COXAL MUSCLE RECEPTORS IN THE CRAB: THE RECEPTOR POTENTIALS OF S AND T FIBRES IN RESPONSE TO RAMP STRETCHES

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INTRODUCTION

The coxal muscle receptors in the crab (see Fig. 1; and Alexandrowicz & Whitear, 1957; Whitear, 1965) are unusual in that they lack all-or-none afferent impulses (Ripley, Bush & Roberts, 1968). Instead, their graded, depolarizing receptor potentials evoked by stretching the receptor muscle spread electrotonically along the two large sensory fibres to the thoracic ganglion, where their cell bodies are situated. These non-impulsive afferent responses are evidently normal for this sense organ, as is demonstrated by their capacity to evoke impulses in motoneurones to the leg promotor muscle (Bush & Roberts, 1968). Since the receptor muscle lies within and in parallel with this muscle (Fig. 1 A), this motoneurone response is presumably the efferent component of a stretch reflex.

The electrical properties of the sensory fibres and the ionic dependence of their resting and receptor potentials have been described (Roberts & Bush, 1971). As in most other nerves and receptors studied, the resting membrane potential depends largely upon permeability to K^+ ions, while the receptor current in normal crab saline is carried principally by Na^+ ions, though other ions evidently contribute to both types of potential. Unlike most other neurones, however, the voltage–current relations of the sensory fibres are almost ohmic, showing little or no active response to depolarizing currents. The occasional apparently 'active' component seen in the neurone's response to current or receptor muscle stretch is always small and graded with stimulus intensity, and at least in the case of stretch is reversibly abolished by tetrodotoxin, indicating that it too is due to Na^+ current. Transmission along the sensory fibres is largely passive and decremental, with a mean length constant of 3·5 mm in fibres averaging about 4·0 mm long and 50 μm in diameter (cf. λ of about 2·5 mm in crustacean motor axons of similar diameter.)

The two main, large-diameter sensory neurones, the 'S fibre' innervating the elastic 'strands' flanking the receptor muscle proximally, and the 'T fibre' ending in its proximal 'tendon' (see Fig. 1 B), respond to controlled stretch of the de-efferented receptor muscle with similar basic forms of receptor potential. In each fibre a 'dynamic component', concurrent with the dynamic phase of the stimulus and varying in height with velocity of stretching, precedes a less depolarized 'static component' related in amplitude to, and lasting for the duration of, maintained stretch (Ripley *et al.* 1968). In the present paper the relations between these responses and the parameters of the

mechanical stimulus are analysed in detail, and the differences between the S and T fibres are assessed (see Bush & Roberts, 1970, for a preliminary report of this work).

METHODS

The shore crabs used throughout this study, *Carcinus maenas*, were kept in re-circulating aerated and filtered sea water at 10–12 °C. Specimens with carapace widths of 30–50 mm were selected, the tougher sheath surrounding the sensory fibres
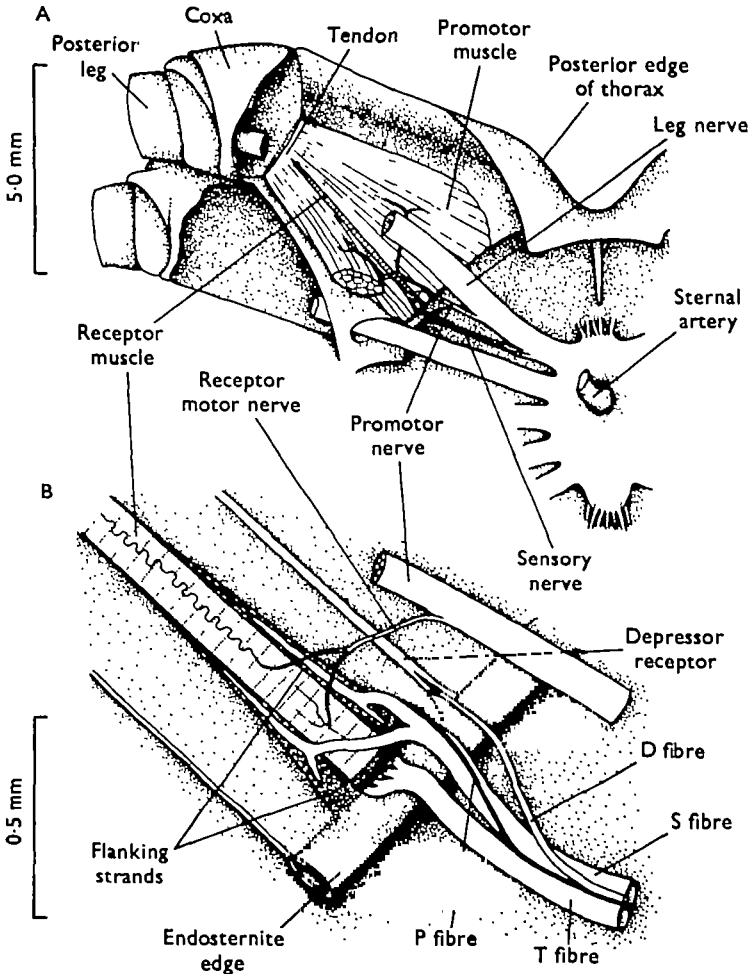

Fig. 1. (A) Semi-diagrammatic dorsal view of the thoracico-coxal muscle receptor *in situ* in the thoracic segment of the posterior right leg, as normally dissected for experiments. (B) An enlarged view of the base of the receptor, showing the gross anatomical relationships of the T-fibre and S-fibre endings to the receptor muscle and flanking strands, respectively.

of larger crabs being more difficult to penetrate with micro-electrodes. The receptor of either posterior leg (fifth pereiopod) was always used, this being the most readily prepared and having the longest sensory fibres (3–5 mm; and a receptor muscle of 4–7 mm mean length *in situ*).

Preparation. After severing all legs and chelipeds distal to their coxae, the dorsal carapace and viscera were removed under running crab saline (see Roberts & Bush, 1971). The cleaned ventral thorax was then pinned on a U-shaped cork block in a dish containing crab saline, for further dissection by transmitted light. In some experiments (e.g. Figs. 2, 11) the receptor system was simply exposed and left *in situ* in the ventral thorax (Fig. 1 A), the receptor nerve being supported for micro-electrode penetration by a suction electrode or fine hook. In most cases, however, the receptor, promotor muscle and thoracic ganglion were isolated and transferred to a small perspex bath containing saline. The ganglion and promotor muscle were pinned out on a small polyethylene plate, with the interconnecting receptor nerve supported by a fine glass rod or stainless-steel pins, and illuminated obliquely from below. Except for the experiments on the motor control of the receptor muscle (section 6) the motor nerve supplying the receptor and promotor muscles were cut, so that the receptor was de-efferented and in open-loop conditions.

Mechanical stimulation. The distal insertion of the receptor muscle, *in situ* or in the isolated preparation, was cut loose on a small piece of tendon, and clamped in a slit in the nylon tip of a probe attached to a Pye–Ling V47 vibrator (the 'puller'). This was mounted nearly horizontally on a longitudinal rack-and-pinion, whose position was monitored by a dial gauge, for control of the resting length of the receptor muscle. Transverse and vertical screw adjustments enabled accurate positioning of the puller. Ramp-function movements of the puller probe, variable in velocity, amplitude and duration, were produced by an electronic function generator, and monitored by an RCA 5734 transducer coupled to the probe by a flexible steel pin. This open-loop system was adequately linear and with adjustable mechanical damping gave reasonable ramps up to about 10 mm/sec, but faster ramps became increasingly rounded (see Figs. 2, 3). Stretch amplitudes generally did not exceed about 0·5 mm, 0·3–0·4 mm being the commonest size of pull employed (cf. receptor muscle length of 5–6·5 mm over the full arc of joint movement in a crab 40 mm wide).

