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FURTHER EVIDENCE FOR SYNAPTIC ACTIONS OF MUSCLE SPINDLE SECONDARIES IN THE MIDDLE LUMBAR SEGMENTS OF THE CAT SPINAL CORD

BY P. J. HARRISON*, L. JAMI AND E. JANKOWSKA

From the Department of Physiology, University of Göteborg, P.O. Box 33031, S-400 33 Göteborg, Sweden and Laboratoire de Neurophysiologie, Collège de France, Place Marcelin Berthelot, 75231 Paris, Cedex 05, France

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SUMMARY

1. The aim of this study has been to investigate the receptor origin of postsynaptic actions evoked by group II muscle afferents in mid-lumbar segments of the cat spinal cord. The experiments tested the hypothesis that the afferents involved were the secondary endings of muscle spindles.

2. Spindle afferents were activated by contractions of intrafusal muscle fibres which were induced by electrical stimulation of fusimotor axons in the distal parts of transected ventral roots by one to three stimuli at 150–500 stimuli/s. A separate series of experiments has shown that such stimuli are effective in activating a considerable proportion of muscle spindle secondaries when contractions of extrafusal muscle fibres are eliminated by differential fatigue of these fibres, provided that several fusimotor axons are stimulated simultaneously.

3. Extracellular field potentials were recorded in the dorsal horn, at such locations where synaptic actions were evoked by electrical stimulation of group II but not group Ia muscle spindle or group Ib tendon organ afferents of pretibial flexors. Effects of activation of spindle afferents following stimulation of fusimotor axons were then compared with effects evoked by electrical stimulation of group II afferents of anterior tibial or extensor digitorum longus nerves and by small stretches of these muscles.

4. Distinct field potentials were evoked by stimulation of ventral root fibres at all locations at which field potentials were obtained from group II afferents stimulated electrically. The latencies of these field potentials were in both cases shorter in the dorsal horn than in the ventral horn.

5. The appearance of these field potentials was not related to contractions of extrafusal muscle fibres and was also observed when these contractions were practically eliminated. Furthermore, their threshold and similar dependence on a potentiating effect of two to three stimuli, as found for single secondaries, allow them to be attributed to secondary endings of muscle spindles.

* Present address: Department of Physiology, University College London, Gower Street, London WC1E 6BT.

INTRODUCTION

Group II muscle afferents have been recently found to evoke very powerful synaptic actions in the 3rd, 4th and 5th lumbar (L3, L4, L5) segments of the cat spinal cord (Edgley & Jankowska, 1987*a*, *b*, 1988). These actions are much stronger than in more caudal segments of the lumbosacral enlargement. They are also stronger than actions of other afferents in L3–L5 segments, and are exerted on interneurones of spinal reflex pathways (Cavallari, Edgley & Jankowska, 1987) as well as on ascending tract neurones (Edgley & Jankowska, 1988; Edgley & Gallimore, 1988). The mid-lumbar segments thus appear to be specialized to a great extent in processing information from group II muscle afferents.

With respect to the receptor origin of these afferents there have been several indications that the majority belong to muscle spindle secondaries (see Discussion in Edgley & Jankowska, 1987*a*). The strongest of these indications were based on observations that extracellular field potentials are evoked not only by electrical stimulation of group II afferents in a muscle nerve but also by stretching this muscle (see Fig. 10 of Edgley & Jankowska, 1987*a*, and Fig. 3 of Edgley & Jankowska, 1988). However, muscle stretches might also activate some other receptors. The aim of the present study has therefore been to extend these observations by investigating the effects of activating muscle spindle afferents in a more specific way, by contractions of the receptor-bearing intrafusal muscle fibres.

To our knowledge this approach has been used in only one series of experiments which, in addition, have not been fully published (C. C. Hunt, personal communication), although at least two techniques of activation of spindle afferents via fusimotor axons are available. The first technique involves functional isolation of single γ-axons in small ventral root filaments, and their electrical stimulation. The second technique involves stimulation of similarly isolated skeleto-fusimotor axons (β-axons; Bessou, Emonet-Denand & Laporte, 1965), during a selective block of neuromuscular transmission to extrafusal fibres. Such a block can be produced either by curare (Bessou *et al.* 1965) or by prolonged repetitive stimulation leading to 'differential fatigue' of extrafusal fibres (Emonet-Denand & Laporte, 1974). Because intrafusal neuromuscular junctions are more resistant to paralysing agents and to fatigue, a stage may be reached when the effects of extrafusal contractions are eliminated and activation of spindle afferents elicited by stimulation of a β-axon may be ascribed exclusively to intrafusal contractions.

The method used in the present study was derived from the second technique: it involved stimulation of several γ-axons (and possibly also β-axons) together with α-axons, and elimination of most, if not all, of the extrafusal contractions by differential fatigue. Since we wanted to compare synaptic actions of the total population of group II afferents in a muscle nerve with synaptic actions observed after activation of spindle secondary endings by fusimotor axons, it was necessary to activate as large a fraction as possible of the spindle population within the selected muscle. This in turn implied stimulation of the largest possible fraction of the fusimotor-axons supply to this muscle, in thick ventral root filaments or whole ventral roots, to avoid the risk of damaging a number of γ- or β-axons inherent in the process of their isolation in small ventral root filaments. Control experiments

were carried out to verify that under such conditions single stimuli or very short trains of stimuli applied to ventral root filaments effectively activate secondary spindle endings.

METHODS

Two series of experiments were performed, their experimental arrangements being shown in Fig. 1.

