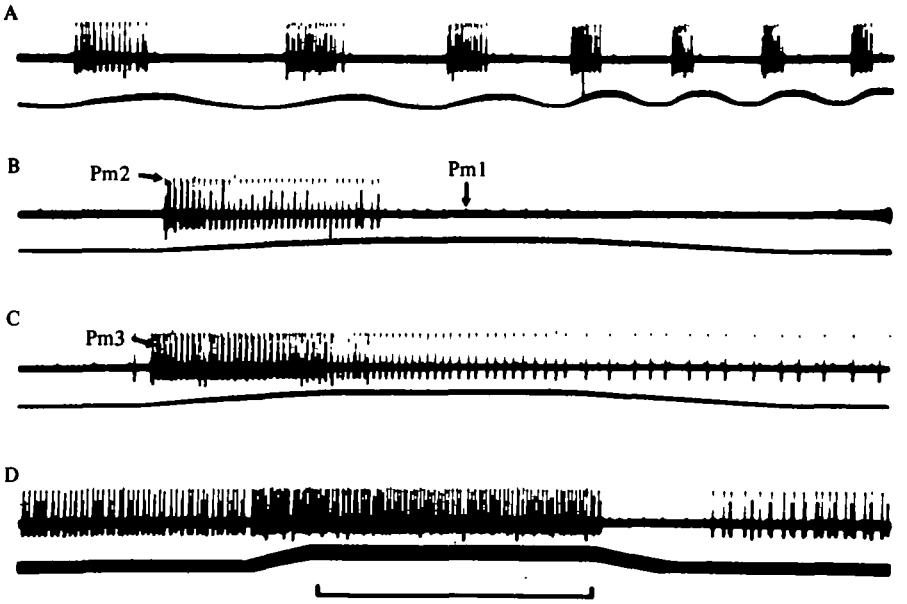


Fig. 2. Reflex responses of ipsilateral promotor motoneurones Pm1, Pm2 and Pm3 to sinusoidal receptor stretch (A); trapezoid stretches at receptor muscle lengths 6·5 mm (B), and 7·5 mm (C); and stretch superimposed upon 160 Hz sinusoidal vibrations of 0·3 mm amplitude (D). The lower trace of each record is the stimulus monitor: stretch upwards, 1·0 mm excursions. Note that all motoneurones respond phasically during the positive dynamic phase of the trapezoid length change in (B) and (C); reflex response frequencies increase with an increase in initial receptor length (compare B and C); all motoneurones show sustained firing in response to sinusoidal vibration alone, the phasic response to stretch is virtually suppressed, and there is suppression of the activity of all motoneurones during and immediately following the release of stretch (D). The pre-stretch tonic activity of Pm1 in (C) is probably of central origin rather than peripherally determined by the increased RM initial length, since there is evidence of 'post-stimulus excitation' (cf. section 6 for explanation). Calibration: 1 s.

(Fig. 2D). Such a sinusoidal vibration was in itself a potent stimulus, causing strong reflex firing of Pm1, Pm2 and Pm3. Moreover, whereas the 'release' or negative dynamic phase of the trapezoid stimulus alone often causes only a small reduction in discharge frequency (Fig. 2C), the simultaneous presence of sinusoidal stimulation results in pronounced suppression of activity during and immediately following a release (Fig. 2D). A consistent feature of the reflex response to a maintained static phase of the stimulus is a rapid decline in impulse frequencies, indicating central and/or peripheral components of adaptation in the sensori-motor reflex system. Figure 2B also illustrates the 'initial component' of the phasic response to stretch. This takes the form of a short, high frequency burst of impulses at the commencement of a ramp stretch of moderate velocity. These and other features of the resistance reflex will be further characterized in the sections which follow, with particular emphasis on the relationship between sensory response and reflex form.

(1) *Tonic motor activity and its dependence on receptor length*

In the majority of preparations, particularly early on while the preparation is in a better condition in terms of excitability, Pm1 is tonically active even at fully relaxed

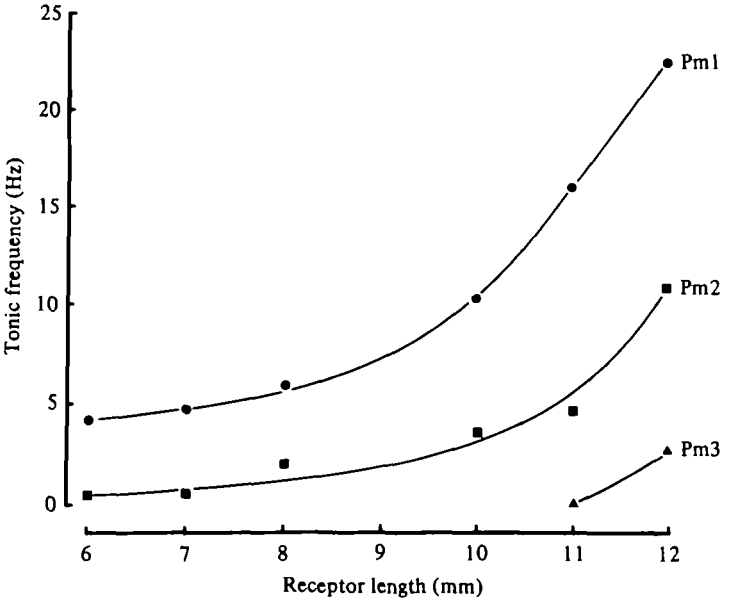


Fig. 3. Steady state impulse frequencies of Pm1, Pm2 and Pm3 in relation to receptor muscle length, up to and including the maximum *in situ* length of 12 mm measured at full remotion of the thoracic–coxal joint. The mean tonic discharge frequency was determined for a 3 s period, 20 s after each length increment of 1 mm. Data for the 9 mm length is omitted due to the intrusion of a spontaneous, centrally originating burst of impulses.

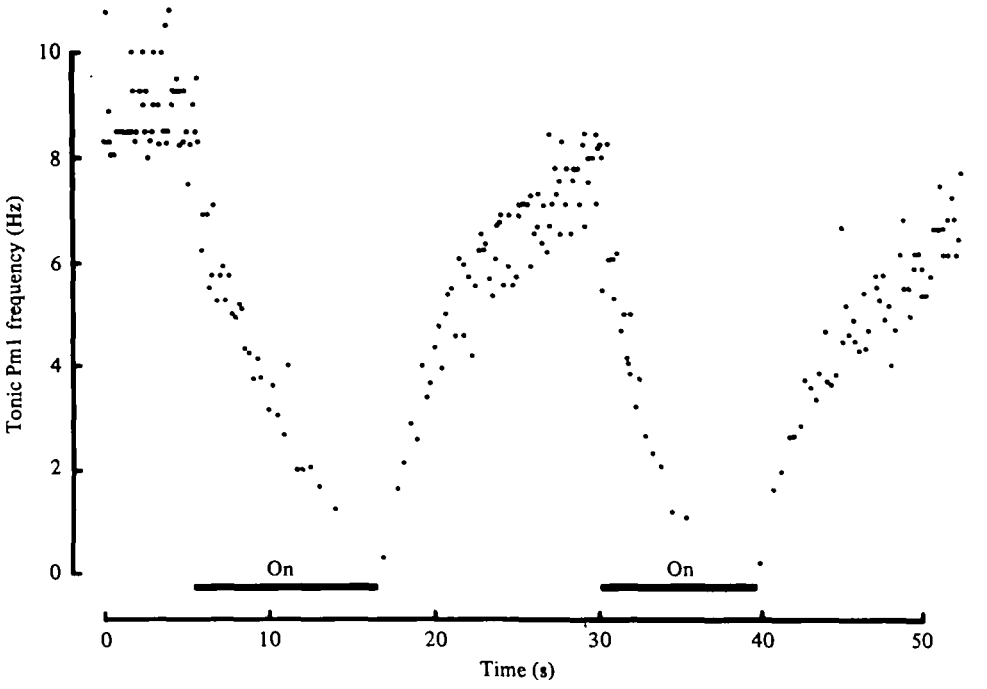


Fig. 4. Suppression of the tonic reflex discharge of Pm1 following excessive saline perfusion flow rates of 6·5 ml/min. (On). Perfusion rates of 1·5–2·5 ml/min do not appear to affect the frequency of tonic discharge (see Methods).

