J. exp. Biol. (1980), **86**, 305–331
With 13 figures
Printed in Great Britain

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

II. REFLEX DISCHARGE EVOKED BY CURRENT INJECTION

BY A. J. CANNONE* AND B. M. H. BUSH

University Department of Physiology, School of Veterinary Science,†
Park Row, Bristol BS1 5LS

(*Received* 19 *October* 1979)

SUMMARY

1. Injection of depolarizing and hyperpolarizing currents into the non-impulsive S and T sensory fibres has allowed a quantitative analysis of the input–output relationships of this sensory-reflex system.

2. Graded depolarization of the T fibre typically results in sigmoid voltage–frequency relationships for motoneurones Pm1–3, the maximum 'slopes' in the most sensitive preparation being 31, 47·5 and 33 Hz/mV respectively, *without taking into account further afferent attenuation within the thoracic ganglion.*

3. Graded depolarizations of the S fibre recruit four motoneurones (Pm1–4) common to the T fibre reflex pathway, although activation thresholds are much higher and the maximum slope or 'gain' of the voltage–frequency relationships is much reduced compared to that of the T fibre reflex pathways. Thus, although the S fibre synapses either directly or indirectly with promotor motoneurones, typical 5–10 mV stretch-induced receptor potentials remain sub-threshold for a contribution to the reflex output, albeit under experimental conditions and at all but the most extended receptor muscle lengths.

4. Differentiating between motor impulse amplitudes, and using simultaneously recorded promotor muscle junction potentials as a further aid, establishes at least nine motoneurones as being reflexly recruited by the S and T fibres: Pm1–8 by the T fibre and Pm1–4 and Pm9 by the S fibre.

5. Hyperpolarization of the T and S fibres confirms that ongoing tonic motor activity is peripherally determined by receptor length prescribed T fibre 'resting' potentials, rather than by the more linearly related S fibre 'resting' potentials at different receptor muscle lengths. Moreover, suppression of the reflex response by sensory fibre hyperpolarizations coincident with stretch stimuli leaves little doubt that it is the T rather than the S fibre that provides the sensory drive for the stretch reflex *in vitro.*

6. By using long duration, constant value T fibre depolarizing potentials, the postsynaptic component of adaptation for Pm1–3 has been found to be slight, in contrast to the more rapid adaptation of the higher threshold

* Permanent address: Department of Zoology, University of the Witwatersrand, Johannesburg 2001, South Africa.
† Address for reprints.

Pm5–8 motoneurones. Moreover, adaptation rates for Pm1–3 discharges are lower the smaller the afferent drive. It is suggested therefore that reflexly activated promotor MNs can be differentiated into 'tonic' and 'phasic' categories.

7. Motoneurone Pm2 sometimes discharges in the form of 'rigid' (7–8 ms intervals) impulse couplets. At low mean reflex frequencies all Pm2 impulses are locked into couplets ('complete' patterning). While both 'rigid' and 'complete' patterning break down abruptly as mean frequencies increase, re-establishment is more gradual (hysteretic) with decreasing mean frequencies. Patterning, albeit a particularly labile phenomenon, is almost certainly an intrinsic, if unknown, property of the motoneurone itself.

INTRODUCTION

In the jointed crustacean limb, proprioceptors are associated with all joints and provide the central nervous sytem with information concerning the position of limb segments, the rate and direction of joint movements, and, in the case of tension receptors (Macmillan, 1976, for review), information on the muscular forces produced during movements is also provided. It is generally accepted that the central nervous system, in the absence of sensory feedback, can generate the cyclical patterns of neuronal activity that form the basis of locomotion (Kennedy & Davis, 1977). Nevertheless, proprioceptive feedback is known to modulate motoneurone (MN) output in several arthropod locomotory systems (e.g. Wilson & Gettrup, 1963; Davis, 1969; Pearson, 1972; Paul, 1976). Resistance reflexes are an important consequence of proprioceptor activation, notwithstanding the fact that, opposing as they do an ongoing locomotory cycle, they are evidently suppressed during active movements (Barnes, Spirito & Evoy, 1972; Field, 1974; Vedel & Clarac, 1975). It is arguable, therefore, that resistance reflexes in the Crustacea are functionally restricted to controlling posture (Fields, 1966; Sokolove, 1973; Macmillan, 1975); to dampening joint movement at the extremes of joint angle during walking (Evoy & Cohen, 1971; Fourtner & Evoy, 1973), and to facilitating the initiation of an antagonistic component of a movement cycle as proposed for the lobster swimmeret system (Davis, 1969); to load compensation during unexpected deviations in locomotory movements (Barnes *et al.* 1972); and finally, in the case of 'distributed' (intersegmental) reflex effects, such reflexes can be seen as contributing to the coordination of the activity of muscles having an essentially synergistic function in limb walking movements (Ayers & Davis, 1977, 1978). A more recent finding is the unambiguous (cf. Moody, 1970) reflex modulation by one proprioceptor (C-B chordotonal organ) of the efferent input to a muscle receptor organ (the accessory flexor muscle of the M-C myochordotonal organ) in a different leg segment (Bush, Vedel & Clarac, 1978).

In order to fully comprehend the contribution of proprioception, and hence proprioceptive reflexes, to the neural regulation of movement or posture, a necessary prerequisite is the establishment of the qualititative and quantitative relationships between sensory feedback and motoneurone output. Moreover, such a study should extend to the innervation patterns, and the electrical and mechanical properties of muscle fibres in the limb muscle reflexly activated (Cannone & Bush, unpublished observations; cf. Atwood, 1967, 1976, for reviews). In the case of the thoracic–coxal

muscle receptor organ (T–C MRO), sensorimotor input–output relationships are of added interest by virtue of the non-impulsive (graded), decrementally conducting nature of the sensory feedback to the thoracic ganglion (Ripley, Bush & Roberts, 1968; Bush & Roberts, 1971; Bush, 1976, 1977; Mirolli, 1979). In this (Bush & Cannone, 1973; Bush, 1976, 1977), and in only a few other preparations (Mendelson, 1971; Pearson & Fourtner, 1975; Maynard, & Walton, 1975; Baylor & Fettiplace, 1976; Burrows & Siegler, 1978) has it been possible to examine information transfer between neurones where the absence of impulses has not been artificially induced. In a recent detailed investigation Blight & Llinás (1980), recording pre- and post-synaptically *within the thoracic ganglion* in the T sensory fibre and in promotor MNs in the crab *Callinectes* while passing current across a sucrose gap into the T fibre, analysed the transmission characteristics of this 'tonic' sensorimotor synapse.

In our previous paper (Cannone & Bush, 1980*a*) the close similarity between the trapezoid stretch-induced receptor potential waveform of the in-series T sensory fibre and the 'frequency envelope' of reflexly activated promotor MNs was established. Several promotor MNs were found to discharge in response to receptor stretch with a predictable order of recruitment dependent upon effective stimulus parameters such as receptor muscle length, stretch velocity and stretch amplitude, all of these parameters being reflected jointly or on their own in the degree of depolarization of the T fibre. Moreover, in the most 'sensitive' preparations and fairly early on in the course of an experiment, the three commonly activated (lowest threshold) MNs, Pm1–3 are tonically active at maintained receptor lengths. The series of experiments described here, involving the injection of constant current pulses of both depolarizing and hyperpolarizing sign into the S and T sensory fibres, were designed to answer several fundamental questions such as (*a*) a possible role for the displacement sensitive S fibre in the resistance reflex; (*b*) whether the tonic activity of promotor MNs is determined purely by receptor length prescribed sensory fibre 'resting' potentials; (*c*) the shape and slope or 'sensitivity' of the input–output relationships for individual MNs; (*d*) the numbers of promotor MNs reflexly activated; (*e*) the characteristics of adaptation of MNs during constant value sensory fibre depolarizations. In addition, discharge patterning in the form of discrete impulse couplets in one of the promotor MNs (Pm2) is described.

METHODS

Crabs were prepared as reported previously (Cannone & Bush, 1980*a*). Direct intracellular stimulation of the S and T fibres was effected using a constant current pulse generator with a stimulus isolation facility. Five current ranges between 0·1 nA and 1·0 mA could be selected, a multi-turn digital readout potentiometer giving a resolution of 10^{-3} within each of these ranges.

Two well-spaced intracellular recording micropipettes in the same sensory fibre allowed determination of the attentuation factor from the decrement in the afferent responses to a stretch stimulus applied to the receptor muscle. From this the fibre length constant, λ, was estimated, using simple infinite cable assumptions (cf. Roberts & Bush, 1971). This in turn can be expressed as the dimensionless electrotonic length, L (= fibre length/λ; Rall, 1969), of the fibre, allowing a more meaningful assessment of current induced reflex activity.

Subsequently, one of the two microelectrodes was used to inject current into the fibre, the membrane potential being monitored by the other. Usually the current passing electrode was positioned proximal (i.e. nearer the thoracic ganglion) to the recording electrode. This ensured that the current flowing from the stimulating micropipette to the synaptic area in the thoracic ganglion did not meet an area of damaged membrane with a consequently lowered resistance giving exaggerated attenuation, as might have resulted from a more proximal recording pipette. The distance between stimulating and recording electrodes was chosen to approximate that between the former and the edge of the ganglion (Fig. 1). Assuming uniform electrical properties along the extra-ganglionic length of the fibre, the signal attenuation between current electrode and ganglion would then be similar to that 'seen' by the distal recording electrode. Thus only the unknown intra-ganglionic attenuation need be taken into account in assessing the true input–output relations, including the gain or 'sensitivity' of the sensory-motoneurone reflex. This central component of the overall attenuation may well be greater than the peripheral contribution, owing to the evident narrowing and branching of the sensory fibres within the ganglion (Bush, 1976) and any associated changes in electrical properties (but cf. Blight & Llinás, 1980).

RESULTS

(1) *Reflex connexions of S and T fibres*

As expected from previous work (Bush & Roberts, 1968) and the first paper of this series (Cannone & Bush, 1980*a*), depolarization of the T fibre evokes reflex discharge of one or more promotor MNs (Fig. 2). In view of the close similarity between the stretch-evoked reflex pattern and the T fibre response waveform, it was surprising to find that S fibre depolarization also resulted in reflex firing of promotor MNs. The T fibre, however, is considerably more potent in its effects than the S fibre, and the individual motoneurones show characteristic differences in their responsiveness to either sensory input. Furthermore, hyperpolarization of the T fibre but not the S fibre inhibits any ongoing or reflexly evoked promotor activity. The input–output properties of the sensory-promotor system have been analysed quantitatively by means of graded current pulses, usually within the range 0–200 nA and of either polarity, injected into the T and S fibres in turn, so as to determine the relationship between sensory fibre membrane potential and resultant reflex discharge frequency for each afferent and efferent unit.