The first series of experiments (on two cats). These were aimed at examining the conditions of activation of secondary endings of tibialis anterior muscle spindles upon stimulation of thick ventral root filaments. These experiments were done under sodium pentobarbitone (Nembutal)

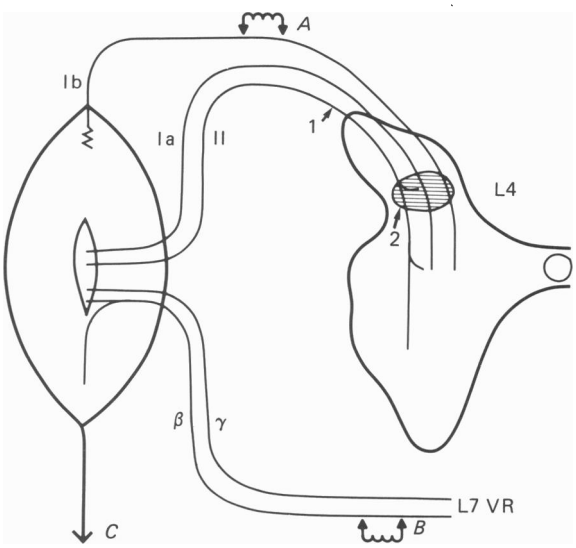

Fig. 1. Diagram of the experimental arrangement. Arrows 1 and 2 indicate the site of the recording in the first and second series of experiments, respectively. The group II afferents were activated either by electrical stimulation of the muscle nerve (*A*), by stimulation of the ventral root (*B*), or by muscle stretch (*C*). The shaded area indicates the area from which the group II field potentials were most often recorded.

anaesthesia (initial dose of 40 mg/kg given I.P., supplemented whenever necessary by additional doses of 10 mg given I.V.). The anterior tibial muscle and its tendon were dissected free without damaging the blood supply and the tendon was attached to a strain gauge rigidly fixed on an electromagnetic puller. Muscle length was set manually at physiological maximum, except when stated otherwise. The muscle nerve was dissected free over a length of 15–20 mm and mounted on a recording electrode via which action potentials of the stimulated motor fibres were monitored. After an extensive denervation of the hip, tail and hindlimb, single afferent fibres from secondary endings of the tibialis anterior spindles were functionally isolated by splitting dorsal root filaments. These afferents were initially identified on the basis of their conduction velocity (below 60 m/s), the regularity of their discharge when the muscle was held at a constant length and their responsiveness to stretch associated with a low dynamic sensitivity. Later in the experiment it was verified that these afferents could be activated by stimulation of γ-axons. The portions of L6 and L7 ventral roots containing motor axons to the tibialis anterior muscle were divided into thick filaments, each of which upon stimulation elicited contractions developing about one-fifth of the total muscle tension. The effects of stimulating these filaments on each of the prepared afferents were observed

before and after fatigue of the extrafusal muscle fibres had developed; a stimulus strength largely above that required for maximal contractions was then used in order to recruit γ-axons, as illustrated in Fig. 2*A*. The fatigue of the extrafusal muscle fibres was induced by stimulation of one ventral root filament at a time, at 100–150/s, for periods of 10 s separated by resting intervals of equal duration. After ten to twelve periods of stimulation, the extrafusal contractions were usually either reduced by 90–95%, or totally suppressed, while activation of spindle endings persisted (Jami, Murthy & Petit, 1982). In the course of these experiments only some aspects of the behaviour of spindle secondaries were analysed after a complete abolition of the extrafusal contractions because intrafusal fatigue often followed after a short while. However, for all the secondary endings that were found to be activated by ventral root stimulation it was verified that their activation persisted after the total suppression of the extrafusal contractions. This provided evidence that the activation of secondary endings observed during combined stimulation of α- and γ-axons with the selected stimulus parameters did not depend on mechanical excitation of spindles by extrafusal contractions.

The second series of experiments. In the second series of experiments the effects evoked by spindle afferents, activated in the same way, were tested in four cats anaesthetized with chloralose (60–80 mg/kg initial dose; 7–9 mg kg^{-1} h^{-1} total dose, the dissection being done under ether anaesthesia) supplemented with 5–10 mg/kg of sodium pentobarbitone (Nembutal). The afferents studied were those of either anterior tibial or extensor digitorum longus muscles. The tendons of these muscles were attached to a muscle puller–myograph (Fetz, Jankowska, Johannisson & Lipski, 1979), either together or separately. Thereby, both the initial tension and the tension developed during stimulation of the ventral root fibres were monitored. The nerves to the same muscles were mounted on pairs of recording electrodes, via which the action potentials of motor fibres were monitored. A third pair of electrodes in contact with the common peroneal nerve, a few centimetres more proximally, was used for electrical stimulation of afferent fibres. The dissection of the muscles and their nerves and the denervation of the rest of the limb was done as in the first series of experiments. The fatigue of the extrafusal muscle fibres likewise was induced with the same experimental paradigm. However, a considerable degree of fatigue appeared even during the prelimimary tests after stimulation of motor axons which accompanied stimulation of sensory fibres within the common peroneal nerve.

The effects of afferents of the anterior tibial and extensor digitorum longus muscles, evoked by electrical stimulation of either the ventral root or these muscle nerves, were judged from the appearance of focal field potentials in L4 or L5 segments and, in a few cases, also from extra- or intracellular records from neurones in these segments. Other procedures relating to the care of the animals were as described previously (see e.g. Edgley & Jankowska, 1987*a*).

RESULTS

Activation of muscle spindle secondaries by stimulation of fusimotor axons with single, double or triple stimuli

The parameters of stimulation of fusimotor axons, which are usually used because they are most effective for activating muscle spindle afferents, i.e. relatively long trains of stimuli at frequencies of 50–150 stimuli/s, were not suitable for the purposes of this study. To allow a comparison with the synchronous effects of group II afferents activated by single electrical stimuli, it was preferable to activate spindle secondary endings by single or at most two to three electrical stimuli applied to fusimotor fibres, but we had first to verify that such stimuli would be effective and would produce effects at fixed latencies. When the muscle is held at a constant length near its physiological maximal, a stimulus applied to a single γ-axons is usually not sufficient to elicit activation of spindle afferents. However, the effectiveness of fusimotor stimulation increases when (i) the muscle is stretched, (ii) the number of stimuli is increased and (iii) several fusimotor axons are stimulated simultaneously (Hunt & Kuffler, 1951; Bessou, Laporte & Pages, 1968; Bessou & Pages, 1969). All these factors were therefore taken into consideration.

