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A QUANTITATIVE STUDY OF SKELETOFUSIMOTOR INNERVATION IN THE CAT PERONEUS TERTIUS MUSCLE

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SUMMARY

1. Physiological tests were used to identify skeletofusimotor or β axons to the cat peroneus tertius muscle in order to assess the proportion of β axons in the motor supply to this muscle.

2. Static β axons (βS) were identified by: (*a*) observation of a delay between the complete block of extrafusal contraction and the failure of spindle activation upon prolonged stimulation, (*b*) increase of spindle excitation with stimulation frequencies above that eliciting maximal extrafusal contraction, (*c*) observation of 'unfused' frequencygram of spindle primary afferent discharge during stimulation of the axon at frequencies above that eliciting complete fusion of extrafusal contraction and (*d*) static action exerted on the response of the spindle afferent to ramp stretch.

3. Dynamic β axons (βD) were identified by the persistence of spindle activation after selective block of extrafusal neuromuscular junctions and by their dynamic action on spindle primary endings.

4. The actions of 116 motor axons (conduction velocity 56–104 m/sec) on ninety-five spindle afferents (fifty-seven from primary and thirty-eight from secondary endings) were examined in ten experiments. Thirty-six β axons (31 % of the total sample) were identified: twenty-four βS (conduction velocity 69–104 m/sec) and twelve βD (conduction velocity 56–91 m/sec).

5. Twenty (35 %) primary endings were activated by a βS and sixteen (28 %) by a βD axon. Nineteen (45 %) secondary endings were activated by a βS and five (13 %) by a βD axon. Convergence of βD and βS axons on the same spindle occurred in 10 % of instances. β-innervated spindles were also supplied by γ axons.

6. Most of the βS motor units were of the fast-fatigue resistant (FR) type, with a few units of the fast-fatiguable (FF) type, and nearly all the βD motor units were of the slow (S) type.

INTRODUCTION

In addition to specific fusimotor axons (γ axons), the motor supply to mammalian muscle spindles includes skeletofusimotor, or β axons distributing terminal branches to both extra- and intrafusal muscle fibres. It is now established that there are two different types of β axons, dynamic and static.

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Dynamic β axons were originally found in cat muscles by Bessou, Emonet-Dénand & Laporte (1963, 1965) using physiological tests that provided conclusive evidence of the skeletofusimotor distribution of such axons. By these criteria, β axons were later demonstrated in several muscles of various mammals (see the review by Emonet-Dénand, Jami & Laporte, 1980), and most of them were found to exert a dynamic action on spindle primary endings. Dynamic β axons innervate intrafusal muscle fibres of the bag_1 type (i.e. effectors of dynamic action) and extrafusal muscle fibres of the slow-oxidative type (Barker, Emonet-Dénand, Harker, Jami & Laporte, 1977). Most dynamic β axons have relatively slow conduction velocities, between 40 and 85 m/sec.

Static β axons were first identified in rabbit lumbrical muscles (Emonet-Dénand, Jankowska & Laporte, 1970), but in cat muscles they were detected only exceptionally (Emonet-Dénand & Laporte, 1975; Emonet-Dénand, Jami & Laporte, 1975). However, histophysiological studies using the glycogen-depletion method of Edström & Kugelberg (1968), suggested the existence of static β innervation in cat muscles (Harker, Jami, Laporte & Petit, 1977). These studies were aimed at verifying whether there are β axons with conduction velocities above 85 m/sec. A number of motor axons to a given muscle, with conduction velocities ranging from 85 to 110 m/sec, were isolated and stimulated together for long periods of time, and subsequently the whole muscle was examined for intrafusal glycogen depletion. The presence of glycogen depletion in some spindles (in addition to the expected extrafusal depletion) proved that some of the stimulated axons distributed terminal branches to these spindles. Furthermore, since motor axons with conduction velocities above 50 m/sec have always been found to supply extrafusal muscle fibres (Kuffler, Hunt & Quilliam, 1951; Ellaway, Emonet-Dénand, Joffroy & Laporte, 1972), it followed that the axons innervating the depleted spindles had a skeletofusimotor distribution. About 25% spindles were thus found to be innervated by fast-conducting β axons in the cat peroneus tertius (Harker *et al.* 1977) and tenuissimus muscles (Jami, Lan-Couton, Malmgren & Petit, 1978). The zones of glycogen depletion observed in these spindles occurred essentially in nuclear chain fibres (i.e. effectors of static action), suggesting that fast β axons were static. So far this has been confirmed only for a very small sample of single fast β axons identified by histophysiological criteria, namely the presence of extra- and intrafusal glycogen depletion after stimulation of a single motor axon (Jami, Lan-Couton, Malmgren & Petit, 1979).

In summary, although in cat muscles static β axons have hitherto escaped physiological identification, evidence was available of their co-existence with dynamic β axons. This evidence prompted the present investigation in an attempt to assess the total incidence of β axons, dynamic and static, in the motor supply to the peroneus tertius muscle (also termed peroneus digiti quinti). This muscle has a large proportion of spindles with p_1 plate innervation (Barker, Stacey & Adal, 1970), and recently this type of intrafusal motor ending was shown to be supplied by β axons (Barker, Emonet-Dénand, Laporte & Stacey, 1980).

A preliminary account of the results has been presented (Jami, Murthy & Petit, 1980).

METHODS

The experiments were carried out on adult cats (2–3·5 kg) anaesthetized with pentobarbitone sodium (Nembutal, Abbott Laboratories, 45 mg/kg I.P.).

The peroneus tertius muscle and its tendon were freed without damaging their blood supply and the tendon was attached either to a puller giving ramp or sinusoidal stretches (in ten experiments) or to a Grass FTO3 tension transducer (in four other experiments) or to a Kulite strain gauge rigidly fixed on the puller (in the last ten experiments). The gauge had a compliance of 160 μm per 300 g. After amplification, the signal-to-noise ratio allowed tensions of 20 mg to be recorded. The peroneus tertius has a very long tendon, and in order to avoid possible interference of tendon compliance with measures of muscle tension, the strain gauge was attached immediately below the tendinous insertion of the longest muscle fibres.

The nerve to peroneus tertius was dissected over 10–15 mm and mounted on a recording electrode. The second recording electrode was movable and could be placed in contact with the preparation at variable distances from the peroneus tertius. When it was close to the muscle, the nerve and muscle action potentials elicited by the stimulation of motor axons in ventral roots could be recorded through the same amplifier.