(*a*) *Input–output relations for T fibre to promotor neurones Pm1–3*

Typical reflex frequency curves (Fig. 3) reveal a sigmoid voltage–frequency relationship for promotor motoneurones Pm1, Pm2 and Pm3, although a linear relationship for both Pm2 and Pm3 is sometimes obtained. Usually, the increases in discharge frequency of these three MNs with incrementally increasing depolarizing currents are comparable, the slopes for Pm1, Pm2 and Pm3 being similar (Fig. 3A). Sometimes however, the slopes of the curves for Pm2 and Pm3 may be considerably steeper than that for Pm1, and may even overlap that of Pm1 (Fig. 3B). The 'gain' (or 'sensitivity') of the reflex pathways varies from one preparation to another. Over

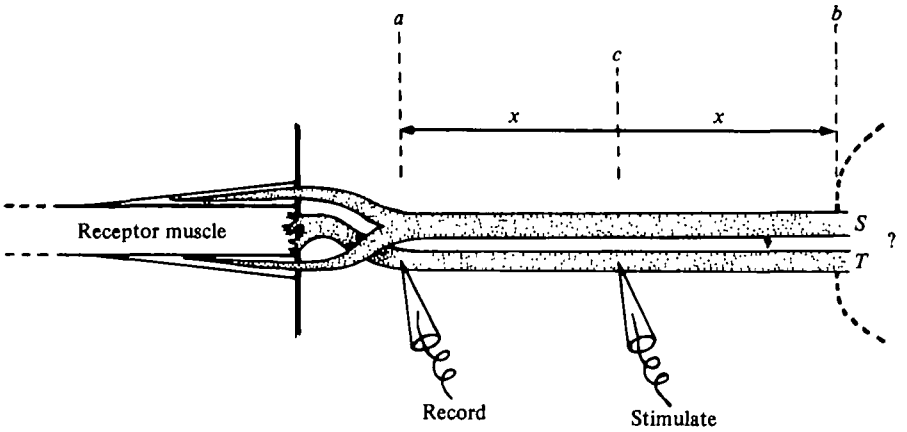

Fig. 1. Diagram of optimal arrangement of current-passing and recording micropipettes. Between the receptor muscle and the edge of the thoracic ganglion the diameters of the S and T sensory fibres are approximately uniform. With the current-passing micropipette at *c*, proximal to the recording electrode and equidistant between *a* and *b*, the stimulus current is conducted to the ganglion unimpeded and, presumably, with similar attenuation to that between *c* and *a*. This peripheral component of attenuation can therefore be largely discounted in any assessment of reflex input–output relationships. There remains an unknown degree of attenuation presynaptically within the ganglion (indicated by the '?'), dependent upon the electrical properties of the intraganglionic portion of the T (or S) fibre (see Bush, 1976, for the neuronal geometry).

the steepest part of the voltage–frequency curves the output–input relationships obtained in 19 preparations tested in this way ranged from 3·6, 4·2 and 3·2 Hz/mV (Fig. 4B) to as high as 31, 47·5, 33 Hz/mV (Figs 2A, 3B) for Pm1, Pm2 and Pm3, respectively. Factors that could be contributing to such marked variation in sensitivity are the relative state of reflex excitability, or 'liveliness' (cf. Cannone & Bush, 1980*a*), of a particular preparation at the time of graded current injections, and the varied electrotonic lengths found for sensory fibres in different preparations (Table 1).

Preparations in which fibre Pm1 was tonically active provided the opportunity of investigating the effects of T fibre *hyperpolarization* on such tonic activity. Even with low tonic frequencies, large hyperpolarizing currents are required to abolish this activity (Fig. 4), of much larger intensities than the depolarizing currents required to add equivalent frequency increments to the initial tonic discharge. The transition from hyperpolarized to depolarized states in the voltage–frequency relationship is usually smooth (Fig. 4A), but occasionally displayed a 'step' or plateau in the relationship (Fig. 4B). Such a step might result from nonlinear summation of subthreshold inputs from sources other than the T fibre, including for instance undetermined central influences and/or a small S fibre contribution. Since the voltage–frequency curves of Fig. 4B were obtained with the receptor muscle at its maximum *in situ* length (12 mm in this preparation), the S fibre would have been considerably depolarized, probably beyond its activation threshold for Pm1 (see below). In any event, the fact that hyperpolarizing the T fibre from its 'resting' membrane potential suppresses the receptor length-dependent tonic discharge of Pm1 (cf. inset to Fig. 4B) clearly implicates the T fibre as providing the dominant, if not the sole, sensory drive to Pm1 – as also to Pm2 and Pm3.

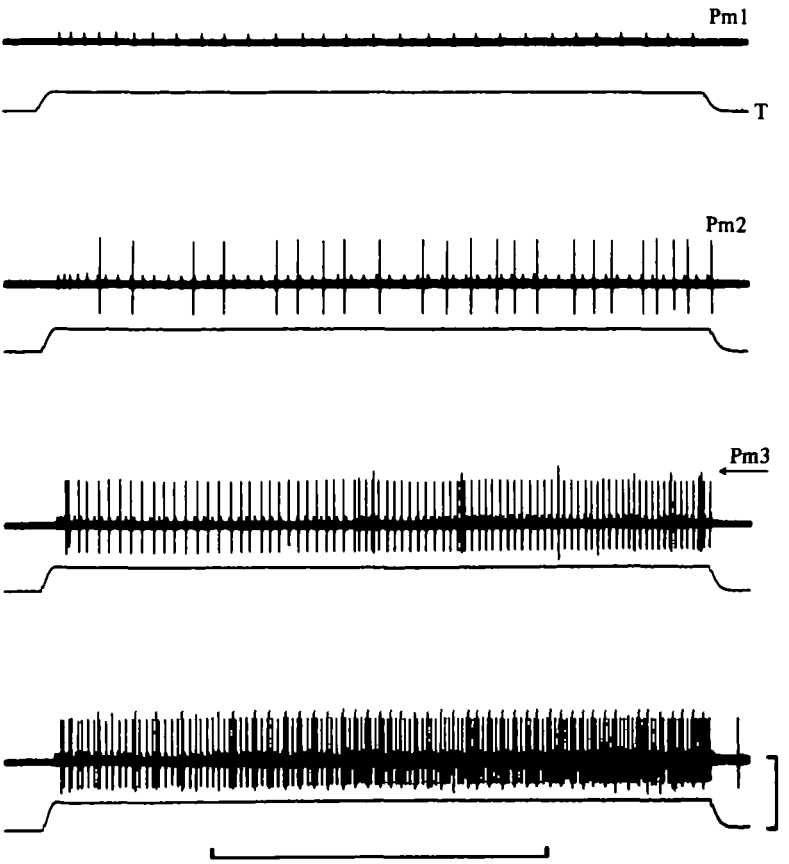

Fig. 2. A series of depolarizing, constant current pulses of increasing intensity injected into the T sensory fibre (lower traces – T fibre potential change) causes a progressive recruitment of promotor neurones Pm1, Pm2 and Pm3, together with a rapid increase in discharge frequencies (cf. Fig. 3B – same experiment). Calibrations: 1 s, 5 mV.

(b) Input–output relations of the S fibre to motoneurone pathways

Depolarizing the S fibre (Fig. 5A) elicits firing of the same three motoneurones as are reflexly activated by stretch alone or by T fibre depolarization. However, the membrane potential threshold for activation of motoneurones by current induced S fibre depolarizations are usually considerably higher than those for the T fibre. Not only are the activation thresholds higher, but the maximum slopes or 'gains' of the voltage–frequency relationships of the individual reflex pathways are much less for the S fibre than they are for the T fibre (Fig. 5B). This is particularly striking for Pm1, and when direct comparisons are made between the S and T fibre induced reflexes in the same preparation within a short period (Fig. 6).

In further contrast to the situation in the T fibre, hyperpolarizing current, however strong, injected into the S fibre has no effect on any ongoing tonic promotor activity. This is perhaps not surprising, however, since the S fibre depolarization thresholds for promotor activation were invariably well beyond its 'resting potential' at RM lengths within the *in situ* physiological length range (Fig. 6). Indeed, in most experi–

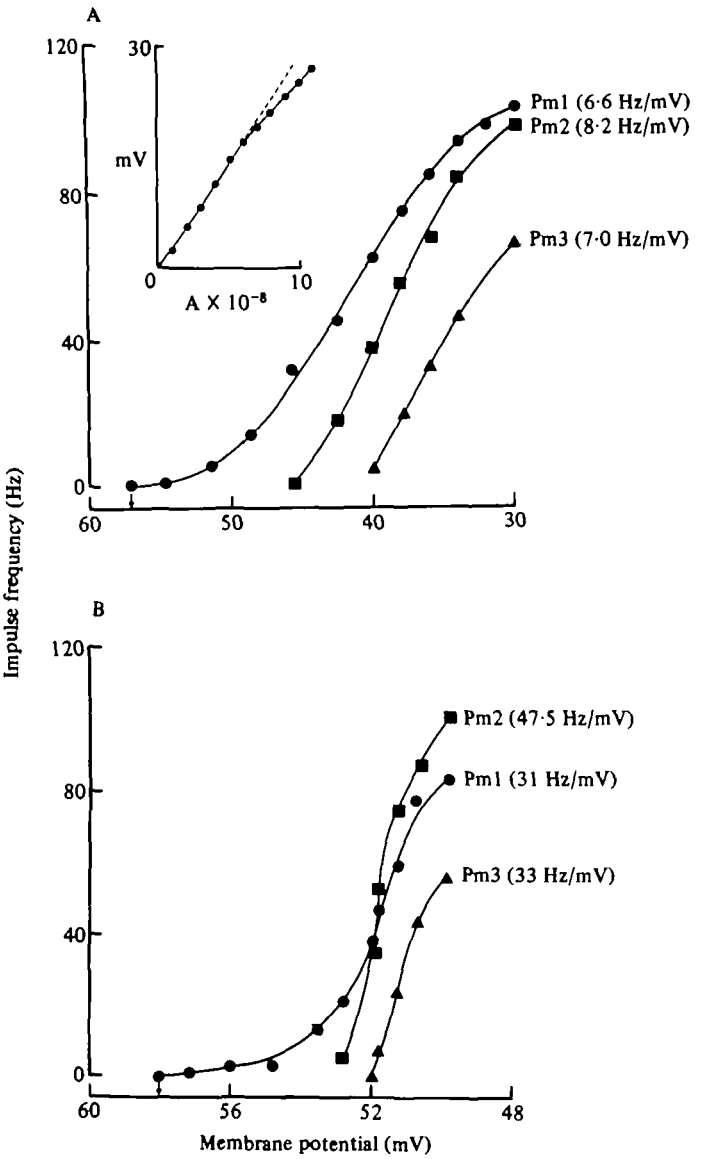

Fig. 3. The input–output relationships of the T fibre to promotor motoneurones Pm1, Pm2 and Pm3. Stated values are from the part of the curves giving the steepest slope. (A) The relationship is typically sigmoid with similar slopes for the three motoneurones. Inset graph (linear scales) shows the current–voltage relationship for the T fibre, in this case showing a slight departure from linearity. The resting membrane potential (arrowed on abscissa) of 57 mV pertains to a receptor muscle length of 9 mm. (B) This preparation, with a T fibre electrotonic length of 0·35 (cf. Table 1), yielded the most sensitive input–output relationships for this reflex system. The resting membrane potential of 58 mV pertains to a receptor length of 6 mm (maximum *in situ* RM length, 11·0 mm).