Effectiveness of activation of spindle secondaries. To estimate the proportion of spindle secondary endings that could be activated with the parameters of stimulation optimal for this study, two experiments were performed in which the responses of single secondary spindle afferent fibres from tibialis anterior muscle were examined. Of nineteen such fibres (conduction velocities 30–49 m/s), eight responded to one to three stimuli applied to thick ventral root filaments or the whole ventral root.

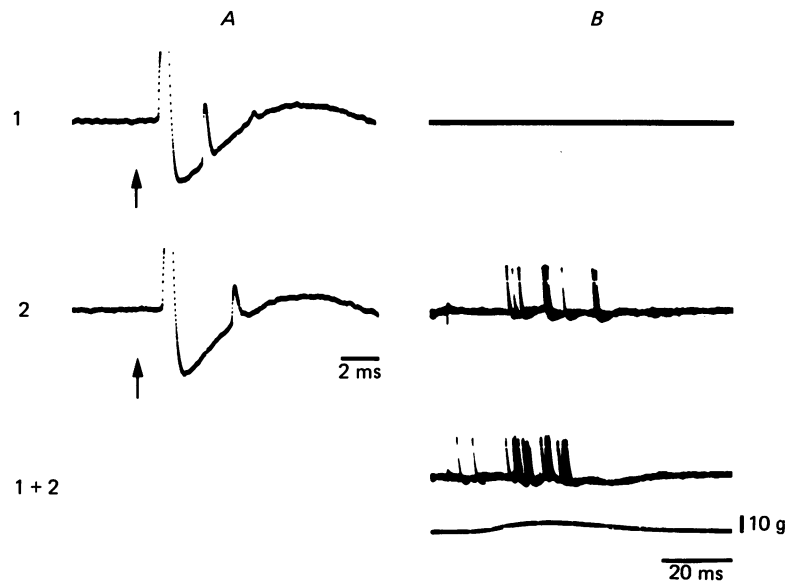

Fig. 2. Effects of stimulation of two filaments of the L7 ventral root, separately or together, on responses of a group II fibre (conduction velocity 47 m/s) innervating a secondary spindle ending of the anterior tibial muscle. *A*, records from the muscle nerve. Arrows indicate the time of stimulation. Both filaments contained a number of α-axons (and possibly also β-axons). In addition, two γ-axons (conduction velocities, 39·5 and 24·6 m/s) were stimulated in filament 1 and one (conduction velocity, 30·3 m/s) in filament 2. *B*, upper row, absence of response of the secondary ending when a single stimulus was applied to filament 1; middle row, response to stimulation of filament 2; bottom row, response to stimulation of both filaments together and simultaneously recorded tension (7 g). Five superimposed traces. The extrafusal contraction was reduced by fatigue (see text). The muscle length was set at 2 mm below maximum physiological length in order to reduce the frequency of the secondary ending resting discharge.

Threshold. With only a few exceptions the threshold for the responses was at a stimulus strength supramaximal for α-axons and above threshold for γ-axons. Weaker stimuli elicited either no response, or a pause in the resting discharges of the secondary ending if some extrafusal muscle contractions occurred.

Spatial and temporal facilitation of fusimotor actions. Single stimuli could be effective (record 2 in Fig. 2*B*) but not when applied to all ventral root filaments (record 1 in Fig. 2*B*). However, simultaneous stimulation of two filaments, one effective and one ineffective, could produce a stronger response than that obtained from the effective filament alone (record 1+2 in Fig. 2*B*). Such facilitation suggests that also the ineffective filaments may have some subliminal actions on the secondary endings.

The responses of the secondaries were enhanced when two or three stimuli were applied instead of one. Records 2 and 3 in Fig. 3 (from the same ending as in Fig. 2, here activated by filament 2 alone) show that the ending responded with shorter latency and with a larger number of impulses, as if the 2nd and 3rd stimulus potentiated the effects of the first one.

Role of muscle length. Another factor of importance for activation of the secondaries was the muscle length. Figure 4 illustrates the combined influence of muscle extension and of triple stimulation, showing the responses of the same ending to stimulation of filament 1. With a single stimulus, this filament was ineffective (Fig. 2*B*), whatever the muscle length, whereas with three stimuli a response appeared,

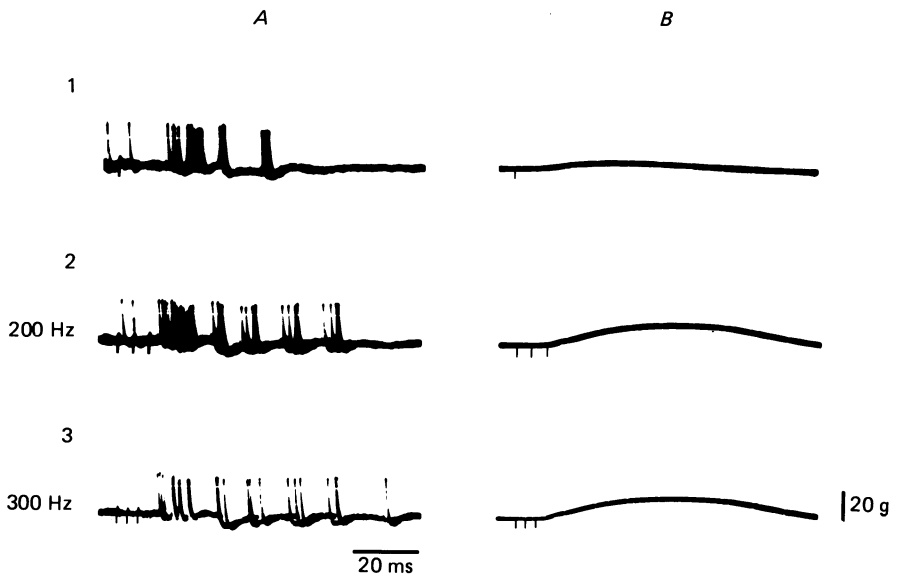

Fig. 3. Effects of increasing the number (from one to three, as indicated by shock artifacts) and the frequency of the stimuli applied to the ventral root filaments. *A*, responses of the same ending as in Fig. 2 to stimulation of filament 2. *B*, simultaneously recorded tension (after the extrafusal contraction had been reduced by fatigue, see text). Five superimposed traces. Muscle length at physiological maximum.

though with a rather long latency (over 50 ms) when the muscle was held at the maximal length. Extension of the muscle then appreciably reduced the latency of the response (to 23 ms after extension of 4 mm, see bottom record in Fig. 4*A*).