After an extensive denervation of the hip, tail and hind limb, a number of group I and group II afferent fibres connected to peroneus tertius spindles were isolated in dorsal root filaments. They were identified on the basis of their responsiveness to muscle stretch and their conduction velocities. The conventional limit of 70 m/sec was taken to separate group I and group II fibres. In the course of each experiment, it was observed regularly that γ axons stimulated in ventral roots could evoke discharge in the prepared afferent fibres, thus ascertaining that these fibres were connected with spindles and not with tendon organs.

Single motor axons were isolated by splitting ventral roots until stimulation of a filament elicited an all-or-none action potential in the muscle nerve together with an all-or-none action potential of the motor unit. The action of each isolated motor axon was tested on each of the prepared afferent fibres. On average ten motor axons were studied in each experiment, which represents one third of the α motor supply to peroneus tertius muscle (Barker *et al.* 1970). An axon was tentatively considered as β, and submitted to identification tests, if its stimulation at 150/sec elicited an increase in the discharge frequency of one or several of the prepared afferent fibres. Great care was taken to ensure that no γ axon was present in a filament containing a presumed β axon. The filament was systematically stimulated at increasing intensities in order to recruit any small-diameter axons it could possibly contain. Detection of γ action potentials was facilitated by the withdrawal of the second recording electrode at a distance from the muscle where muscle action potentials appeared with a reduced amplitude so that they would not mask the small amplitude potentials of γ axons.

For each identified β axon, the fatiguability of the extrafusal portion of its motor unit was examined following the procedure described by Burke, Levine, Tsairis & Zajac (1973). The motor axon was stimulated by trains of 330 msec duration at a rate of 40/sec, repeated every second over a period of 2 min. The fatigue index, defined as the ratio of the maximal tension produced after 2 min of stimulation (i.e. during the 120th tetanus) to the tension produced during the first tetanus, allowed identification of the motor unit type as either fast-fatiguable (FF) or fast-fatigue resistant (FR) or slow (S).

RESULTS

Physiological detection of β axons starts with the observation that the stimulation of some single motor axons elicits the activation of spindle sensory endings together with the contraction of extrafusal muscle fibres. This in itself is not sufficient since the possibility exists that the contraction of an ordinary motor unit induces some mechanical excitation of a spindle. Indisputable identification of a β axon requires the ascertainment of its intrafusal action under conditions in which interference of any extrafusal action can be ruled out (Bessou *et al.* 1965). This is achievable for dynamic β axons because their intrafusal neuromuscular junctions are much more resistant than the extrafusal ones either to curarizing agents (Bessou *et al.* 1965) or

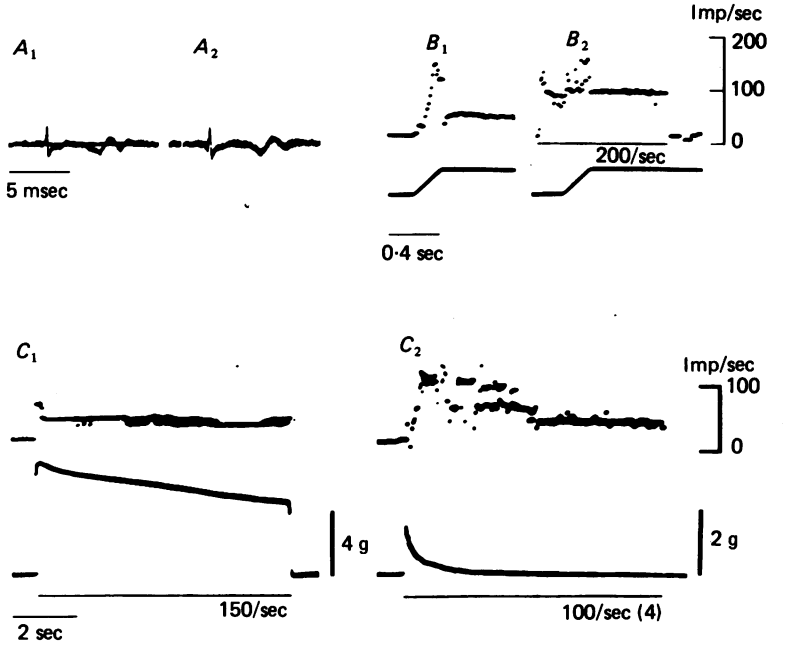

Fig. 1. Identification of a static β axon. A, action potentials of the β axon (conduction velocity 89 m/sec) and of its motor unit, led from the peroneus tertius nerve during stimulation of a ventral root filament. A_1, stimulus intensity near threshold, showing the all-or-nothing behaviour of the potentials. A_2, supramaximal stimulation, showing the absence of any other axon in the filament. B, effect exerted by the axon on the response of a primary ending (conduction velocity of the afferent fibre 91 m/sec) to ramp stretch. B_1, passive response of the ending recorded with an instantaneous frequency meter. The lower trace represents the muscle length: ramp of 2 mm at 8 mm/sec. B_2, response during stimulation of the axon at 200/sec. C, persistence of intrafusal action after abolition of extrafusal contraction. Upper traces: discharge of the primary ending. Lower traces: isometric tension recorded at the muscle tendon. C_1, first period of stimulation at 150/sec. C_2, fourth period of stimulation at 100/sec.

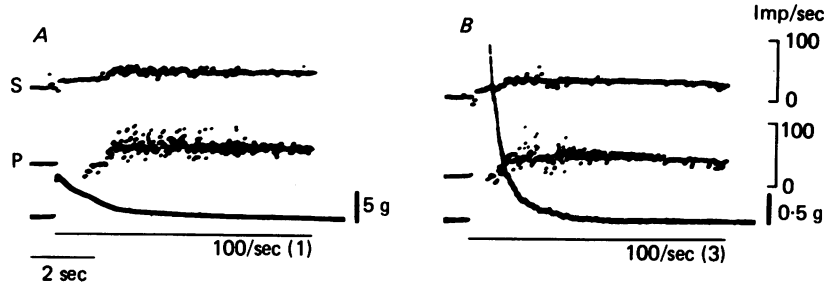

Fig. 2. Activation of spindle discharge by a static β axon outlasting the abolition of extrafusal contraction. This axon (conduction velocity 98 m/sec) activated two spindle endings, a secondary (S, conduction velocity of the afferent fibre 56 m/sec) and a primary (P, conduction velocity of the afferent fibre 112 m/sec). A, first period of stimulation at 100/sec. B, third period of stimulation at 100/sec. Note the increase in the gain of tension recording in B.

to fatigue caused by high-frequency prolonged stimulation (Emonet-Dénand & Laporte, 1974). A selective block of extrafusal junctions can thus be obtained and used to identify dynamic β axons by observing the persistence of their intrafusal action during the block. By means of this procedure, dynamic β axons to the peroneus tertius muscle were readily identified. But if some β axons have extra- and intrafusal junctions displaying similar sensitivities to curare and/or repetitive stimulation, it is clear that they cannot be detected by the same method.