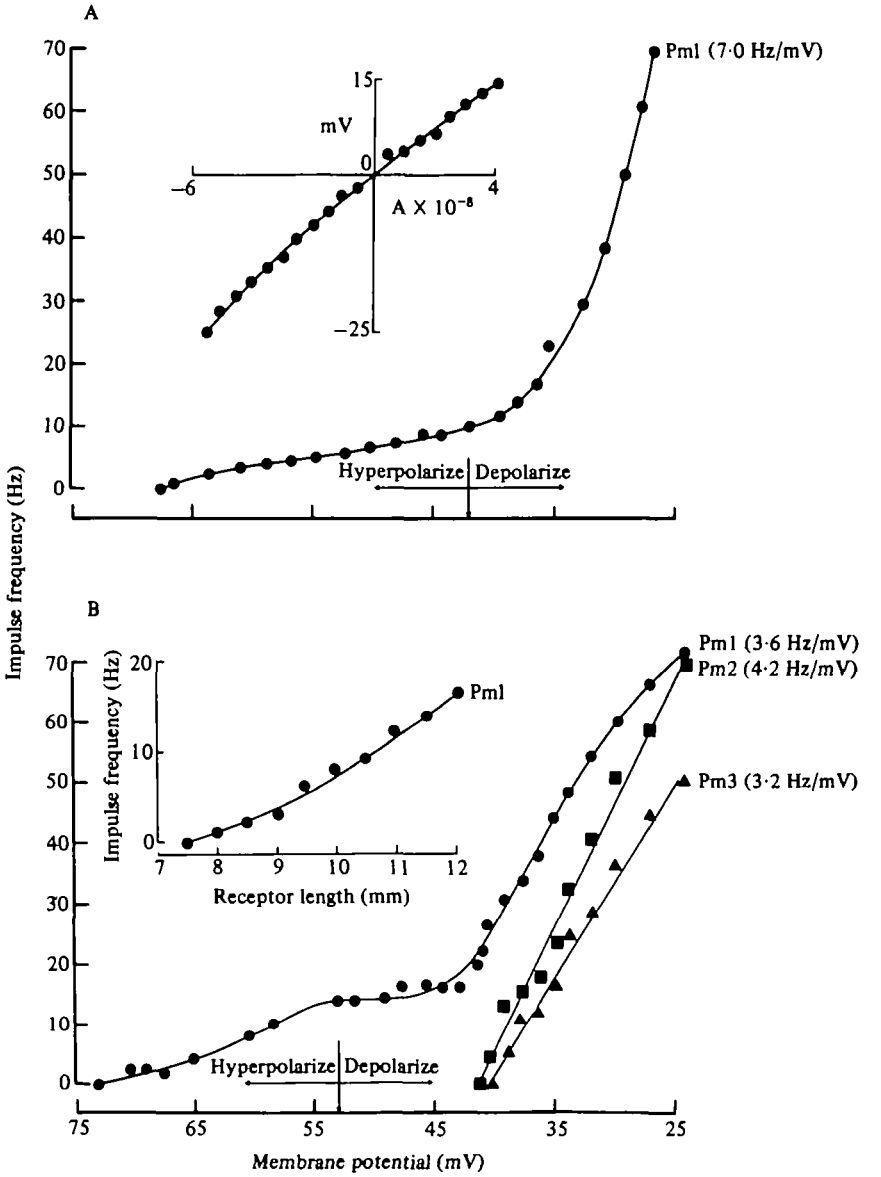

Fig. 4. The tonic firing frequency of fibre Pm1 is added to by T fibre current-induced depolarization, and abolished by T fibre hyperpolarization. (A) A 'smooth' transition in Pm1 impulse frequency occurs on either side of the 'resting' membrane potential (42 mV at a RM length of 11·0 mm) following depolarizing and hyperpolarizing current pulses. The inset graph (linear scales) illustrates the current–voltage relationship of the T fibre over the same current range. (B) The 'activation' threshold for fibre Pm1 is 4–5 mV more depolarized than the T fibre 'resting' potential of 53 mV at the fully extended *in situ* receptor muscle length of 12 mm. The significance of this 'step' in the voltage–frequency relationship is discussed in the text. Note that the input–output relationships of fibres Pm2 and Pm3 in this preparation are both approximately linear. The inset graph relates Pm1 tonic frequency to RM length in the same preparation.

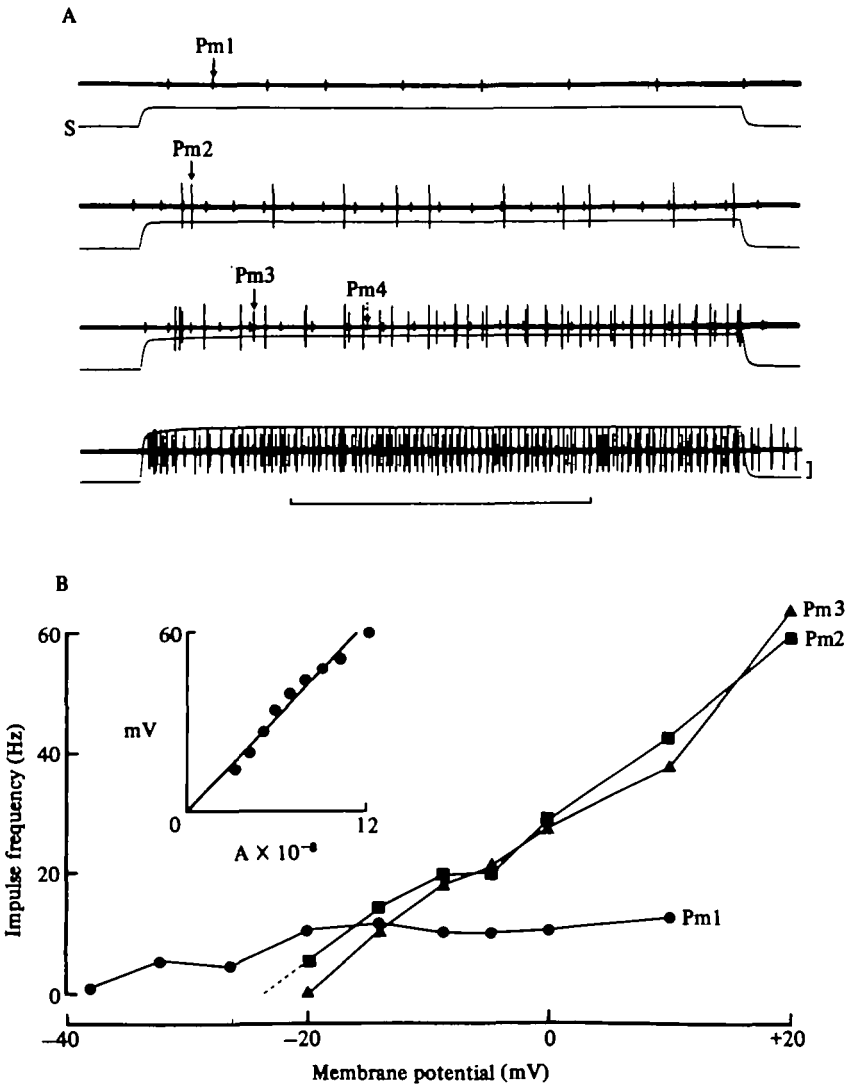

Fig. 5. Depolarization of the S sensory fibre by constant current pulses leads to reflex activation of promotor motoneurones. (A) Graded depolarizations of the S fibre results in the successive recruitment of Pm1, Pm2 and Pm3 (solid arrows). Note the recruitment of a fourth motoneurone (not plotted in B) in the third record (hatched arrow), with impulses smaller in amplitude than Pm1 (cf. Fig. 10). Calibrations: 1 s, 20 mV. (B) Reflex discharge frequencies of Pm1, Pm2 and Pm3 from the same experiment as A, related to the S fibre membrane potential. Inset graph (linear scales) plots the depolarizing current–voltage relationship for the S fibre over the same voltage range. The 'resting' membrane potential at the moderate receptor length of 9·0 mm (*in situ* maximum: 12·0 mm) was recorded as 50 mV, and the electrotonic length of this S fibre was 0·40 (cf. Table 1).

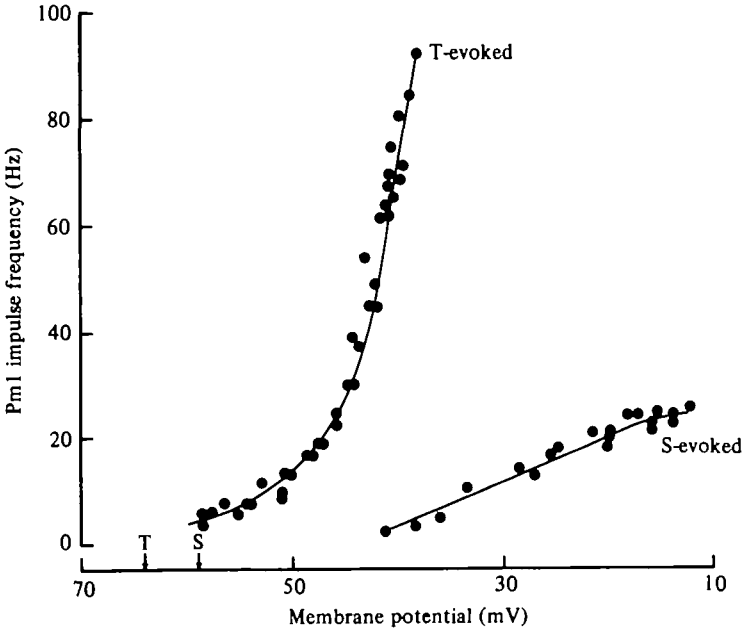

Fig. 6. The impulse frequency of motoneurone Pm1 related to the S and T fibre membrane potentials obtained by depolarizing current injections in the same preparation. The resting membrane potentials (arrowed on abscissa) of 59 mV (S) and 64 mV (T) pertain to RM lengths of 10·0 and 11·0 mm respectively. Electrotonic lengths (cf. Table 1) were 1·11 (S) and 0·74 (T). Note that Pm1 activation via the S sensory fibre required a depolarization of at least 18 mV even at the extended RM length of 10·0 mm (*in situ* maximum, 11·0 mm).

ments these thresholds were higher even than the peak potential achieved by the stretch-induced S fibre receptor potential at maximal receptor lengths (except at high stretch velocities). These observations are consistent with the view expressed in the previous paper (Cannone & Bush, 1980*a*), that the S fibre probably does not contribute directly to the stretch reflex. However, the presence of (direct or indirect) synaptic connexions between the S fibre and promotor motoneurones Pm1–3, implied by the excitatory action of (strong) S-fibre depolarization, leaves open the possibility of summation of S and T inputs to these MNs *in vivo*, particularly at the more extended receptor lengths. This possibility is reinforced by the observation that the only experimental S fibre whose depolarization threshold for Pm1 activity was within the range of stretch-evoked receptor potentials (Fig. 5B), was also the only one with a high length constant (preparation 5, Table 1). This allows greater confidence in the accuracy of the measurements on this preparation – notwithstanding the still high activation thresholds for fibres Pm2 and Pm3, of 25–30 mV more depolarized than the −50 mV resting potential recorded at the moderate receptor length of 9 mm. The question of possible summation of S and T fibre reflex effects will be discussed later.

Table 1. *Length constants* (λ) *and dimensionless electrotonic lengths* (L) *of S and T sensory fibres:* $L = l/\lambda$ (*Rall*, 1969), *where l is taken as the length of the nerve cylinder from the receptor muscle to the edge of the thoracic ganglion, and* λ *is calculated on simple infinite cable assumptions*

(Although data for the S fibre is lacking for preparation 3, it is included since it yielded the highest λ value for the T fibre.)

Preparation	S and T fibre lengths (l, mm)	T fibre		S fibre	
		λ (mm)	L	λ (mm)	L
1	5·5	7·46	0·74	4·95	1·11
2	5·2	4·96	1·05	3·04	1·71
3	4·8	16·02	0·30	—	—
4	4·5	15·60	0·29	3·28	1·37
5	4·4	8·01	0·55	10·97	0·40
6	4·2	11·88	0·35	4·89	0·86
7	—	10·35	—	1·44	—

(*c*) *Afferent hyperpolarization effects on the stretch reflex*

The reflex response to a normally effective stretch of the receptor muscle can be partially or completely suppressed by applying graded hyperpolarizations to the T fibre during the period of the stretch stimulus (Fig. 7A). In contrast, hyperpolarizing the S fibre has no effect upon the stretch-evoked reflex, however large the hyperpolarizing current (Fig. 7B). This constitutes decisive evidence that it is the in-series T fibre rather than the in-parallel S fibre that provides the principal if not sole sensory drive for the stretch reflex (see Cannone & Bush, 1980*a*). As expected, the amplitude of the afferent fibre response to stretch increases progressively with increasing hyperpolarization, since the displacement of the membrane potential is away from the equilibrium potential for ions contributing to the receptor potential, and the 'driving force' (Hodgkin & Huxley, 1952) therefore increases.

An intriguing effect of T fibre hyperpolarization in preparations exhibiting spontaneous, centrally originating promotor MN activity is the evident suppression, partial if not complete, of a centrally originating burst of impulses (Fig. 7C, 1st record). This again raises the question of how hyperpolariziation of the T fibre exerts an inhibitory effect, in this case upon a centrally determined firing pattern rather than upon a peripherally determined tonic one (see Discussion).

