

PROPRIOCEPTIVE INPUT FROM TWO BASAL JOINT STRETCH RECEPTORS TO LEG MOTONEURONES IN THE ISOLATED THORACIC GANGLION OF THE SHORE CRAB

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Summary

The reflex effects and interactions of two proprioceptors upon motoneurones supplying the four basal leg muscles of the shore crab *Carcinus maenas* have been studied in a new *in vitro* preparation consisting of the thoracic-coxal muscle receptor organ (TCMRO) and the coxo-basal chordotonal organ (CBCO) isolated together with the whole thoracic ganglion complex to which they were still connected by their afferent nerves. Each receptor strand was stimulated mechanically, while recording intracellularly from motoneurones in the ganglion, and extracellularly from the cut motor nerves innervating the promotor and remotor muscles of the thoracic-coxal (T–C) joint and the levator and depressor muscles of the coxo-basal (C–B) joint.

Stretch of the TCMRO evoked reflex firing in several units in the promotor motor nerve, confirming previous studies. In addition to this ‘intrajoint’ reflex, however, TCMRO stretch also elicited ‘interjoint’ reflex responses in motoneurones of both the levator and depressor muscles. Similarly, stretch and release of the CBCO produced intrajoint resistance reflexes in levator and depressor motoneurones, respectively, as well as interjoint reflexes in promotor and remotor motoneurones. In general, the CBCO produced stronger reflex effects in all four motor nerves than did the TCMRO.

Intracellular recordings from individual motoneurones of all four muscles revealed that the majority of them received convergent input from both proprioceptors. The importance of such convergent input *in vivo* is discussed.

Introduction

Decapod Crustacea have sensory receptors that provide the central nervous system (CNS) with information concerning joint position and movement, muscle contraction and tension, and limb loading (reviewed by Bush and Laverack, 1982; see also relevant chapters in Mill, 1976). The proprioceptors that have been extensively studied are the chordotonal organs, which occur at most joints of the

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legs, and several forms of muscle receptor organ, each of which has a receptor muscle under efferent control in addition to its own distinctive sensory supply. This paper is concerned with the central interaction of afferent input from one example of each proprioceptor type, namely the coxo-basal chordotonal organ (CBCO) of the second joint of a walking leg and the thoracic-coxal muscle receptor organ (TCMRO) of the first, most proximal joint of the same leg. The CBCO has many peripherally located bipolar sensory neurones responding phasically or tonically to stretch or release of the receptor strand (Bush, 1965a); the TCMRO has two large, non-spiking afferents whose cell bodies are situated in the ipsilateral segmental thoracic ganglion, the dynamically sensitive 'T fibre' and the length-sensitive 'S fibre' (Bush and Roberts, 1971).

Both these proprioceptors mediate various reflexes that are presumed to be involved in motor control of the limbs during posture and in locomotion (Bush, 1977; Clarac, 1985). These include 'resistance reflexes' (Bush, 1962; Bush and Roberts, 1968), which resemble mammalian stretch reflexes in that they tend to resist imposed movements at the same joint, and intersegmental (Clarac *et al.* 1978) or 'distributed' reflexes (Ayers and Davis, 1977), which affect neighbouring or more distant joints. Since the term 'intersegmental' is often used to refer to different body segments rather than limb segments, and the term 'distributed' is even less specific, we have decided to use the term *intrajoint* to distinguish reflex effects on motoneurons of muscles controlling the same joint as that monitored by a particular proprioceptor, while the term *interjoint* is used for reflex effects on motoneurons of muscles controlling the neighbouring joint. Thus, for example, in both the crab and the rock lobster, stretch and release of the CBCO evokes *intrajoint* resistance reflexes in the coxo-basal levator and depressor muscles and *interjoint* reflexes in most other muscles of the same leg (Bush, 1965b; Clarac *et al.* 1978). Similarly, the TCMRO in both crabs and crayfish evokes *intrajoint* reflexes in the thoracic-coxal promotor and remotor muscles and *interjoint* reflexes in the levator and depressor muscles (Cannone and Bush, 1980; DiCaprio and Clarac, 1983; Skorupski and Sillar, 1986).

The extensive divergence of proprioceptive influences implied by these *interjoint* reflexes indicates that there must also be convergence of inputs from several proprioceptors upon the motoneurone pool for each muscle, and probably onto individual motoneurons. Indeed, in the intact animal, sensory reafference from the large array of proprioceptors in each leg must cooperate in the reflex control of motoneurone output. Much of the previous work on the reflex effects mediated by crustacean limb mechanoreceptors, however, has focused upon single proprioceptors (see references cited above). Furthermore, most studies of proprioceptive function in Crustacea have been performed on intact or only partially isolated preparations, with the thoracic nervous system remaining *in situ* and the limb nerves often being left intact, so that sensory inputs other than those being stimulated could influence the results (e.g. the set position of one joint can affect the reflex response to movement of another joint: Bush, 1962). Finally, at least some of the proprioceptive input to a given motoneurone is likely to be

subthreshold and, therefore, not registered in extracellular motor nerve recordings. Intracellular recordings from identified motoneurones during activation of leg proprioceptors are necessary to assess any such subthreshold influences.

This paper presents results of a study exemplifying one approach to the analysis of convergent input from different mechanoreceptors in a single limb. The sensorimotor system selected for study constituted the two major proprioceptors of the basal region of a walking leg, the TCMRO and the CBCO, with the motoneurones of the two sets of antagonistic muscles controlling the first two joints, the promotor and remotor muscles of the thoracic-coxal (T-C) joint and the depressor and levator muscles of the coxo-basal (C-B) joint. Using a recently developed isolated preparation of the thoracic ganglion of the shore crab with only these two receptor organs of one leg kept intact (Bush and Head, 1985), we have characterised their reflex effects and interactions upon the motoneurones of the four basal limb muscles. Only recordings made from stable, tonically active or quiescent preparations, either lacking any endogenous rhythmicity or made between periods of spontaneous rhythmic motor activity, are considered here. A second paper deals with the interactions between proprioceptive input and rhythmic motor output of central origin (Head and Bush, 1991*b*; see also Head, 1986).

Materials and methods

All experiments were performed on the shore crab *Carcinus maenas* (L.). Male crabs measuring 30–70 mm across the carapace were obtained from the Plymouth Marine Laboratory, and maintained at 12–15°C in tanks containing filtered and aerated artificial sea water.

The physiological saline used had the following ionic composition: (in mmol l⁻¹) NaCl, 500; KCl, 12; CaCl₂, 12; MgCl₂, 20; buffered to pH 7.4 with Tris base (10 mmol l⁻¹) and maleic acid (2 mmol l⁻¹).

Preparation

The experiments in this study were carried out on a totally isolated nervous system preparation, made up of the complete multisegmental thoracic ganglion complex together with the two proprioceptors (TCMRO and CBCO) from the first two basal joints of the right back leg (Fig. 1). The preparation was continuously superfused with crab saline at 14°C. Prior to the dissection, the crab was chilled to 4°C in a refrigerator.