Selectivity of fusimotor actions. The responses of the secondaries were obtained both when the stimulation of the ventral root filaments elicited muscle contractions which developed measurable tensions and after the extrafusal tension was abolished by differential fatigue. The amounts of muscle tension in response to triple stimuli to filaments 1 and 2, shown in the records of Figs 3 and 4, were 14 and 10 g, and represented less than 5% of the initial tension developed by the muscle (320 and 350 g, respectively, in response to similar stimuli). The tension record in Fig. 2 corresponds to a further stage of fatigue, when the twitch elicited by stimulation of filaments 1 and 2 together did not develop more than 7 g, against 205 g before fatigue.

Latencies. The latencies of the responses of the eight secondaries activated by stimulation of ventral root filaments ranged from 15 to 30 ms for single stimuli at the maximal muscle length. Shorter latencies were obtained with triple stimuli and at longer muscle lengths. The shortest latency observed is illustrated in the bottom record, labelled 3, of Fig. 3*A*. The first impulse of this record appeared with a latency of 13·5 ms after the first stimulus; given the conduction times along the group II afferent fibre (3·8 ms) and the γ-axons (6 ms; see lower record in Fig. 2*A*), such a latency leaves only 3·7 ms of intramuscular delay for both the spindle activation and the generation of impulses in the secondary ending, which appears extremely short.

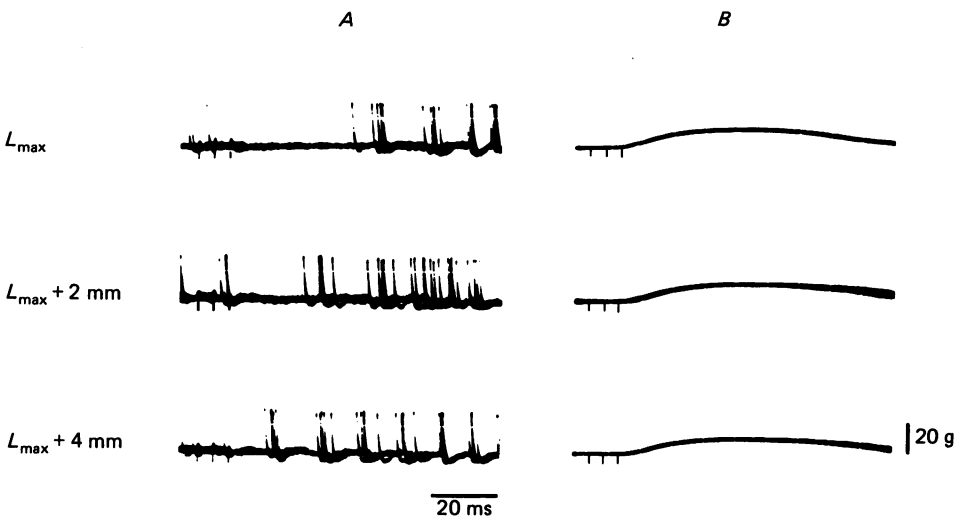

Fig. 4. Effects of an increase in muscle length from maximum physiological length (L_{max}) on responses to triple stimulation at 200 Hz. *A*, responses of the same ending as in Figs 2 and 3 to stimulation of filament 1. *B*, simultaneously recorded tension (with extrafusal contraction reduced by fatigue). Five superimposed traces.

If, however, this spindle was activated by a static β-axon (with a conduction time of about 2 ms; see record 2 of Fig. 2*A*) the intramuscular delay would amount to 5·5 ms and the short latency would be accounted for (Jami, Petit & Scott, 1985). The relative contributions of γ- and β-axons to the effects of stimulation of ventral root filaments in the present experiment could not be assessed, except in this particular case, where, in addition to the short latency of the responses, the response appeared for a stimulation strength just above threshold for α-axons.

Postsynaptic effects of muscle spindle secondaries activated via fusimotor fibres

The experiments described above show that a certain proportion of secondary endings of muscle spindles can be activated by one to three stimuli applied to ventral root fibres. The same stimuli would also activate primary endings (Bessou *et al.* 1968). However, when the effects of the secondaries were tested at locations where no synaptic actions were evoked by electrical stimulation of group I afferents (Edgley & Jankowska, 1987*a*), the activation of muscle spindle primaries was of no consequence. The same was true for any actions of Golgi tendon organs which might be activated by any residual contractions of extrafusal muscle fibres.

Parallel postsynaptic actions of group II afferents activated by electrical stimuli and via fusimotor axons. Figure 5*B* illustrates the main results of this study. It shows that distinct extracellular field potentials can be evoked in the L4 segment following stimulation of a ventral root. These occurred at the same locations at which large field potentials were evoked by electrical stimulation of group II fibres in the anterior tibial nerve (Fig. 5*A*), although they were much smaller, appeared later and had a different time course. It will be noted that the more ventrally recorded field potentials appeared with a somewhat longer latency, whether they were evoked by the direct or the indirect method of activating the afferents. Ventral root stimulation