(2) *Constant current effects at different receptor lengths*

For both the S and T fibres, injecting depolarizing current pulses of constant intensity at different resting lengths of the receptor muscle emphasizes the role of *level* of depolarization rather than voltage change *per se* in determining the reflex response. Thus an identical current pulse (or stretch: Cannone & Bush, 1980*a*) applied at an extended receptor length results in a stronger reflex response compared to that evoked at a more relaxed receptor length, manifested as an increase in discharge frequency and number of activated promotor MNs. This is notwithstanding the fact that current pulses of constant intensity injected into the afferent fibre at increasing receptor lengths result in membrane potential changes of *decreasing*

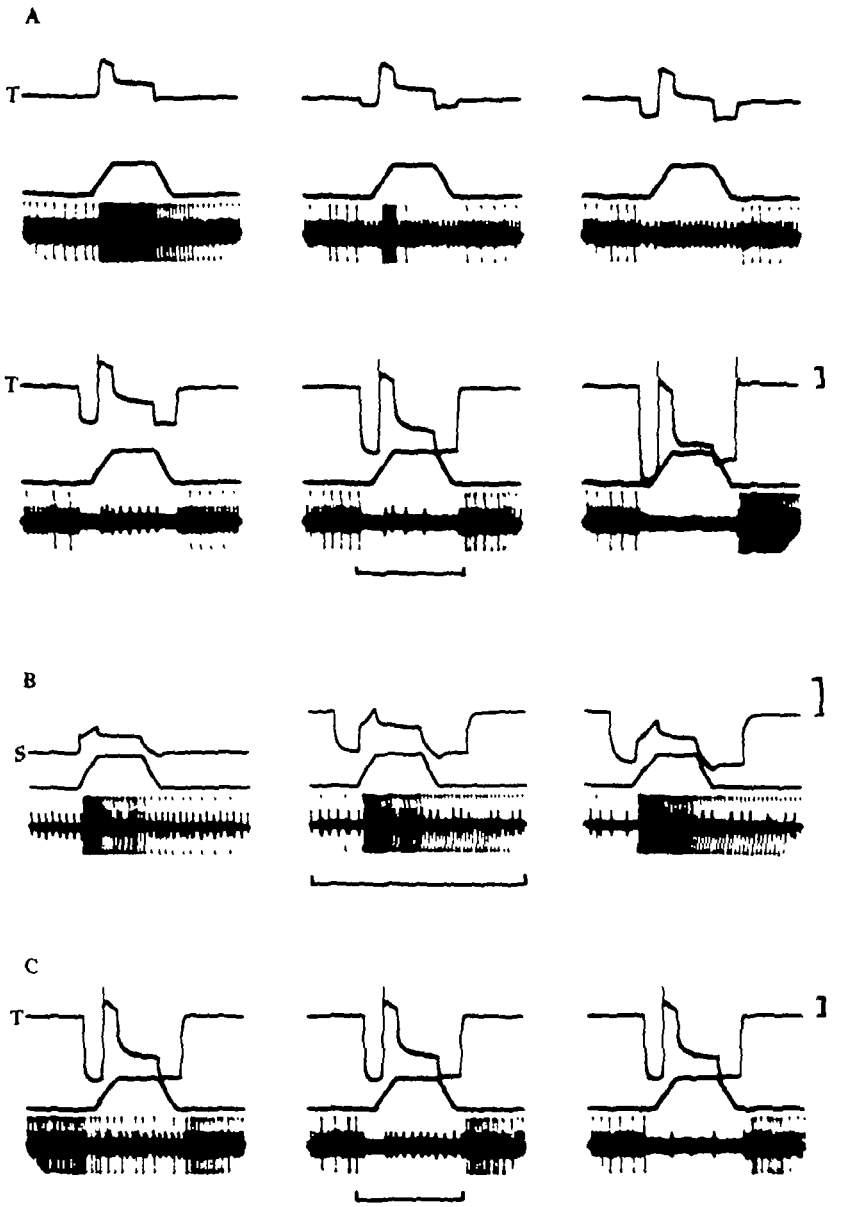

Fig. 7. The effects of hyperpolarizing the T and S fibres. (A) Superimposing graded intensity, hyperpolarizing current pulses into the T fibre upon identical trapezoid stretch stimuli (middle traces), progressively abolishes both the reflex response to stretch and tonic activity of Pm1 and Pm2. (B) With the T fibre intact, superimposing hyperpolarizing current pulses into the S fibre upon trapezoid stretch stimuli fails to affect the reflex response. (C) A series of three stretches superimposed upon hyperpolarizing pulses which are of adequate intensity to suppress the reflex response to stretch (3rd record). A spontaneous, centrally originating burst of impulses (1st record) is partially suppressed during the period of the current pulse (compare a pre-stimulus Pm2 frequency of 51 Hz reduced to 11 Hz during the hyperpolarization in the 1st record, with a pre-stimulus frequency of 11 Hz in the 3rd record). Calibrations: 1 s, 20 mV.

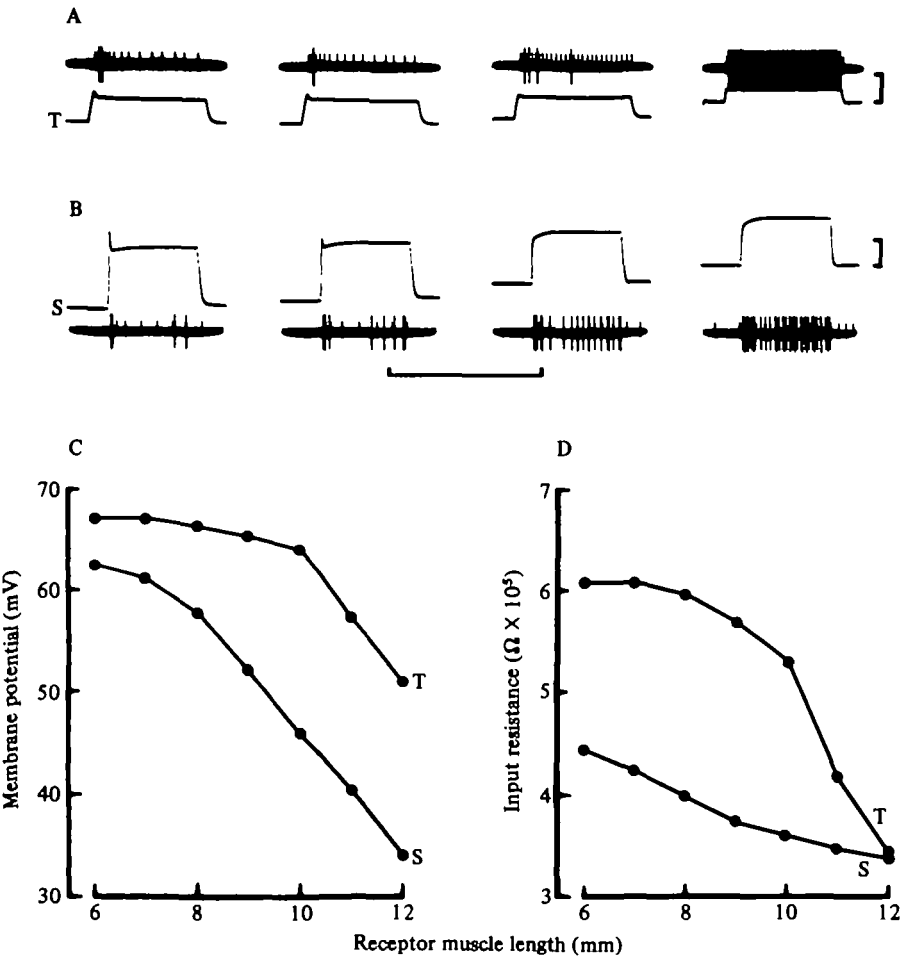

Fig. 8. The effects of constant current pulses applied to (A) the T and (B) the S fibre at increasing receptor lengths. Recordings and graphs (C, D) are directly comparable (same preparation, identical receptor lengths) except that 30 nA were delivered to the T fibre and 120 nA to the S fibre. Since the voltage excursions become progressively smaller, it is apparent that the input resistance of both sensory fibres decreases with an increase in receptor length. Note that the initial 'spiky' transient in the sensory response, if present, is larger the greater the initial membrane potential and that this feature of the sensory response is transmitted to the reflexly activated motoneurones. Calibrations: 1 s, 20 mV. (C) Graph of resting (initial) membrane potential of the S and T fibres related to receptor length. (D) Graph of input resistance of S and T fibres (corrected for λ, taking into account the distance between current-passing and recording electrodes) related to receptor length.

magnitude (Fig. 8A, B). This implies a decrease in input resistance of the fibre with increase in receptor length (Fig. 8D), due presumably to an increase in conductance of the transducer terminals with stretch (cf. Lowe, Bush & Ripley, 1978), as in other stretch receptors (e.g. Brown, Ottoson & Rydqvist, 1978). The functional significance of this effect can be appreciated when considering the reflex response to receptor displacement of a particular magnitude superimposed upon a variable receptor length, or thoracic–coxal joint angle (Cannone & Bush, 1980*a*).

(3) *Adaptation of reflex responses*

Adaptation of the sensory response at presynaptic stages (mechanical and transduction levels) has been dealt with previously (Bush & Roberts, 1971; Bush & Godden, 1974; Bush, Godden & Macdonald, 1975; Cannone & Bush, 1980*a*). There remains the possibility of postsynaptic adaptation at the levels of the central synapses in the reflex pathways and the site of impulse generation in the motoneurones. In many preparations, fibre Pm1 is tonically active at frequencies related to the length of the receptor muscle, and fibres Pm2 and Pm3 may also fire tonically, evidently at least partly as a result of afferent input within the physiological length range of the receptor muscle (fig. 3, Cannone & Bush, 1980*a*). It is therefore important to determine the ability of the central transmission process to maintain a constant frequency impulse output in the motoneurones in response to an invariant membrane potential in the sensory fibres, particularly in the case of the T fibre. A postural role for the peripherally driven 'tonic' motoneurone output cannot be convincingly argued if in fact there is a significant decline in impulse frequencies in the face of a constant sensory input.

It is clear that the motoneurone response is particularly sensitive to the early phase of a current induced depolarization (Figs. 8, 9). Even when an initial fast transient is absent in the intracellular recordings, as in the T and S fibre responses at the longer receptor lengths (Fig. 8), the reflex response nevertheless shows a high-frequency burst during the early phase of current pulse. This is commonly followed by some depression in frequency before a second increase to a relatively constant level maintained over a longer time course. Further, during the first few seconds of a prolonged (e.g. 20 s duration) constant current pulse, the T fibre membrane potential may decline by several millivolts (Fig. 9A) so that an accurate indication of the rate of central adaptation of motoneurone impulses during this early phase cannot be obtained from this data. With the T fibre membrane potential fairly stable during the remainder of a 20 s pulse however, the impulse frequency of all three promotor MNs under consideration (Pm1, Pm2 and Pm3) still declines steadily. The greater the *level* of depolarization of the T fibre, and consequently the higher the resulting reflex frequencies, the greater the rate of adaptation in all three MNs (Table 2). With smaller, more physiological depolarizations of the T fibre, however, such as would presumably occur following slight postural adjustments of the thoracic–coxal joint in the living animal, the rate of adaptation may be very small (Fig. 9B).

The three promotor MNs, Pm1, Pm2 and Pm3, differ in their rates of adaptation, these differences being more evident with larger current induced depolarizations (especially at unphysiologically high levels of depolarization). Thus, both within and beyond probably normal physiological limits, Pm1 shows the least and Pm2 the greatest rate of adaptation to constant current pulses. Accordingly, of the three motoneurones Pm1 has properties best fitted for a tonic role, a role appropriate for the maintenance of the postural stance of the thoracic–coxal joint. The continued discharge of fibres Pm2 and Pm3 over equally long periods of time with only slightly greater rates of adaptation, suggests that they too may contribute towards this function. This view is reinforced by the observation in one particularly 'excitable' preparation (see Fig. 3, Cannone & Bush, 1980*a*) of maintained tonic discharge of all three motoneurones at the maximum *in situ* length of the receptor muscle.