Stimulation and recording

The two receptor strands were attached at their distal ends to separate electromechanical stimulators (Fig. 1B). The stimulus form most commonly used was a constant-velocity stretch–hold–release, or ‘trapezoidal’ function (see Cannone and Bush, 1980), with variable-duration ramp-and-hold phases and

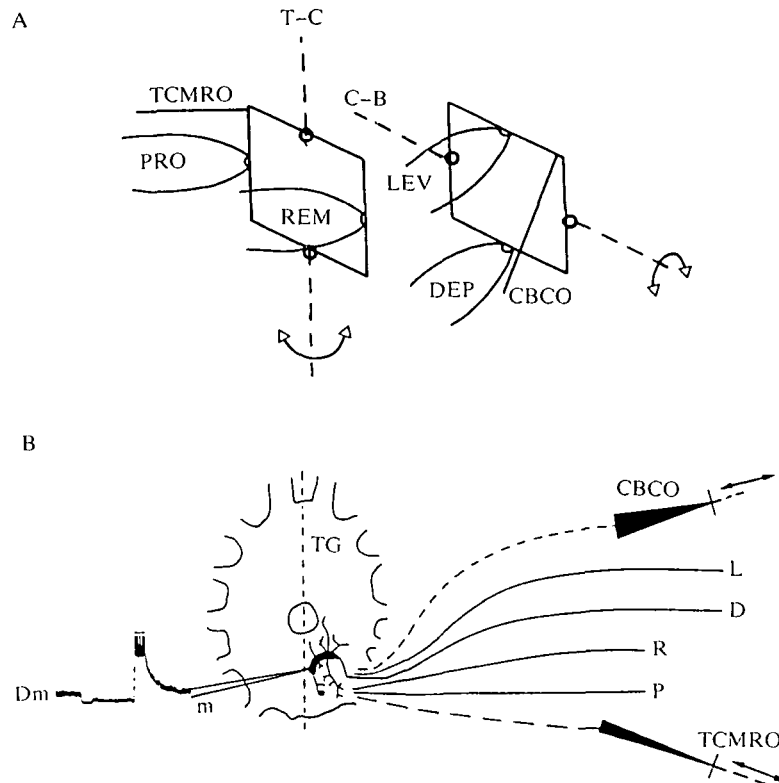


Fig. 1. A schematic diagram of the crab preparation used in the present study. (A) The promotor (PRO) and remotor muscles (REM) of the thoracic-coxal joint (T-C) move the leg in the horizontal direction; the thoracic-coxal muscle receptor organ (TCMRO) is stretched by retraction of the leg. The levator (LEV) and depressor (DEP) muscles move the leg in the vertical plane about the coxo-basal (C-B) joint; the coxo-basal chordotonal organ (CBCO) is stretched by depression of the leg. (B) Isolated nervous system preparation of a thoracic ganglion (TG) with the levator (L), depressor (D), remotor (R) and promotor (P) motor nerves that innervate the two antagonistic muscle groups responsible for moving the two basal joints. The two proprioceptors, TCMRO and CBCO, from the first two basal joints are attached to electromechanical pullers. An intracellular microelectrode (m) is shown recording from the central neurite region of a motoneurone; the cell was stained with Lucifer Yellow. The intracellularly recorded response of a depressor motoneurone (Dm) to a stretch-hold-release stimulus of the CBCO is illustrated.

amplitudes of 0.5–1.5 mm (or 5–20% of the mid *in situ* length of either receptor strand).

The electrophysiological techniques were conventional. Polyethylene suction electrodes were used to record from the cut ends of the motor nerves. The intracellular micropipettes had resistances of 30–100 M Ω when filled with 5% Lucifer Yellow in 1 mol l⁻¹ LiCl. Penetrated cells lacking a stable resting

membrane potential of at least -55 mV were rejected. The data from each experiment were stored on a seven-channel FM tape recorder for subsequent analysis and photography. At the end of the experiment any presumed motoneurones from which recordings had been obtained were ionophoresed with Lucifer Yellow for subsequent anatomical identification. The ganglion was fixed in 4% formol saline, and whole-mount preparations were viewed with a fluorescence microscope.

Results

A rigorous quantitative analysis of the reflex responses to TCMRO and CBCO stimulation was not undertaken. It would, in any event, be of doubtful value in view of the inherent variability in the recorded responses, both within and between preparations. A major factor underlying this response variability appeared to be subliminal fluctuation in central excitability, even in the absence of any overt, rhythmic or tonic motor output. Accordingly, no attempt has been made in this study to determine accurately the numbers of motoneurones involved in the various reflexes or to quantify the intensities of the responses obtained.

However, qualitative descriptions will be given in terms of the relative strengths of the most prevalent responses, without distinguishing between the numbers of units involved and the frequency of firing of individual units. Thus, for instance, a 'strong' response to stretch of either proprioceptor (see Table 1) might involve one or more phasic units (often distinguishable by their relatively large spikes in the extracellularly recorded trace) discharging at a high overall (i.e. combined) frequency during the dynamic phase of stretch (i.e. lengthening). This could be followed during the hold phase either by fairly steady firing at a lower frequency in one or more tonic units (see Fig. 2) or by an adapting discharge of 'phasic-tonic' units (see Fig. 3).

In most cases, the actual number of units discharging probably fell short of the maximum number present in the respective motor nerves. Bevingut *et al.* (1983) reported some 7–10 motoneurone somata for each of the four main muscle pools in *Carcinus maenas*, a figure consistent with our own provisional observations using the cobalt backfilling method (see also Bush, 1977). In general, it seemed from our extracellular recordings that more motor units were recruited by the intrajoint stimuli than by the interjoint ones (see Figs 2, 3A). Furthermore, a significant proportion of the units seen to respond in the extracellular traces showed some degree of tonic activity, even in the more quiescent preparations.

Motor nerve responses to the stimulation of individual receptors

Reflex effects of the TCMRO

Stretch–hold–release stimuli applied to the isolated distal end of the TCMRO commonly evoked reflex responses in the promotor, remotor and levator motor nerves; depressor responses were less common (Fig. 2; Table 1). The promotor nerve generally showed the strongest and most consistent response, with several

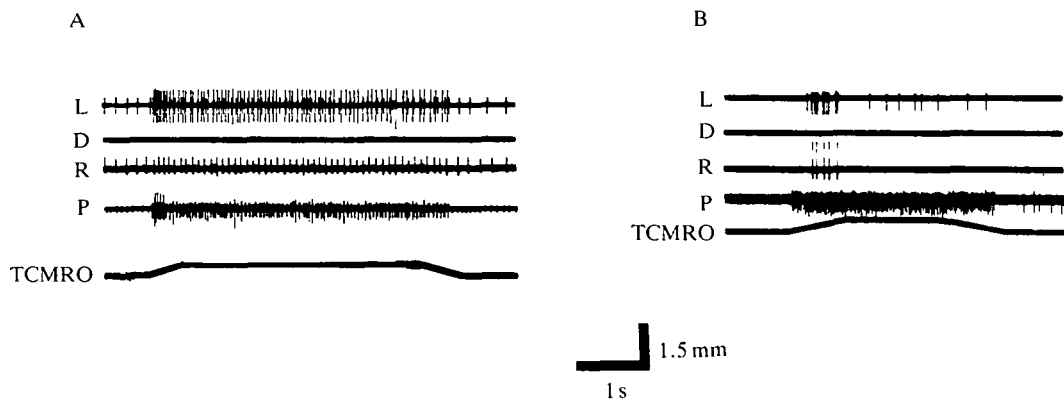


Fig. 2. Typical reflex responses recorded extracellularly in the four motor nerves to a stretch-hold-release stimulus applied to the TCMRO (from two different preparations). Traces from top to bottom: levator (L), depressor (D), remotor (R) and promotor (P) motor nerve recordings; the bottom trace is the length monitor attached to the TCMRO. In this and all subsequent figures, stretch is denoted by an upward deflection, release by a downward deflection; the amplitude of stretch was 0.5 mm. (A) In the levator nerve a single small spike unit is tonically active; during the stretch phase its firing frequency is increased, and two larger units are recruited; all three units remain active throughout the hold phase, but at lower frequencies than during the stretch. The promotor nerve responds in a similar way. In the remotor nerve there is no noticeable dynamic response, but the frequency of a tonic unit is increased for the duration of the stimulus. (B) None of the four nerves was tonically active in this experiment. In the levator nerve two units are active by stretch of the TCMRO, one of which continues during the hold phase of the stimulus. One remotor unit is activated by stretch, while at least three promotor units are active throughout stretch and hold. In neither experiment was there any activity in the depressor nerve.

units firing during the dynamic phase of stretch, followed by a somewhat reduced overall response during the hold phase. This is the classical stretch reflex, or 'resistance reflex', described previously in this crab (Bush and Roberts, 1968; Cannone and Bush, 1980). More surprisingly, perhaps, one or more remotor units were often also excited by TCMRO stretch (Fig. 2), although the same stimulus occasionally had the opposite effect on the remotor nerve (Table 1).