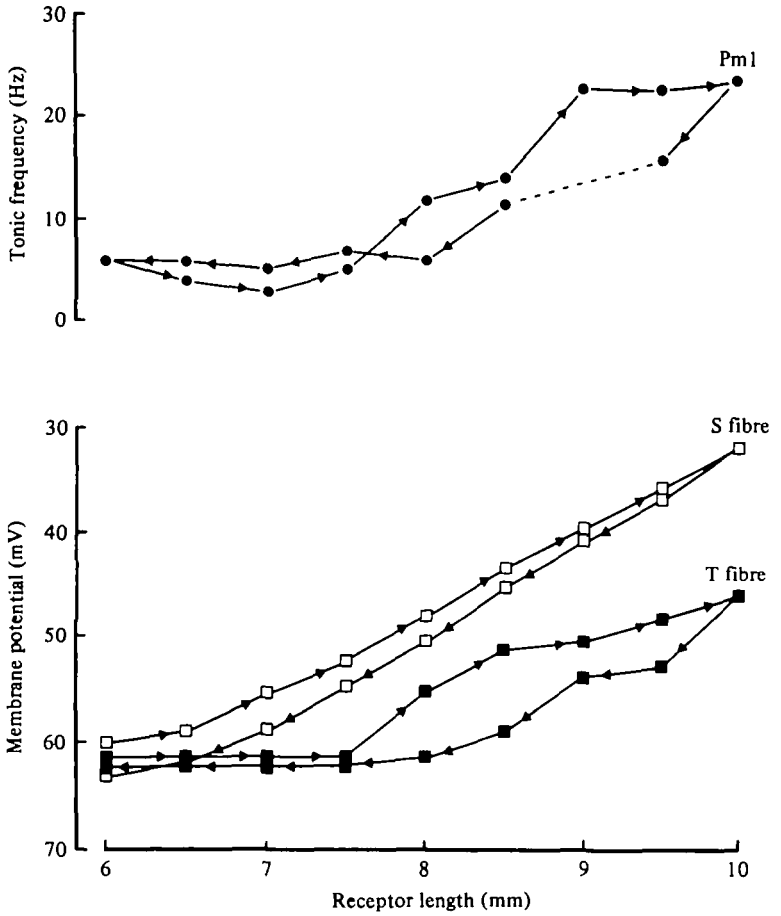


Fig. 5. Tonic discharge of Pm1 following incremental (both increasing and decreasing) receptor length changes at 20 s intervals, in relation to the simultaneously recorded length-dependent membrane potentials of the S and T sensory fibres. The direction of length change is indicated by arrows on all three graphs. At 9 mm (decreasing length change) a central burst interrupted this series of tonic frequency measurements and its effects probably elevated the frequency of Pm1 discharge at 8·5 mm as well.

RM lengths. The tonic discharge frequency increases with increments in RM length (Fig. 3), being remarkably constant at any given length. When promotor nerve activity is recorded as early as possible and prior to intracellular recordings from the sensory nerves, Pm2 and Pm3 are occasionally found to be tonically active at RM lengths within the physiological range (Fig. 3). Not surprisingly, tonic activity declines progressively during the course of an experiment, this being particulary evident when sensory responses are monitored intracellularly and direct comparisons of voltage-related threshold levels can be made with respect to time. The inescapable conclusion is that reflex responses represented in this and subsequent papers in the series (Cannone & Bush, 1980*a*, *b*, *c*) are unlikely to reflect the normal *in vivo* level of reflex excitability of the system.

The rate of decline of reflex sensitivity varies considerably from one preparation to another. In some cases tonic activity of Pm1 is retained for many hours, whereas

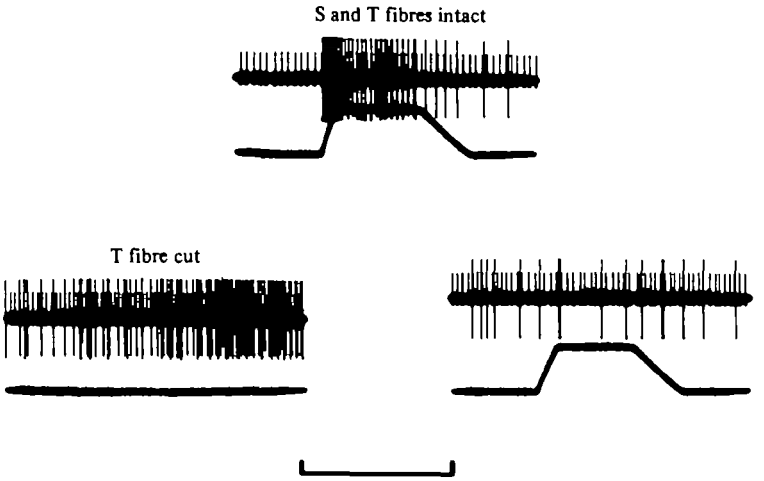


Fig. 6. The effect of selectively cutting the T sensory fibre. Cutting the T fibre results in a long-lasting, high frequency burst of impulses in reflexly active motoneurones. A subsequent stretch stimulus is totally ineffective in evoking a reflex response. Calibration: 1 s.

in others no tonic activity is observed at all. The reasons for this variability are difficult to define in view of the rigid and consistent experimental conditions adhered to. A possible variable is the relative effectiveness with which the perfusant irrigates central synaptic areas in different preparations. Figure 4 illustrates the importance of perfusion rate in one preparation tested. A reversible inhibition of Pm1 tonic activity could be effected by maximal perfusion pressures yielding a flow rate of 6·5 ml/min. The reason for this effect is not clear, nor is it known what are the physiologically normal vascular pressures and motoneurone tonic discharge rates.

Both the S and T sensory fibres are depolarized by increments in RM length, the former in a highly linear manner (Fig. 5; see also Bush & Roberts, 1971). The membrane responses of these two fibres differ even more when the RM is incrementally shortened. The S fibre response retains its linear relationship to RM length, whereas the T fibre repolarizes non-linearly and with significant hysteresis (at least in the absence of receptor motor activity: cf. Cannone & Bush, 1980*c*). The tonic response of Pm1 under these conditions reflects the behaviour of the T rather than the S fibre membrane potential (Fig. 5). This suggests that the T fibre may contribute more directly than the S fibre to the tonic reflex control of the promotor motoneurones by the muscle receptor organ.

(2) *Selective section of S and T sensory fibres: effects on the stretch reflex*

The sensory drive for the promotor stretch reflex can be established by selectively cutting the S and T sensory fibres. Cutting the T fibre alone results in a prolonged high frequency discharge of the same MNs as those participating in a stretch induced reflex. However, subsequent stretch stimuli applied to the RM now fail to evoke any reflex responses in the promotor neurones (Fig. 6). Selectively cutting the S fibre also results in a high frequency promotor discharge, which gradually declines. This time, however, the reflex response to a subsequent stretch is evidently unaffected (Fig. 7).

The high frequency discharges that follow the cutting of either S or T sensory

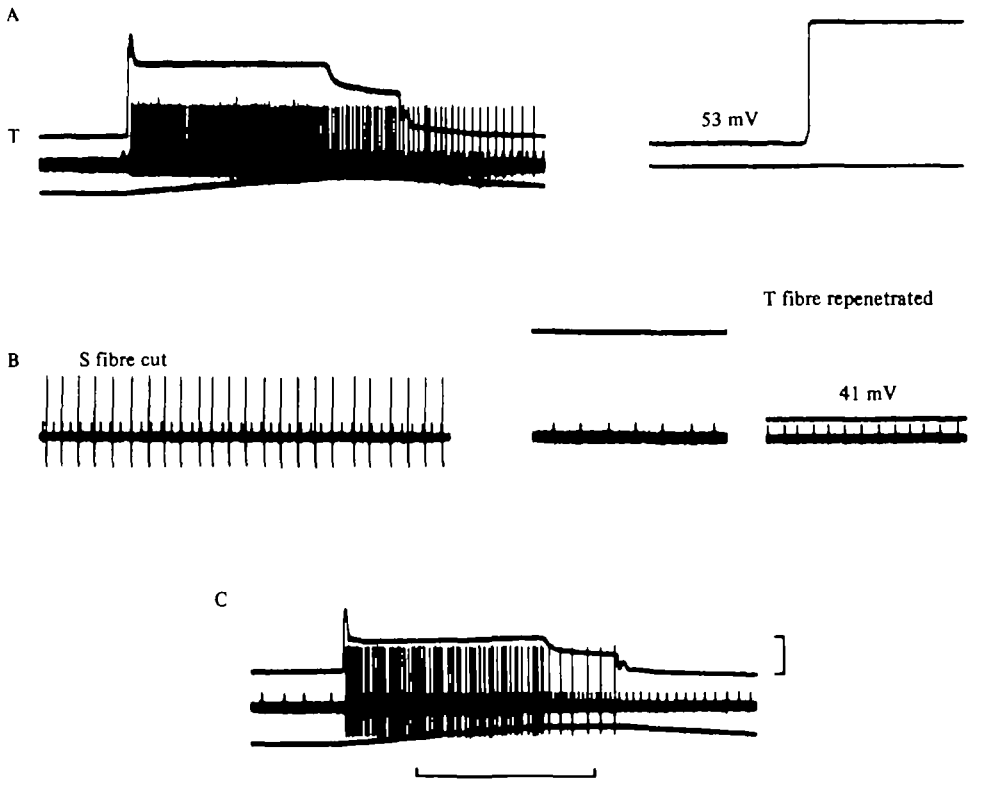


Fig. 7. The effect of selectively cutting the S sensory fibre. (A) The receptor potential of the T fibre in response to a stretch (monitored on lower trace) is recorded together with the reflex response (middle trace) involving Pm1 and Pm2. Withdrawal of the micropipette from the T fibre reveals a resting membrane potential of 53 mV. (B) Cutting the S fibre elicits a burst of impulses in Pm1 and Pm2 which gradually declines until only Pm1 remains active (7 Hz in sample record). Repenetration of the T fibre yielded a resting membrane potential of 41 mV. This lower value, together with an increased tonic impulse frequency (12 Hz) in Pm1, indicates a degree of damage inflicted on the membrane during repenetration. (C) A stretch stimulus identical to that in (A) evokes a qualitatively similar reflex response. The lower impulse frequencies can be attributed to the reduction in the T fibre receptor potential amplitude, consequent upon the injury induced increase in 'resting' ionic conductances. Calibrations: 1 s, 20 mV.

fibres can be attributed to sustained depolarizing injury potentials conducted in the sensory fibres to the synapses. The fact that a stretch induced S fibre depolarization does not evoke a reflex response, whereas depolarization induced by damage to the same fibre does, indicates a high threshold link between the S fibre and reflexly active promotor MNs. This conclusion is reinforced by more quantitative experiments involving depolarizing and hyperpolarizing current injections into the sensory fibres (Cannone & Bush, 1980*a*). Nonetheless, it is clear that the T fibre provides the dominant, if not the exclusive, drive for the stretch evoked promotor reflex.