Table 2. *Table relating the 'adaptation' rates of fibres Pm1, Pm2 and Pm3 to the T fibre membrane potential, at two different receptor lengths and stimulating current intensities. (Same preparation as Fig. 9A)*

Length of receptor muscle (mm)	Current (nA)	T fibre potential: Pre-stimulus (mV)	T fibre potential: During stimulus (mV)	Rate of frequency decline from 2–20 s (Hz/s): Pm1	Pm2	Pm3
7·0	120	50	14·5	1·0	2·0	1·33
11·0	70	40	19·5	0·79	1·11	0·94

(4) *High-threshold promotor motoneurones showing rapid adaptation*

In a few exceptionally 'lively' preparations, receptor stretch alone has revealed that motoneurones of higher thresholds than Pm3 can be activated (Fig. 10A). These fibres, although active only during the positive dynamic phase of the trapezoid stimulus, cannot on this basis alone be characterised as rapidly adapting or phasically responding. Injection of current pulses into the S and T fibres has proved more valuable in establishing (*a*) the total number of motoneurones that may be reflexly activated, (*b*) the differential recruitment of certain of these motoneurones by depolarization of the S and T fibres, and (*c*) the phasic nature of the response characteristics of at least some of these motoneurones.

A fourth promotor MN can occasionally be discriminated in the reflex responses to moderate depolarizing currents applied to either S or T fibres, in addition to the three commonly active MNs Pm1–3 (Fig. 10B, D). The recorded impulses of this motoneurone (Pm4) are smaller than those of Pm1, so that relatively high gain, good signal-to-noise recordings are needed to see it. It has a higher threshold than Pm1–3 for either S or T fibre depolarization (hence Pm4), and in the absence of current stimuli has never been observed to be tonically active within the physiological range of receptor lengths.

Large depolarizing currents injected into the T fibre will, in some fresh and active preparations, evoke discharges of large-amplitude spikes that decay in frequency fairly rapidly. By simultaneously recording T fibre membrane potentials, promotor nerve activity, and intracellular junctional potentials in different promotor muscle fibres, it can be clearly established that these larger spikes represent further MNs recruited in addition to Pm1–4, and are not merely the result of compounding effects at the high reflex frequencies in force (Fig. 10C). At least three separate junctional events, each attributable to one of the additional large-amplitude impulses (termed 'Pm5', 'Pm7' and 'Pm8'; pairs of solid arrows in each of three different sections of the record), can be identified in the same polyneuronally innervated muscle fibre. Moreover, in this recording a further motoneurone ('Pm6') can be identified on the basis of impulse amplitude (broken arrows) although it does not appear to innervate this particular muscle fibre. Other muscle fibres recorded from in the same preparation all responded with large amplitude junction potentials to one or more of these additional large amplitude impulses. Significantly, whereas fibres Pm1, Pm2 and Pm3 discharge at high maintained frequencies throughout the long duration current pulse, the large amplitude impulses adapt rapidly in frequency and are absent altogether at the end of the stimulus pulse.

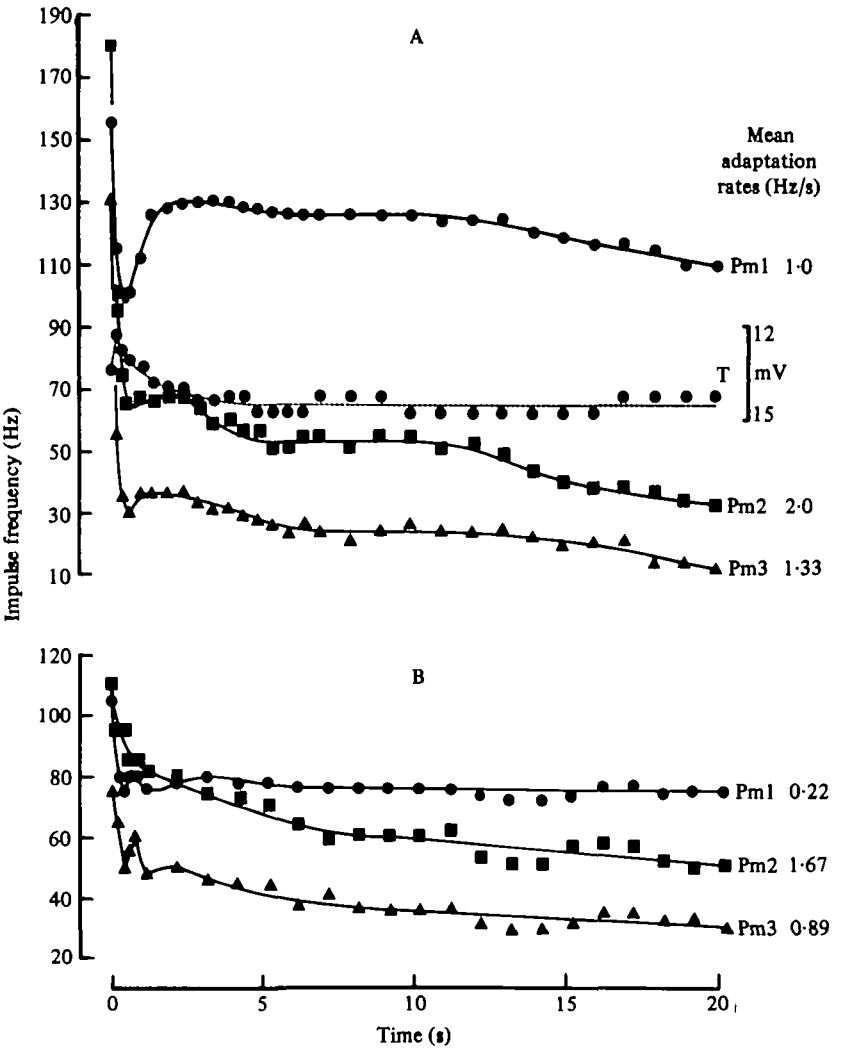

Fig. 9. Adaptation in the discharge frequencies of motoneurones Pm1, Pm2 and Pm3 in two preparations (A, B) during a constant intensity depolarizing current pulse of 20 s duration delivered to the T sensory fibre. Due to the effects of the initial 'spiky' transient (refer to Fig. 8) the first two seconds are not included in the determination of mean adaptation rates indicated on the right for each motoneurone. In (A) the T fibre membrane potential is also plotted (dashed line, ordinate on right); in (B) this is omitted since it was not accurately determined, though as in (A) it was stable from 2–20 s.

Thus the same T fibre depolarization recruited at least eight different motoneurones (Pm1–8), each identifiable on the basis of clearly distinguishable impulse amplitudes and/or muscle junction potentials. It is also clear that for many muscle fibres of the promotor muscle it is the high threshold, rapidly adapting, large-amplitude impulses (presumably large-diameter motoneurones) that produce the most marked electrical and mechanical responses. The large-amplitude muscle junction potentials may rapidly summate at the beginning of the current pulse, and may even cause a depolarization of the muscle fibre membrane supra-threshold for the generation

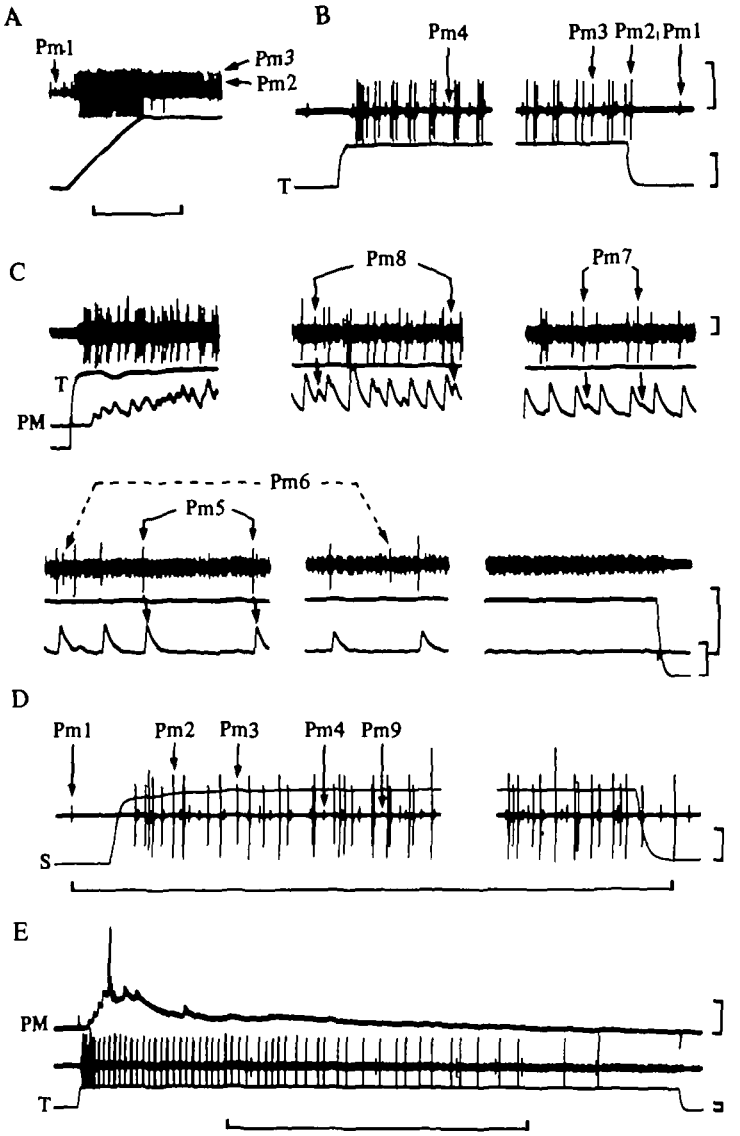

Fig. 10. Promotor motoneurones reflexly activated by (A) receptor stretch, (B, C, E) T fibre depolarization and (D) S fibre depolarization. (A) In exceptionally 'lively' preparations receptor stretch activates not only Pm3, but also motoneurones giving larger amplitude impulses. (B) Moderate T fibre current-induced depolarizations often recruit four motoneurones, Pm4 impulses being smaller in amplitude than those of Pm1–3. (C) Large-amplitude depolarizations of the T fibre recruit at least four additional motoneurones (Pm5–8) with large amplitude impulses. Note that (B) and (C) are from the same preparation, with the extracellular promotor recording in (B) being 2·5 times the gain of that in (C). Simultaneous intracellular recording of junctional potentials in a promotor muscle fibre (PM) enables unequivocal matching of junctional potentials to motoneurone impulses (pairs of solid arrows). Impulses of a motoneurone not innervating this particular muscle fibre are indicated by dashed arrows. (D) S fibre depolarization recruits Pm1–4 and a further motoneurone (Pm9) with impulse amplitude less than half that of Pm4, and apparently unique to the S fibre reflex. (E) The large-amplitude Pm5–8 impulses of (C), although rapidly adapting compared to those of Pm1–3, may nevertheless cause a twitch contraction of a promotor muscle fibre by virtue of facilitation and summation of the large-amplitude junctional potentials to threshold for the generation of a muscle action potential (PM). Calibrations: 1 s (bar under D also applies to B and C), 20 mV, 0·5 mV (extracellular).