In addition to these intrajoint reflexes, the TCMRO also has interjoint reflex effects upon the levator and depressor motor nerves (Fig. 2; Table 1). Stretch and hold stimuli dynamically activated levator units in all trials, and tonic firing continued through the hold phase in some cases. In contrast, the same stimuli only occasionally activated the depressor units.

Reflex effects of the CBCO

In all experiments, the CBCO produced strong reflex effects in the four motor nerves from which recordings were made (Table 1; Fig. 3). In most experiments the CBCO caused stronger intra- and interjoint reflex effects than the TCMRO

Table 1. Analysis of reflex responses recorded (as in Figs 2, 3) in the promotor, remotor, depressor and levator motor nerves to stretch–hold–release stimuli of the thoracic-coxal muscle receptor organ (TCMRO) and the coxo-basal chordatonal organ (CBCO)

	Intrajoint reflexes		Interjoint reflexes	
	Promotor	Remotor	Depressor	Levator
TCMRO stimulus				
Stretch	+++ (100 %)	+ (70 %)/– (10 %)	+ (20 %)	+ (100 %)
Hold	+ (100 %)	+ (40 %)/– (10 %)	0 (100 %)	+ (40 %)
Release	0 (100 %)	– (10 %)	0 (100 %)	0 (100 %)

	Intrajoint reflexes		Interjoint reflexes	
	Levator	Depressor	Remotor	Promotor
CBCO stimulus				
Stretch	++ (100 %)	– (100 %)	+ (100 %)	++ (90 %)
Hold	+ (90 %)	– (100 %)	+ (42 %)	+ (32 %)
Release	+ (10 %)/– (10 %)	++ (100 %)	+ (10 %)/– (20 %)	+ (84 %)

A single sign denotes an increase (+) or a decrease (–, where tonic activity was present) in the overall firing frequency of all active units in each nerve, a double sign (++) denotes a strong response during the dynamic part of the stimulus; (0) indicates that there was no response to the stimulus. Opposing signs show that different responses were seen in different preparations.

The percentages indicate the total number of times a given result occurred in response to at least 20 similar stimuli, any shortfall from 100 % indicates no response (e.g. 20 % of the total number of stretches failed to evoke a response in the remotor nerve and 80 % produced no depressor response). *N*=66 preparations.

(Table 1). The main intrajoint effect was that stretch of the CBCO excited units in the levator motor nerve and inhibited any activity in the depressor nerve, while release of the CBCO excited the depressor motor units (Fig. 3A,B). These are resistance reflexes that would tend to oppose movement about the coxo-basal joint (see Bush, 1965c).

Stretch, hold and release of the CBCO also excited one or more units in the motor nerves to both the remotor and the promotor muscles of the proximal, thoracic-coxal joint (Fig. 3A,C). This interjoint reflex involves simultaneous activation of antagonistic muscles when the CBCO is stretched. Note, however, the variability seen in Table 1, so that CBCO release may lead to the excitation of levator and remotor firing in some cases and to its inhibition in others.

Reflex interaction between the two receptors

Different proprioceptors must function in concert *in vivo*, so each of the individual ‘reflexes’ described above will be subject to modification both by the inputs from other proprioceptors and by central control. The precise nature and extent of any such interactions will depend upon the relative timing and the type of

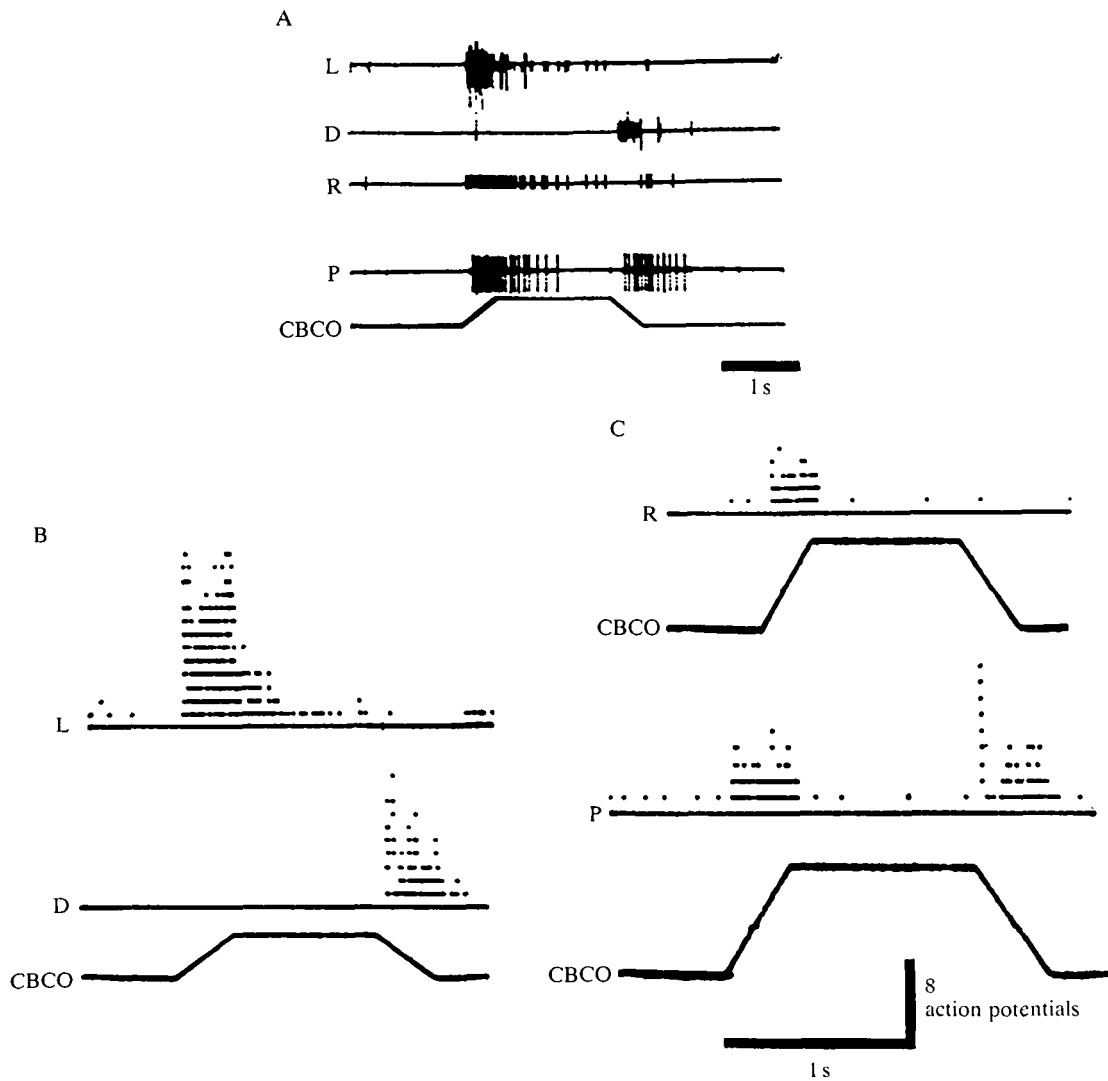


Fig. 3. Responses of the levator (L), depressor (D), remotor (R) and promotor (P) motor nerves to stretch-hold-release stimuli applied to the CBCO. (A) Simultaneous extracellular recordings from all four nerves of a typical response to a single stimulus to the CBCO. At least three levator units are activated during stretch, while one or two continue to fire during the hold phase. Four or five depressor units are excited upon release of the CBCO. Remotor and promotor units in this preparation are activated by both the stretch and release phases of the stimulus in a compound interjoint reflex. (B) Peri-stimulus time histograms (i.e. the recording is made throughout the stimulus) illustrating the intrajoint reflexes in response to CBCO stretch, hold and release. Levator motoneurons (L) are activated on stretch while depressor motoneurons (D) are activated on release. (C) Interjoint reflexes of remotor (R) and promotor (P) units in response to two separate CBCO stimuli. The remotor units are activated on stretch while the promotor units are excited by both stretch and release. Eight successive stimuli are combined to produce the histograms, and all active units are included.

stimulation of the individual proprioceptors, as well as on the prevailing central activity. Combined inputs from the CBCO and TCMRO with different phase relationships produced additive responses in each of the four motor nerves. For example, stretch of either the TCMRO or the CBCO alone excited one or more units in the promotor nerve (see Figs 2, 3 and Table 1), while simultaneous stimulation of both receptors evoked a significantly stronger response. Similarly, when TCMRO stretch coincided with CBCO release, the resulting promotor nerve response was again enhanced, to an extent comparable to the summed individual responses.