Recording techniques were conventional. Glass micropipettes filled with 3 M-KCl with d.c. resistances of 20–50 MΩ were used to record intracellularly from the sensory fibres, and Polythene suction electrodes for *en passant* extracellular recording in some early experiments. Stimulus and response were displayed on a triggered multi-beam oscilloscope and photographed on a 35 mm oscilloscope camera with automatic frame advance. In each record the sensory responses appear on the upper traces, while the bottom trace records the output of the movement transducer, stretching of the receptor muscle being indicated by upward deflexion of this trace and releasing by downward deflexion (except in Figs. 4B–D and 10, where the movement trace is inverted.)

RESULTS

1. *Preliminary observations*

The main features of the responses of the S and T fibres to brief, fast pulls on the receptor muscle are illustrated in Fig. 2, substantiating our previous observations (Ripley *et al.* 1968). The absence of all-or-none impulses in the afferent fibres is confirmed by extracellular records from the whole receptor nerve (Fig. 2A, B), as well as by intracellular recording from each fibre (A, C–E). Only slow, graded responses

with the basic dynamic-static form of typical depolarizing receptor potentials are seen. Instead of generating impulses, these receptor potentials spread decrementally along the S and T sensory fibres towards the thoracic ganglion (B–E). The gradation of the dynamic and static amplitudes with velocity and magnitude of receptor-muscle stretch is illustrated in Fig. 2A, F and G.

Similar graded responses without impulses occur in the 30–40 μm diameter 'D fibre', when the non-muscular, elastic depressor-receptor strand which it inner- vates (Alexandrowicz & Whitear, 1957) is stretched (Fig. 8, inset records).
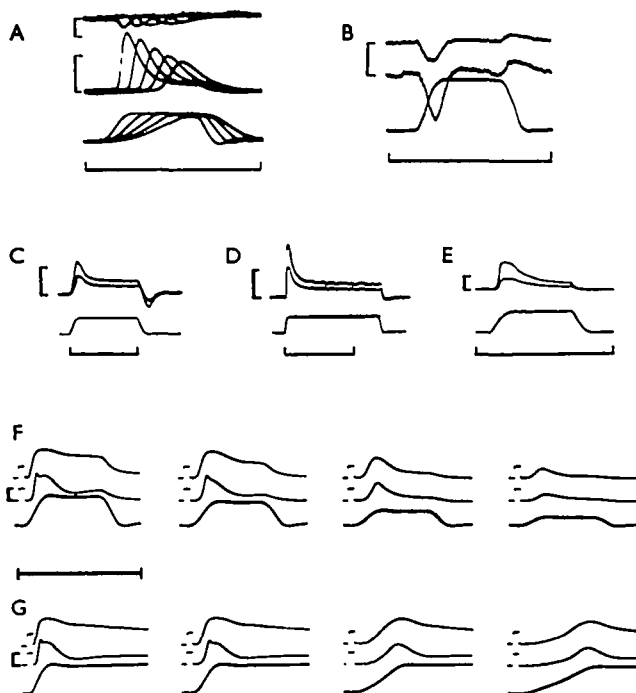

Fig. 2. S-fibre and T-fibre responses to brief pulls (lower traces, stretching upwards) applied to the receptor muscle *in situ*. (A) Gradation in dynamic response with velocity of stretching, five responses superimposed: middle traces, intracellular T-fibre recording; upper traces, simul- taneous extracellular recordings from whole receptor nerve. (B) Simultaneous *a.c.* recordings (time constant, 0·3 sec.) with one extracellular electrode near the receptor muscle (middle trace), and another about 1·5 mm proximal (upper trace); (C–E) Intracellular records from two electrodes about 2 mm apart in an S fibre (C) and two T fibres (D, E). (F–G) Simultaneous S (upper traces) and T (middle traces) responses to decreasing stretch amplitudes (F) and velocities (G). Calibrations: 20 mV (intracellular), 100 μV (extracellular), and 100 msec.

There is, however, a good deal of variability between preparations, both in the form of the receptor potentials in each fibre and in the differences of form between S and T fibres (cf. Figs. 2 and 3A–D). Furthermore, even successive responses to a constant stimulus within a single preparation can vary significantly in form. Fig. 3E, F shows a not uncommon effect of repetition of a constant fast stretch – the development of a small spike-like transient on the rising phase of the dynamic component. Its rate of development depends upon the rate of repetition, longer intervals being less effective than shorter ones. As will be seen later (section 5), variation of this kind is probably

due to progressively changing resting tension in the receptor muscle, as a result here of the repetitive stimulation, even though it is de-efferented.

2. *Characterization of S and T response waveforms*

Most of our earlier observations on S and T fibres, like those in Figs. 2 and 3, were made with relatively fast and brief pulls. However, characteristic and more readily distinguishable response waveforms are revealed by using slower stretches, which also allow clearer separation of the different temporal components of the responses. Even
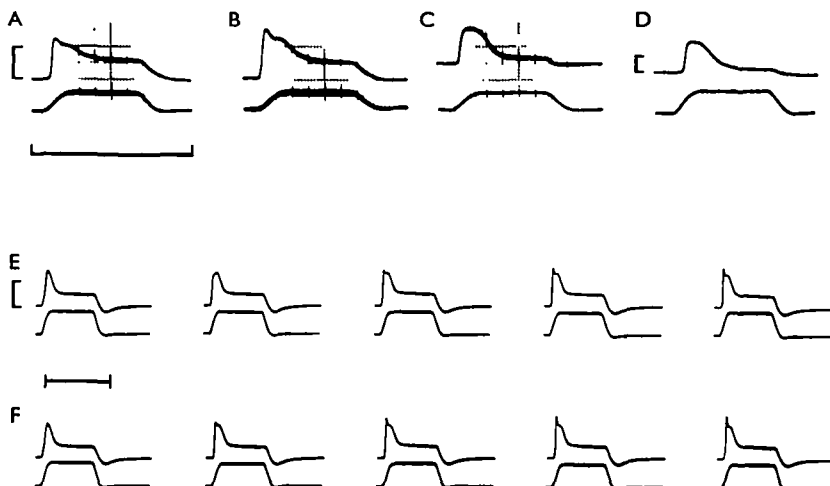

Fig. 3. Variability of receptor potentials in response to fast stretches. (A–D) Responses of two S fibres (A, B) and two T fibres (C, D) in three different isolated preparations (A; B and C; D). (E, F) T-fibre responses to constant pulls repeated at intervals of 1·7 sec. (E) and 0·7 sec (F). Calibrations: 20 mV and 100 msec.

here, the response waveforms vary somewhat between preparations. More or less typical waveforms of S and T responses to such stimuli are shown in Figs. 4 and 5. (Note that the time-base is 5–10 times slower than in Figs. 2 and 3 and in most of the records shown in our previous papers.)

A first point to notice is that T-fibre responses are often larger than the corresponding S responses (Figs. 4B, 5), though the degree of difference seen in Fig. 5 is unusual. However, in some preparations the responses of the S fibre are larger than those of the T fibre (Fig. 4A), while on occasions the S and T responses have been observed to vary in amplitude reciprocally (see later).