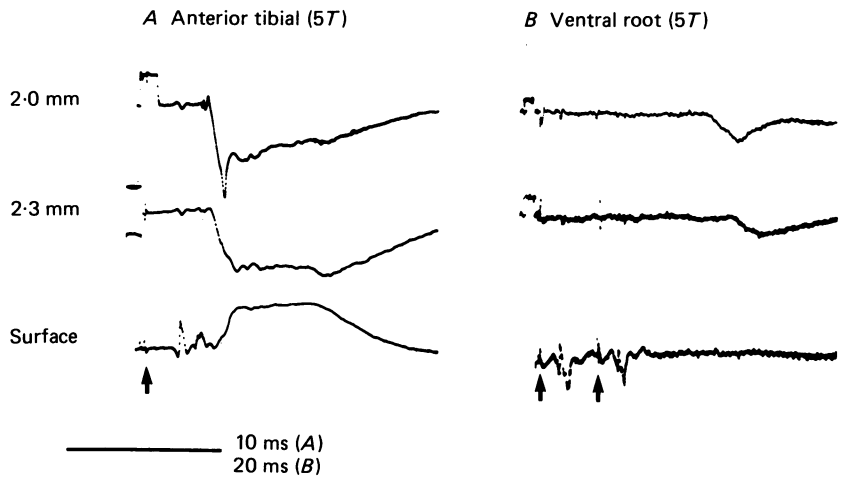

Fig. 5. Comparison of extracellular field potentials evoked by stimulation of the anterior tibial nerve (*A*, with single shocks) and of the L7 ventral root (*B*, with two shocks), the extensor digitorum longus nerve being previously sectioned. The potentials were evoked in the L4 segment at the indicated depths, the lowermost records being from the surface of the spinal cord in L5. Shock artifacts are indicated with arrows. Averages of 256 responses. No visible contractions of the anterior tibial muscle were evoked by the ventral root stimulation. For further explanations see text. The calibration pulses are 200 μV.

was effective in evoking field potentials, like those illustrated in Fig. 5, in all five preparations in which they were tested, provided that appropriate stimulus parameters were used.

The records from the surface of the spinal cord in Fig. 5*A* show the incoming afferent volleys; they preceded the extracellular field potentials with an appropriate time interval to be responsible for them. In contrast the surface records of Fig. 5*B* show two volleys which occurred too early to be attributed to afferent volleys from the anterior tibial and extensor digitorum longus muscles, appeared to be related to small twitches of back muscles which were difficult to deafferent and were not related to the group II field potentials which followed them (as will be shown later).

Threshold. Figure 6*A* shows that some field potentials appeared at a quite low stimulus intensity (1·2 times threshold for α-fibres) but distinct field potentials with a sharp onset required stimuli which were 4–5 times α-fibre threshold, and well above γ-fibre threshold.

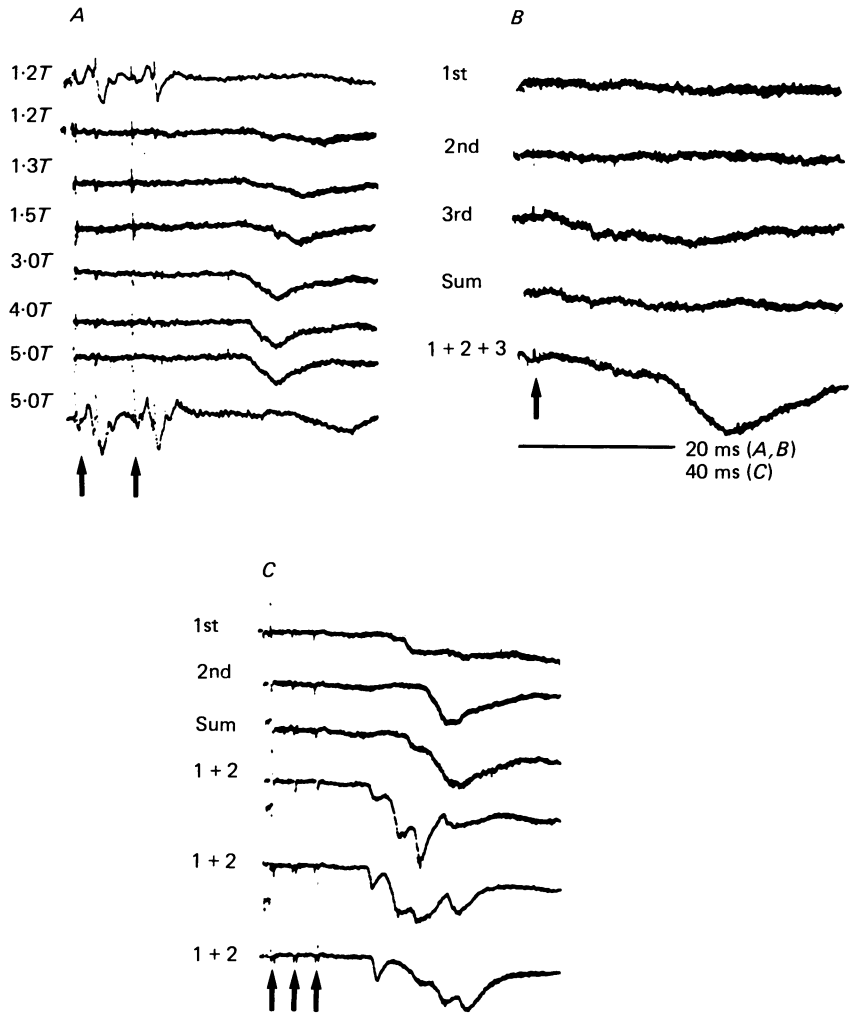

Fig. 6. Threshold and spatial facilitation of synaptic actions evoked via fusimotor fibres. Records in *A* show effects of an increase in intensity of stimulation of the L7 ventral root from 1·2 to 5·0 times threshold (*T*, for α-fibres) or the amplitude of the extracellular field potentials recorded at the same site as records of Fig. 5. The top and the bottom records are from the surface of the spinal cord (to the weakest and to the strongest stimuli, respectively). In *B* and *C* are extracellular field potentials recorded at a similar location in two other cats. These were evoked by stimulating each of the three (*B*) or two (*C*) ventral root filaments separately or together (1 + 2 + 3; 1 + 2). Note that in the latter cases the field potentials were much larger than the sum of those evoked by stimulation of single filaments. All records are averages from 256 responses. The calibration pulses are 200 μV in *C* and 100 μV for *A* and *B*. Arrows indicate shock artifacts. Two stimuli were used in *A*, one stimulus in *B* and three stimuli in *C*. No measurable muscle tension was evoked when records in *A* and *B* were obtained while the muscle tension was of about 8% of that developed before the fatigue records in *C*.