A simpler form of interaction between these two joints concerns the effect of the position of one joint upon the response to movement of the other. This is exemplified by an experiment on the effect of TCMRO length upon the interjoint response of the promotor nerve to CBCO stimulation. The TCMRO was set to two different lengths, one relatively short (within the lower half of its *in situ* length range) and the other relatively extended (in the upper half of this range). The extended length selected was sufficient to cause maximal tonic activation of a single unit in the promotor nerve (Fig. 4A). This unit was identified as Pm1 (Cannone and Bush, 1980), since it had the smallest spikes in the promotor nerve recording, was tonically active more often than other promotor units, and had the lowest threshold for reflex activation by the TCMRO. Fig. 4B,C compares the effect of these two TCMRO lengths upon the CBCO-promotor reflex.

When the TCMRO was relaxed, Pm1 was not tonically active and responded only to the dynamic phases of the CBCO stimulus, on both stretch and release (Fig. 4B, top trace). When the TCMRO was stretched, the peak dynamic response frequency of Pm1 to both stretch and release of the CBCO was approximately doubled and was superimposed upon a tonic background discharge (Fig. 4A,B, lower trace). During the hold phase its frequency remained a little above the background rate.

The same analysis was repeated (Fig. 4C) for the second unit normally recruited by TCMRO stretch, termed Pm2 (Cannone and Bush, 1980). This unit is more velocity-sensitive than Pm1, and was not usually tonically active at either of the two TCMRO lengths used here, although at the stretched length there was sometimes a low level of tonic activity (Fig. 4C). The main effect on Pm2 of setting the TCMRO to a stretched length was to increase its dynamic responses to CBCO stretch and release. In this experiment, the responses of Pm2 to stretch rose by 140% and those to release by 110%, over their firing rates at the more relaxed length.

Intracellular recordings

Intracellular recordings were made from individual motoneurons to establish the nature and relative potency of convergent inputs from the two basal limb proprioceptors.

Most of the intracellular recordings described here are presumed to have been from the neuropilar regions of the secondary neurites and integrating segments

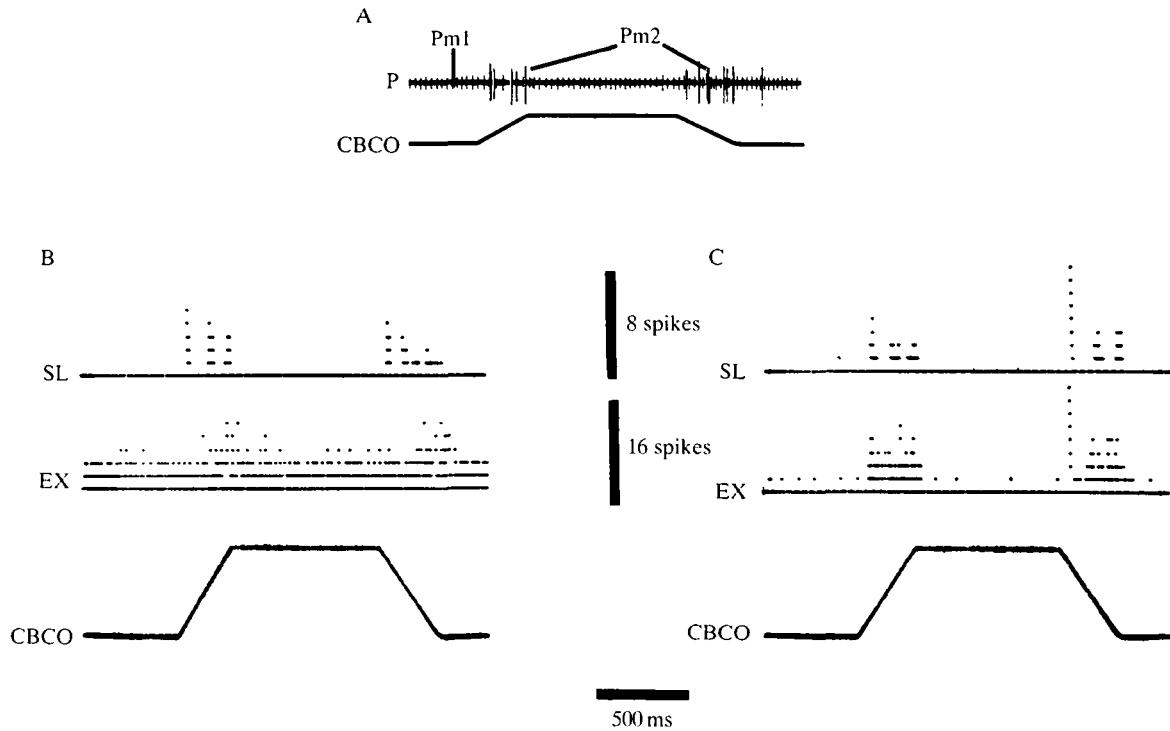


Fig. 4. The responses of promotor motoneurons to standard stretch-hold-release stimuli (bottom traces) applied to the CBCO with the TCMRO set at two different lengths. (A) In this sample recording the TCMRO is set within the upper 50% of its *in situ* length. A single small spike unit, Pm1, is tonically active; one or two additional units, termed collectively Pm2, are recruited on both stretch and release of the CBCO. (B) Peri-stimulus time histograms of the response of Pm1 only, selected by means of a window-discriminating circuit, to standard CBCO stimuli with the TCMRO set to the lower 50% of its *in situ* length (SL) and to the extended (EX) length. At the extended length, Pm1 is tonically active, and the dynamic responses to both the stretch and the release phases of the stimulus are also increased over those at the shorter length (note the different vertical scales). (C) With the larger Pm2 unit(s) selected by the window discriminator, the dynamic responses to both stretch and release are again increased at the extended length, to a greater extent than those of Pm1, and there is also some tonic activation of Pm2. Eight successive responses to identical stimuli at 10-s intervals are summed in each SL trace, but only four were used for the EX traces.

(Fig. 1B). Spike-like potentials recorded in different neurones and preparations ranged widely in amplitude (5–30 mV) and rise time and were probably electrotonic reflections of impulses originating at more peripheral spike initiation zones. Where cell bodies were seen to be impaled, the recorded 'spikes' seldom reached 5 mV and decay times exceeded 10 ms, resulting in an inadequate temporal resolution for the purposes of this study. Discrete transients identifiable as postsynaptic potentials resulting from individual presynaptic impulses were

seldom discernible, possibly because they usually originated at some distance from the site of penetration. In contrast, the occurrence of relatively large, complex potential changes that sometimes closely resembled the presumed receptor potentials in one of the two non-spiking afferents of the TCMRO, or similar response waveforms resulting from CBCO stimulation, suggested that the synaptic inputs from these proprioceptors may, in many cases, have been located much nearer to the recording microelectrode, particularly in those cases where the response waveform had a very sharp onset.

Some 40 or more cells penetrated within the right posterior thoracic ganglionic neuropile yielded stable membrane potentials of at least -55 mV in the 'resting' condition (i.e. in the absence of any peripheral input or central drive). Of these, 25 were positively identified as motoneurones of one of the four basal limb muscles, according to the usual criteria, namely (a) 'spikes' in the intracellular trace were correlated 1:1 with orthodromic impulses in a unit recorded extracellularly in the relevant muscle nerve; (b) antidromic stimulation of the motor nerve elicited spikes in the impaled cell and (c) Lucifer Yellow injection from the recording microelectrode showed an axon in the appropriate motor nerve. Impaled cells not meeting all three criteria are not considered here: these included some motoneurones of more distal leg muscles, in addition to further motoneurones that almost certainly innervated the basal leg muscles.