(3) *Trapezoid RM stretch: sensory-promotor correspondence*

(*a*) *Adaptation.* The stretch induced receptor potential of the T fibre reflects the resultant tension change in the RM more closely than the primary length change (Bush & Godden, 1974). Not surprisingly therefore, the T fibre receptor potential

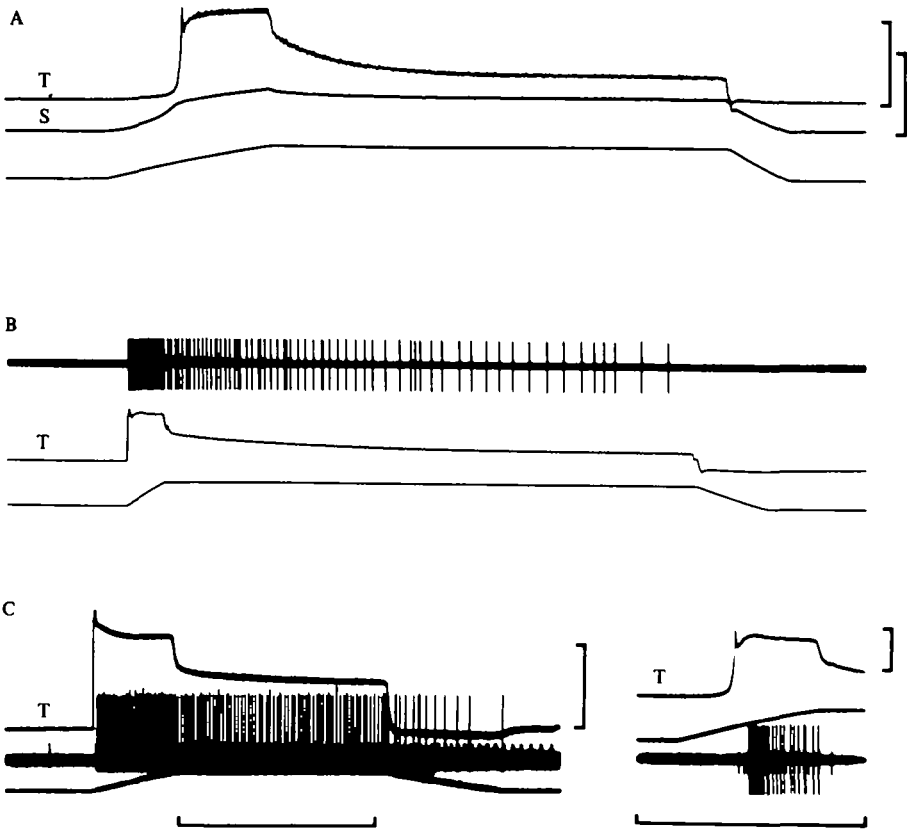


Fig. 8. The marked adaptation of the T fibre receptor potential, compared to that of the S fibre during the maintained 'plateau' in the trapezoid stretch stimulus (A), can largely be held responsible for the adaptation seen in the tonic reflex response (B). Under conditions where the positive dynamic component of the T fibre response shows a decline, this too is reflected in the reflex response (C). Calibrations: 1 s, 20 mV.

declines steadily during the static phase (or plateau) of a trapezoid stimulus (Fig. 8A; see also Bush & Roberts, 1971), as does the tension in the receptor muscles. This decline or adaptation in the sensory response is thus evidently due to the viscoelastic properties of the RM, since the T fibre ends in series with the RM (Bush, 1976). In contrast, the anatomical arrangement of the S fibre endings in parallel to the RM ensure response characteristics of this fibre largely independent of RM tension, so that the S fibre static component displays little or no adaptation (Fig. 8A). A further form of 'adaptation' in the T fibre receptor potential occurs during the positive dynamic component of the response at the higher stretch velocities (Fig. 8C). At low stretch velocities, however, the amplitude of the positive dynamic component either increases slowly during the ramp (Figs 8A; 10) or more commonly remains at an almost constant level (Figs 7; 9B; 11E, F). The reflex response of the promotor neurones to trapezoid receptor muscle stretch reflects both these forms of adaptation in the T fibre response (Figs 8B, C). In addition, however, the overall adaptation in the reflex discharge evidently includes a central nervous component of adaptation, i.e. at the level of synaptic transmission and/or at the MN spike initiation

site. This is most readily investigated by constant current depolarizations of the sensory fibres and will be considered elsewhere (Cannone & Bush, 1980*a*).

(*b*) *Frequencygrams.* From the foregoing sections the form of the promotor reflex appears to be related, qualitatively at least, much more closely to the T fibre receptor potential than to that of the S fibre. A more direct comparison between the S and T fibre receptor potentials and the concomitant promotor discharge can be obtained from the simultaneously recorded instantaneous frequencies ('frequencygrams') for each promotor unit (Figs 9; 11E, F). The frequency envelopes of all MNs activated by a range of stretch velocities clearly reflect the waveform of the T fibre receptor potential, and accordingly differ considerably from that of the S fibre. The close similarity of the reflex firing frequencies to the T fibre depolarization is most obvious during the positive dynamic phase of the stimulus. In contrast to the S fibre but like the T fibre receptor potential, the promotor discharge level either remains fairly constant throughout this phase when the stretch velocity is low or, with higher stretch velocities, steadily declines in frequency. On cessation of the dynamic phase of stretching the reflex discharge again declines with a time course similar to that of the T fibre depolarization, rapidly at first and then more gradually, giving the adaptation referred to above.

(*c*) *Stretch velocity.* The relationship between the amplitude of the positive dynamic component of the T fibre receptor potential and stretch velocity within the range 0·5–15 mm/sec is essentially logarithmic, with a slope of about 20 mV/10-fold change in velocity – considerably steeper than that for the S fibre (Bush & Roberts, 1971). Similarly, the reflex discharge frequencies of the promotor neurones during the dynamic phase of stretch also vary nearly logarithmically with stretch velocity and, like the T (but not the S) fibre, show a high sensitivity to stretch velocity (Fig. 9). Even quite low stretch velocities can evoke high frequency reflex responses. In the experiment represented in Fig. 9C, for example, Pm1 responded with a slope of approximately 60 Hz for a 10-fold change in stretch velocity over the range 0·2–2·00 mm/sec. A more detailed quantitative analysis of the relationship between afferent input and reflex response is presented in the second paper in this series (Cannone & Bush, 1980*a*).

(*d*) *RM length and the stretch reflex.* Trapezoid stimuli of identical amplitude, duration and stretch or release velocities evoke qualitatively and quantitatively different reflex responses if imposed at different 'rest' lengths of the RM. The discharge frequency and recruitment of motoneurones during both dynamic and static phases of the stimulus is increased with increasing rest lengths of the RM, while the latency of the reflex response may be considerably increased at the more relaxed RM lengths (Fig. 10). As before, these features reflect the simultaneously recorded T fibre receptor potentials much more closely than the S fibre responses. An important additional parameter is the direction of change in rest length prior to a test stretch. The reflex response is markedly reduced if the length at which the stretch is applied is approached from a longer rest length rather than from a shorter one. This hysteresis in the stretch evoked reflex closely parallels the previously reported hysteresis in the T fibre receptor potentials (Bush & Roberts, 1971). This phenomenon depends critically upon the interval between the new receptor rest length and the test stretch imposed at that rest length (see section 6).