Spatial and temporal facilitation. Stimulation of the whole L7 or L6 ventral root was much more effective than stimulation of small ventral root filaments, even when these contained several γ-fibres, and the effects from two to three filaments potentiated each other's actions considerably. This is illustrated in Fig. 6*B*, which shows that a distinct field potential was evoked by simultaneous stimulation of three L7 ventral root filaments, but not by stimulation of each of them separately. Simultaneous stimulation of two L6 ventral root filaments in another cat similarly resulted in a much earlier and larger response (Fig. 6*C*) than when they were stimulated separately. A very slight change in the timing of the stimuli applied to the two filaments greatly changed the configuration of the potentials (two lower records

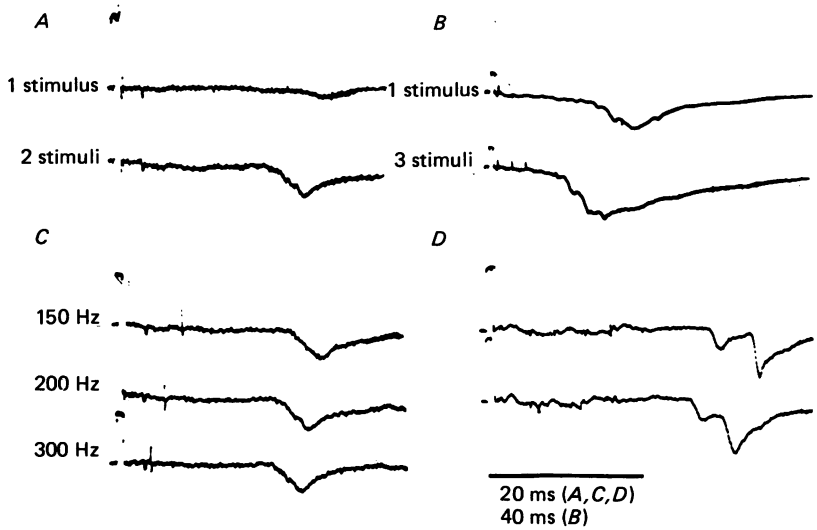

Fig. 7. Temporal facilitation of synaptic actions evoked via fusimotor fibres. *A* and *B* show a greater effectiveness of two or three stimuli than of single stimuli (at 500 Hz in *A* and 300 Hz in *B*). *C* and *D* illustrate a similar effect of an increase in the frequency of the stimuli. Averaged records of 256 extracellular field potentials. *A* and *C* are from the same experiment as Fig. 5 and *B* and *D* are from the same experiment as Fig. 6*B* and *C*, respectively, before the fatigue of the extrafusal fibres has developed. The calibration pulses are 200 μV.

of Fig. 6*C*) suggesting that their different components were evoked by relatively few single secondaries, each of which was dependent on combined actions of fusimotor axons of the two filaments.

Single stimuli were effective in all preparations but they usually evoked only very small field potentials (Fig. 7*A*, top). These were greatly enhanced, and appeared earlier, when two or three stimuli were used instead (compare upper and lower pairs of records in Fig. 7*A* and *B*). A decrease in the interstimulus intervals from 6·6 to 3·3 ms reduced the latency of the responses (Fig. 7*C* and *D*), in most cases without changing their amplitude or configuration. The use of higher frequencies of stimuli (within 150–300 stimuli/s) appeared thus to facilitate activation of the same afferents without helping to recruit a larger number of them. At a higher frequency (400 stimuli/s), the facilitatory effect of the 2nd stimulus disappeared or decreased.

Latency. The shortest latencies of the field potentials recorded in the four preparations were 16·5, 17, 18·5 and 20 ms. These were within the range of latencies of activation of single spindle secondaries found in the first series of these experiments. The minimal latencies of the field potentials were only 3 ms longer than the minimal latency of activation of one of the secondaries which were attributed to β actions. Considering that the records from single secondaries were made some 50 mm caudal of the records of field potentials, that ascending and terminal axon

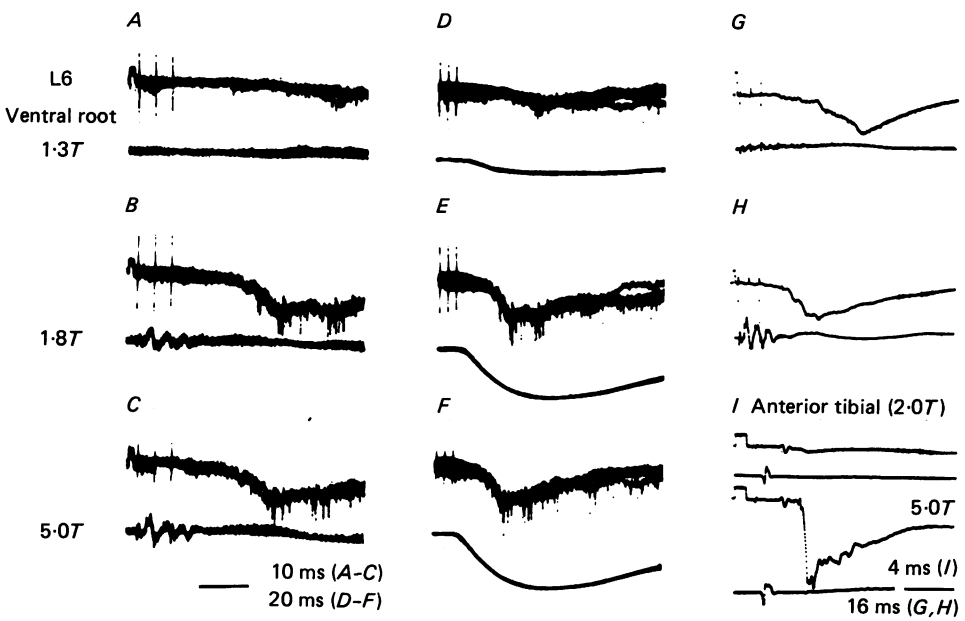

Fig. 8. Field potentials and extracellularly recorded neuronal responses (upper traces of each pair) following L6 ventral root stimulation. The records show the effects of the ventral root stimulation before any fatigue of the extra- or intrafusal fibres of the extensor digitorum longus or anterior tibial muscles, both with intact innervation, developed. The responses which were then evoked by electrical stimulation of group II afferent fibres in the anterior tibial nerve (at 5*T*) but not by group I afferents (at 2·0*T*) are shown in *I*. They are displayed together with records from the surface of the spinal cord in *A–C* and *G–I* and with records of muscle tension in *D–F* (at a slower time base). Single superimposed records are shown in *A–F* and averages of 128 responses in *G–I*. Data from the same experiment as in Figs 6*B* and 7*B*. The calibration pulses are 200 μV.

collaterals have a much lower conduction velocity than within the peripheral nerves (for references see Edgley & Jankowska, 1987*a*) and that one has to consider the synaptic delay of the field potentials, the two groups of data are well in agreement.