The 25 identified neurones were six promotor, four remotor, 10 depressor and five levator motoneurones. Apart from four of the depressor motoneurones, which lacked an interjoint input from the TCMRO, the remaining 21 motoneurones showed both intra- and interjoint reflexes, responding to stimulation of both the TCMRO and the CBCO, i.e. they had convergent inputs from these two proprioceptors. In some cases, the intrajoint reflex appeared to involve direct, monosynaptic connections between the afferents and motoneurones, but a rigorous analysis was not attempted.

Representative intra- and interjoint responses of promotor, remotor, depressor and levator motoneurones to TCMRO and CBCO stimulation will now be described.

Promotor neurones

Fig. 5 shows typical intracellular recordings from three of the six positively identified promotor neurones that responded to both proprioceptors.

Intrajoint reflex. As expected from the extracellular recordings summarized in Table 1, all the promotor neurones recorded intracellularly were depolarized by TCMRO stimulation (Fig. 5A,C,D). A striking feature of these promotor responses was how closely they resembled the T fibre afferent response to the same stimulus (Fig. 5A). In some cases, one or more relatively tonic promotor units, monitored by simultaneous extracellular recordings, continued to fire during the hold phase (Fig. 5C), suggesting that the cells most commonly penetrated in these experiments were the larger, more phasic motoneurones.

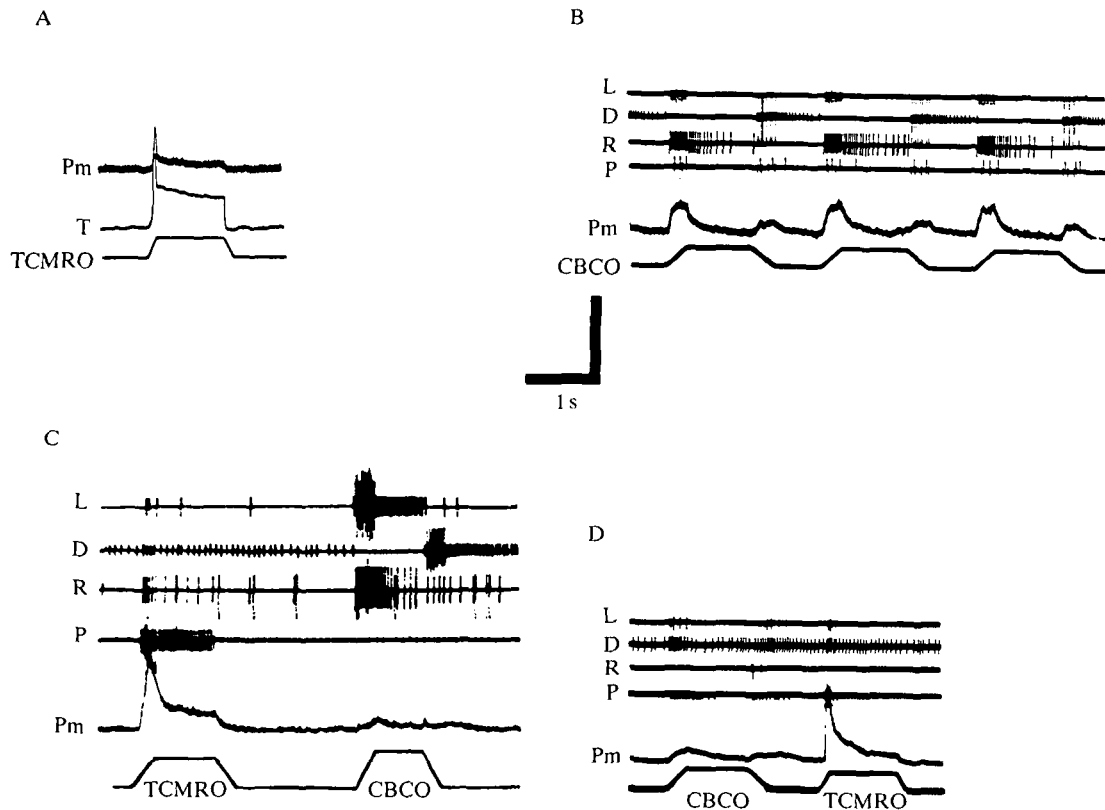


Fig. 5. Intracellular recordings from three promotor motoneurons (Pm), illustrating their responses to proprioceptive input from the TCMRO and CBCO. (A) During stretch of the TCMRO, the promotor motoneurone receives a depolarising input, which closely resembles the TCMRO afferent response (T) recorded simultaneously from the central intraganglionic region of the T fibre in this experiment. (B) This same promotor motoneurone also receives a subthreshold depolarising input on both stretch and release of the CBCO. (C,D) Two other promotor motoneurons receive convergent inputs from both the TCMRO and the CBCO. The input from the TCMRO, however, had a much stronger effect and evoked spiking of the impaled cell during the dynamic phase of stretch. In B, C and D the simultaneous extracellular recordings from the levator (L), depressor (D), remotor (R) and promotor (P) nerves are also shown. Vertical scale (intracellular traces only), A, 7 mV (Pm), 25 mV (T); B, 4 mV; C, D, 25 mV.

Interjoint effects. Fig. 5B,C,D shows the promotor neurones being depolarized on both stretch and release of the CBCO. Although these interjoint inputs remained subthreshold in these examples, two other promotor units were, in fact, suprathreshold for this CBCO input, as can be seen from the extracellular recordings in Fig. 5B,D. As noted above, any such subthreshold responses *in vivo* could sum with other depolarizing inputs, causing the cell to fire.

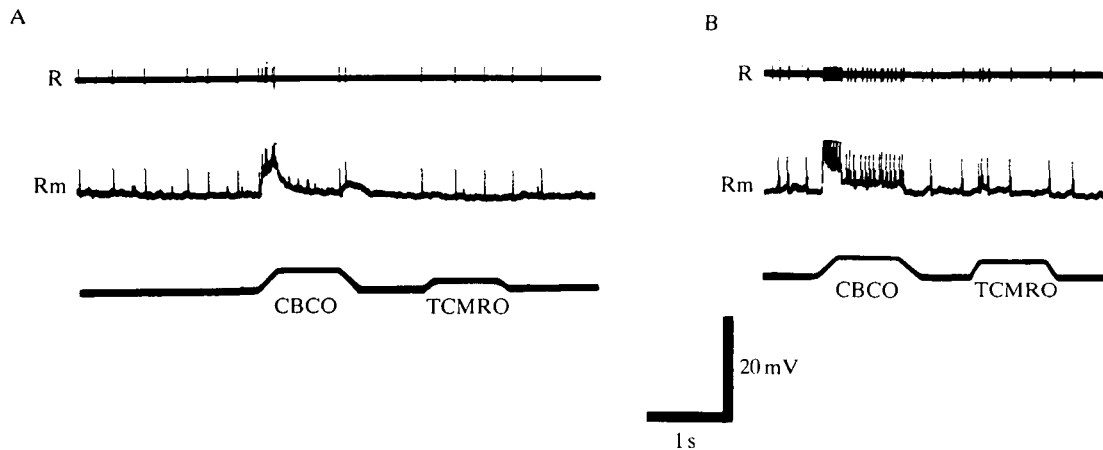


Fig. 6. Intracellular recordings from two remotor motoneurons (Rm), illustrating their responses to stretch–hold–release stimuli of the CBCO and TCMRO; the extracellular remotor nerve recording is also shown (R). (A) The remotor motoneurone is depolarized on both stretch and release of the CBCO, but note the lack of input from the TCMRO. (B) This cell is depolarized by CBCO stretch, it remains depolarized compared with resting levels during the hold phase and it repolarises on release. In contrast to the first cell, this one is also dynamically excited, albeit weakly, by stretch of the TCMRO. Both cells discharge strongly on CBCO stretch.

Remotor neurones

Contrasting examples of intracellular recordings from two of the four remotor neurones that met all three criteria and responded to both proprioceptors are illustrated in Fig. 6.