The differences in shape of the S-fibre and T-fibre responses seen in Fig. 4B, C were consistent throughout the experiment, and are fairly representative. The S response generally resembles an algebraic sum of analogue potentials reflecting receptor length and rate of change (first derivative) of length (see Fig. 4E). Thus the positive dynamic component of the S response – i.e. that portion of the response occurring during the positive dynamic phase of the stimulus, or stretching – comprises a positive velocity response superimposed upon a length response, and often also a rather variable initial transient or 'delta function' spike which might be an acceleration response (see below). Similarly, during the negative dynamic phase (shortening), an almost mirror-

image, negative velocity response is generally seen in the S fibre, sometimes preceded by a small 'negative acceleration' transient (which when present is less variable than the positive acceleration spike at onset of stretch).
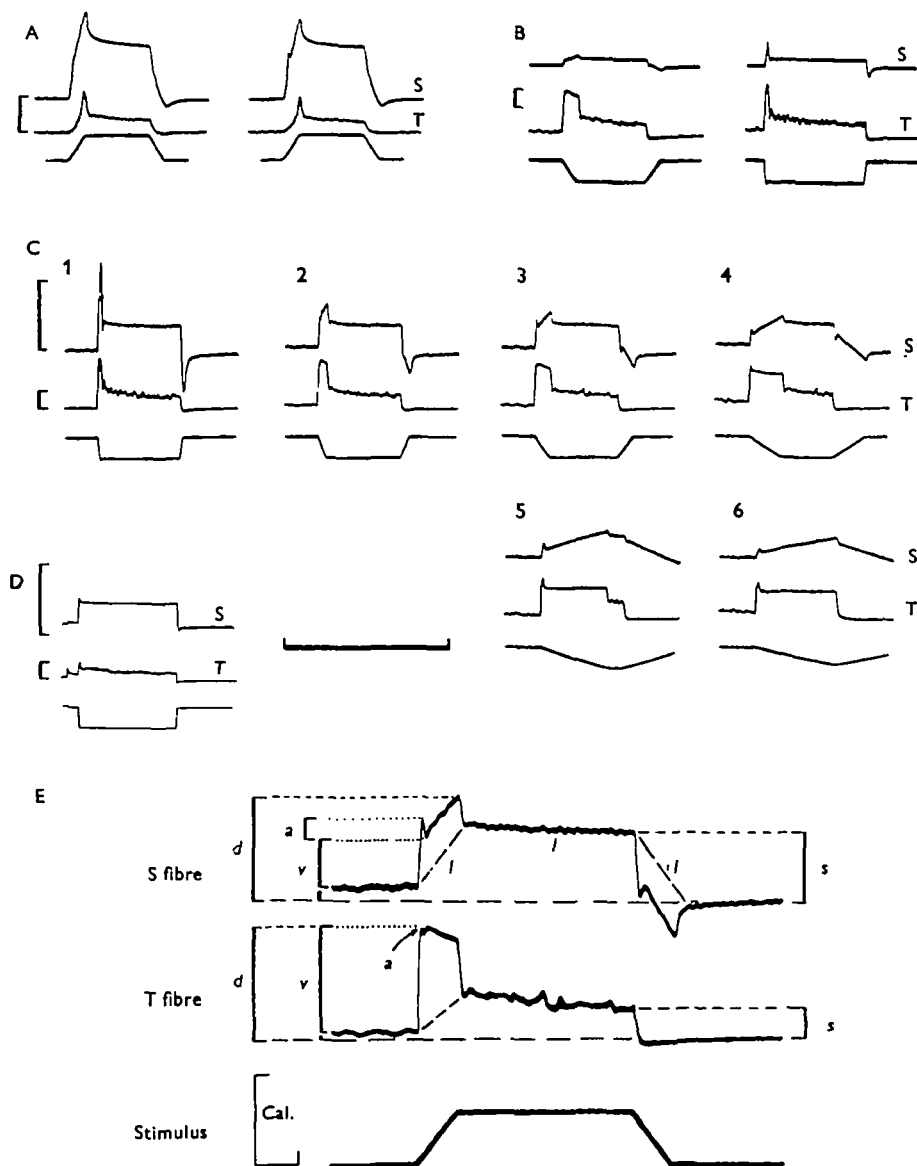

Fig. 4. Simultaneous S-fibre and T-fibre responses to relatively slow stretches of the receptor muscle (lower traces: stretching upwards in A, E; downwards in B–D). (A) Large S and small T responses, for comparison with (B) small S and large T responses, all to similar stimuli. (C) Successive responses (1–6) to decreasing velocities of stretch. (D) Relatively large and well-sustained static component of S response, compared with marked dynamic response in T fibre even to the small initial step. B–D are from one preparation. Calibrations: 20 mV and 1 sec (50 sec in D). (E) ' Typical' response to a 5 mm/sec stretch, showing the contributions of length (*l*), velocity (*v*) and 'acceleration' (*a*) components; '*d*' indicates the dynamic amplitudes plotted in Fig. 6 (see text), and '*s*' the 'static response' to stretch. Calibration 10 mV (S), 40 mV (T) and 100 msec.

By contrast, the T-fibre responses do not show a true negative dynamic component, but only a relatively small and simple hyperpolarizing 'after-potential', whose amplitude varies little if at all with rate of shortening, but rather with degree of stretch or depolarization prior to release (Fig. 5 C). Moreover the positive dynamic component in the T fibre is not a simple function of stretch velocity. Instead, it commonly declines throughout the dynamic phase of the stimulus, at a rate which decreases with decreasing stretch velocity until, at a particular fairly low velocity, it may remain at a constant level and at still lower velocities it may increase during stretching (Figs. 4 C, 5 A). The
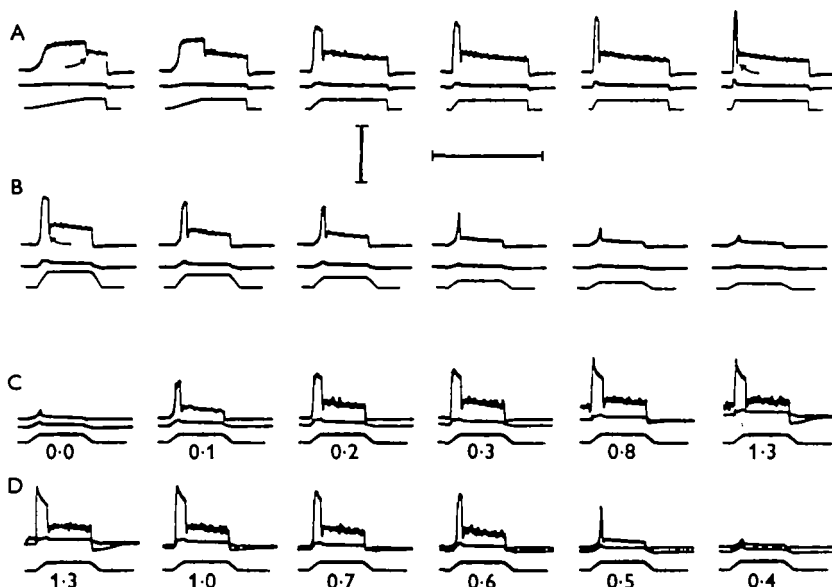

Fig. 5. T-fibre and S-fibre responses in one preparation to variation in stimulus parameters: (A) increasing stretch velocity; (B) decreasing stretch amplitude; and (C) increasing, followed by (D) decreasing receptor-muscle lengths, in steps of 0·1 mm at 10 sec intervals. Only selected records are shown in C and D; the subscripts indicate the length increment in millimetres over the minimum length *in vivo* (normal length range: 5·0–6·5 mm). Stretch amplitude in A, C and D was 0·3 mm. Calibrations: 50 mV and 1 sec.

initial rate of decline is also enhanced by increasing the resting length of the receptor muscle (Fig. 5 C). Whether this decline in the T-fibre dynamic component is due to 'adaptation' of the velocity response, or to an acceleration component with a long time-constant of decay, is not clear from these experiments.

An alternative and perhaps more likely 'acceleration response', in the T as well as the S fibre, is the much briefer spiky transient at the onset of the dynamic component (Fig. 4 C, E). This is more evident with slower stretches, being increasingly obscured by the velocity component with faster stretches. However, it is a particularly labile portion of the response in both fibres, and appears to vary with the starting length, or tension, in the receptor muscle (cf. Figs. 5 C, D, 9, 10). Immediately following cessation of stretching, particularly at the higher velocities and extents of stretch, the T-fibre response commonly, and sometimes also the S-fibre response, shows a small transient repolarization (arrowed in Fig. 5). This could result from the deceleration at this

moment, or it might be a consequence of K^+ activation. Clearly, further studies are needed to assess the effects both of acceleration and of deceleration.