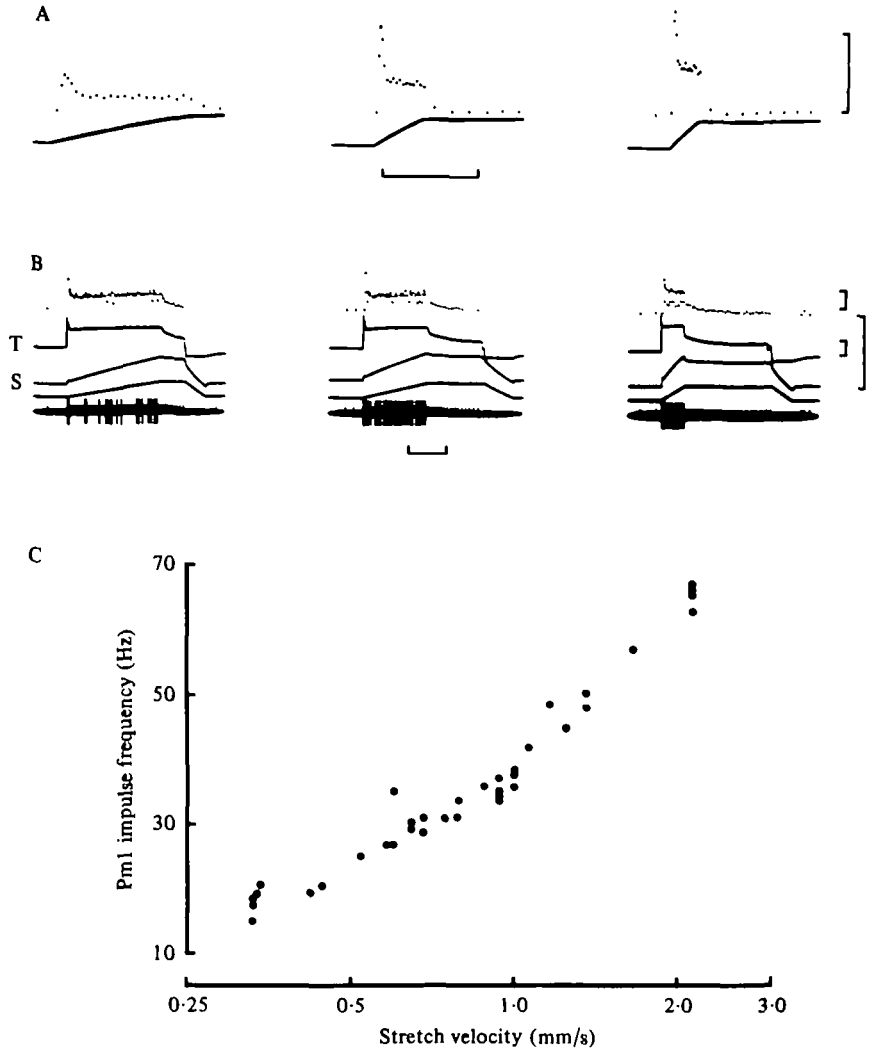


Fig. 9. The correspondence of Pm1 reflex impulse frequency envelopes to the waveform of the T fibre receptor potential, and the velocity sensitivity of the positive dynamic component and reflex response. (A) Frequencygrams of Pm1 at three different stretch velocities. (B) Frequencygrams of Pm1 with the simultaneously recorded T and S fibre receptor potentials evoked at increasing stretch velocities. Note the increase in amplitude of the T fibre dynamic component following higher stretch velocities; and the relative stability thereof, consequent upon the well stretched initial RM length (9·5 mm) compared to the maximum *in situ* extended length (11 mm) in this preparation. (C) Graph of Pm1 frequencies during the dynamic 'plateau' in response to varying stretch velocities. A 60 Hz change in frequency occurs in response to a tenfold change in stretch velocity, a very nearly logarithmic relationship over the velocity range 0·2–2·0 mm/sec. Calibrations: 1 s, 20 mV, 100 Hz.

(*e*) *Negative dynamic component.* A final feature which merits consideration in the present context of the relation between sensory and promotor reflex responses is the 'negative dynamic component' – during the release phase of a trapezoid stretch. Here again the reflex response shows a much closer parallel with the T fibre response than with the S fibre receptor potential. This is most clearly seen when there is some

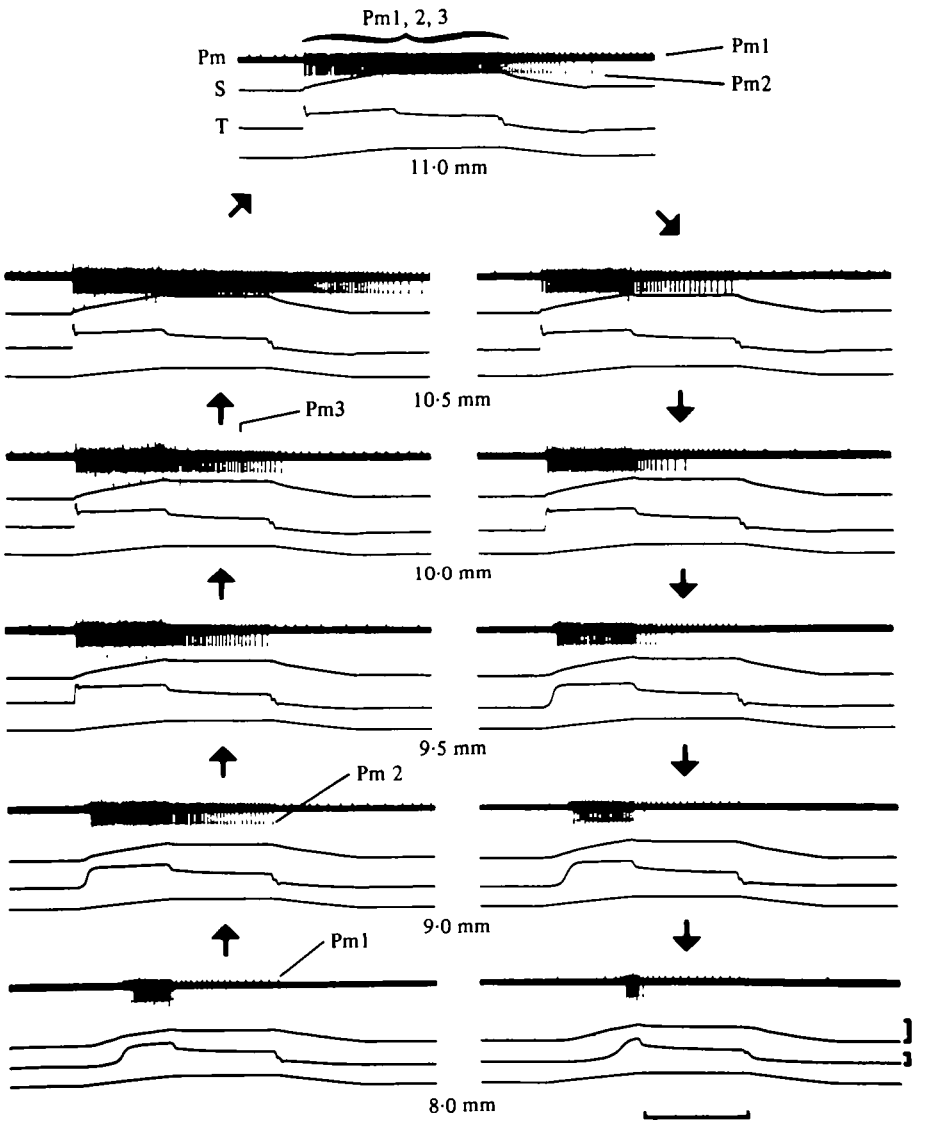


Fig. 10. The reflex response following identical trapezoid length changes applied to the receptor muscle at increasing followed by decreasing initial lengths (direction indicated by arrows), with simultaneously recorded T and S fibre receptor potentials. The time interval between successive length changes is 20 s. Note the progressive recruitment of promotor neurones Pm1–3, and development of the T fibre initial response, for stretches at increasing initial RM lengths. Calibrations: 1 s, 20 mV.

tonic background firing of the promotor MNs, as in Figs 9B (final record) and 14C. The firing frequency of the active MNs generally drops abruptly, like the T fibre depolarization, rather than gradually as in the S fibre. When there is a distinct hyperpolarizing negative dynamic component in the T fibre – as when the RM is at a relatively long initial length – the promotor MN firing frequency may drop considerably during the release and then resume at its earlier tonic level. Similar effects

are seen with hyperpolarizing currents or during tetanic stimulation of the RM (Cannone & Bush, 1980*a*, *c*).