Intrajoint reflexes. The response of remotor neurones to TCMRO input in these quiescent preparations was invariably weak, unlike the intrajoint reflexes recorded in the other three motoneurone groups. In some instances there was virtually no response to TCMRO stimulation (Fig. 6A), whilst in others the impaled remotor neurone was weakly excited during stretch of the TCMRO (Fig. 6B). Moreover, there was little indication of an underlying shape resembling a TCMRO afferent receptor potential, as noted for promotor neurones (Fig. 5A).

Interjoint effects. All four fully identified (and several other presumed) remotor neurones received convergent inputs from the CBCO. This input was always stronger than the intrajoint effect, in contrast to the other three sets of motoneurones where the intrajoint reflex tended to be the stronger. Both cells responded to CBCO stimulation with a strong depolarization during the dynamic phase of stretch; this declined during the hold phase to a level still somewhat depolarized compared with resting levels. On release, the first remotor neurone received a further small depolarizing input (Fig. 6A).

Depressor motoneurones

Of ten cells identified as depressor motoneurones in the present study, six were

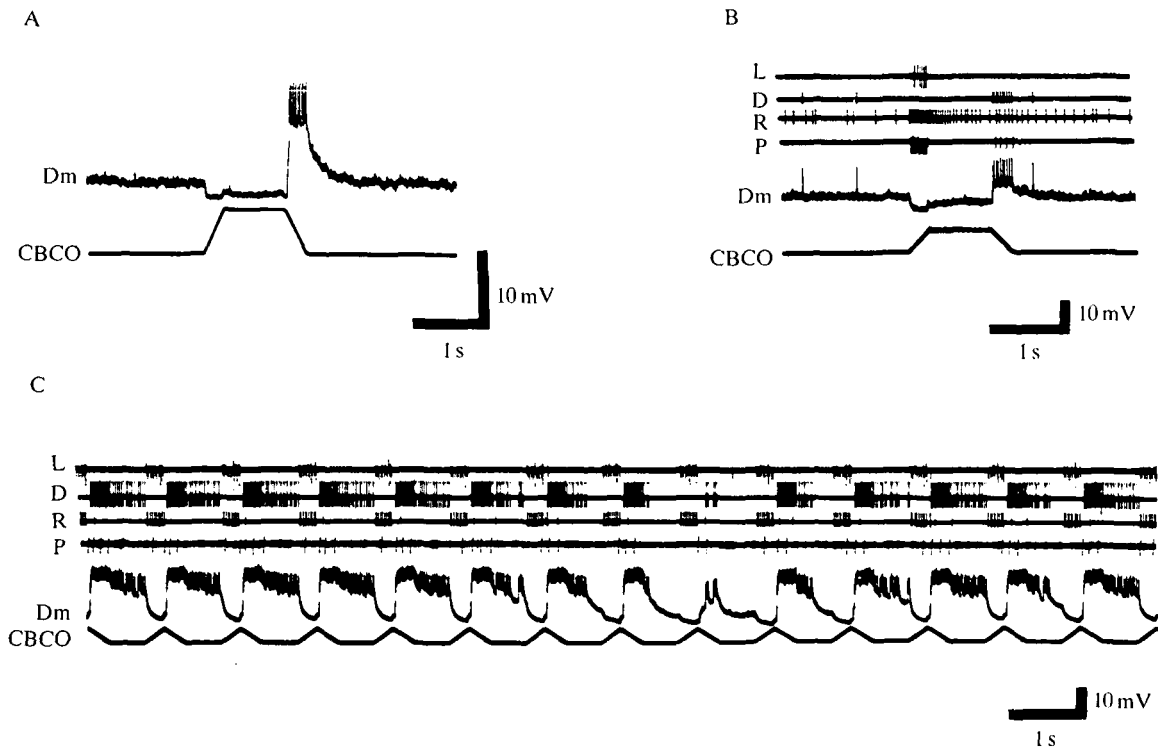


Fig. 7. Examples of intracellular recordings from three depressor motoneurones (Dm) and their responses to stimulation of the CBCO, with simultaneous extracellular recordings (in B and C) from the levator (L), depressor (D), remotor (R) and promotor (P) motor nerves. (A,B) Stretch of the CBCO produces a hyperpolarization of the membrane potential of the motoneurone; during the hold phase it repolarizes somewhat; on release, there is a large depolarization, at the height of which spiking is initiated. (C) With repetitive stretch–release stimuli (at 1 s^{-1}), the depolarization and spiking of this depressor motoneurone outlast each release and, throughout most of this sequence, are only terminated by the inhibitory input on the next stretch.

clearly influenced by both proprioceptors (Figs 7, 8), and the other four lacked an interjoint input from the TCMRO.

Intrajoint reflexes from the CBCO. The typical response of a depressor motoneurone to a stretch–hold–release stimulus of the CBCO is illustrated in Fig. 7A,B. During stretch, a strong inhibitory input was evident in the form of a distinct hyperpolarizing IPSP, and this continued throughout the hold phase, though at a somewhat less hyperpolarized level.

One of the 10 depressor motoneurones in this study lacked any response during stretch but showed a marked hyperpolarization during the plateau. All 10 responded to CBCO release with a large depolarizing shift in the membrane potential (Fig. 7A). Spikes were usually superimposed on this depolarization, leading to impulses that were recorded in the depressor motor root (Fig. 7B). In about half the recordings, the spiking outlasted the release phase, often being

terminated only by the inhibitory input from an ensuing stretch. This is well illustrated in Fig. 7C, where repetitive stretch–release stimuli elicited prolonged bursts in the impaled motoneurone. Following the last release of such a series, this cell continued firing at reduced frequency for up to 4 s; in the absence of stimulation, however, there was no tonic activity.

Interjoint effects from the TCMRO. As noted above, six of the ten depressor motoneurones studied also received a convergent input from the TCMRO. In these cells, stretch of the TCMRO evoked a transient depolarization in response to the dynamic phase of stretch, falling to a small, almost indiscernible, plateau during the hold phase (Fig. 8A). This reflex was not readily predictable from the extracellular depressor nerve recordings, where an interjoint effect from the TCMRO was rarely seen (Table 1). This can be explained by the fact that the depolarizing input from the TCMRO was seldom large enough to drive the cell past its spiking threshold. Where it was large enough, the responses of additional units in the extracellular nerve recording (Fig. 8C) confirmed the potential efficacy of this interjoint reflex. After a period of rest, the first stretch of a series in some cases evoked a spike in the cell (Fig. 8B). Subsequent responses, however, often failed to reach threshold, but instead declined progressively to a new equilibrium level (Fig. 8A).

Two depressor motoneurones from different preparations also showed a small transient depolarization following TCMRO release (Fig. 8C). This ‘off’ response was only observed with high ramp velocities, and only after the first stimulus following several minutes of rest. It is unlikely to have been a movement artefact since, after unhooking the TCMRO, the same movement failed to elicit the off response.

Levator motoneurones

Five cells that met each of the criteria for levator motoneurones were studied in different preparations. All five responded to input from both proprioceptors. Two are illustrated in Fig. 9.