The static components of both S and T fibres decline slowly in amplitude initially, with a time constant of the order of 1 sec (Figs. 4, 5). (*Note*: The term 'static component' as used here – see Introduction – does not imply a completely static or constant membrane potential, but refers to that portion of the response occurring during the
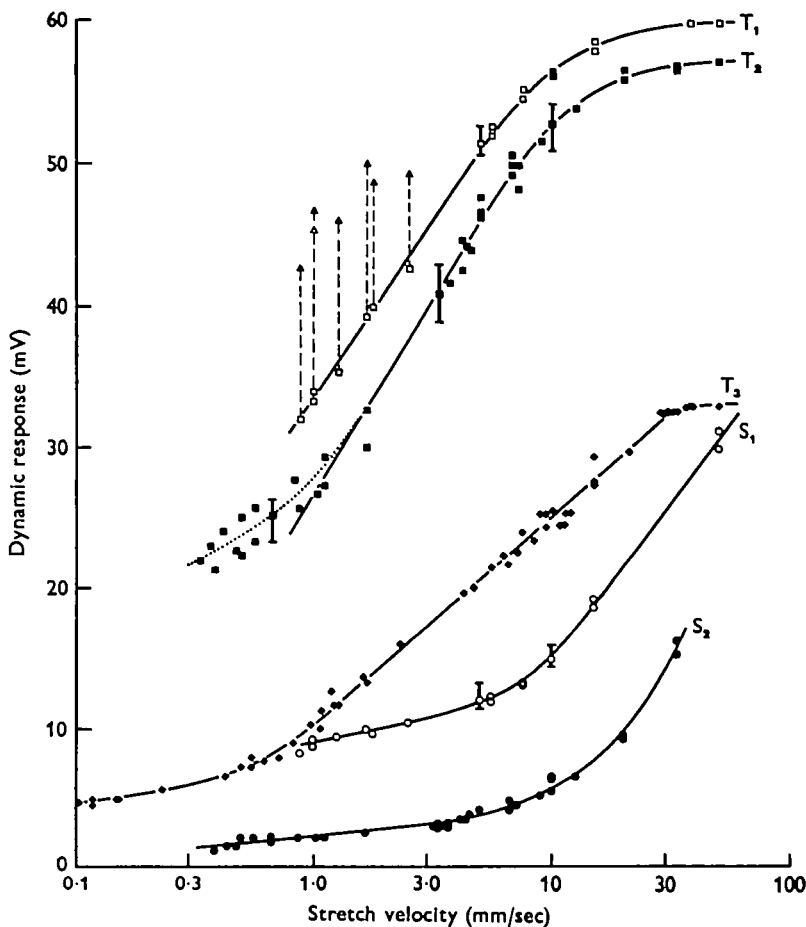

Fig. 6. Relation between velocity of stretch and amplitude of the dynamic component in two S fibres and three T fibres; S_1 and T_1 are from the preparation represented in Fig. 4C, S_2 and T_2 from that of Fig. 5, and T_3 from that of Fig. 9B. Triangles represent the peak amplitudes of the initial spiky transients ('acceleration components'?), squares the beginnings of the true velocity components, except at the lower velocities in T_2 (dotted line: see text), and circles the maximum amplitudes of the S-fibre dynamic components. (See Fig. 4E.)

static phase of the stimulus, in accordance with muscle spindle terminology; e.g. Matthews, 1964.) This 'adaptation' in the first second or two of the static components of both fibres does not appear to vary in extent or rate with velocity of the preceding stretch (except perhaps in the T fibre immediately after fast stretches in some cases). Thus it can be regarded as a true adaptation in the static component, rather than a decline in some after-effect of the dynamic component. Whether its origin is mechanical

▶ neural, however, remains to be investigated. The former seems more likely at present, since steady outward currents applied intracellularly to the S and T fibres result in steady depolarizations, with little if any indication of neural accommodation. If anything, the degree of adaptation during the static phase was generally rather less in the S than in the T fibre, suggesting a further source of adaptation in the T fibre.

A common difference between the S and T fibres was the frequent presence of small oscillations, or 'noise', superimposed on the static component (or steady-length potential) of the T but not the S fibre (Figs. 2D, 4C, 5, 7C). These oscillations commonly vary in amplitude directly with degree of sustained depolarization in the T fibre, and they appear to depend primarily on the tension in the receptor muscle. Thus they generally increase with receptor length (Figs. 5B, C, 7C), but they may also vary at any one length, perhaps due to spontaneous variations in resting tension, albeit in the de-efferented receptor muscle (see later). In addition, the amplitude of any potential oscillations in the T fibre is greatly enhanced by any slight mechanical vibrations at the puller probe tip. That such vibrations are not normally the primary (or even a necessary) causal factor in the oscillations, however, is indicated by their occasional occurrence in the complete absence of any known vibrations (e.g. just before stretching in Figs. 4B, C and 5C).

3. *The dynamic response and stretch velocity*

In Fig. 6 the relation between velocity of receptor-muscle stretch and the amplitudes of the dynamic components is plotted for the two S fibres and three T fibres represented in Figs. 4C, 5 and 9. Over the range from around 0·5 to 10–30 mm/sec. the T-fibre responses varied nearly logarithmically with stretch velocity, with a slope of 15–25 mV per tenfold change in stretch velocity. In contrast, S fibres generally showed relatively low sensitivity to rate of stretch over most of the range of velocities studied, even when their responses were larger than those represented here. Above 5–10 mm/sec S fibres with small responses showed a marked increase in velocity sensitivity, as seen in Fig. 6.

It should be noted that the dynamic amplitudes plotted in Fig. 6 are not strictly equivalent for the S and T fibres, or even for each of the T-fibre curves, since in most cases the value plotted was the maximum height of the dynamic component (above the mean resting membrane potential just before stretching or 200–300 msec after release). As indicated above and illustrated in Fig. 4C, E, the peak of the S response occurs at the end of the dynamic phase of the stimulus, whereas that of the T response commonly occurs near the beginning of stretch. Thus the S-fibre values plotted in Fig. 6 represent the sums of the velocity and length responses. But since the static component of this fibre on cessation of stretching would be virtually constant for all points from a given series of responses at different velocities (see section 4, below), its contribution to the S-fibre dynamic values plotted would not alter the shapes of the curves as plotted here (with a linear ordinate), but only their position on the vertical scale.

On the other hand, the values plotted in Fig. 6 for the T fibres probably represent either the true velocity components, or in some cases perhaps the sums of velocity and acceleration components. Only in curve T_2, from the preparation of Fig. 5, does the static component probably contribute to some of the values plotted, namely those for the lower velocities, as indicated by the dotted line. Owing to the slow sigmoid

rising phase of the dynamic component in these responses (e.g. Fig. 5A1), the amplitudes could not be measured satisfactorily at the onset of stretching, as was done for curves T_1 and T_3. Thus throughout this series of records only the maximum height was plotted even when this occurred at the end of stretching, as was the case for the slower stretches.
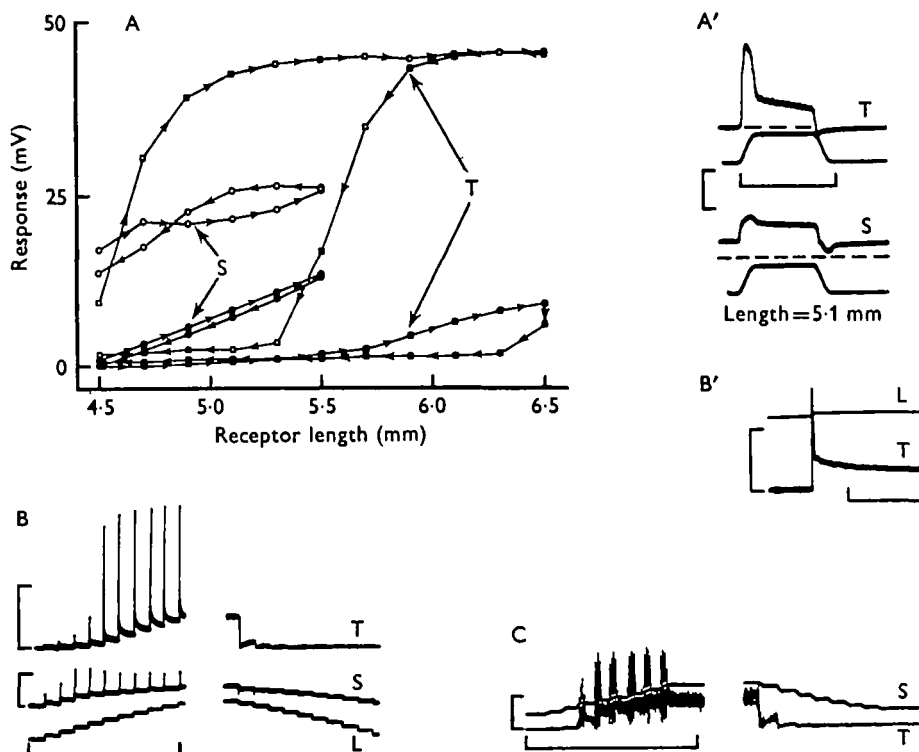