In the present experiments the attempts to secure a durable selective block of extrafusal junctions, whether by curare or high-frequency stimulation, often appeared inconclusive with presumed static β axons. It was therefore necessary to devise some additional tests for the identification of static β axons. In a first series of fourteen experiments that were performed for this purpose, we did not attempt to identify as many β axons as possible but rather to obtain reasonable certainty for each identification of a static β axon. In a second series of ten experiments the tests thus established were applied to the systematic searching for β axons.

Section I. Physiological identification of static β axons

The stimulation of a dynamic β (βD) axon at high frequencies (300–500/sec) rapidly results in the disappearance of muscle action potentials, indicating a block of extrafusal neuromuscular junctions, while the spindle activation persists for several seconds (Emonet-Dénand & Laporte, 1974). Static β (βS) axons rarely lent themselves to this test because, when high frequencies of stimulation were used, the spindle activation they elicited hardly outlasted the elimination of extrafusal contraction. But a more slowly progressing decline of spindle activation could be induced using lower stimulation frequencies (100–250/sec) during periods of 3–8 sec separated by resting intervals of equal duration. Under these conditions the extrafusal contraction usually disappeared after three to ten periods of stimulation whereas spindle activation occasionally persisted for sometime, as illustrated in Fig. 1. A fast-conducting motor axon (Fig. 1*A*) activated a primary ending on which it exerted a static action (Fig. 1*B*). Fatigue of extrafusal contraction appeared during stimulation at 150/sec (note the fall in the tension record of Fig. $1C_1$) but it took four periods of stimulation at 100/sec to observe disappearance of extrafusal tension with persistence of spindle activation (Fig. $1C_2$). Upon further stimulation (not illustrated) the discharge of the activated spindle started to display a high variability while its mean level tended to subside. During intervals between two periods of stimulation the ending resumed its regular resting discharge. After suppression of the extrafusal contraction it took five additional periods of stimulation of this axon to completely abolish its effect on the spindle. In summary, a delay occurred between the block of extrafusal contraction and the disappearance of spindle activation by this axon. During the first seconds of this delay (4 sec in Fig. $1C_2$) the persistence of spindle activation was similar to that observed with a βD axon during a selective block of extrafusal contraction, which allowed the identification of the axons as β. When such selective blocks could be obtained with βS axons they differed from those obtained with βD axons not only by the fact that they required lower stimulation rates but also by their much briefer duration. After a few seconds of selective block, spindle activation by a βS axon usually showed signs of failure (i.e. variability and

decline of discharge frequency). However, we considered that, provided spindle activation outlasted the extrafusal block by at least 2 sec, the delay of spindle activation failure with respect to the disappearance of extrafusal contraction could be regarded as an evidence for identification of βS axons. For the sake of brevity this test will be hereafter termed 'test of delayed failure of spindle activation'.

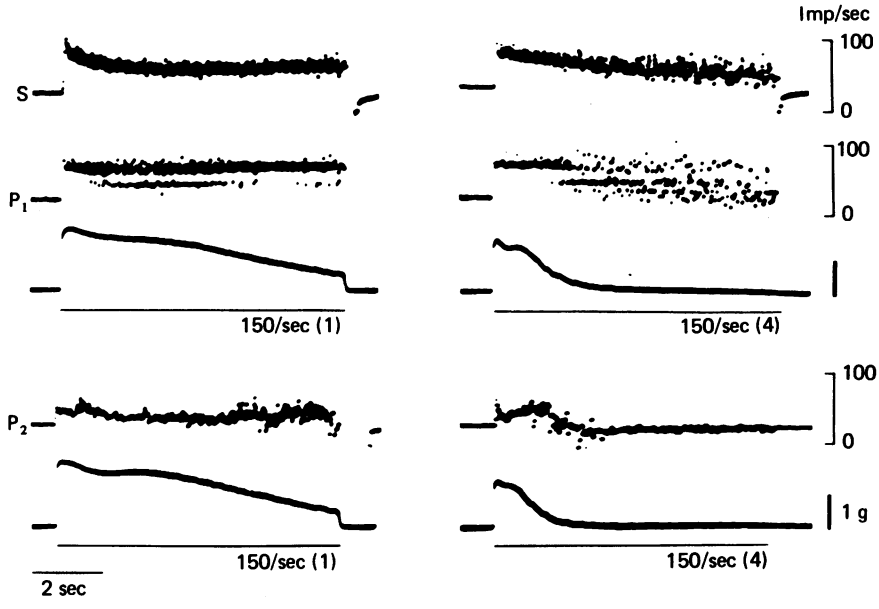

Fig. 3. Test of delayed failure of spindle activation after abolition of extrafusal contraction. Activation of three spindle endings by the same motor axon (conduction velocity 85 m/sec). S, action of the axon on a secondary ending (conduction velocity of the afferent fibre 41 m/sec). P_1, action of the axon on a primary ending (conduction velocity of the afferent fibre 105 m/sec). P_2, action of the axon on another primary ending (conduction velocity of the afferent fibre 95 m/sec). The first and fourth periods of stimulation at 150/sec are illustrated. See text for further description.

Another example is shown in Fig. 2, illustrating the action of a βS axon on two spindle endings, a primary and a secondary. For both endings the activation persisted after abolition of extrafusal tension, although with a slightly lower discharge frequency. In this instance, as in that of Fig. 1, the test of delayed failure of spindle activation could by itself give a reasonable certainty to the identification of a βS axon. This was the case for two thirds of the identified βS axons acting on primary endings (fifteen out of twenty-three actions reported below in Section II) and almost all the βS axons acting on secondary endings (fifteen out of seventeen actions reported below in Section II).

However, other instances appeared questionable because signs indicating failure of spindle activation appeared very early, as illustrated in Fig. 3 which shows the action of an axon on three spindle endings, a secondary and two primary ones. The activation of the secondary ending was maintained after disappearance of extrafusal tension but with an increase in the variability of the ending discharge whose mean frequency tended to subside. However, its minimum level remained for a few seconds about 10–20 impulses/sec above that of the resting discharge, which possibly

indicated a β effect. The activation of the first primary ending (P_1) showed a much higher variability together with a faster decline of spindle excitation after suppression of extrafusal contraction. The discharge of the second primary ending (P_2) closely followed the fall of extrafusal tension, quickly returning to its resting level after the suppression of extrafusal contraction. The fact that the discharges of endings P_1 and P_2 displayed different behaviours after extrafusal block suggested that they might be activated by different mechanisms. Extrafusal activation was plainly identifiable for P_2 but the cause of the high variability in the discharge of P_1 was not clear. As mentioned above, identified βS axons did elicit such variability when their stimulation was continued beyond the brief stage during which spindle activation persisted while extrafusal contraction was blocked. For these reasons, the possibility that the activation of the secondary ending and the first primary ending of Fig. 3 might be due to a β axon was not discarded, but it appeared indispensable to collect additional evidence of the fusimotor action of this axon.