Intrajoint reflexes from CBCO. Two of the five levator neurones were purely phasically sensitive, responding to CBCO stimulation only during the dynamic phase of stretch (Fig. 9A). Several spikes were usually superimposed upon an underlying slow wave depolarization. They were correlated with the impulses of a large unit in the extracellular levator nerve trace. It is possible that these cells received input only from velocity-sensitive CBCO afferents. Indeed, no levator motoneurones receiving tonic excitatory input from the CBCO were found in this study (see Bush, 1965c; Clarac *et al.* 1978). One of the levator motoneurones recorded intracellularly exhibited evidence of inhibitory input: on stretching the CBCO the cell responded normally, with spikes superimposed upon a graded membrane depolarization, but during the hold phase all existing activity was inhibited and the membrane potential returned to its resting level (Fig. 9B). Upon CBCO release, the membrane depolarized again and the synaptic activity returned, and indeed was enhanced during, and for a short period after, the

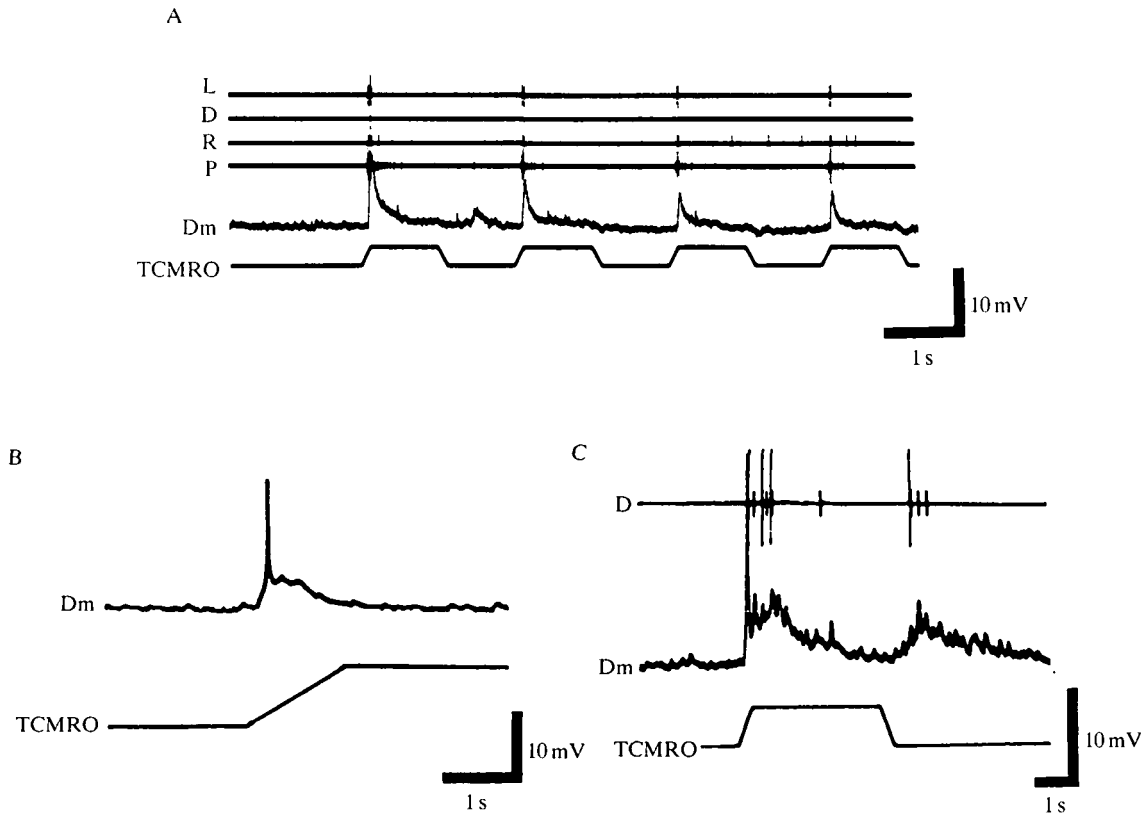


Fig. 8. Depressor motoneurons receive a convergent interjoint input from the TCMRO. (A) The depressor motoneurone (Dm) responds to the first of a series of TCMRO stimuli with a large dynamic depolarization on stretch, and returns to slightly above its resting level during the hold phase; some 'habituation' of the response occurs over the following three stimuli. The general shape of the response of this depressor motoneurone to the TCMRO stimulus resembles that of promotor motoneurons and reflects the T fibre afferent receptor potentials (cf. Fig. 4A,C,D), which often show a comparable habituation to repetitive stimuli. Simultaneous extracellular recordings from the levator (L), depressor (D), remotor (R) and promotor (P) show relatively weak reflexes in this instance (cf. Fig. 2), with evidence of habituation in each nerve. (B) Following a period of rest in another preparation, the first stretch of the TCMRO initiates a spike in the depressor motoneurone. (C) This depressor motoneurone was excited on both stretch and release, and the extracellular depressor nerve recording (D) shows two units responding in the same way.

release phase. This type of response may underlie the inhibition of levator spiking during the hold phase, and the subsequent increase in activity on release seen in some extracellular recordings from some levator motoneurons in other preparations (see Table 1).

Interjoint effects of the TCMRO. All five levator motoneurons examined

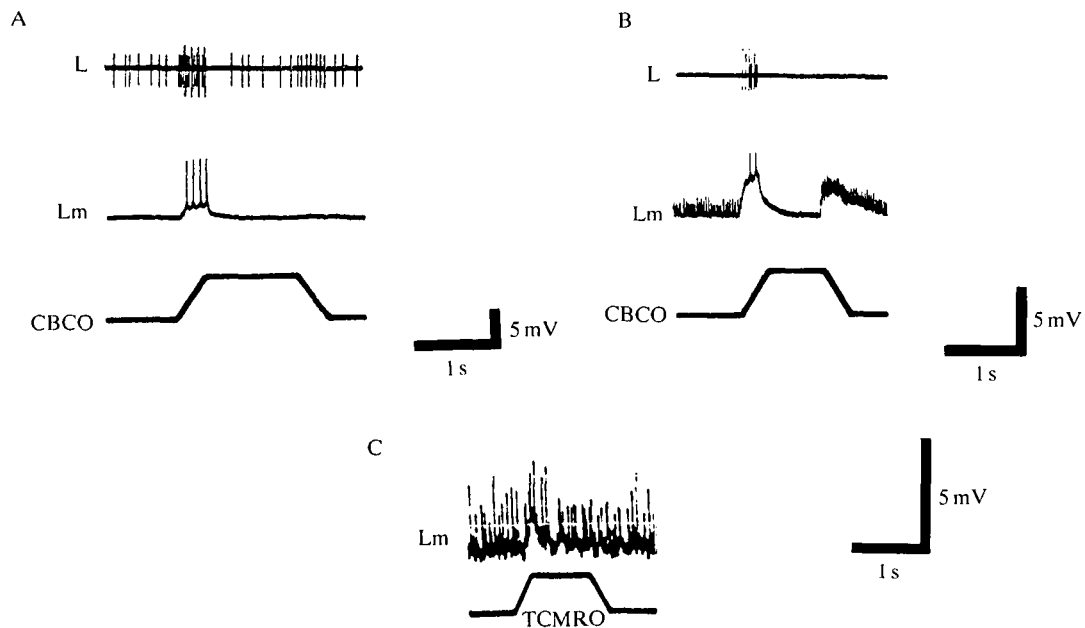


Fig. 9. Intracellular recordings from two levator motoneurones (Lm), illustrating their responses to CBCO and TCMRO stimulation. (A) A levator motoneurone (Lm) responding only to stretch of the CBCO. The simultaneous extracellular recording from the levator nerve (L) shows the purely phasic response of the same unit (four larger spikes), together with a tonically active unit that is also excited by CBCO stretch, but appears to be slightly inhibited during the hold phase. (B) A cell that responds to both stretch and release of the CBCO: two spikes occur at the peak of the large stretch-induced depolarization, but the tonic synaptic activity before and after the stimulus is inhibited during the stretch and hold phases. (C) The same cell as in B receives a small depolarizing input on stretch of the TCMRO.

intracellularly received a convergent interjoint reflex input from the TCMRO (Fig. 9C). Stretch of the TCMRO resulted in a relatively weak response and only rarely caused the cell to spike.

Discussion

The reflex effects of the thoracic-coxal muscle receptor organ and the coxo-basal chordotonal organ upon the muscles of the two basal joints of the back leg of the crab have been characterised by means of extracellular recording from the motor nerve roots and intracellular recording from individual motoneurones innervating these muscles. Each proprioceptor evoked intrajoint reflexes confined to the joint containing the receptor and also influenced the motor output to the muscles of the other joint (interjoint reflexes). Usually the intrajoint reflexes were stronger and more consistent than the interjoint ones. In the quiescent or tonically active

preparations considered here, these intrajoint reflexes were predominantly resistance reflexes (see Bush, 1962, 1965c; Bush and Roberts, 1968). *In vivo* they would tend to oppose any movement about the joint, and thus help to maintain the postural status of the limb. The same reflexes can function during locomotor behaviour in intact crayfish to counteract unintended movements or unexpected loading of their respective joints (Barnes, 1977). Normally, during centrally generated rhythmic motor activity, however, at least in the isolated crayfish nervous system, these proprioceptive reflexes are strongly modulated in a phase-dependent manner (Skorupski and Sillar, 1986).