Fig. 7. Relation between receptor-muscle length and static and dynamic responses in the S (circles) and T fibres (squares). (A) Plots of the initial membrane potential ('static response' – closed symbols) and peak amplitude of the dynamic response (open symbols) to constant pulls (0·3 mm and 400 msec duration – examples (A') shown beside graph applied every 10 sec at different receptor lengths, first increasing then decreasing as indicated by arrows. (B) Responses from the same preparation to stepwise changes in length (L): inset record (B') shows a response to one increment in length at × 50 the sweep-speed of the stepwise recordings. (C) Responses from a different preparation (same as Fig. 5) to a similar sequence of length changes, but produced manually by rack-and-pinion (see text; no movement trace). Calibrations: 20 mV and 500 msec (in A' and B'), 50 sec (in B and C).

In contrast, the elevated points at the lower velocities in curve T_1 (triangles) indicate the peaks of the initial fast spike (the putative 'acceleration component' – see Fig. 4 C, E). Similar initial transients are often discernible at the higher velocities but are exceeded in height by the main dynamic component. These points clearly do not fit a simple relationship with stretch velocity. The principal T-fibre values plotted in Fig. 6 therefore (represented by the squares and solid curves, T_1, T_2, and T_3) are the peak amplitudes (measured from the unstretched base-line) of what appear to be the true velocity components, excluding the initial spiky element.

4. *Membrane potential and receptor-muscle length*

The membrane potential of both S and T fibres varies with the length of the receptor muscle and, presumably, with the tension in it (Figs. 5 B–D, 7). When the receptor is clearly slack, the membrane potential in both fibres is maximal (-50 to -70 mV in different preparations). Sustained stretch causes a maintained depolarization of S and T fibres, by an amount directly related to the extent of stretch, but depending also upon the degree of contraction or tonus in the receptor muscle.

When there is no evidence of active muscle tension or spontaneous variation in response (see later), the steady state or static response of the S fibre, measured at least 1 sec after any length change, is an approximately linear function of receptor length (Fig. 7). This relationship is generally true regardless of how a given length is reached, whether before or after a slow or rapid increase or decrease in length. Thus it is seen in the S fibre not only with varying amplitude stretches of longish duration (cf. Fig. 5 B), but is particularly clear with stepwise changes in length (Fig. 7 B, C). In contrast to the S fibre, however, the T fibre's membrane potential is related to receptor length in a markedly non-linear way (Figs. 5 C, D, 7).

Moreover the T fibre shows pronounced hysteresis to increasing and then decreasing lengths, in both its dynamic and its static responses. The hysteresis in its static responses is clearly seen with stepwise increments and decrements in length at, for example, 5 or 10 sec intervals (Fig. 7 B). It is also evident in the dynamic responses to constant pulls applied every 10 sec at different lengths, first increasing and then decreasing (Fig. 7 A). By contrast, little if any hysteresis is present in the S-fibre responses to the same stimuli.

Fig. 7 C shows simultaneous stepwise responses of the S and T fibres in another preparation to slow lengthening and shortening movements, produced by hand manipulation of the rack-and-pinion holding the puller. These rather shaky stretching movements evoked in the T fibre bursts of quite large spike-like potentials, which however were highly variable in amplitude and did not reach zero potential. At the greater steady lengths between stretching movements the T fibre appeared very 'noisy', perhaps partly but not wholly due to slight vibrations in the probe holding the receptor muscle. As already seen (section 2), similar 'noise' is evident in the T fibre's responses to electronically controlled stretch-release stimuli (Fig. 5). Its source will be discussed later. In spite of the slow movements and 'noisy' T-fibre response in Fig. 7 C, hysteresis is pronounced in this fibre but absent from the S-fibre trace, which closely reflected the length changes (not monitored in this record).

Intracellular records have also been obtained from the somewhat smaller diameter 'D fibre', which ends in the non-muscular 'depressor receptor' (Alexandrowicz & Whitear, 1957) alongside the muscle receptor (Fig. 8). In the dependence of its response amplitudes upon receptor length and amplitude of stretch, the 'D fibre' resembles the S more than the T fibre. In particular there is little hysteresis in either its dynamic or its static components in response to constant stretch-release pulls at different receptor lengths. This, like its relatively linear behaviour, is in accordance with its lack of any muscle tissue (cf. the S fibre).

5. *Response variability and the effect of the receptor muscle*

As already noted, the S-fibre and T-fibre responses to standard stimuli to the receptor muscle are by no means constant. Differences between preparations are sometimes quite marked (see Figs. 2–5), and may be accentuated by the occasional occurrence of graded active responses of the sensory fibre membrane (Roberts & Bush, 1971, figs. 4, 5). One important reason for such differences may well be uncontrolled variability in the contractile condition of the de-efferented receptor muscles, and associated variability in their visco-elastic properties and stiffness. Likewise much of the variability in the responses to similar stimuli seen in single preparations can probably be attributed to 'spontaneous' changes in resting tension or contracture in the receptor muscle. In some preparations, for example, slight changes in degree of
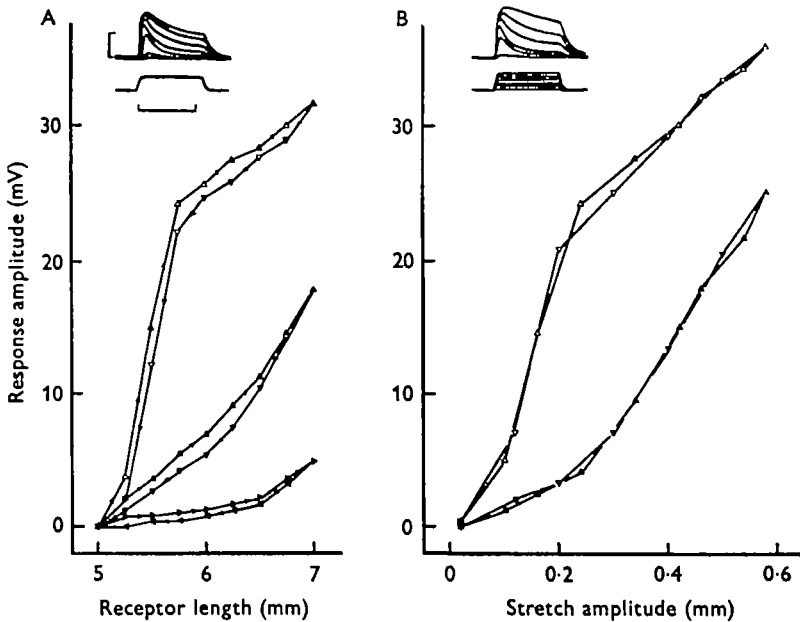

Fig. 8. 'D fibre' responses to stretching the elastic depressor-receptor strand, represented graphically and by superimposed sample responses. (A) Responses to a constant stimulus applied at different receptor lengths, at 10 sec intervals; and (B) to different amplitudes of stretch at a constant length. Open triangles: peaks of dynamic components; closed triangles: amplitudes of 'static components' just before release; lowest triangles in A: membrane potential just before stretch. Arrows and directions of triangles indicate sequence of responses. Calibrations: 20 mV and 100 msec.

contracture were noticed under the dissecting microscope, particularly in the proximal third of the receptor, even ½–1 h following section of its efferent nerve supply in the promotor motor nerve.