Increase of spindle excitation for frequencies of stimulation above that eliciting maximal extrafusal contraction

Bessou *et al.* (1965) observed that when a βD axon is stimulated at increasing frequencies, the acceleration of spindle discharge continues to augment for frequencies of stimulation above that producing maximal extrafusal contraction. Since this further augmentation cannot be due to extrafusal action, it is considered as evidence of the skeletofusimotor distribution of the stimulated axon (see Matthews, 1972). In the present study, this feature was sought for all the presumed βS axons.

To begin with, the frequency of stimulation producing the maximal extrafusal contraction, or tetanic fusion frequency, had to be determined for each presumed βS motor unit. This was done by applying short trains of stimulation (150–300 msec duration) at increasing frequencies, with the gain of the tension-recording device set at its maximal value. Fusion was considered as complete when the level of the tension plateau reached a maximum and showed a smooth outline, without any ripples in phase with stimulation.

The test for an increase of spindle excitation with frequencies of stimulation above that eliciting maximal extrafusal contraction was applied as follows: periods of stimulation lasted 150–300 msec and during each period the frequency of stimulation was increased in a step fashion, both initial and final frequencies being well above the fusion frequency of the extrafusal portion of the motor unit. This sequence was repeated ten times and the records of spindle discharge frequency were superimposed on the oscilloscope screen. As shown in Fig. 4, a step increase in the frequency of stimulation of a βS axon produced an increase in the discharge frequency of the activated spindle, contrasting with the absence of increase in the simultaneously recorded extrafusal tension.

Comparison of the spindle discharge and tension records of Fig. 4 shows, in addition, that after the cessation of stimulation, the activation of spindle endings subsided before decline of extrafusal tension. This fact provides a further indication that spindle activation was not a consequence of extrafusal contraction (see also Fig. 5 below).

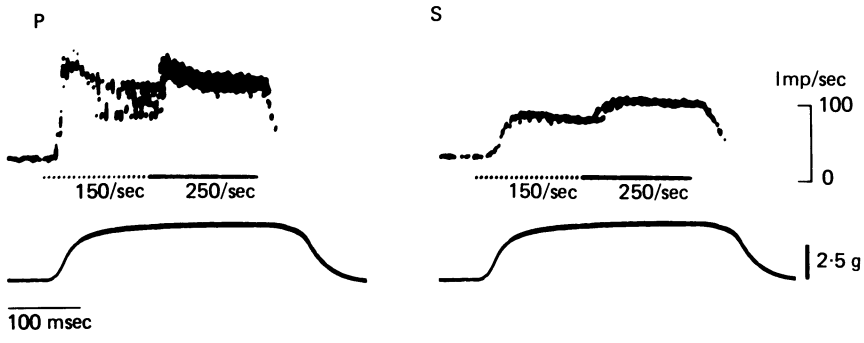

Fig. 4. Increase of spindle excitation when a static β axon is stimulated at frequencies above that producing fused extrafusal contraction. Activation of two spindle endings by the same β axon (conduction velocity 88 m/sec). P, primary ending (conduction velocity of the afferent fibre 92 m/sec); S, secondary ending (conduction velocity of the afferent fibre 31 m/sec). In each graph the upper trace represents ten superimposed records of the ending discharge during 300 msec periods of stimulation. The stimulation frequency was increased abruptly from 150/sec to 250/sec during each period of stimulation. The lower trace represents the isometric tension of the motor unit, recorded at the muscle tendon. The fusion frequency of this motor unit was 120/sec.

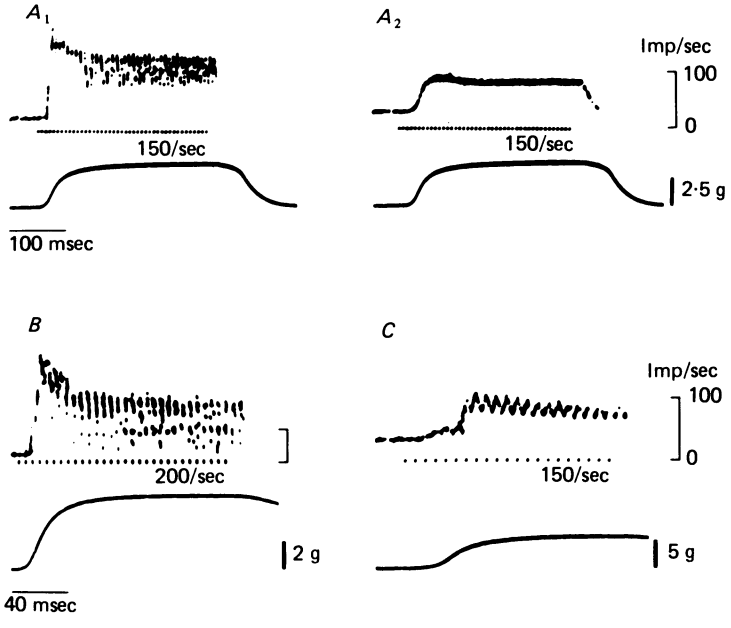

Fig. 5. Frequencygrams of spindle afferent discharge recorded during stimulation of static β axons. Each graph represents the superimposition of twenty records of spindle afferent discharge during stimulation of a β axon (upper trace). The isometric tension developed by the motor unit is recorded at the muscle tendon (lower trace). *A*, same endings as in Fig. 4 activated by the same axon. A_1, primary ending; A_2, secondary ending. *B*, primary ending (conduction velocity of the afferent fibre 103 m/sec) activated by a static β axon (conduction velocity 92 m/sec; fusion frequency of the motor unit 166/sec). *C*, secondary ending (conduction velocity of the afferent fibre 53 m/sec) activated by a static β axon (conduction velocity 92 m/sec; fusion frequency of the motor unit 120/sec).