The role of the interjoint reflexes is more difficult to assess but, when these reflexes, recorded in the isolated nervous system, are considered in the context of studies on intact animals, a probable function can be attributed to at least one of them. During sideways walking, the preferred direction in crabs, the thoracic-coxal (T-C) joint is held relatively immobile so as to form a semi-rigid strut to support the body while the coxo-basal (C-B) joint moves actively (Clarac and Barnes, 1985). The dynamic co-activation of the promotor and, to a lesser extent, the remotor muscle on both stretch and release of the CBCO would effectively stiffen the T-C joint, so enabling it to function as a strut during sideways walking, with the tension of the T-C muscles always being potentiated in phase with the C-B movements. The intrajoint reflex from the TCMRO to the promotor and remotor nerves would act to amplify the effect of the input from the CBCO.

From the intracellular recordings obtained in this study it can be predicted that the majority of motoneurons innervating the muscles of the two basal leg joints receive convergent inputs from both the principal proprioceptors monitoring these joints. At least one of these inputs (CBCO stretch to the depressor motoneurons) is inhibitory, so its effect would only be registered in extracellular nerve recordings (or by the muscle itself in the intact animal) against a background of impulse activity in these motoneurons. Several other inputs are subthreshold (e.g. the TCMRO input to some depressor motoneurons), so that inputs from one receptor alone will not initiate spikes in the motoneuron and, therefore, would not affect the target muscle. However, convergent depolarising input from both receptors at the same time could summate to elicit propagated spikes. The implication of this is that the phase and timing of the input from the two proprioceptors is critical in determining the motor output.

It has been proposed that convergent input of hyper- and depolarising inputs on single motoneurons may play an important role in motor coordination (Sandeman, 1969; Hoyle and Burrows, 1973; Burrows and Horridge, 1974). This can be directly illustrated in our preparation by taking as an example a typical depressor motoneuron that receives convergent input from both the CBCO and the TCMRO (see Figs 7 and 8). When stretch-hold-release stimuli are applied separately to the two receptor strands, the responses recorded from the integrating segment of the motoneuron might resemble that shown in the second trace of Fig. 10A, where the action potential output to the depressor muscle is represented in the top trace. With simultaneous stimulation (Fig. 10B), the excitatory

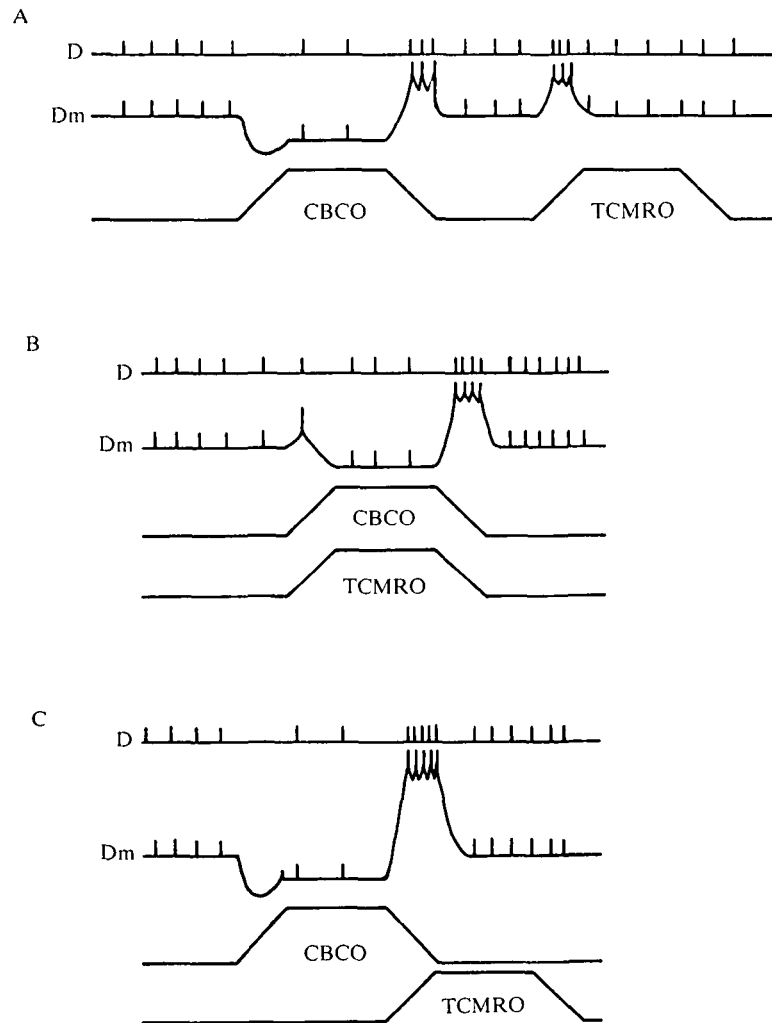


Fig. 10. Diagrammatic representation of convergent inputs onto a depressor motoneurone from the TCMRO and the CBCO. The motoneurone's membrane potential (D_m) and the consequent extracellularly recorded impulse output in the depressor motor nerve (D) depend upon the relative phase of the combined proprioceptive inputs. (A) Separate inputs converge onto the depressor motoneurone. (B) Both receptors are now in phase: note how the depolarizing input from the TCMRO cancels out some of the hyperpolarizing input from the CBCO. (C) CBCO release (falling phase) coincides with the rising phase of the TCMRO stimulus: the two depolarizing inputs now summate, evoking a higher-frequency burst of action potentials in the extracellular recording.

influence of the TCMRO will counteract to some extent the inhibitory input from the CBCO, thus reducing the fall-off in action potential frequency during both the dynamic and hold phases of CBCO stretch. When CBCO release coincides with

TCMRO stretch (Fig. 10C), the two convergent depolarising inputs summate and the resulting impulse output exceeds that evoked by either receptor alone.

The length of the TCMRO affects the gain of the interjoint reflexes from the CBCO to the promotor neurones. Thus, when the leg is remoted, so stretching the TCMRO, there will be a stronger interjoint reflex from the CBCO to the promotor muscle, tending to move the leg forwards. In this way, the CBCO can exert a powerful influence in regulating the position of the more basal T-C joint, in addition to its primary action on the C-B joint.

The results from this study indicate that *in vivo* the positioning and movement of the first two basal joints of the leg will be subject to combined reflex control by both the TCMRO and the CBCO. These act on the motoneurones innervating the two major antagonistic muscle groups in these joints, with the CBCO probably having the dominant role in positioning the two joints in relation to each other.

Many of the excitatory reflex influences observed in this study, interjoint as well as intrajoint, could be mediated by monosynaptic connections between the respective afferent and motoneurones, but direct proof of this must await dual intracellular recordings. One reason for this proposition is the quite close resemblance that is seen between the shape of the intracellularly recorded motoneurone response and that of one or other of the concomitant afferent receptor potentials, e.g. the reflex response of some promotor neurones to TCMRO input (see Fig. 4A; see also Blight and Llinas, 1980). Similarly, the depolarizing responses in the depressor motoneurones to TCMRO stretch resembled T fibre receptor potentials. Moreover, the observation that the depolarizing response is confined largely to the stretch phase of the TCMRO stimulus, and depends upon its velocity (Fig. 7C), reflects the marked velocity sensitivity of the T fibre (in contrast to the amplitude-sensitive S fibre). Furthermore, the decline (or 'habituation') in the response to successive stretches may be due to a parallel decline in the afferent response, resulting from the mechanical arrangement of the sensory endings of the T fibre in series with the receptor muscle of the TCMRO (Bush and Cannone, 1985).

A further complication in the system derives from the occurrence of interjoint reflex actions upon the efferent motor innervation of the TCMRO, which is normally co-activated with the promotor neurones (Head and Bush, 1991a). To simplify the system in this study the efferent pathway was opened. The effect of the pathway being closed is outside the scope of this paper and is dealt with in a previous paper (Head and Bush, 1991a).

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