Indirect evidence for such contractural changes is illustrated in Fig. 9. (A) shows variations in response to a constant-velocity stretch at a constant receptor-length in one preparation. The changes in the dynamic components of these responses are similar to those produced in another preparation (Fig. 9B) by imposed length changes. This strongly suggests that the resting receptor muscle tension increased progressively

the first four responses in Fig. 9A, by amounts comparable to the tension increases produced in the lower records by the increasing receptor lengths.

Since the T-fibre dendrites are in series mechanically with the whole receptor muscle, variations in contractile tension should directly affect T-fibre responses to applied stretch. However, it is *a priori* less clear how tension in the receptor muscle should affect S-fibre responses to stretch.

In the experiment from which the records in Fig. 4B, C were obtained, the S-fibre responses to several identical stretch-release stimuli applied at irregular intervals within a 10 min period were virtually identical except for the height of the initial transient ('acceleration'?) peak at the onset of stretch. Further analysis revealed that this initial spike height in the S fibre was well correlated with the resting membrane potential in the T fibre just preceding each pull. As Fig. 10 shows, the amplitude of
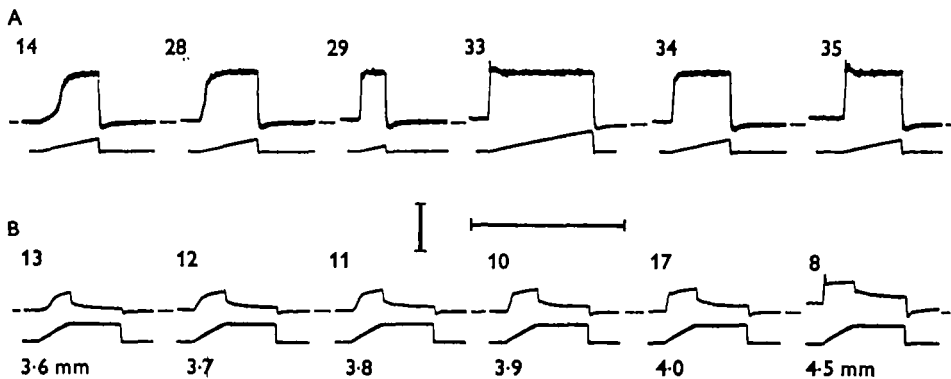

Fig. 9. 'Spontaneous' variation in form of T-fibre dynamic responses to constant velocity pulls (A), compared with selected responses to constant stimuli applied at different receptor muscle lengths (B). The figures indicate the number of each response in the series (evoked at about 10 sec intervals), and in (B) the resting length of the receptor muscle. (A) is from the same preparation as Fig. 5, and (B) from a different preparation. The base-lines between records indicate the 'unstretched' resting potential. Calibration: 20 mV and 1 sec.

the initial S spike (relative to the S membrane potential at the end of the record) is inversely related to approximately the square root of the degree of depolarization of the T fibre immediately preceding each pull (relative to its maximum membrane potential at that receptor-muscle length). If it is assumed that the resting membrane potential in the T fibre is some function of the tension prevailing in the receptor muscle, then this observation suggests an inverse dependence of this initial peak of the S response upon receptor tension.

Another, again spontaneous, 'reciprocal' variation in S and T responses involved the complete receptor potentials (Fig. 11). Constant, fast pulls of 100 msec duration were applied to the receptor muscle at 1 min intervals, without any known variation in experimental conditions. At first the S-fibre dynamic component decreased steadily in amplitude while the T-fibre static component increased somewhat. After the 27th pull the whole T response suddenly decreased, and simultaneously the whole S response increased to its former size.

As noted previously, the S and T responses may also differ in size reciprocally in

different preparations. Thus in the experiments represented in Figs. 4B–D and 5 the S-fibre responses were considerably smaller than the corresponding T responses whereas the reverse was true in the preparation yielding Fig. 4A. This variability between preparations may well depend upon the same factors as the corresponding variation within a single preparation.

6. *Electrical behaviour of the receptor muscle*

The receptor muscle resembles typical slow crustacean muscle in its electrical response to impulses in its motor nerve (Fig. 12). Single-shock stimulation of the whole (promotor + receptor) motor nerve while recording intracellularly from single fibres in the receptor muscle evokes depolarizing junctional potentials of 2–6 mV amplitude. Repetitive stimulation at various frequencies causes facilitation and summation of the j.p.s, the extent of each varying in the usual manner with stimulation frequency and
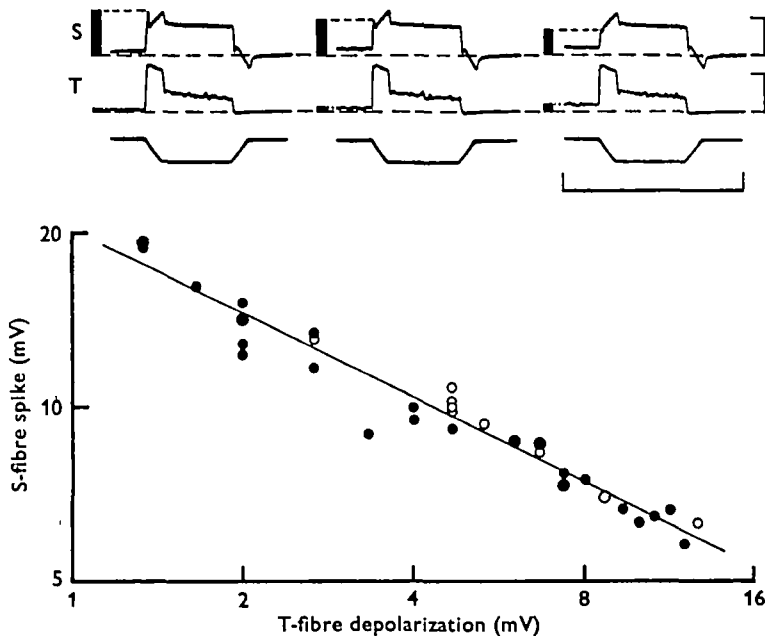

Fig. 10. Inverse relation between the amplitude of the initial fast spike in the S response and the level of depolarization in the T fibre. Both values are measured from the post-stimulus resting potential, as indicated by the vertical bars before each sample record. From the same series as Fig. 4c. Stretch velocities were 5·0–5·6 mm/sec (solid circles; large ones = two points each), 7·5–10 mm/sec (small open circles), and 2·5 mm/sec (large open circle). Calibrations 10 mV (S), 40 mV (T) and 1 sec.

time course of the individual j.p.s. Different muscle fibres in the same or different receptors show a range of time courses, as exemplified in Fig. 12 (compare A and B). Single muscle fibres never exhibited more than one type of junctional potential, even with fine gradation of motor nerve stimulation. Whether there is more than one motor axon to the whole receptor muscle, however, is still uncertain.

Apart from one exceptional case, no clearly active responses were encountered from any of the intracellular recordings made in the posterior leg receptor muscles of six

abs. The exception, from one of three penetrations in one preparation, constituted brief (2 msec), large (70 mV), non-facilitating and non-summating all-or-none impulses. In view of their proximal location in the receptor muscle, short latency (1 msec), and one-to-one following to 200/sec, it seems probable that they resulted from a fortuitous penetration of a motor axon near its entry into the receptor muscle. However, the possibility of there being one or more muscle fibres with propagated impulses cannot be excluded at this stage.