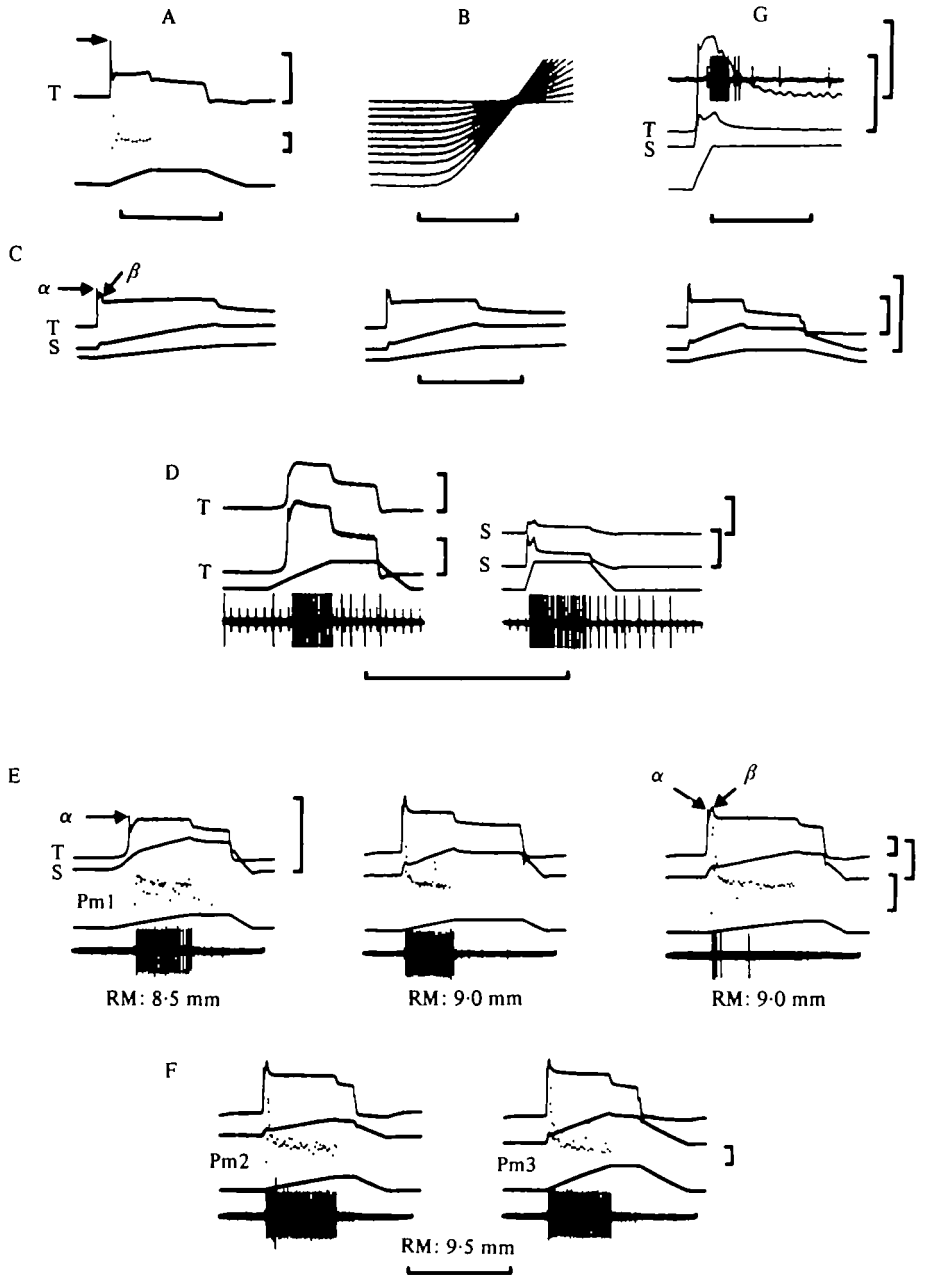
It is clear from the foregoing analysis, then, that the main characteristics of the reflex response of the promotor neurones to trapezoid stretch of the receptor muscle reproduce the principal features of the concomitant T fibre receptor potentials. This holds true for a wide range of stretch velocities and (though not explicitly described here) amplitudes, as well as at different initial lengths of the RM, however approached. Any changes in shape of the T fibre response, whether normal physiological ones or abnormal or artificially induced modifications, are mirrored by the instantaneous frequencies of the reflexly responding promotor MNs. At the same time, the reflexly evoked discharge 'waveforms' differ radically from those of the simultaneously recorded S fibre receptor potentials. There can be little doubt, therefore, that it is the T fibre rather than the S fibre which provides the major, if not the only, sensory drive for the promotor stretch reflex.

(4) *The 'initial response' to RM stretch: reflex latency*

The sensory responses to ramp stretches commonly display a brief initial transient preceding the velocity dependent positive dynamic component (Fig. 9, see also Bush & Roberts, 1971). In the T fibre, this initial response is generally more depolarizing than the main dynamic component, and occasionally achieves a level of depolarization more than double that of the succeeding dynamic component (Fig. 11 A). The possibility that this response is due to a stimulus aberration contributed by the puller can be discounted, since at all stretch velocities the monitor trace of the imposed stretch is artifact free (Fig. 11 B). A variety of factors which affect the initial tension or stiffness of the receptor muscle influence the initial response. Thus, for example, it increases progressively with (*a*) stretch velocity (Figs 9 B; 11 C), (*b*) RM length (Fig. 10), (*c*) the passive development of tonus in a relaxed, unstimulated RM (Figs 12; 13), and (*d*) efferent 'conditioning' at short initial lengths (Bush & Godden, 1974; Cannone & Bush, 1980*c*).

Particularly at low stretch velocities the initial response can often be differentiated into two separate components, a brief 'spiky' transient, the α (alpha) component followed by a longer duration β (beta) component (Fig. 11 C, E). The former probably represents an active, voltage dependent membrane response of the afferent nerve fibre, whilst the β component can be attributed directly to the mechanical properties of the receptor muscle prevailing at the onset of stretch, and may therefore be regarded as the primary 'initial response' of the sensory transducer (Bush, 1976, 1977). The

Fig. 11. Sensory and reflex responses to ramp stretches – the 'initial' response. (A) The initial response (arrowed) of the T fibre receptor potential is sometimes more than double the amplitude of the velocity dependent dynamic component. (B) Highly amplified monitor traces of a series of ramp stretches of varying velocities show that the initial response of the T fibre cannot be attributed to an abberration of the stimulus waveform. (C) The initial response can be differentiated into an early 'spiky' alpha (α) component, followed by a longer duration beta (β) component which increases progressively in amplitude with an increase in stretch velocity, often surpassing the amplitude of the α component (see also E and F). (D) The α component of the T fibre receptor potential may attenuate rapidly (distance between electrodes, 2·0 mm; length constant, 5·0 mm), and in contrast to the frequencygram in (A) no initial burst of reflex activity apparently ensues, providing the β component is also reduced (cf. first record, E). Attenuation of the S fibre potential is also marked (distance between



electrodes, 1·8 mm; length constant, 3·0 mm). (E) The β component of the T fibre response is only evoked at receptor lengths which ensure a dynamic response over the entire ramp duration (cf. Figs 8A, 12). The initial burst of Pm1 activity (frequencygrams) reflects the β component, absent in the first record. Note that at a reduced stretch velocity (3rd record) the β component alone achieves the activation threshold for Pm2 impulses (large spikes, lower trace). (F) Frequencygrams for Pm2 and Pm3 also indicate a high frequency initial burst for a taut receptor muscle at the onset of stretch. (G) Responses to a fast stretch show the T fibre – reflex latency to be approximately 10 ms. Calibrations: A, C, D, E, F, 1 s; B, 10 ms; G, 100 ms; 20 mV; 100 Hz.

amplitude of the β component varies directly with stretch velocity, and at the higher velocities it may exceed and even obscure the α component, hence dominating the overall initial response (Fig. 11 C). At the maximum velocities used here, it eventually comes to occupy the whole dynamic response, apart from the early α spike (Fig. 11 G). The α component varies directly with the rate of depolarization at the onset of the dynamic response, being particularly evident in conditions when the β component is small or absent, as in the slack receptor after a longish rest interval (Fig. 12). Clearly, then, caution is called for in correlating any early component in the promotor reflex response to RM stretch with the initial response of the receptor, and where possible a distinction should be made between responses to the α and β components, respectively.

In view of the often large 'initial response' and dominant reflex role of the T fibre, it is not surprising that the three responsive promotor MNs Pm1–3, commonly show a prominent, albeit brief, high frequency burst of impulses at the onset of their stretch evoked reflex discharge (Figs 9 A, B; 11 E, F). Like the T fibre initial response, these 'initial bursts' vary in amplitude (i.e. instantaneous frequency) directly with stretch velocity, and with the initial length and tension (or stiffness) of the receptor muscle. Close inspection of a variety of records suggests that the initial burst is largely a response to the β rather than the α component. Thus when the β component is lacking (e.g. Fig. 12), the latency of the reflex discharge is significantly longer than when it is present in the same preparation (cf. Fig. 13, last record). Conversely, in conditions when there is a large β initial response (or would be if it were recorded) – as when the response begins sharply at the onset of a ramp stretch – there is generally a large (i.e. high frequency) yet brief, short latency initial burst (Figs 9 A, B; 10; 11 E, F).

Furthermore, in experiments with two electrodes impaling the T fibre at separate points along its length, the α component is commonly much reduced or even absent at the proximal electrode (Fig. 11 D). Thus it appears possible that due to the rapid attenuation of the α component (cf. Mirolli, 1979 for the S fibre) there is little or no central synaptic transmission of this feature of the sensory response. It is nevertheless clear that in the absence of a β component and with an exceptionally large amplitude α component, the attenuation is not so great as to prevent entirely an initial burst, albeit exceptionally brief, by promotor MNs (Fig. 11 A). The α component, although subject to gross attenuation, may still exert a significant influence in 'speeding up' the depolarizing phase of the decrementally conducted receptor potential. In any event, it may tentatively be concluded from this analysis that the main initial response (i.e. the β component) of the T fibre's stretch evoked receptor potential provides a potent component to the overall afferent input to the stretch reflex. For a resistance reflex it can only be advantageous to ensure that a high frequency burst of impulses impinges upon the contractile machinery at the very commencement of the reflex motor programme.