Duration. The duration of the field potentials greatly varied, depending on the number of stimuli applied to the ventral roots (see Fig. 7*B*), the number of stimulated fusimotor fibres (see Fig. 6*C*) and possibly also the degree of fatigue of the intrafusal muscle fibres, since the longest ones were recorded at the beginning of testing (e.g. as in Fig. 8*E* and *F*). Similar variability has been found in records from single afferents, the shortest bursts of discharges evoked in them not exceeding two

spikes at 7 ms interval, or one spike. The durations of the field potentials and of discharges of secondary spindle afferents appear thus to be comparable, both for the shortest and for the longest ones (compare for instance Figs 6*B* and 2*B* or Figs 7*B* and 3*A*2).

Selectivity of fusimotor actions. The amplitudes of responses evoked by stimulation of the ventral root fibres varied during experiments. They were as a rule highest at the beginning of the testing, and sometimes comparable to those of field potentials evoked by electrical stimulation of group II muscle afferents. For instance, the area of the potential of Fig. 8*H* almost equals that of the electrically evoked potential of Fig. 8*I* (at the same amplification but at a 4 times faster time base). At the stage of muscle fatigue illustrated, they were also quite distinct without averaging (Fig. 8*A–F*). All the procedures leading to fatigue of the extrafusal muscle fibres turned out to have some effect on these responses; they resulted in a decrease of their

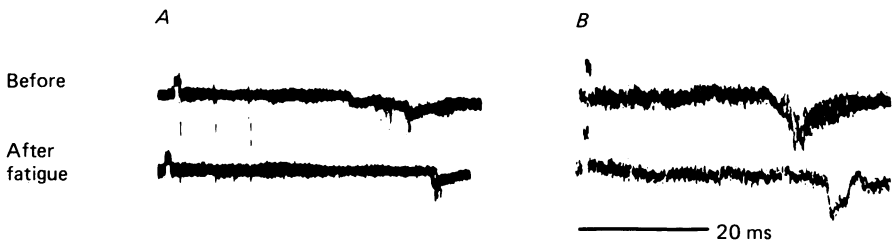

Fig. 9. Effects of fatigue on the effects evoked via fusimotor fibres. *A* and *B* show single superimposed records of extracellular field potentials evoked by stimulating two L7 ventral root filaments (1st and 2nd filament of Fig. 6*C*, respectively) before and after fatigue of the extrafusal muscle fibres has developed. The calibration pulses are 200 μV.

amplitude and in an increase of their latency. Two examples of such changes are shown in Fig. 9*A* and *B*. It was thus impossible to obtain estimates of the maximal effects evoked via fusimotor fibres under conditions when contractions of extrafusal muscle fibres were totally eliminated. However, we wish to stress that although much larger effects were obtained by ventral root stimulation when it produced some muscle contraction, distinct responses were also evoked by the stimuli which were not followed by any recordable tension (in the two experiments illustrated in Figs 5, 6*A* and *B*, 7*A* and *C* and 9, lower traces), or by stimuli which induced less than 10% of the original tension (in the two experiments illustrated in Figs 6*C*, 7*B* and *C* and 10).

Effectiveness of fusimotor actions. Despite the fact that less than half of the single secondaries were activated by stimulation of the ventral roots, as reported in the first section, the ventral root stimulation appeared to be quite effective in discharging neurones (see Figs 8*B*, *C* and 9*A*, *B*) at locations where no group I synaptic actions were detected. Intracellular records from a few neurones at such locations revealed very large unitary postsynaptic potentials induced by stimulation of the fusimotor fibres (Fig. 10*G*), as has been previously reported for the effects of electrically stimulated afferents (Lundberg, Malmgren & Schomburg, 1987; Edgley & Jankowska, 1987b) and is seen in Fig. 10*A–D*. Only very few afferents may thus be sufficient to discharge individual neurones. Furthermore, in the record of Fig. 10*G*

two or three distinct EPSP profiles can be distinguished, and not many more unitary EPSPs would be needed to account for the EPSPs evoked by electrical stimulation of the anterior tibial nerve (Fig. 10*D*).

Additional control experiments. In one experiment it was verified that at the same location at which extracellular field potentials were evoked by electrical stimulation of group II, but not of group I, afferents of the extensor digitorum longus muscle, both a rapid stretch of this muscle and ventral root stimulation induced field potentials with an appropriate latency. The responses following the two procedures, both of which were expected to adequately activate the secondaries, showed amplitudes of the same order and similar time courses.