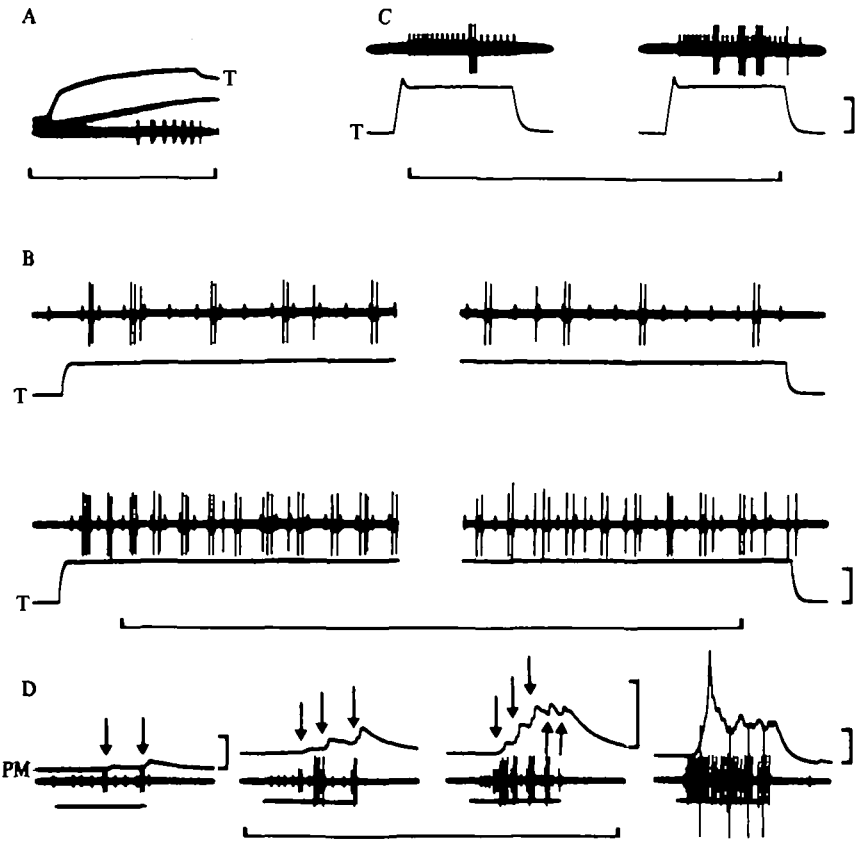

Fig. 11. Pattern generation in promotor motoneurone Pm2, usually in the form of discrete impulse couplets, evoked by receptor stretch (A) and by injecting current pulses into the T fibre (B–D). In (A) the upper trace is the frequency meter output of the impulse discharge (lower trace). T fibre positive dynamic component and stretch monitor are also recorded. (B) Recordings of the effects of two different intensity current stimuli showing both 'rigid' and 'complete' patterning of Pm2 impulses (refer to text and Figs. 12, 13 for explanation of terms). (C) An unusual form of Pm2 impulse patterning in which well-separated bursts of 2–4 impulses rather than couplets are evoked. (D) The effect of couplet patterning on the electrical responses of a promotor muscle fibre (PM). Current stimuli to the T fibre indicated by bars (lower trace). The muscle-fibre membrane undergoes a stepwise depolarization which, at adequately high couplet frequencies, may result in a muscle action potential (last record). Arrows above indicate couplets; arrows below indicate single (uncoupled) Pm2 impulses. Note that for the two uncoupled impulses, although the junction potentials are facilitated and large, the overall muscle depolarization stabilizes, pointing to the efficacy of couplet patterning. Calibrations: 1 s, 20 mV.

of a muscle action potential (Fig. 10E), in turn resulting in a visually apparent twitch-like contraction of the muscle fibre.

Of the eight motoneurones (Pm1–8) recruited by T fibre depolarization, four (Pm1–4) are common to S fibre depolarizations. In addition, the S fibre uniquely activates another high threshold motoneurone ('Pm9'), impulses of which are smaller than those of Pm4 (Fig. 10D). The four large-amplitude, rapidly adapting impulses (Pm5–8) detected by strong T fibre stimulation are not evoked by S-fibre depolarization.

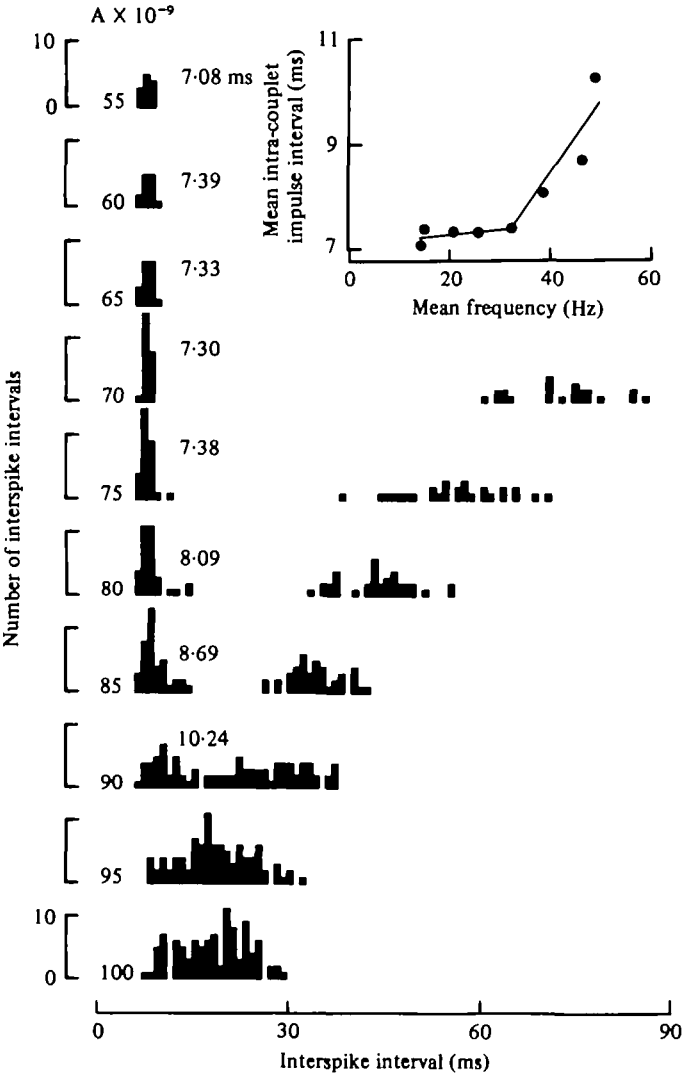

Fig. 12. A series of histograms illustrating some features of motoneurone Pm2 impulse patterning evoked by graded intensity, current-induced depolarizations of the T sensory fibre. For each current pulse within the range 55–100 nA (column on left) the number of Pm2 interspike intervals (ordinates) is related to the interspike interval (abscissa). The intra-couplet impulse interval populations appear on the left with the mean values in ms indicated (column on right). The inter-couplet impulse interval populations appear on the right for current pulses of 70 nA and larger. With the larger current stimuli the intra- and inter-couplet interval populations become inseparable. Records in Fig. 11B pertain to this graph. Inset graph emphasizes the abrupt increase in intra-couplet impulse interval as related to mean impulse frequencies.

(5) *Patterning of Pm2 motor impulses*

The generation of a patterned discharge has been well documented for a variety of crustacean motor systems (e.g. Wilson & Davis, 1965; Burrows & Horridge, 1968; Gillary & Kennedy, 1969*a*). It has also been established that grouped motor impulses can produce enhanced postsynaptic effects in crustacean muscle fibres, compared to

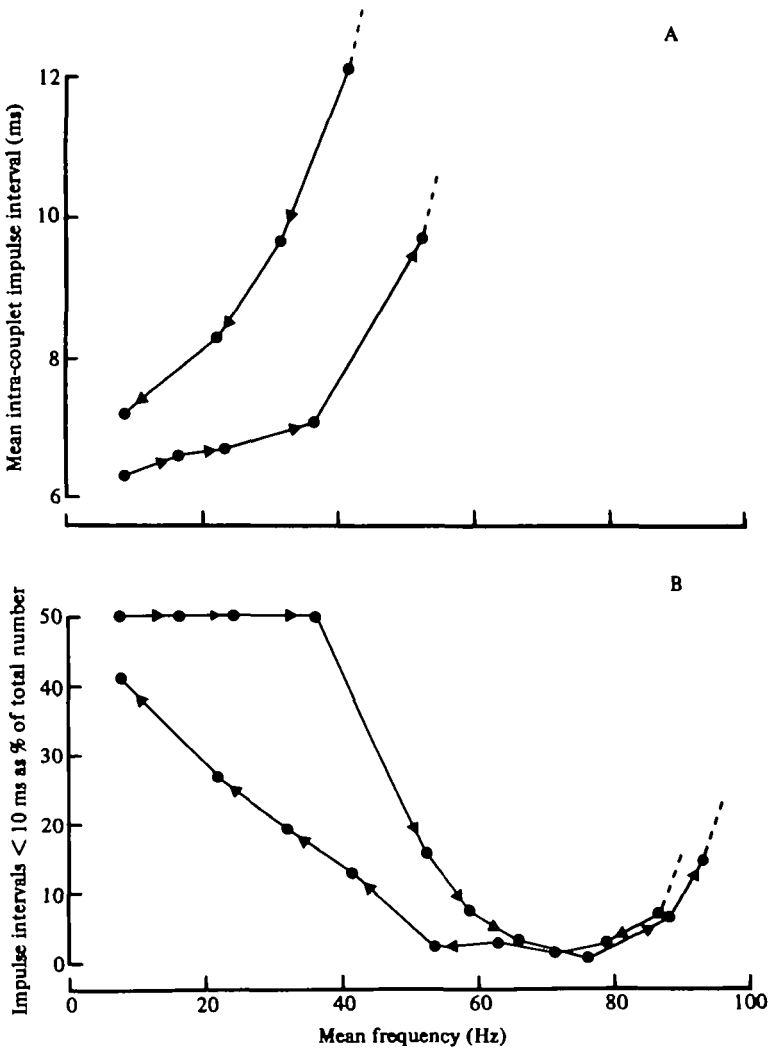

Fig. 13. Graphs illustrating the two features of patterning and their breakdown following an increase in mean frequency of Pm2 discharge (not the same experiment/series as Fig. 12) and the re-establishment of both 'rigid' and 'complete' (refer to text for explanation of terms) patterning following a decreasing series of current intensities, and hence mean Pm2 frequencies. (A) Rigid patterning abruptly breaks down at mean frequencies of 35–40 Hz, but the re-establishment of rigidity does not seem to be linked to mean Pm2 frequencies, a marked hysteretic effect being apparent (direction of change in stimulus regimen indicated by arrows). Since this series of stimuli included several of high intensity during which it was not possible to distinguish between intra-couplet and inter-couplet impulse intervals, the graph is 'open loop'. (B) Complete patterning similarly breaks down at a mean frequency of 35–40 Hz, and re-establishment thereof likewise shows a hysteretic behaviour unrelated to mean frequency.

evenly spaced unpatterned outputs at the same average frequency (Wiersma & Adams, 1950; Ripley & Wiersma, 1953; Gillary & Kennedy 1969*b*). In the present study, evidence has been obtained of pattern generation in fibre Pm2, though the effects of such patterning on the development of tension in the innervated muscle fibres has not yet been evaluated. Pattern generation was observed in relatively few preparations,

Table 3. *Variability in the reflex response to S fibre depolarizations, evoked by injection of repetitive* (20 *s intervals*), *constant intensity* (10 *nA*) *current pulses at the same receptor length* (11 *mm*, in situ *maximum length*)

(The receptor length imposed tonic frequency of Pm1 at this length was 8–9 Hz. Pm2 was not tonically active).

Pre-stimulus frequency (Hz)	Frequencies during current pulse (Hz)	
Pm1	Pm1	Pm2
16	21	10
11	17	2·5
9	14·5	2

suggesting that the central mechanism or motoneurone properties responsible for pattern generation are particularly labile under the experimental conditions in force.

Patterned output in response to either receptor stretch (Fig. 11 A), or to depolarizing currents injected into the T sensory fibre (Fig. 11 B), is usually in the form of impulse couplets with an interval of 7–8 ms between impulses of a couplet. Occasionally, however, patterning may take the form of well-separated bursts of impulses, each burst comprising 2–4 impulses (Fig. 11 C). Recording the electrical response of a promotor muscle fibre to a current induced patterned output of Pm2 has shown a step-like depolarization of the membrane which may, provided high couplet frequencies are evoked, lead to the generation of a muscle action potential triggering a twitch contraction of the muscle fibre (Fig. 11 D).