In several micro-electrode traverses through the middle of these receptor muscles, an average of 3–4 successive intracellular potentials were noted, ranging from −40 to −70 mV. Taken together with anatomical findings of Whitear (1965; see especially fig. 4), this suggests that there may be 8–16 muscle fibres in the receptor.
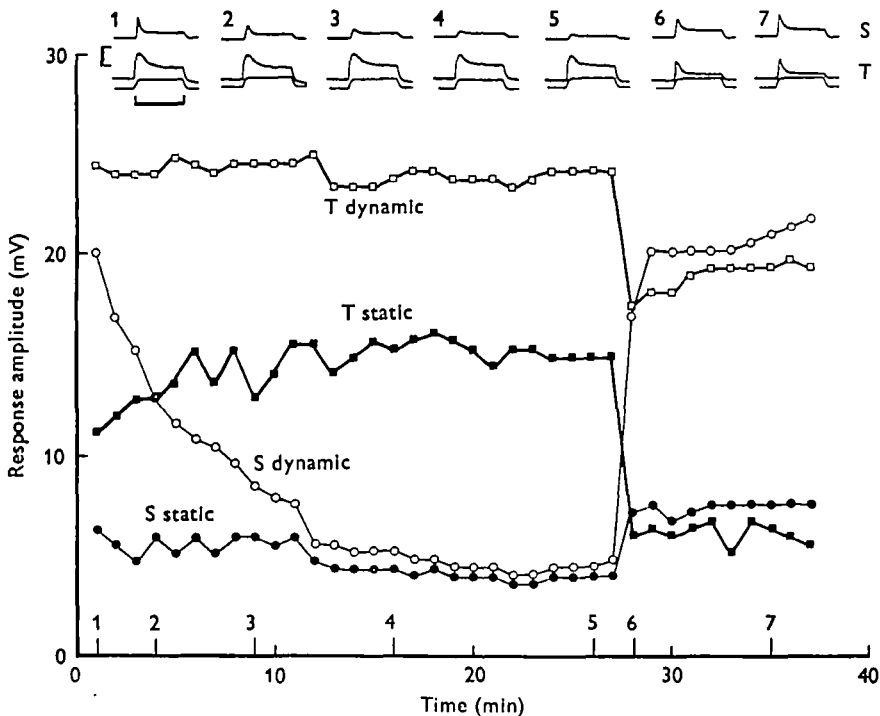

Fig. 11. Reciprocal variation with time in the peak dynamic and final 'static' amplitudes of the S and T responses to pulls of constant 100 msec duration applied to the receptor muscle every minute. The numbers above the abscissa indicate the times at which the corresponding records were obtained. Calibrations: 20 mV and 100 msec.

In a relatively fresh preparation with intact motor supply spontaneous centrally originating discharges of varying frequency can be recorded in the receptor muscle (Fig. 12 C). This provides a basis for the prediction that, as with other muscle receptors efferent modulation of the sensitivity of the receptor can and does occur naturally in the intact animal.

The slow, facilitating junctional potentials seen in these records of both evoked and spontaneous efferent activity suggest that the receptor muscle may well be mechanically slow too, capable of sustained contractures of the kind that could underlie the 'spon-

taneous' variations in receptor-potential amplitudes described in the preceding section. The possible occurrence of some phasic, mechanically fast muscle fibres in the receptor cannot yet be ruled out however. Further study involving tension measurements is needed to establish the mechanical properties of the receptor muscle and its linkages with the S-fibre and T-fibre dendrites.
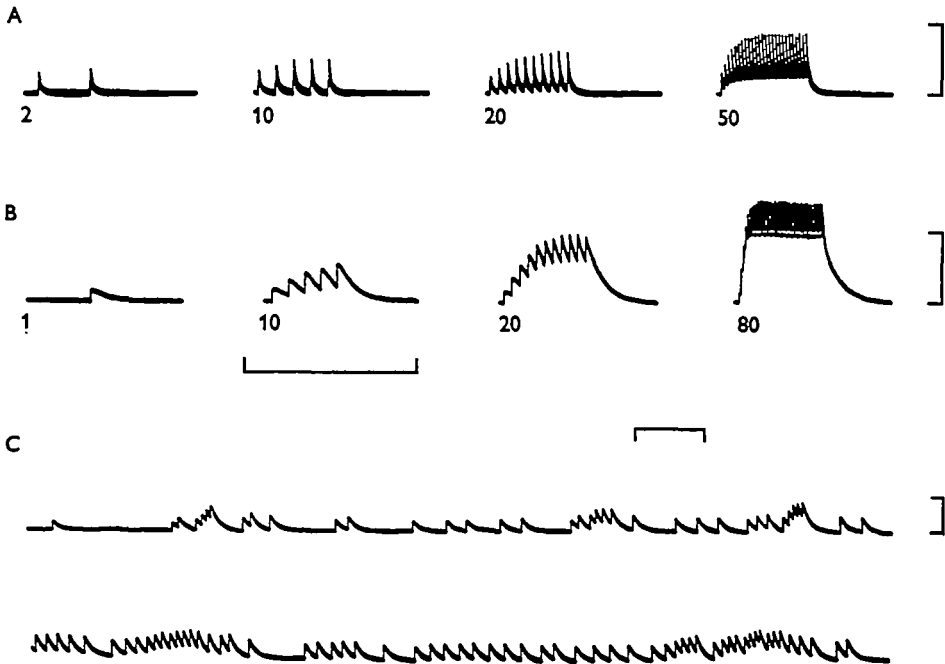

Fig. 12. Intracellular records from the receptor muscle. (A, B) Responses of two muscle fibres in different preparations to electrical stimulation of the (promotor + receptor) motor nerve at the frequencies shown (Hz). (C) Spontaneous, centrally originating activity in a third preparation with intact motor connection between ganglion and receptor muscle. Calibrations: 20 mV and 1 sec.

DISCUSSION

Mechanisms underlying the differences between S-fibre and T-fibre responses

In the general features of their responses, outlined in the Introduction, the S and T fibres of the crab coxal muscle receptor behave quite similarly. However, several important differences have emerged from the present study. These are outlined in the Summary. Their possible origins are considered here.

The ultrastructure of the dendritic terminals of the S and T fibres appears very similar, with elongated T-shaped branchlets embedded in identical-looking connective tissue and collagen 'strings' (Whitear, 1965). The receptor potentials evoked by stretch are affected similarly in both S and T fibres by removal of Na^+ from the bathing saline (Roberts & Bush, 1971). No consistent differences between S and T fibres were found in their electrical properties and responses to depolarizing or hyperpolarizing currents. It therefore seems likely that the response differences noted above are due to the differences in anatomical relationships of the S and T fibres with the receptor muscle.

As illustrated in Fig. 1, and in Alexandrowicz & Whitear (1957) and Whitear (1965), the two large dendrite branches of the S fibre lie in elastic strands flanking the receptor muscle in the proximal 20–30 % of its length. These strands are connected relatively loosely to the basal sheath tissue of the receptor muscle, but more distally they appear to merge with the sheath which surrounds the receptor muscle intimately throughout its length. Thus the S-fibre endings evidently lie in parallel mechanically with the proximal portion of the receptor muscle, but seem to be in series with the distal 70–80 % of its length. In contrast, the T-fibre endings appear morphologically to lie in series with the whole receptor muscle. Thus there is probably a larger viscous element in the mechanical linkages of the T than of the S fibre. This could provide a structural basis for the relatively large dynamic component and pronounced hysteresis in the T fibre as compared with the S fibre. Other response differences, such as the 'adaptation' in the T-fibre dynamic component, the 'noisy' oscillations on the T-fibre static response, and the negative velocity response in the S fibre, may also depend upon such differences in the mechanical connexions of the S-fibre and T-fibre endings with the receptor muscle. The 'noise' on the T-fibre 'static' potential might perhaps be a consequence of slight oscillations in receptor-muscle tension. Such tension oscillations could presumably affect directly the membrane potential of the in-series coupled T fibre, particularly when the receptor muscle is well stretched, which is when the 'noise' is most evident. The cause of any such oscillations in tension, however, is as yet unknown.