Frequencygrams of spindle afferent discharge recorded during stimulation of static β axons

Frequencygrams (Bessou, Laporte & Pagès, 1968*a*) are constructed by superimposing successive records of the instantaneous frequency of discharge of spindle primary endings during stimulation of single fusimotor axons. This method is considered as providing information on the size and time course of the contraction of intrafusal muscle fibres. Frequencygrams of primary ending discharge recorded during stimulation of static γ axons have shown that fused tetanic contraction of certain intrafusal muscle fibres (presumably the chain fibres) requires very high stimulation rates, above 180–200/sec (Bessou, Laporte & Pagès, 1968*b*). As fused tetanic contraction of most extrafusal motor units is observed at lower rates of stimulation, frequencygrams of spindle afferent discharge recorded during stimulation of presumed βS axons were examined with the purpose of detecting possible differences between the fusion frequencies of the intra- and extrafusal portions of β motor units. These frequencygrams were systematically constructed with stimulation rates above that producing the complete fusion of the extrafusal motor unit in question.

Fig. 5A_1 shows a typical frequencygram of a spindle primary ending activated by an axon that was eventually identified as a βS axon. The fusion frequency of the extrafusal motor unit was 120/sec and the frequencygram was constructed with 150/sec stimulation. Regular oscillations of the primary ending discharge frequency appeared in phase with stimulation, suggesting that some mechanical events synchronous with stimulation took place in the spindle. A similar instance is illustrated in Fig. 5*B* with stimulation at 200/sec of a βS axon whose extrafusal motor unit showed complete fusion for stimulation at 166/sec. Such 'unfused' frequencygrams indicated the occurrence of intrafusal contraction because, if the spindle activation were only due to mechanical excitation resulting from extrafusal contraction, the frequencygram should appear fused for stimulation rates producing complete fusion of extrafusal contraction.

Bessou & Pagès (1969) showed that frequencygrams of secondary endings appear fused for rates of stimulation of γ axons much lower than those producing fusion of primary ending frequencygrams. Most of the identified βS axons gave fused frequencygrams with secondary endings (Fig. 5A_2). Unfused frequencygrams of secondary afferent discharge were seen rarely and displayed only small oscillations of the afferent discharge frequency in phase with stimulation (Fig. 5*C*).

Type of fusimotor action exerted by fast-conducting β axons on the response of activated spindles to muscle stretch

Ramp stretches were used to test the type of fusimotor action exerted by presumed β axons. In agreement with the results of previous histophysiological studies (Harker *et al.* 1977; Jami *et al.* 1978, 1979) nearly all the fast-conducting β axons were found to exert a static action on the primary and secondary endings they activated (Fig. 6). Actions exerted on the response of spindle endings to sinusoidal stretch (1 Hz, amplitude 0·5 mm) were also examined and, as was reported for γ static axons (Emonet-Dénand, Laporte, Matthews & Petit, 1977), β axons exerting a static action on responses to ramp stretches were quite often found to increase the modulation of a spindle afferent discharge during sinusoidal stretch.

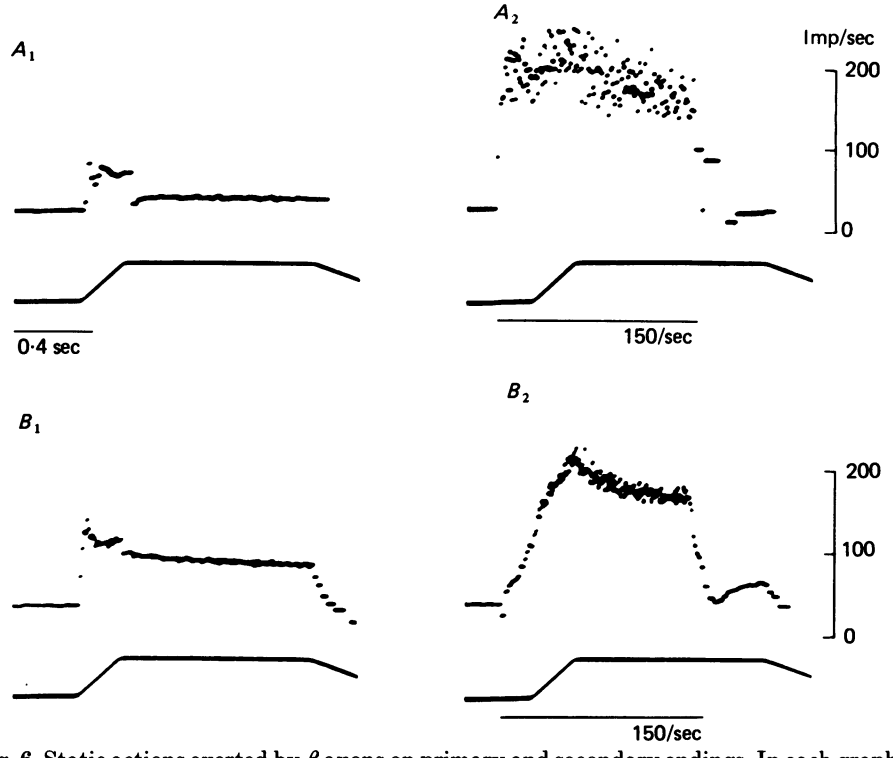

Fig. 6. Static actions exerted by β axons on primary and secondary endings. In each graph the upper trace is the discharge of a spindle ending and the lower trace represent the muscle length. *A* and *B* are from the same experiment. *A*, primary ending (conduction velocity of the afferent fibre 103 m/sec) activated by a static β axon (conduction velocity 90 m/sec). A_1, passive response of the ending to a stretch of 2 mm at 8/sec. A_2, response during stimulation of the axon at 150/sec. *B*, secondary ending (conduction velocity of the afferent fibre 36 m/sec) activated by an other static β axon (conduction velocity 94 m/sec). B_1, passive response. Same stretch as in *A*. B_2, response during stimulation of the axon at 150/sec.

Conjunction of criteria required for identification of static β axons

In addition to the test of delayed failure of spindle activation, three other criteria were systematically examined for each presumed βS axon: (i) significant increase of spindle excitation when the motor axon was stimulated at frequencies higher than that eliciting a maximal extrafusal contraction (Fig. 4), (ii) 'unfused' frequencygram of the spindle afferent discharge during stimulation of the motor axon at a rate above the frequency of tetanic fusion for the extrafusal tension (Fig. 5) and (iii) static effect of the presumed β axon on the response of the spindle afferent to a ramp stretch (Fig. 6). It was observed that most of the βS axons that could be identified by the test of delayed failure of spindle activation (Figs. 1 and 2) also satisfied the three other tests. Each of these tests, by itself, was quite suggestive of the fusimotor action of the axon under study (although it did not provide absolute proof) but whenever the three criteria were satisfied in conjunction this strengthened their value. Moreover it appeared that when a presumed βS axon satisfied the three criteria, the test of delayed failure of spindle activation never showed an immediate cessation of spindle

activation upon abolition of extrafusal contraction. It was therefore considered that axons for which the test of delayed failure of spindle activation gave dubious results (see S and P_1 in Fig. 3) could nevertheless be identified as β axons provided they satisfied *all* the three other criteria. Fig. 7 shows the application of this scheme to the axon of Fig. 3: its action on the secondary ending and on the first primary ending (P_1) was accepted as a β effect, but not its action on the second primary ending (P_2). In the final counts, only eight βS actions on primary endings and two βS actions on secondary endings were identified on such a basis. These actions were elicited by six axons of which three were positively identified as β (by the test of delayed failure of spindle activation) for their action on another ending.