In view of the non-impulsive, decremental nature of conduction and the long membrane time constant of the afferent nerve fibres in this sensory system (Bush, 1976; Mirolli, 1979), an accurate measure of afferent–efferent, and hence synaptic, latency is difficult to achieve with peripheral recordings. This difficulty is exacerbated by the uncertainty of the intra-ganglionic electrical properties and degree of attenua-

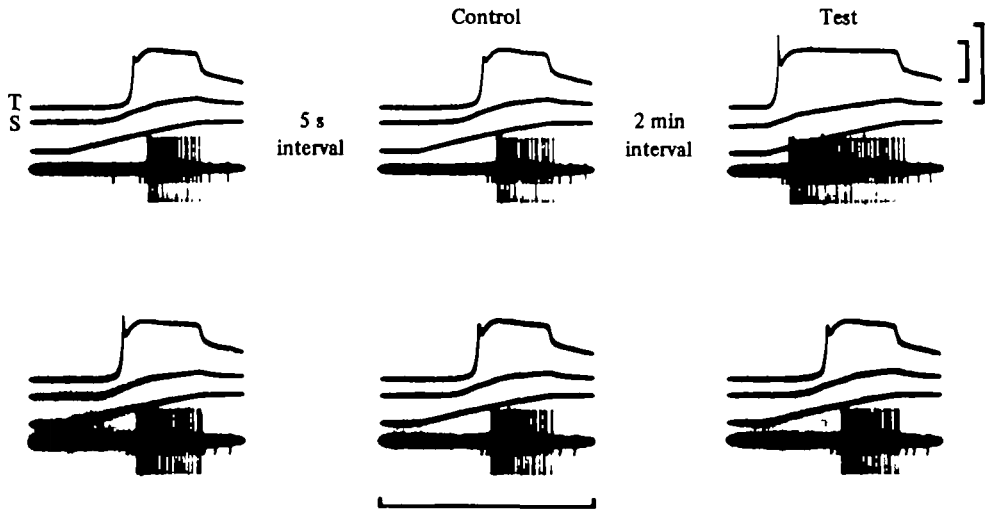


Fig. 12. The influence of the previous history of receptor length change on the response, both sensory and reflex, to subsequent test stretches. A period of 2 min free of stretch stimuli is interposed into a series of stretches performed at 5 s intervals. Note that the dynamic component of the T fibre occupies almost the entire ramp duration in the 'test' record, and that with succeeding stretches, this enhanced dynamic response declines to its pre-test or 'control' duration of approximately half the total ramp duration. Moreover, the rest period enhances the spiky alpha component of the T fibre dynamic response, and the duration of the S fibre dynamic response is also increased. The beta component is absent due to the 'slackness' of the receptor muscle (7·5 mm: maximum *in situ* length, 12·0 mm). Calibrations: 1 s, 20 mV.

tion of the afferent signal, given the evident reduction in afferent diameter and the branching of the S and T neurones within the thoracic ganglion (Bush, 1976). Information about post-synaptic activation thresholds is also lacking. The minimum afferent–efferent latency of about 9–10 msec (Fig. 11G) observed in the present experiments does not, therefore, permit any definitive conclusion about the number of synapses in the RM-promotor stretch reflex. In the light of the foregoing discussion of the relative efficacy of the α and β components of the receptor's initial response, this figure of about 10 msec probably represents an overestimate of the true afferent–efferent latency. Simultaneous intra-ganglionic recording from both sensory and motor neurones will be necessary to answer this question.

(5) *Temporal factors in the response to repeated stretches*

An important aspect of reflex adaptation is a form of 'depression' occurring in response to identical stretch stimuli imposed under certain conditions. Bush & Roberts (1971) attributed 'spontaneous' variation in the form of the T fibre dynamic response following constant velocity stretches of the RM to variations in resting tension within the RM. The experiments described here emphasize the influence of the previous history of receptor length change on the response, both sensory and reflex, to RM stretch.

At a fairly relaxed but constant RM rest length, ramp stretches applied at regular time intervals – say one stretch every 5 s – result in sensory and reflex dynamic responses of constant duration but occupying only a part of the total ramp duration

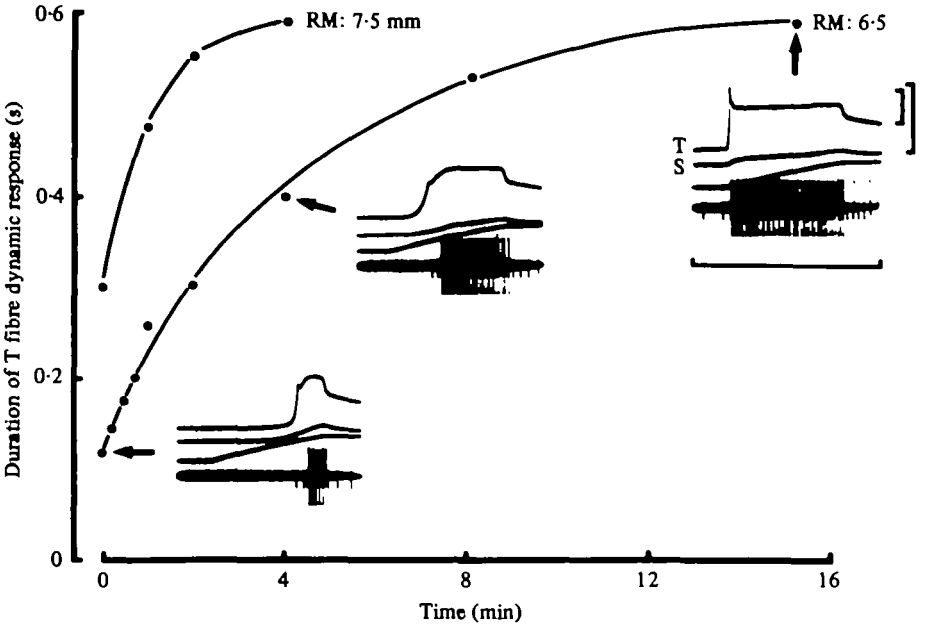


Fig. 13. Time dependent changes in the sensory and reflex responses to constant stretches applied at initial receptor lengths of 6·5 mm and 7·5 mm (maximum *in situ* RM length, 12·0 mm). The duration of the T fibre dynamic component is plotted as a function of the time between two stretches, the pre-test 'control' stretch and the 'test' stretch (cf. Fig. 12). After each test stretch following increasing periods of rest, the receptor was stretched repetitively at 5 s intervals to restore the sensory response to its 'control' level (bottom record, arrowed on graph). The records for the 4 min and 15 min rest periods are also presented. As illustrated in these sample records, the reflex response closely follows the T fibre response waveform. Calibrations: 1 s, 20 mV.

(Fig. 12). Interposing a rest period longer than the 5 s repeat period results in dynamic sensory responses of longer duration. The longer the rest period the longer the dynamic component of the subsequent 'test' response to stretch (Fig. 13). Following this test stretch, several stretches are required to once again restore the dynamic component to its constant duration, 'depressed' form (Fig. 12). As before, the phasic reflex response faithfully follows the T fibre dynamic response. This effect is most marked at the extreme lower end of the RM physiological length range, depending as it does on the 'slackness' of the RM. In contrast to its duration, the amplitude of the T fibre receptor potential, and therefore the concomitant reflex frequencies, are not significantly influenced by such variations in the temporal sequence of constant velocity and amplitude stretches – with the exception of the initial response which increases in amplitude following a rest period. Moreover, when the resting length of the RM is sufficient to ensure that the T fibre dynamic response commences at the beginning of the ramp stretch, variation in inter-stretch interval now has little or no effect on either the T (or S) fibre or reflex responses to constant stretches.

The significant feature revealed by these experiments is the decrease in the latency of the T fibre and reflex responses at relatively slack RM lengths, consequent upon increases in the rest period preceding repetitive length changes. This effect can only be attributed to an increase in the mechanical resistance of the RM to stretch, that is, to time-dependent changes in the visco-elastic properties of the RM, in circum-

stances where the RM receives neither efferent input nor imposed length changes. The most ready explanation is that cross-bridges between RM contractile proteins are spontaneously formed during periods of inactivity, and are broken by repeated stretch until ultimately the full 'plasticity' of the RM, as seen with repeated stretch at short time intervals, is restored (see Hill, 1968; Brown, Goodwin & Matthews, 1969; Hunt & Ottoson, 1976). From a functional point of view, it is clear that these time-dependent variations in sensory and resulting reflex responses to constant stretches would, if present in the living animal, lead to uncertainty and ambiguity in the proprioceptive signal. Subsequent papers in this series (Cannone & Bush, 1980*b*, *c*) however, will suggest that this problem may be resolved *in vivo* by continuous efferent control of RM tension. Such receptor motor activity could serve to maintain the gain of the receptor under conditions of varying load at different RM lengths.

(6) *Spontaneous activity and post-stimulus excitation*

Many preparations reveal evidence of a central drive onto the same MNs as are reflexly activated. This phenomenon is usually manifested by variable duration bursts of activity which often intrude into and summate with peripherally induced reflex firing patterns (Figs 5; 14A, B, Cannone & Bush, 1980*b*). It is unlikely that these spontaneous, centrally originating bursts of activity in these highly dissected preparations reflect 'normal' *in vivo* patterns of behaviour. In those preparations with all nerves to the basal leg segments intact, visual observation shows that the spontaneous firing of promotor MNs is correlated with vigorous, synchronized movements of the thoracic–coxal and coxobasal joints of all pereiopods, a form of activity not seen in the intact animal. It is interesting that central excitation does not involve any more MNs than are normally reflexly activated, notwithstanding the fact that 22 neurones have been detected histologically in the 'motor' nerve supplying the promotor muscle (Cannone & Bush, 1980*b*).