S–T reciprocity

The structural model outlined above offers a possible hypothesis to account for the apparent 'reciprocity' in response amplitudes sometimes observed between the S and T fibres. A stretch applied to the distal end of the receptor muscle would result in an approximately proportional increase in length of the flanking strands containing the S dendrites, depending upon the relative compliance per unit length along the receptor. Any increase in receptor muscle tension, provided it occurred uniformly over its whole length, should, according to the model, cause an increase in the T-fibre response to the same stretch but little change in the S response. However, if the tension or 'stiffness' of the portion of the receptor muscle in parallel with the flanking strands increases more than that of the more distal regions, then the S response to the same pull would decrease. Conversely, a greater reduction in stiffness of the basal portion than of the rest of the receptor would result in a relative increase in the S response compared with the T response. Indeed, non-uniform variation in stiffness along the receptor muscle could provide the basis for considerable lability in the responses of both S and T fibres.

There is at present little evidence for this hypothesis. However, the slow, electrically passive neuromuscular properties of the receptor muscle, as in other slow crustacean muscle, could permit localized activation through, for example, regional variation in synaptic density or proportion of strongly facilitating to poorly facilitating junctions (cf. Atwood, 1967). Given such regional variation, the foregoing model could account for reciprocal variations in S and T responses when the receptor's efferent supply is intact. In the experiments described here, however, the promotor-receptor motor nerve was cut. Despite this, slow 'spontaneous' changes in receptor tension evidently

did occur in some experiments (e.g. Figs. 3E, F, 9A, 10, 11). Such 'contractural' tension variations might have resulted, for instance, from gradual ionic changes due to slow leakage from cut or damaged muscle fibres. Or they might have been due to direct mechanical effects of repeated stretching on the motor terminals or on the receptor muscle itself; or in some cases to progressive or sudden loss of contractural tone in the course of an experiment. This last explanation could underlie the rapid decrease in the T response and reciprocal increase in the S response after 27 min in the experiment illustrated in Fig. 11 – provided the resulting decrease in stiffness was greater in the basal portion than in the more distal regions of the receptor muscle.

In any event, preliminary support for the above 'reciprocity' hypothesis comes from the observation that, following damage by pinching or fortuitous breakage of the receptor muscle proximally, without apparent injury to the adjacent flanking strands, the S response increased in amplitude while the T response diminished. Further discussion of this problem, however, is best deferred until recordings of receptor tension, and measurements of relative length or tension changes along its length, have been completed. Other experiments in progress, in particular on the effects of controlled motor stimulation, should throw more light on the role of the receptor muscle and its effects on the S and T responses to receptor length changes.

Possible functional significance of the S and T fibres

Two general interpretations of the functional roles of the S and T fibres can be suggested from the present results. One is that the T fibre provides sensory information predominantly about the *dynamic* phase of any receptor length change, and hence thoracic-coxal joint movement, whereas the S fibre provides information primarily about its *static* condition, or joint position. Thus the T fibre normally appears to be more sensitive to variation in stretch velocity, whereas the steady membrane potential of the S fibre is a more reliable index of receptor length. However, the S fibre does show characteristic velocity components, negative as well as positive; while the T fibre's membrane potential clearly is dependent upon receptor length, albeit with marked hysteresis. The other interpretation, then, is that the S fibre gives reliable information of both dynamic and static phases of *passive* joint movements, while the T fibre is directly subject to efferent control and thus gives information primarily concerned with *active* movements. But the evident 'reciprocity' between S and T fibres discussed above suggests that the S fibre may also be affected by receptor contraction. Perhaps, therefore, the true role of the S and T fibres will incorporate both of these functions, possibly in varying degrees depending upon the crab's locomotory or postural condition or 'state of alertness'.

Comparisons with other muscle receptors

The general functional similarity with other muscle receptors under efferent control is obvious enough – for example, the myochordotonal organ of the mero-carpopodite joint in crab legs (Cohen, 1963), the abdominal muscle receptor organ in crayfish (e.g. Fields, 1966) and caterpillar (Weevers, 1966), and the vertebrate muscle spindle (e.g. Matthews, 1964). The form of the S response closely resembles the variation with time in the sensory impulse frequency of the caterpillar MRO to slow ramp-function stretches. With faster stretches this MRO's response changes in form

nd comes increasingly to resemble the T fibre's declining dynamic response. Thus the caterpillar MRO's response to ramp stretches appears in this respect to be intermediate between the S and T fibres' responses, perhaps not surprisingly since there is only one bilateral MRO cell per segment. The greater dynamic sensitivity of T fibres suggests comparisons with fast crustacean abdominal receptors and primary spindle endings, and the greater static sensitivity of S fibres with slow abdominal or secondary spindle receptors.

The dependence of T-fibre responsiveness upon receptor muscle tension and efferent control indicates a functional analogy with each of these systems in its probable involvement in some form of servo control of posture and movement (cf. Fields, Evoy & Kennedy, 1967). The possibility that the S fibre is relatively independent of receptor tension (except under certain conditions) suggests analogy with the chordotonal organs of the more distal joints of crab's legs. These lack any direct efferent control, but give rise to negative-feedback resistance reflexes (Bush, 1965), similar to vertebrate stretch reflexes, and are evidently important in the reflex co-ordination of limb movement (Evoy & Cohen, 1969).

Thoracico-coxal muscle receptors with two large-diameter sensory neurones are also present in other decapod Crustacea (Alexandrowicz & Whitear, 1957; Alexandrowicz, 1967). In Astacura they differ from those of crabs in that the dendritic arborizations of the two fibres intermingle at the base of the receptor muscle, and there are no flanking strands. Moreover, lying alongside the muscle receptor in astacurans is a thoracico-coxal chordotonal organ. Here a clear functional distinction of these two receptor organs seems likely, analogous perhaps to that between the myochordotonal and chordotonal organs of the mero-carpopodite joint referred to above. In crabs, the comparatively much modified S fibre may have largely 'taken over' the functions of the astacuran thoracico-coxal chordotonal organ. A structurally somewhat intermediate situation exists in Palinura, which lack either separate flanking strands or a chordotonal organ for this joint, but have more spatially differentiated 'S'-fibre and 'T'-fibre homologues than Astacura. Comparative studies on all of these groups of Decapoda should help towards an overall understanding of the mechano-receptor mechanisms involved.

SUMMARY

1. Intracellular and extracellular recordings from the two large-diameter S and T sensory fibres of the posterior thoracico-coxal muscle receptor in shore crabs confirm the graded, dynamic-static nature of the receptor potentials evoked by stretching the receptor muscle, and the lack of afferent impulses.

2. Slow ramp-function stretches evoke receptor potentials with characteristic shapes, which differ between the two fibres in several respects:

(i) The dynamic component in the S fibre resembles an algebraic sum of length and velocity responses and a variable initial 'acceleration' (?) transient, while in the T fibre it commonly declines ('adapts') during stretching, especially at greater velocities and starting lengths.

(ii) On release of stretch the S fibre usually exhibits a 'negative velocity response', but the T fibre repolarizes rapidly often with a slight hyperpolarization.

(iii) The dynamic response of the T fibre is generally greater than that of the S fibre,

and increases more steeply and approximately logarithmically with stretch velocity over a 10- to 50-fold range.

(iv) The 'static response' or degree of depolarization increases fairly linearly with receptor length in the S fibre but very non-linearly in the T fibre.

(v) The T fibre displays pronounced hysteresis in its dynamic and static responses at increasing and decreasing lengths, but the S fibre shows little hysteresis.

(vi) The T fibre but not the S fibre commonly shows small rapid oscillations or 'noise' superimposed upon strongly depolarized 'static' potentials.

(vii) The S and T responses may be affected reciprocally by some forms of receptor muscle contraction.

3. Graded receptor potentials evoked in the 'D' fibre by stretching the non-muscular depressor-receptor strand of the coxo-basal joint show little hysteresis.

4. Receptor muscle fibres respond to motor nerve stimulation or spontaneous motor impulses from the thoracic ganglion with slow, facilitating and summating excitatory junctional potentials.

5. The mechanisms underlying the differences between S and T responses, and their functional significance to the animal, are discussed, and comparisons are drawn with other muscle receptors.

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