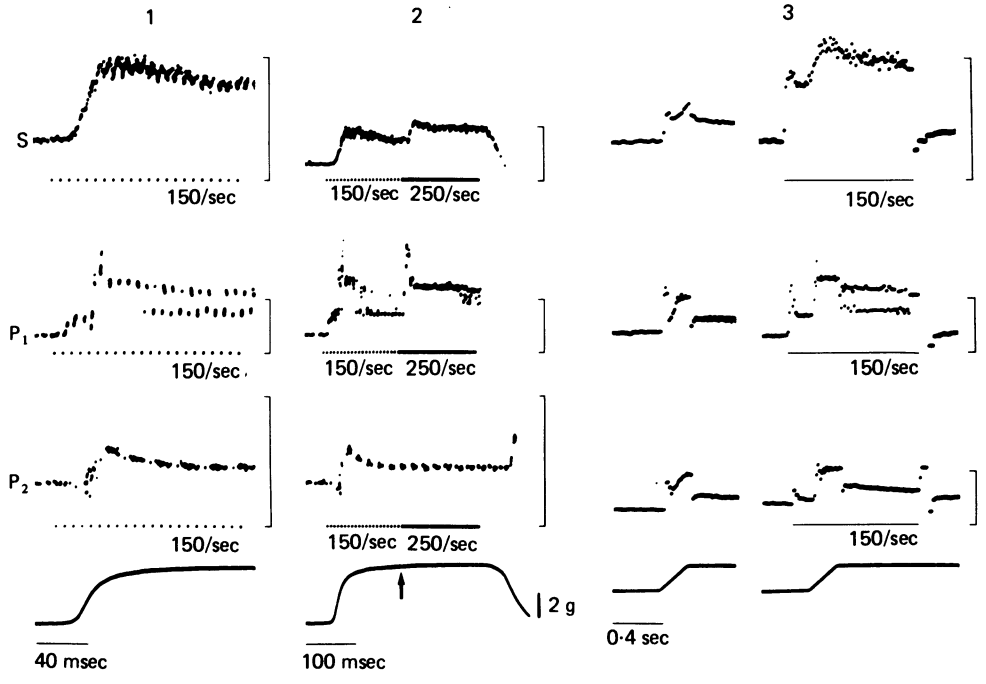

Fig. 7. Identification tests applied to the same axon as in Fig. 3 (fusion frequency of the motor unit 100/sec). Column 1: the frequencygrams appear 'unfused' for S and P_1 but not for P_2. Column 2: an abrupt increase in stimulation frequency from 150 to 250/sec caused an abrupt increase in the discharge frequencies of S and P_1 but not of P_2. The tension developed by the motor unit was recorded together with the discharge of the ending in each test, but since it did not vary it is shown here only once, at the bottom of columns 1 and 2. In the tension record of column 2, the arrow points to the moment of the increase in stimulation frequency. Column 3: static action exerted by the axon on the response of S and P_1 to a ramp stretch. Absence of static action on the response of P_2. At the bottom of the column is represented the muscle length; stretches of 2 mm were applied at 8 mm/sec. Vertical bars near each graph are calibrations for instantaneous frequency: 0–100 impulses/sec.

Section II. Incidence of static and dynamic β innervation in the peroneus tertius muscle

Table 1 summarizes the results, giving the numbers of spindle afferents examined in each experiment and how many of them were activated by a β axon, as well as the numbers and conduction velocities of the examined motor axons and how many of them were identified as βD or βS axons. In the course of each experiment γ axons

TABLE 1

Expt.	Spindle afferents examined: Group I Number	Group I β-activated	Group II Number	Group II β-activated	Motor axons examined: Number (conduction velocities)	Identified as β axons	Number of γ axons identified
1	6	5	4	2	10 (70–93 m/sec)	2D+3S	9
2	4	1	4	1	13 (69–91 m/sec)	1D+1S	11
3	2	2	5	2	15 (56–93 m/sec)	1D+3S	14
4	8	4	2	2	12 (61–92 m/sec)	4D+1S	7
5	6	3	4	2	10 (59–90 m/sec)	1D+2S	9
6	7	3	3	2	10 (81–104 m/sec)	4S	14
7	7	2	3	1	10 (81–96 m/sec)	3S	15
8	5	2	5	4	14 (75–98 m/sec)	1D+3S	28
9	6	2	4	1	11 (66–100 m/sec)	1D+1S	11
10	6	4	4	2	9(67–98 m/sec)	1D+4S	17
Total	57	28	38	19	114	12D+24S	135

were also identified and some of them were observed to excite one or several of the prepared spindle afferents. They were not particularly sought for, and the figures given in Table 1 (last column) merely represent the γ axons that were met incidentally during the process of isolating α and β motor axons. These figures show that each of the examined muscles received a normal supply of γ axons, so that its skeletofusimotor innervation regularly came in addition to (not instead of) a purely fusimotor innervation.

In total, we examined 1067 instances of the action on a spindle afferent of a motor axon supplying extrafusal muscle fibres (626 actions on primary endings and 441 on secondary endings). Acceleration of spindle discharge during stimulation of the motor axon at 150/sec was observed in 127 instances (eighty with primary and forty-seven with secondary afferents) of which sixty-three (only 5·9 % of the total) were finally identified as β effects (forty-one on primary and twenty-two on secondary endings).

Static and dynamic β axons were identified in all but two experiments (numbers 6 and 7), in which only βS axons were found. In these two experiments the conduction velocities of the examined motor axons ranged from 81 m/sec upwards and it is well known that βD axons usually have conduction velocities below 80 m/sec (Emonet-Dénand & Laporte, 1975; Emonet-Dénand *et al.* 1975; Jami *et al.* 1978). Therefore, the absence of βD axons in these two experiments should not be taken as indicating that dynamic β innervation is inconstant in peroneus tertius.

Of the 114 examined motor axons, thirty-six (31 %) were identified as β axons, of which twelve were dynamic and twenty-four static. The histogram in Fig. 8 shows the remarkable segregation of conduction velocities between static and dynamic β axons. All the βS axons had conduction velocities above 80 m/sec except two axons with conduction velocities of 79 and 69 m/sec respectively.