Graded depolarizations of the T fibre allow a quantitative analysis of impulse patterning (Fig. 12). This series of histograms portrays the intervals between Pm2 impulses for a series of current injections of 55–100 nA. In this particular experiment, with mean impulse frequencies of up to 32 Hz (55–75 nA stimulus pulses), an overall mean intra-couplet impulse interval of 7·3 ms with little variation was retained. Larger currents, and therefore more elevated mean impulse frequencies, result not only in the mean inter-couplet impulse intervals becoming progressively shorter, but also in a gradual increase in the *intra*-couplet interval. Eventually the two inter-spike interval 'populations' (intra-couplet and inter-couplet intervals) merge, at a current intensity of 95 nA, obscuring the couplets altogether. Thus, the higher the mean impulse frequency the less rigid the interval between impulses of a couplet.

While these experiments do not reveal the mechanism responsible for pattern generation in motoneurone Pm2, they do indicate that patterning can be experimentally altered in a manner that possibly also occurs *in vivo*. Moreover, patterning in this sytem is almost certainly a property of the motoneurone itself, since it is restricted to only one of eight or more reflexly activated motoneurones.

(6) *Centrally imposed variability in the reflex response to current injections*

More so than in the case of the T fibre, S fibre depolarization often leads to variable reflex response frequencies even when spontaneous, centrally originating impulse activity is not evident. This is more readily apparent when constant intensity current pulses are injected repetitively at the same receptor muscle length. For example, an overt pre-stimulus central drive onto Pm1 is reflected not only in an increased

discharge of Pm1 during a current stimulus, but also in the effect of elevating Pm2 discharge frequencies where no pre-stimulus discharge of this MN occurred (Table 3). A likely explanation for this behaviour is that the central nervous system imposes a variable, often sub-threshold excitatory influence upon promotor MNs. This effect can therefore be seen to be related to the previously described loose coupling between central drive and the phenomenon of post-stimulus excitation (Cannone & Bush, 1980*a*). Such variability in the reflex response, occurring as it does *during* sensory fibre activation, can have important implications if it can be shown to occur in the *in vivo* situation.

DISCUSSION

The experiments described here and in our previous paper (Cannone & Bush, 1980*a*) make it abundantly clear that there is a quantitative relationship between sensory response and reflex strength. By implication, as the T–C joint angle increases on remotion, there is both a recruitment of reflexly active promotor MNs and an increase in their discharge frequencies, similar to the situation in the more distal leg segments (e.g. Evoy & Cohen, 1969). The quantitative basis for these effects is the level of depolarization achieved by the in-series T sensory fibre. However, such a simplistic view fails to take into account the fact that muscle receptor organs not only respond to joint movement, but also to efferent modulation of receptor muscle tension. Although in some conditions the S fibre may respond to strong isometric contraction of the receptor muscle (cf. Cannone & Bush, 1980*c*), it is the T fibre that is specially responsive to active tension (Bush & Godden, 1974) generated by impulse discharge in the two excitatory motoneurones innervating the receptor muscle (Bush & Cannone, 1974).

A reflex role for the S fibre?

In general, signal attenuation in the S fibre was found to be much greater than that in the T fibre, with an electrotonic length (L) of 0·4 (λ, 11 mm) being an exceptional value compared to 'normal' values > 1·0 (λ, 3·5 mm). Earlier determinations of length constants for the S fibre (Roberts & Bush, 1971; Bush, 1976) were similarly significantly lower than our upper values for the T fibre (λ, 16 mm; L, 0·3). The apparent non-contribution of the S fibre to the stretch-evoked reflex (Cannone & Bush, 1980*a*; Fig. 7, this paper) needs to be reconciled with the established central connectivity between the S fibre and at least five promotor MNs. Several factors need to be taken into account. Firstly, the progressive decline in reflex excitability of these highly dissected preparations (cf. Cannone & Bush, 1980*a*) would suggest that the thresholds for motoneurone activation by the S fibre *in vivo* could be lower than those determined experimentally. Secondly, although the dynamic components of the stretch-evoked receptor potentials in the S fibre are relatively small in amplitude due to a low sensitivity to velocity of stretch, there is a substantial change in the steady-state membrane potential of the S fibre over the physiological length range of the receptor muscle; this can be as much as 30–40 mV depolarization over an effective length change of 4–6 mm (cf. Fig. 5, Cannone & Bush, 1980*a*). Thirdly, in the one experimental S fibre for which a relatively large length constant was found (λ = 11

mm, cf. Table 1), stretch-evoked receptor potentials at the more extended receptor lengths achieved a level of depolarization greater than the activation threshold for Pm1 as determined by injecting currents into the same S fibre. Finally, S fibre connectivity has been established for the low threshold, slowly adapting promotor MNs, Pm1–4, but *not* for the higher threshold, rapidly adapting promotor MNs, Pm5–8. Taking all these factors into consideration, we conclude that the S fibre almost certainly contributes to the resistance reflex *in vivo*. Any such contribution is probably a small one, however, and might well be limited to the more extreme, 'remoted' joint positions when the receptor muscle approaches its fully extended length *in situ*. Moreover, the fact that S fibre connectivity seems to be confined to the lower threshold, slowly adapting promotor MNs suggests that it may be primarily concerned with a postural role of the T-C MRO. In any event, it must be concluded from this and the preceding paper that the promotor resistance reflex *in vivo* is predominantly, but probably not exclusively, mediated by the T fibre. Accordingly the gain and effective range of the reflex will be much more amenable to central control via the receptor motor supply than if the S fibre provided the principal sensory input (cf. Cannone & Bush, 1980*b*, *c*).

Inhibition by T fibre hyperpolarization – a subthreshold effect?

That as many as nine promotor MNs are reflexly recruited by T and S fibre sensory input is beyond dispute. In contrast, it is not clear whether the sensorimotor connexions are monosynaptic and whether they are chemically transmitting (Bush, 1977; Cannone & Bush, 1980*a*). However, in the absence of any evidence to the contrary, chemical synaptic transmission in this reflex system is indicated (Emson, Bush & Joseph, 1976), and monosynaptic connexions occur in *Callinectes* (Blight & Llinás, 1980).

There is considerably less doubt as to the source of the tonic firing of Pm1–3, since T fibre receptor-length-induced depolarization adds to the frequency thereof, and T fibre hyperpolarization decreases this tonic output, at least for Pm1. This latter effect is explicable in terms of our proposal that there is a continuous 'tonic' release of transmitter by the T fibre even at fully relaxed receptor lengths (Cannone & Bush, 1980*a*), in much the same way that locust interneurones release transmitter at their 'resting' potentials (Burrows & Siegler, 1978). However, the reason why T fibre hyperpolarization can suppress the frequency of an apparently centrally originating spontaneous burst of impulses is not immediately obvious if purely chemical synaptic transmission is assumed. In this context too the failure of T fibre hyperpolarization – as during a pronounced negative dynamic response to a trapezoid length change – to affect the 'post-stimulus excitation', a phenomenon coupled to spontaneous central drive (Cannone & Bush, 1980*a*), needs to be accounted for. Although spontaneous bursts in promotor MNs probably do not reflect *in vivo* patterns of centrally determined activity (cf. Cannone & Bush, 1980*a*), reconciling these observations should provide a useful insight into central connectivity patterns and transmission characteristics.

Assuming that synaptic transmission is chemical, the simplest explanation of the suppression of centrally originating bursts of impulses in promotor MNs is that there is a continuous subthreshold excitatory drive onto these promotor MNs by the T

fibre. This is not indicated for the S fibre since there is no effect of S fibre hyperpolarization on centrally originating spontaneous activity. The T fibre drive could conceivably sum with a variable, even subthreshold, central drive, explaining why such central bursts of impulses involve only those promotor MNs which are active in that particular preparation. Hyperpolarization of the T fibre would then remove the peripheral component of synaptic drive onto promotor MNs, thereby effectively raising the threshold for the central component of synaptic drive onto MNs so affected. The concept that a major contribution of sensory input associated with movements is to provide the central nervous system with a source of (generalized) excitatory input from which movement can be 'moulded' (Cohen, 1965) is consistent with the subthreshold excitatory effects proposed here for the T fibre input.

Factors influencing the nature of the reflex response

Adaptation to maintained stretch of the receptor muscle, as evidenced by a decline in the T fibre level of depolarization, can be at least partly attributed to viscoelasticity of the receptor muscle (Bush & Roberts, 1971; Cannone & Bush, 1980*a*). Similarly, receptor muscle properties are responsible for the time-dependent variability seen in repetitive responses to identical stretch stimuli. Thus, all peripheral components contributing to adaptation are duly reflected in the reflex output, the T fibre response waveform with its prominent positive dynamic component dictating a 'phasic' and a 'tonic' character thereto (Cannone & Bush, 1980*a*).

Clearly, information transfer within a non-impulsive sensory neurone is advantageous in the sense that there is no conversion from analogue to digital signalling, and thus no loss of information content at a spike initiation site. Certain constraints are imposed on such a neurone, however, by virtue of the passive electrical properties dictating signal attenuation and distortion with distance. Nevertheless, in a comparative treatment of non-impulsive neurones, Pearson (1976) concludes that in preparations such as the T–C MRO where high-output frequencies are achieved by small changes in the input, the discharge rate of motoneurones can be *precisely* regulated by an input element that is non-impulsive, rather than by a spiking driver neurone producing discrete excitatory postsynaptic potentials. Thus, in a non-spiking sensory system such as the T–C MRO a 'requirement' for high amplification between sensory feedback and reflex response (cf. Fig. 3) could conceivably have contributed to the evolution of this purely analogue sensory signalling system.

The nature of the T–C MRO reflex response may be additionally determined by factors other than the above, and the possible summatory role of the S fibre input. These could include centrally imposed subthreshold excitatory effects imposing a degree of variability on the reflex output to a constant sensory input; the degree of frequency adaptation *within* a particular MN; the extent to which impulse patterning favours muscular contraction compared to a non-patterned impulse regime; and finally, neuromuscular properties and innervation patterns of the individual muscle fibres making up the promotor muscle (Cannone & Bush, unpublished observations). All of these factors could determine to a large extent the efficacy of the resistance reflex.

The MNs supplying the tonic flexor and extensor muscles of the crayfish abdomen, and the 'slow' (tonic) MNs of crustacean leg muscles, are normally concerned with postural regulation and slow movements (Atwood & Walcott, 1965; Kennedy & Takeda, 1965*a*; Wilson & Davies, 1965). These MNs often show a high level of spontaneous discharge. By contrast, the MNs supplying the 'fast' (phasic) flexor and extensor muscles, and those supplying certain 'fast' crab leg muscles, show little or no spontaneous tonic activity (Kennedy & Takeda, 1965*b*). On the basis of evidence presented here (section 3), we conclude that promotor MNs reflexly activated can themselves be categorized as tonic (Pm1–3, and probably Pm4) and phasic (Pm5–8), partly on the basis of the observed rates of frequency adaptation, and partly on the basis of the relative activation thresholds of these two groups of MNs. The low rates of frequency adaptation of Pm1–3, particularly at moderate (physiological) levels of T fibre depolarization, supports our contention that the reflex activation of these MNs could serve a postural role (Cannone & Bush, 1980*a*). The high threshold, larger-amplitude impulses of Pm5–8 adapt rapidly in frequency in response to a high, constant amplitude, T fibre depolarization. Nevertheless, the large-amplitude, facilitating and summating muscle junction potentials so evoked can lead to the initiation of muscle action potentials, and hence twitch contractions of muscle fibres innervated by these MNs. Thus, during the dynamic phase of an imposed remotion when T fibre depolarization is at its maximum, the recruitment of the phasic category of promotor MNs over and above the firing of the tonic category can be expected to lead to the development of a powerful muscular resistance at the very onset of the movement.