Preparations that display spontaneous activity commonly also demonstrate a phenomenon that can best be described as post-stimulus excitation. This takes the form of a prolonged MN discharge of variable duration at the termination of a stretch stimulus, long after the T fibre has repolarized to its original membrane potential at the particular RM rest length (Fig. 10, RM length 10·5, increasing series). There appears to be a direct relation between the degree of centrally originating activity and the duration and impulse frequency of the post-stimulus excitation. The greater the pre-stimulus central drive the more pronounced the post-stimulus excitation effect (Fig. 14B). It is probable that sensory feedback impinges upon the central driving source, in addition to acting directly on the MNs in question. The variable nature of the post-stimulus excitation can then be viewed as a constant sensory input (i.e. constant stretch stimulus) summing with a variable level of often sub-threshold central excitation (e.g. in Fig. 10 at RM length 10·5, increasing series, pre-stimulus central drive is only apparent on Pm1, yet Pm2 'excitability' during and after the stretch is also enhanced). Such an explanation would account for the fact that whereas hyperpolarization of the T fibre during the negative dynamic component (Fig. 14C) or by current injection (Cannone & Bush, 1980*a*, Fig. 7) normally inhibits tonic reflex firing, similar hyperpolarization during a post-stimulus excitatory pattern of MN discharge has no inhibitory effect (Fig. 14D).

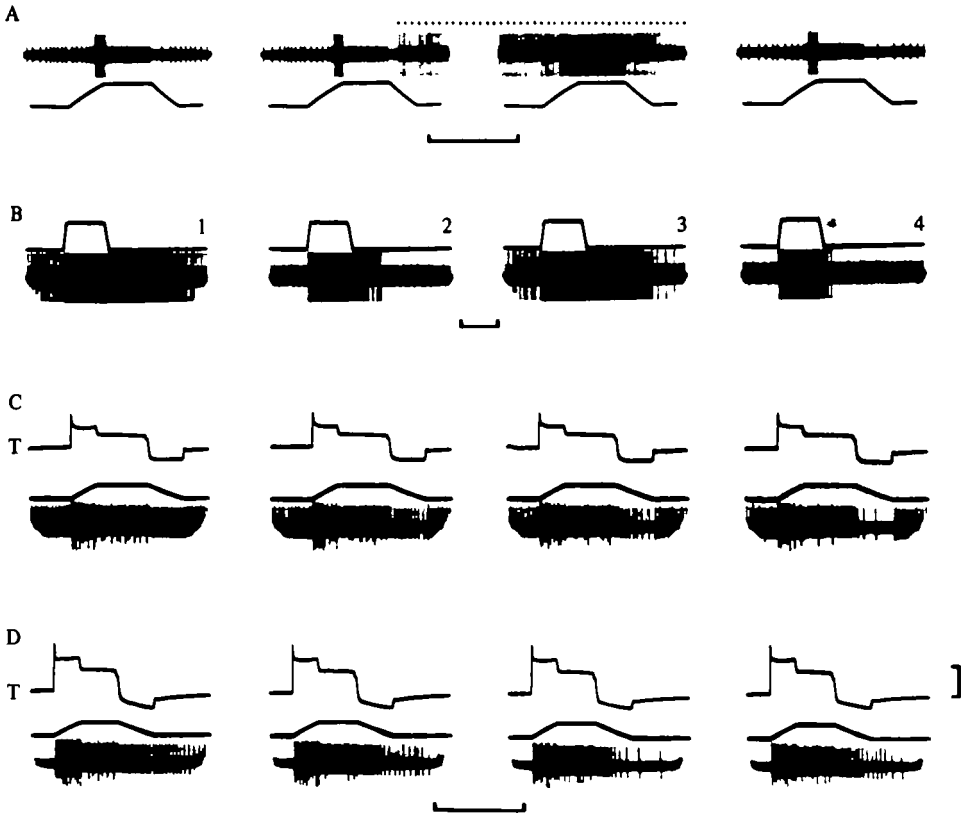


Fig. 14. Spontaneous, centrally originating motor activity and 'post-stimulus excitation'. (A) Spontaneous burst (dotted line) occurs only in MNs that are reflexly activated. (B) The degree of post-stimulus excitation following a stretch is loosely dependent on the 'level' of central excitability in these four successive recordings taken at 10 s intervals. Note that in records 1 and 3 pre-stretch central drive on to Pm2 (large amplitude spikes) coincides with extensive post-stimulus excitation. Post-stimulus excitation of Pm2 is evident in record 2 although there is no pre-stimulus activity in this fibre. A degree of central drive (sub-threshold for Pm2) is nevertheless indicated on comparing pre-stimulus frequencies of Pm1 (record 2, 24 Hz: record 4, 18 Hz). (C) Four successive records at 10 s intervals obtained early in the experiment during which time penetration damage to the T fibre (membrane potential low, small amplitude receptor potentials) resulted in a high level of 'tonic' activity in promotor MNs. The hyperpolarizing negative dynamic component of the T fibre response has a marked inhibitory effect on this peripherally induced 'tonic' activity. (D) Later in the experiment as the T fibre recovers (membrane potential increases, receptor potentials larger in amplitude), 'tonic' activity of Pm2 is lost and T fibre hyperpolarization now exerts no effect on the post-stimulus excitability. The absence of peripheral biasing indicates a purely central origin for the MN impulses occurring after the stretch stimulus (explanation in text). Calibrations: 1 s, 20 mV.

DISCUSSION

The thoracic–coxal receptor muscle in crabs lies in parallel with promotor muscle fibres. It is therefore stretched by limb remotion and shortened by limb promotion. In crabs it is the only known proprioceptor at the T–C joint, although in the Astacura (*Homarus vulgaris* and *Astacus astacus*) there is a chordotonal organ adjacent to, and lying in parallel with it (Alexandrowicz & Whitear, 1957). The T–C chordotonal organ

responds only to limb promotion, there being no sensory neurones found to respond to limb remotion or to stretch of the isolated elastic strand (Bush, 1976; Bush & Cannone, unpublished observations). In addition to the two large diameter S and T sensory fibres, the T–C MRO has a small diameter fibre (4–6 μm) innervating one of the connective tissue flanking strands, with terminations similar to those of the S fibre (Whitear, 1965). The dimensions of this P fibre has thwarted attempts to record from it. Consequently it can only be assumed from the nature of its terminations that it is a sensory element, although the possibility that it is inhibitory in its action, similar to the accessory nerve fibres of the abdominal MROs (cf. Fields, 1976 for a review), cannot be excluded. In any event, attempts to describe fully the physiology, reflex effects, or functional role of the T–C MRO must be considered incomplete in the absence of information on the physiology of the P fibre.

Several promotor MNs can be reflexly activated by receptor stretch (Bush & Roberts, 1968), the results presented here being confined to Pm1–3, the three MNs most commonly recruited in response to stretch stimuli. The main conclusion of these essentially qualitative experiments is that the promotor stretch reflex is driven by the in-series, primarily tension sensitive T fibre, rather than by the in-parallel, primarily displacement-sensitive S fibre. Observations on additional promotor MNs, which have higher activation thresholds and which are sometimes recruited by receptor stretch or, more reliably, by injection of constant current pulses into the S and T sensory fibres, are presented elsewhere (Cannone & Bush, 1980*a*).

Tonic activity and experimental reliability

Although synaptic efficacy in these highly dissected preparations could be maintained for many hours by employing the transfusion and perfusion technique described, a persistent consideration in these experiments, and in those described later (Cannone & Bush, 1980*a*, *b*, *c*), must be the extent to which normal *in vivo* levels of excitability and response are reflected in the recordings obtained, taking into account the time required from sacrificing the crab to the placement of electrodes and the impalement of the sensory fibres. In those preparations in which sophistication of approach is compromised in favour of speed of recording from the promotor nerve, tonic firing of promotor MN Pm1 proceeds even at completely relaxed RM lengths, and Pm2 and Pm3 discharge tonically usually at more extended RM maintained lengths. However, the tonic activity of these MNs declines steadily with time and eventually is lost completely, although the preparation may still show a considerable degree of reflex excitability in response to receptor stretch. This indicates in the first instance that the 'resting' membrane potential of the T fibre, even at fully relaxed RM lengths, probably provides a steady presynaptic drive onto the MNs in question, similar to the behaviour of non-spiking locust interneurones reported by Burrows & Siegler (1978). Two assumptions can reasonably be adopted. Firstly, that the reflex pathway in common with other myotatic or stretch reflexes, is monosynaptic. The intra-ganglionic disposition of sensory fibres and promotor MNs (Bush, 1976, 1977; Blight & Llinás, 1978), and the intra-ganglionic pre- and postsynaptic recordings of Blight & Llinás (1978) support this assumption. Measurements of input–output latency in a non-spiking sensory system such as this are not very helpful in deter-

mining whether connectivity is mono- or polysynaptic (Bush, 1976; Burrows & Siegler, 1978). Whereas a number of different criteria can be used to determine whether transmission between spiking neurones is, or is not, monosynaptic (see Berry & Pentreath, 1976 for a review), the same criteria cannot be applied to a decrementally conducting system, for reasons mentioned previously (cf. section 4, this paper).

A second assumption, that transmission is chemical rather than electrical, is supported by the relatively high concentrations of choline acetyltransferase, the enzyme synthesizing acetylcholine, found in the T–C MRO sensory nerve (Emson, Bush & Joseph, 1976). This strongly suggests cholinergic central transmission for the sensory fibres, as in other crustacean sensory systems (Florey, 1973). Thus in this preparation, as in the non-spiking locust interneurones (Burrows & Siegler, 1978), it seems likely that the T fibre at least releases 'tonic' amounts of transmitter, the amount being dependent upon the RM length prescribed 'resting' membrane potential.