Seventeen of the βS axons innervated motor units of the FR type in agreement with the histophysiological observations of Jami *et al.* (1979). However, six other βS axons (all of which had conduction velocities above 90 m/sec) innervated motor units of the FF type, and the slowest βS axon innervated a motor unit of the S type.

In total the twenty-four βS axons activated twenty primary and seventeen secondary spindle endings. Half of the βS axons acted on a single spindle ending and the others were distributed as follows: nine acted on two endings, two acted on three endings, and one acted on four endings.

With a single exception, the βD axons had conduction velocities below 80 m/sec and innervated extrafusal motor units of the S type, in agreement with the findings of Barker *et al.* (1977). The only βD axon that innervated a motor unit of the FR type, had a conduction velocity of 91 m/sec. In total, the twelve βD axons activated sixteen primary and five secondary endings; four of the βD axons acted on a single spindle ending, six others acted on two endings, and the last two acted on three endings.

β axons, identified as dynamic by the action they exerted on a primary ending, were occasionally observed to excite also a secondary ending (Fig. 9). In none of such cases however, did the β axon exert a dynamic action on the response of the activated secondary ending to muscle stretch (compare rows P and S in Fig. 9*B*).

The histogram of Fig. 10 shows the distribution of β-activated spindle afferent fibres in the studied sample. The proportion of β-activated endings is the same for primaries and secondaries: twenty-eight out of fifty-six primary endings (49 %) and

nineteen out of thirty-eight secondary endings (50%). Among the primary endings, 35% (twenty endings) were activated by βS axons and 28% (sixteen endings) by βD axons. Among the secondary endings, 45% (seventeen endings) were activated by βS axons and 13% (five endings) by βD axons.

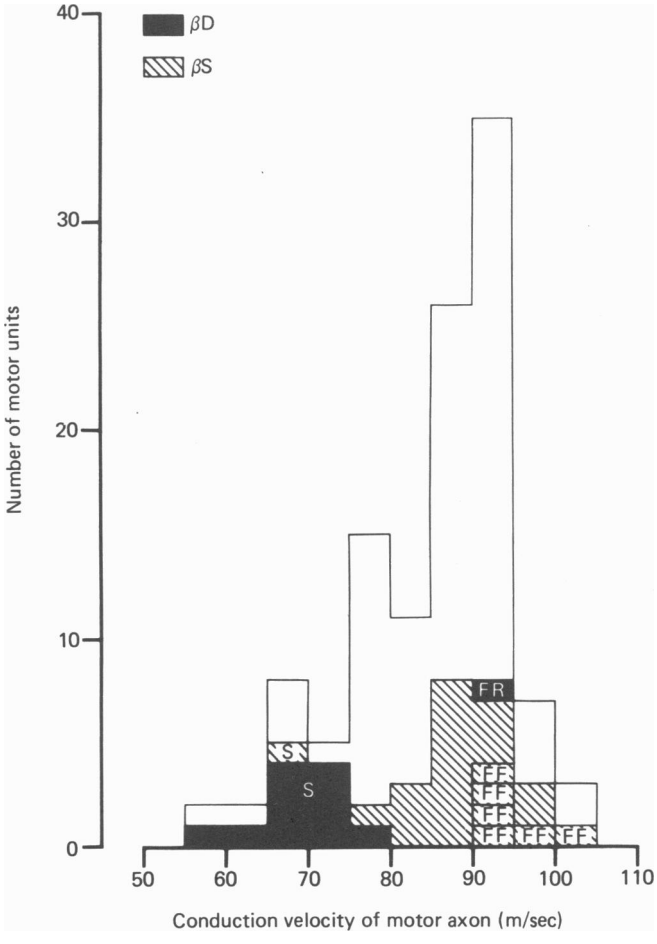

Fig. 8. Histogram of the conduction velocities of 114 motor axons innervating extrafusal muscle fibres in the peroneus tertius muscle. β axons are represented by dark (βD) or hatched (βS) areas. S, motor units of the slow type. All the βD motor units for which there is no individual indication were of the S type. FR, motor units of the fast-fatigue resistant type. All the βS motor units for which there is not individual indication were of the FR type. FF, motor units of the fast-fatiguable type.

Convergence of two, or even three, β axons onto the same spindle was observed in fourteen instances, that is for eleven (19%) primary and three (8%) secondary endings (see also Emonet-Dénand & Laporte, 1975; McWilliam, 1975). One primary afferent was activated by two βS axons. More often, the convergence involved one βS and one βD axon (for six primary and three secondary endings) or even two βS plus one βD axon (for one primary ending) and two βD plus one βS axon (for another

primary ending). By contrast, in cat tenuissimus spindles examined with the glycogen-depletion method, convergence of βS and βD axons was found very rarely (Jami *et al.* 1978).

It is known that βD axons can exert noticeable dynamic effects upon stimulation at 10–40/sec (Emonet-Dénand & Laporte, 1979). In the present study, stimulation at such presumed physiological frequencies was found effective not only for dynamic but also for static β axons. Occasionally, even a single shock to a βS axon appeared

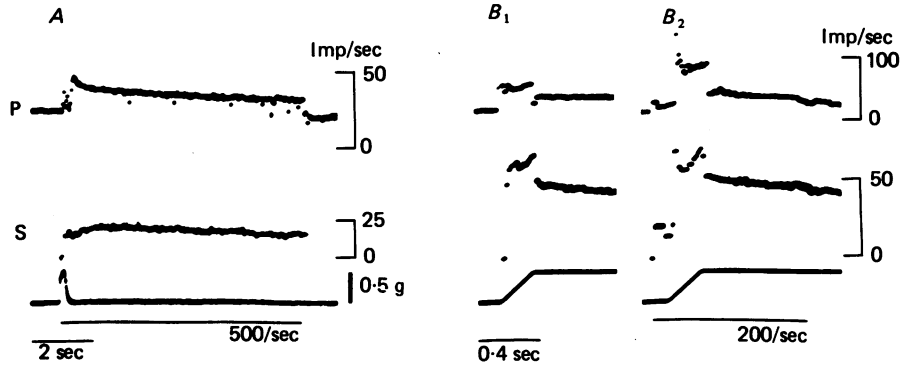

Fig. 9. Dynamic β axon (conduction velocity 66 m/sec) activating a primary (P, conduction velocity of the afferent fibre 92 m/sec) and a secondary ending (S, conduction velocity of the afferent fibre 34 m/sec). *A*, identification of the β axon by the persistence of its activation of P and S after disappearance of extrafusal tension (lowermost trace) resulting from prolonged stimulation of the axon at 500/sec. The secondary ending had no resting discharge for this muscle length. *B*, effect exerted by the β axon on the responses of P and S to ramp stretching of the muscle. B_1, passive responses. The lowermost trace represents muscle length. Ramp of 2 mm at 8 mm/sec. B_2, responses during stimulation of the β axon at 200/sec. The dynamic index of P is doubled while that of S is unchanged.