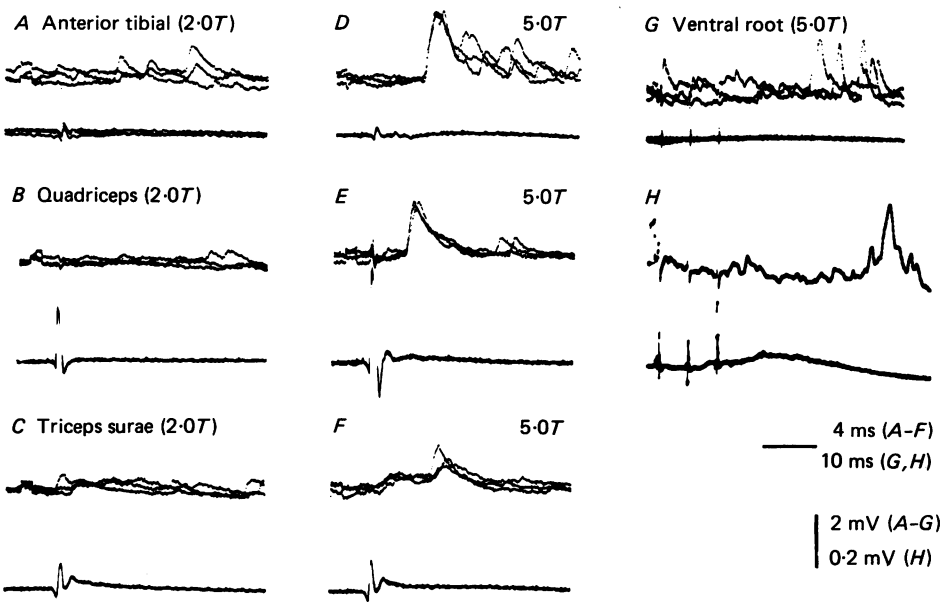

Fig. 10. Comparison of postsynaptic potentials (upper traces) evoked in an interneurone located in the dorsal horn of the L4 segment by electrical stimulation of afferents of anterior tibial, quadriceps and gastrocnemius–soleus nerves (*A–F*, with the intensities indicated) and by afferents of the anterior tibial and/or extensor digitorum longus muscles when they were activated by stimulation of fusimotor fibres in the L7 ventral root (*G–H*). Three superimposed traces in *A–G*. An average of 128 responses in *H*. Data from the same experiment as in Figs 6*C*, 7*D* and 9.

In two experiments it was verified that field potentials evoked in the dorsal horn by ventral root stimulation disappeared after the nerves to the anterior tibial and the extensor digitorum longus nerves were crushed. Nerve impulses simultaneously recorded in a dorsal root filament were then similarly abolished. The field potentials could not thus be due to any spread of current to afferents outside the dorsal roots, to stimulation of sensory fibres within the ventral roots or to twitches of any muscles which escaped the denervation.

The volleys accompanying back muscle twitches which could not be eliminated in two out of the four preparations were concluded to be unrelated to the field potentials since (i) distinct effects of ventral root stimulation appeared when they were absent

(Figs 8*G*, 9 and 10*G* and *H*), (ii) in some cases field potentials required stronger stimuli than for these volleys (Fig. 6*A*) *and* (iii) these volleys remained after transection of tibialis anterior and extensor digitorum longus nerves, which abolished the field potentials.

DISCUSSION

The results of this study show that postsynaptic actions of electrically stimulated group II muscle afferents in mid-lumbar segments of the cat spinal cord are matched by actions evoked via electrically stimulated ventral root fibres and we interpret the latter as actions due to secondary muscle spindle afferents activated by fusimotor γ- and/or β-fibres. Some of the reasons for this conclusion have already been given in the Results section. These will now be briefly summarized and supplemented.

First, the receptors of origin of the extracellular field potentials evoked via stimulation of L7 or L6 ventral roots in our experiments were limited to those in the anterior tibial or extensor digitorum longus muscles because the potentials disappeared after the nerves to pretibial flexor muscles were crushed and appeared in at least some preparations in which stimulation of the L6 or L7 ventral roots did not evoke any visible contractions in other muscles.

Second, any contribution of receptors activated by contractions of the extrafusal muscle fibres was not important since at least some of the effects of ventral root stimulation were obtained in preparations in which no measurable contractions were evoked, the sensitivity of the myograph being sufficient for recording tensions of only a few grams. Furthermore, none of the two types of receptors which might be activated by very small muscle contractions, the Golgi tendon organs and Pacinian corpuscles, could have been involved since the field potentials analysed were recorded at locations in which no field potentials or postsynaptic actions of group Ib afferents were evoked, even by synchronous electrical stimulation of all of them. The effects of Pacinian corpuscles are also rare at such locations (Edgley & Jankowska, 1987*a*, *b*). Free endings (Stacey, 1969; Cleland, Rymer & Edwards, 1982) and other mechanoreceptors (McLennan, 1972) appear on the other hand to have a much higher threshold to muscle stretch or muscle contraction.

Third, the contribution of primary endings of muscle spindles activated by fusimotor fibres will be highly unlikely because no effects of group Ia afferents electrically stimulated are evoked at the same locations in the dorsal horn.

Fourth, field potentials evoked by electrical stimulation of group II muscle afferents were matched not only by field potentials evoked by stimulation of ventral root fibres but also by stretches of the anterior tibial and extensor digitorum longus muscles which were adequate for activation of muscle spindle secondaries (Edgley & Jankowska, 1987*a*, 1988; repeated in this study).

Fifth, the parameters of stimulation of the ventral root fibres which were effective in evoking field potentials in L4 segments were the same as those required for activation of identified spindle secondaries. The comparison of data in the two parts of the results shows that both required fairly strong stimuli, were more easily evoked by stimulation of a larger number of ventral root fibres (or of a whole ventral root) and showed a marked decrease in the latency of responses evoked

by the first stimulus, when this stimulus was followed by one or two stimuli at 200–300 stimuli/s.

Sixth, the latencies of responses evoked by the ventral root stimulation were fully compatible with their mediation via γ- or β-fibres and secondary endings of muscle spindles.

For all these reasons it seems reasonably safe to postulate that the secondaries were the main source of origin of the described phenomena.

To what extent can our observations be generalized? We have only analysed the receptor origin of those group II synaptic actions in the L4 segment which were evoked from pretibial flexors, because of the more convenient anatomical arrangement for these muscles than for other muscles. In particular, in the case of quadriceps and sartorius, the other main source of group II actions in mid-lumbar segments (Edgley & Jankowska, 1987*a*, *b*, 1988), it would be much more difficult to stimulate their fusimotor axons in the L4 and L5 ventral roots (which are considerably shorter than L7 and L6 ventral roots) without a risk of current spread. Likewise it would be much more difficult to denervate other muscles innervated by L4 and L5 roots and to control the tension developed by stimulation of these ventral roots. We propose nevertheless that the conclusions concerning group II afferents of pretibial flexors be generalized to group II afferents of quadriceps and sartorius, in view of comparable effects evoked from all these muscles on electrical stimulation of their afferents (Edgley & Jankowska, 1987*a*).

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