The increase in tonic firing frequency with maintained increases in RM length suggests a postural role for the T–C MRO resistance reflex, or at least for the length-dependent discharge of the promotor motoneurones Pm1–3 (see Cannone & Bush, 1980*a* for a more extensive treatment of this aspect). Compensation for increased load such as when a crab stands on an antero-posteriorly inclined plane, or the rigid maintenance of T–C joint angle during an extension–retraction cycle of the leg in sideways walking, could be effectively accomplished by a receptor length induced tonic output of Pm1–3, or by receptor efferent input effecting a tonic reflex output via the tension sensitive T sensory fibre (Cannone & Bush, 1980*b*, *c*).

Finally, caution is called for when attributing a functional role *in vivo* to the reflex responses recorded in the semi-isolated preparation. For example, a clear constraint is imposed by the progressive decline in reflex excitability of these preparations, manifested as an increase in reflex activation thresholds and concomitant decrease in discharge frequencies and number of promotor motoneurones activated by a given stretch stimulus. Moreover, under certain conditions in specially 'lively' preparations, repeated RM stretch can evoke 'assistance reflexes', tending to reinforce rather than resist imposed length changes (DiCaprio & Clarac, 1980). Thus only when it becomes possible to analyse these various proprioceptive reflexes in relatively intact animals will any definitive conclusion as to their behavioural significance be warranted.

T fibre receptor potential and reflex correspondence

The evidence presented here points overwhelmingly to the T fibre providing the afferent feedback for the reflex response to stretch. Thus, adaptation of the reflex response follows the adaptation of the T fibre receptor potential; the reflex is abolished by selectively cutting the T but not the S fibre; the velocity sensitivity of the reflex response reflects the logarithmic relationship between stretch velocity and T fibre dynamic component amplitude; the hysteresis in the T fibre response to increasing or decreasing receptor length changes is similarly reflected in the reflex response; and the reflex corresponds precisely to the increased duration of the T fibre dynamic component following increasing periods of RM inactivity. Nevertheless, the S fibre may well have a reflex role *in vivo*, particularly in view of the recognized temporal

decline in 'excitability' of these preparations, and since a depolarizing current – albeit a large one – injected into the S fibre is known to reflexly activate certain promotor MNs (Bush & Cannone, 1973; Cannone & Bush, 1980*a*). From the point of view of postural control, the intuitive 'suitability' of a linearly depolarizing S fibre membrane potential with incremental receptor length changes (Bush & Roberts, 1971), compared with the non-linear (tension) sensitivity of the T fibre to RM length change, is not supported by experimental evidence presented here. However, evidence for a possible S fibre reflex role will be considered in our second paper (Cannone & Bush, 1980*a*).

The frequency envelope for each of the three most commonly elicited promotor MNs, Pm1–3, reflects the waveform of the T fibre response to trapezoid stretch stimuli remarkably closely, and accordingly differs substantially from that of the S fibre response. This close correspondence holds true for stretches at all RM lengths within the physiological length range, and for all stretch amplitudes and velocities. Varying these stimulus parameters, moreover, shows that recruitment of promotor MNs is dependent upon the level of T fibre depolarization. Thus, 'phasic' and 'tonic' components of the stretch reflex can be distinguished, determined by the dynamic and static components respectively of the T fibre response. Preceding the phasic reflex response is the initial burst – a high frequency discharge of motor impulses probably elicited by the primary initial response of the T fibre, i.e. the β component, rather than by the brief, spiky α component.

The advantages of the promotor resistance reflex being driven by T rather than S fibre input can readily be appreciated. The promotor muscle of *Carcinus* has muscle fibres that are both singly and polyneuronally innervated by various combinations of motoneurones. The entire range of electrical responsiveness is evident, from muscle fibres that show small amplitude, poorly facilitating excitatory junction potentials, to fibres that are capable of generating muscle action potentials following facilitation and summation of graded potentials contributed by several discharging MNs (Cannone & Bush, 1980*a*; Cannone & Bush, unpublished observations; see also reviews by Atwood, 1967, 1976). Since facilitation and summation of graded junction potentials play an important role in determining both the time course and the extent of the mechanical response of crustacean muscle fibres to motor input, it is apparent that a reflexly evoked initial burst of high frequency impulses impinging upon the promotor muscle would be most effective in promoting *rapid* muscle resistance at the very onset of an imposed movement. By contrast, a S fibre evoked resistance reflex would lack such an initial 'priming' effect. Moreover, the sustained reflex drive by the T fibre during the remainder of the dynamic phase of stretch, and the signalling of stretch velocity by dynamic amplitude – and hence concomitant reflex output in terms of both MN recruitment and impulse frequency modulation – are further advantages when compared to the gradual increase in depolarization of the S fibre during the course of a ramp stretch.

Ambiguity inherent in the T fibre to motoneurone pathways

The ambiguity in the sensory and reflex responses to two identical stretch stimuli applied at the same RM initial length, but with the initial RM length approached from both a shorter and a longer RM rest length (section 3*d*), can be directly related

to the changes in response characteristics seen when the intervals between successive stretches are varied (section 5). Hill (1968) described a short range elastic component (SREC) in frog skeletal muscle which operated over a very small initial length range of a ramp stretch. He attributed this to the existence of stable bonds (cross bridges) between the thick and thin muscle filaments. The SREC is thought to be responsible for a rapid increase in tension at the onset of the ramp stretch, after which tension remains at a constant level for the remainder of the ramp. Subsequently, Brown *et al.* (1969) attributed the ramp stretch induced initial bursts of the mammalian spindle primary ending *in the absence of preceding fusimotor stimulation* to the spontaneous formation of cross bridges in the resting spindle. Moreover, they found that fusimotor stimulation prior to a test stretch enhanced the initial burst, and that repeated stretch progressively abolished this effect. More recently, recordings of generator potential, primary ending discharge and intrafusal muscle tension, particularly under conditions of repeated stretch, have confirmed that initial burst characteristics are imposed by mechanical components in the mammalian system (Hunt & Ottoson, 1976). Since it is the amplitude of the initial component of the primary ending generator potential that is directly related to stretch velocity (Hunt & Ottoson, 1976), it is tempting to draw the analogy between it and the velocity dependent β component of the T fibre initial response, the component almost certainly responsible for the initial burst seen in the reflex response to ramp stretch.

In the muscle fibres of the T–C MRO it is likely that similar mechanical properties are responsible for the observed responses in the in-series, tension-sensitive T fibre. Thus, the hysteresis in the responses to ramp stretches applied at increasing, followed by decreasing, RM lengths (Figs 5, 10) is critically dependent upon the delay between the change in RM length and the application of a stretch at that particular length. It is inferred that the longer this delay the more cross bridges will spontaneously form, and hence the less compliant (SREC re-established) the receptor muscle fibres will be, thereby minimizing the hysteretic effect. Similarly, the responses seen when identical stretches are applied at the same RM rest length, but with varying intervals between stretches (Figs 12, 13), can also be attributed to the spontaneous formation of cross bridges between muscle filaments during a 'rest' period. The subsequent stretching and disruption of bonds following repeated stretches at short intervals restores 'compliance' to the RM, resulting in the 'control' T fibre dynamic and reflex responses occupying once again only a part of the dynamic phase of stretch. The apparent failure of a single 'test' stretch to completely restore the response to its fully compliant 'control' configuration is possibly not irreconcilable with current ideas on short range tension effects and the formation and disruption of bonds between muscle filaments. Components of series elasticity other than the stretching of bonds between filaments (cf. Rack & Westbury, 1974) in a receptor muscle of approximately 12 mm *in situ* extended length can possibly account for the failure of a 1·0–1·5 mm stretch applied to its distal end to disrupt *all* existing cross bridge bonding between filaments. Alternatively, all bonds are disrupted but a proportion reform before the next stretch in the series. On the other hand, it is possible that this type of experiment, to the best of our knowledge as yet unreported for any other muscle receptor, involving as it does an arrangement whereby an 'incomplete' sensory response is evoked consequent upon ramp-stretching a relatively slack receptor muscle, offers the potential

to reveal hitherto unsuspected mechanical properties underlying sensory feedback from a muscle receptor, and by implication properties shared by skeletal striated muscle.

The results obtained in this study (cf. also Cannone & Bush, 1980*c*) confirm and considerably extend the observations made originally by Bush & Roberts (1971) who noted the spontaneous variation in the form of the T fibre dynamic response to constant velocity stretches. This form of mechanically imposed ambiguity in sensory feedback, and hence reflex activity, can possibly be resolved *in vivo* by a tonic excitatory motor input to the RM (Bush, 1976; Cannone & Bush, 1980*b*), and is certainly eliminated or at least reduced by experimental stimulation of the receptor efferent nerve (Cannone & Bush, 1980*c*).

Concluding remarks

In this our first paper on the reflex physiology of the T–C MRO, no attempt is made to integrate fully all aspects of the reflex response. The results of experiments involving the injection of depolarizing and hyperpolarizing currents into the sensory fibres, with or without superimposed stretch stimuli, in addition to providing a quantitative basis to the input–output relationships of this system, provide additional insights into aspects such as a possible reflex role for the S fibre; total numbers of promotor MNs that can be reflexly activated; and central connectivities that are suggested, consequent upon sensory influence on spontaneous, centrally originating MN activity (Cannone & Bush, 1980*a*).

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