A final consideration in the context of the efficacy of the resistance reflex and the factors that contribute thereto is the stretch-induced positive feedback to the receptor muscle (Bush & Cannone, 1974), and the suggested tonic excitatory input to the receptor muscle (Cannone & Bush, 1980*b*). Efferent input to the receptor muscle during an imposed movement can therefore be expected to lead to an enhancement of the gain of the reflex loop, or at least to extend the range of receptor length over which the reflex is activated, and to abolish ambiguities in the reflex response imposed by viscoelastic properties of the receptor muscle (Cannone & Bush, 1980*a*, *c*).

This work was supported by a project grant from the Medical Research Council to B.M.H.B.

REFERENCES

ATWOOD, H. L. (1967). Crustacean neuromuscular mechanisms. *Am. Zool.* **13**, 357–378.

ATWOOD, H. L. (1976). Organization and synaptic physiology of crustacean neuromuscular systems. *Prog. Neurobiol.* **7**, 291–391.

ATWOOD, H. L. & WALCOTT, B. (1965). Recording of electrical activity and movement from legs of walking crabs. *Can. J. Zool.* **43**, 657–665.

AYERS, J. L. & DAVIS, W. J. (1977). Neuronal control of locomotion in the lobster, *Homarus americanus*. II. Types of walking leg reflexes. *J. comp. Physiol.* **115**, 29–46.

AYERS, J. L. & DAVIS, W. J. (1978). Neuronal control of locomotion in the lobster, *Homarus americanus*. III. Dynamic organization of walking leg reflexes. *J. comp. Physiol.* **123**, 289–298.

BARNES, W. J. P., SPIRITO, C. P. & EVOY, W. H. (1972). Nervous control of walking in the crab, *Cardisoma guanhumi*. II. Role of reflexes in walking. *Z. vergl. Physiol.* **76**, 16–31.

BAYLOR, D. A. & FETTIPLACE, R. (1976). Transmission of signals from photoreceptors to ganglion cells in the eye of the turtle. *Cold Spring Harbour Symp. quant. Biol.* **40**, 529–536.

BLIGHT, A. R. & LLINÁS, R. (1980). The non-impulsive stretch-receptor complex of the crab – a study of depolarization–release coupling at a tonic sensorimotor synapse. *Phil. Trans. R. Soc.* B (in press).

BROWN, H. M., OTTOSON, D. & RYDQVIST, B. (1978). Crayfish stretch receptor: an investigation with voltage-clamp and ion-sensitive electrodes. *J. Physiol.* **284**, 155–179.

BURROWS, M. & HORRIDGE, G. A. (1968). Eyecup withdrawal in the crab *Carcinus* and its interaction with the optokinetic response. *J. exp. Biol.* **49**, 285–297.

BURROWS, M. & SIEGLER, M. V. S. (1978). Graded synaptic transmission between local interneurones and motor neurones in the metathoracic ganglion of the locust. *J. Physiol.* **285**, 231–255.

BUSH, B. M. H. (1976). Non-impulsive thoracic–coxal receptors in crustaceans. In *Structure and Function of Proprioceptors in the Invertebrates* (ed. P. J. Mill), pp. 115–151. London: Chapman & Hall.

BUSH, B. M. H. (1977). Non-impulsive afferent coding and stretch reflexes in crabs. In *Identified Neurones and Behaviour of Arthropods* (ed. G. Hoyle), pp. 439–460. New York: Plenum Press.

BUSH, B. M. H. & CANNONE, A. J. (1973). A stretch reflex in crabs evoked by muscle receptor potentials in non-impulsive afferents. *J. Physiol.* **232**, 95–97*P*.

BUSH, B. M. H. & CANNONE, A. J. (1974). A positive feedback reflex to a crustacean muscle receptor. *J. Physiol.* **236**, 37–39*P*.

BUSH, B. M. H. & GODDEN, D. H. (1974). Tension changes underlying receptor potentials in non-impulsive crab muscle receptors. *J. Physiol.* **242**, 80–82*P*.

BUSH, B. M. H., GODDEN, D. H. & MACDONALD, G. A. (1975). A simple and inexpensive servo system for the control of length or tension of small muscles or stretch receptors. *J. Physiol.* **245**, 1–3*P*.

BUSH, B. M. H. & ROBERTS, A. (1968). Resistance reflexes from a crab muscle receptor without impulses. *Nature, Lond.* **218**, 1171–1173.

BUSH, B. M. H. & ROBERTS, A. (1971). Coxal muscle receptors in the crab: the receptor potentials of S and T fibres in response to ramp stretches. *J. exp. Biol.* **55**, 813–832.

BUSH, B. M. H., VEDEL, J. P. & CLARAC, F. (1978). Intersegmental reflex actions from a joint sensory organ (CB) to a muscle receptor (MCO) in decapod crustacean limbs. *J. exp. Biol.* **73**, 47–63.

CANNONE, A. J. & BUSH, B. M. H. (1980*a*). 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. *J. exp. Biol.* **86**, 275–303.

CANNONE, A. J. & BUSH, B. M. H. (1980*b*). Reflexes mediated by non-impulsive afferent neurones of thoracic–coxal muscle receptor organs in the crab, *Carcinus maenas*. III. Positive feedback to the receptor muscle. (In preparation.)

CANNONE, A. J. & BUSH, B. M. H. (1980*c*). Reflexes mediated by non-impulsive afferent neurones of thoracic–coxal muscle receptor organs in the crab, *Carcinus maenas*. IV. Motor activation of the receptor muscle. (In preparation.)

COHEN, M. J. (1965). The dual role of sensory systems: Detection and setting central excitability. *Cold Spring Harbor Symp. quant. Biol.* **30**, 587–599.

DAVIS, W. J. (1969). Reflex organization in the swimmeret system of the lobster. I. Intrasegmental reflexes. *J. exp. Biol.* **51**, 547–563.

EMSON, P. C., BUSH, B. M. H. & JOSEPH, M. H. (1976). Transmitter metabolizing enzymes and free amino acid levels in sensory and motor nerves and ganglia of the shore crab, *Carcinus maenas*. *J. Neurochem* **26**, 779–783.

EVOY, W. H. & COHEN, N. J. (1969). Sensory and motor interaction in the locomotor reflexes of crabs. *J. exp. Biol.* **51**, 151–169.

EVOY, W. H. & COHEN, M. J. (1971). Central and peripheral control of arthropod movements. *Adv. comp. Physiol. Biochem.* **4**, 225–266.

FIELD, L. H. (1974). Sensory and reflex physiology underlying cheliped flexion behaviour in hermit crabs. *J. comp. Physiol.* **92**, 397–414.

FIELDS, H. L. (1966). Proprioceptive control of posture in the crayfish abdomen. *J. exp. Biol.* **44**, 455–468.

FOURTNER, C. R. & EVOY, W. H. (1973). Nervous control of walking in the crab, *Cardisoma guanhumi*. IV. Effects of myochordotonal organ ablation. *J. comp. Physiol.* **83**, 319–329.

GILLARY, H. L. & KENNEDY, D. (1969*a*). Pattern generation in a crustacean neuron. *J. Neurophysiol.* **32**, 595–606.

GILLARY, H. L. & KENNEDY, D. (1969*b*). Neuromuscular effects of impulse pattern in a crustacean motoneuron. *J. Neurophysiol.* **32**, 607–612.

HODGKIN, A. L. & HUXLEY, A. F. (1952). Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J. Physiol.* **116**, 449–472.

KENNEDY, D. & DAVIS, W. J. (1977). Organization of invertebrate motor systems. In *Handbook of Physiology*, sect. 1, vol. 1, part 2 (ed. S. R. Geiger, E. R. Kandel, J. M. Brookhart and V. B. Mountcastle), pp. 1023–1087. Bethesda, MD: American Physiological Society.

KENNEDY, D. & TAKEDA, K. (1965*a*). Reflex control of abdominal flexor muscles in the crayfish. II. The tonic system. *J. exp. Biol.* **43**, 229–246.

KENNEDY, D. & TAKEDA, K. (1965*b*). Reflex control of abdominal flexor muscles in the crayfish. I. The twitch system. *J. exp. Biol.* **43**, 211–227.

LOWE, D. A., BUSH, B. M. H. & RIPLEY, S. H. (1978). Pharmacological evidence for 'fast' sodium channels in nonspiking neurones. *Nature, Lond.* **274**, 289–290.

MACMILLAN, D. L. (1975). A physiological analysis of walking in the American lobster, *Homarus americanus*. *Phil. Trans. R. Soc. Lond.* B, **270**, 1–59.

MACMILLAN, D. L. (1976). Arthropod apodeme tension receptors. In *Structure and Function of Proprioceptors in the Invertebrates* (ed. P. J. Mill), pp. 427–442. London: Chapman and Hall.

MAYNARD, D. M. & WALTON, K. D. (1975). Effects of maintained depolarization of presynaptic neurons on inhibitory transmission in lobster neuropil. *J. comp. Physiol.* **97**, 215–243.

MENDELSON, M. (1971). Oscillator neurones in crustacean ganglia. *Science, N.Y.* **171**, 1170–1173.

MIROLLI, M. (1979). The electrical properties of a crustacean sensory dendrite. *J. exp. Biol.* **78**, 1–27.

MOODY, C. J. (1970). A proximally directed intersegmental reflex in a walking leg of the crayfish. *Am. Zool.* **10**, 501.

PAUL, D. H. (1976). Role of proprioceptive feedback from nonspiking mechanosensory cells in the sand crab, *Emerita analoga*. *J. exp. Biol.* **65**, 243–258.

PEARSON, K. G. (1972). Central programming and reflex control of walking in the cockroach. *J. exp. Biol.* **56**, 173–193.

PEARSON, K. G. (1976). Nerve cells without action potentials. In *Simpler Networks and Behaviour* (ed. J. C. Fentress), pp. 99–110. Sunderland, Mass: Sinauer.

PEARSON, K. G. & FOURTNER, C. R. (1975). Nonspiking interneurons in walking system of the cockroach. *J. Neurophysiol.* **38**, 33–52.

RALL, W. (1969). Time constants and electrotonic length of membrane cylinders and neurons. *Biophys. J.* **9**, 1483–1508.

RIPLEY, S. H., BUSH, B. M. H. & ROBERTS, A. (1968). Crab muscle receptor which responds without impulses. *Nature, Lond.* **218**, 1170–1171.

RIPLEY, S. H. & WIERSMA C. A. G. (1953). The effect of spaced stimulation of excitatory and inhibitory axons of the crayfish. *Physiologia comp. Oecol.* **3**, 1–17.

ROBERTS, A. & BUSH, B. M. H. (1971). Coxal muscle receptors in the crab: the receptor current and some properties of the receptor nerve fibres. *J. exp. Biol.* **54**, 515–524.

SOKOLOVE, P. G. (1973). Crayfish stretch receptor and motor unit behaviour during abdominal extension. *J. comp. Physiol.* **84**, 251–266.

VEDEL, J. P. & CLARAC, F. (1975). Neurophysiological study of the antennal motor patterns in the rock lobster, *Palinurus vulgaris*. II. Motoneuronal discharge patterns during passive and active flagellum movements. *J. comp. Physiol.* **102**, 223–235.

WIERSMA, C. A. G. & ADAMS, R. T. (1950). The influence of nerve impulse sequence on the contractions of different crustacean muscles. *Physiologia comp. Oecol.* **2**, 20–33.

WILSON, D. M. & DAVIS, W. J. (1965). Nerve impulse patterns and reflex control in the motor system of the crayfish claw. *J. exp. Biol.* **43**, 193–210.

WILSON, D. M. & GETTRUP, E. (1963). A stretch reflex controlling wingbeat frequency in grasshoppers. *J. exp. Biol.* **40**, 171–185.