sufficient to activate a primary ending, as was observed for static γ axons by Bessou *et al.* (1968*b*). Some, but not all, of the identified βS axons elicited an early discharge (Hunt & Kuffler, 1951) of the spindle ending they activated. However, this was not a reliable test for identification of βS axons because, in several instances, axons that did elicit early discharges failed to satisfy other criteria and therefore to qualify as β axons. In experiments 6–10, βS axons were systematically examined for their action during 40/sec stimulation. A few of them elicited a pause in the discharge of their activated spindles, as might be expected from the unloading effect of extrafusal contraction. But the majority of the tested βS axons (eleven out of fifteen) were able to excite primary as well as secondary endings, and driving of the sensory ending discharge was often observed in response to stimulation of βS axons at rates ranging between 25 and 50/sec (see Fig. 5 in Emonet-Dénand *et al.* 1975). However, it is difficult to decide whether the effects of βS axons observed for low stimulation rates were due to the intrafusal action of these axons or to their extrafusal action or to an interaction of both.

No relation was found between the action of β axons on spindles observed during low-frequency stimulation and the amount of tension developed by the extrafusal

portion of their motor unit. In the present sample of β motor units the maximal tetanic tensions ranged from 0·25 to 5 g for twelve S type units, from 1·8 to 45 g for eighteen FR type units and from 8 to 62·5 g for five FF type units (one of the FF units got fatigued before its tension could be measured).

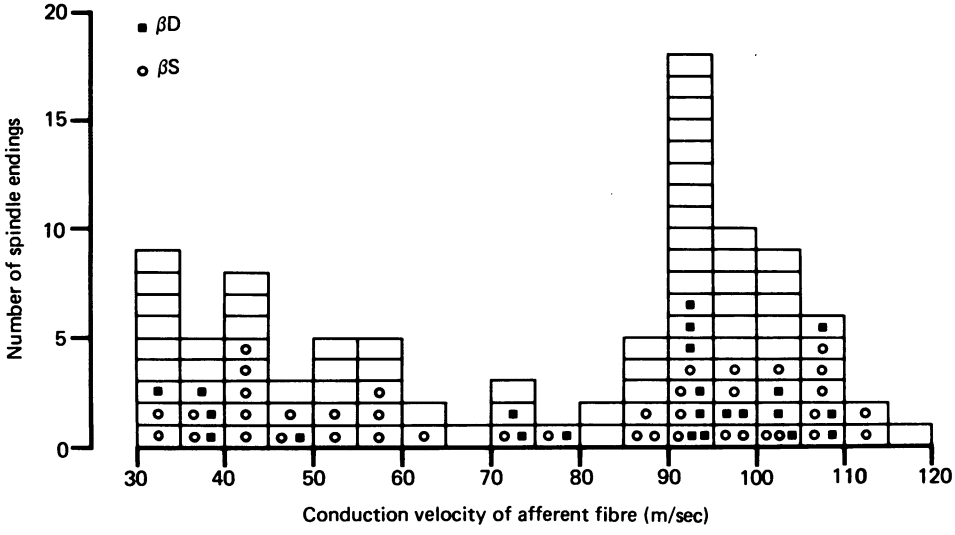

Fig. 10. Histogram of the conduction velocities of ninety-five afferent fibres from peroneus tertius spindles. Each division represents an afferent fibre. Symbols indicate the activation of the spindle ending by one or more β axons.

DISCUSSION

The main finding of this study is that there are at least 31 % of β axons among the motor axons innervating extrafusal muscle fibres in the cat peroneus tertius muscle. This figure may be conservative since it is not possible in each experiment to test the action of every motor axon on all the spindles of the studied muscle. One third of the β axons to the peroneus tertius were dynamic and two thirds were static. Static β axons were identified on criteria that will be discussed to begin with.

Identification of static β axons

Fast-conducting βS axons could not be identified by selective block of extrafusal contraction because their intrafusal action rapidly declined during high-frequency stimulation, so that, at best, what could be observed was a delay between the block of extrafusal contraction and the failure of spindle activation. It is not known whether the 'fatiguability' of the intrafusal action of βS axons is due to synaptic failure or to the muscle effectors themselves. A similar fatiguability was reported for some static γ axons (Emonet-Dénand & Laporte, 1978). However, in rabbit lumbrical muscles βS axons resisted a combination of light curarization with repetitive stimulation at 200/sec (see Fig. 6 in Emonet-Dénand *et al.* 1970). No explanation is available for this difference between βS axons of the cat and rabbit. Another difference is that in rabbit muscles βS and βD axons have conduction velocities in the same range (Emonet-Dénand *et al.* 1970).

A crucial issue in accepting the tests that were used in the present study as criteria for identification of βS axons, is whether the tension recorded at the muscle tendon provides an accurate account of all the mechanical effects produced within the muscle by the contraction of a motor unit. Peroneus tertius is a small unipennate muscle in which muscle fibres run over relatively short distances from their osseous origin to a relatively thick tendon. Given the stiffness of the tendon, and the fact that it was kept very short in the present experiments (see Methods), the structure of the muscle should allow an efficient transmission of pull from contracting muscle fibres to the tension transducer. In fact, 'fibrillation' of fatiguing extrafusal muscle fibres was occasionally detected, displaying minute fast transients of tension that were not synchronized with stimulation.

Some motor units were observed to activate a spindle and give 'unfused' frequencygrams for low stimulation rates, presumably reflecting the unfused tetanic contraction of the motor unit, whereas for rates of stimulation eliciting complete fusion of extrafusal tension the frequencygrams appeared fused. In such instances, the spindle excitation reached a maximum when the motor axon was stimulated at the tetanic fusion frequency of the motor unit. The fact that this kind of observation was made in the same experiments in which βS axons were found to satisfy the two criteria illustrated in Figs. 4 and 5, strengthens the value of these tests for differentiating between extra- and intrafusal effects of a βS axon.

The static action exerted by fast-conducting β axons on primary spindle endings had to be tested before the abolition of extrafusal contraction because of the fatiguability of the intrafusal action of these axons. Possible interactions between extra- and intrafusal contraction could therefore not be avoided and it is known that such interactions may distort the action exerted by a β axon (see fig. 4 in Emonet-Dénand *et al.* 1975). This raises the question of whether the incidence of static axons among fast-conducting β axons was not over-estimated. However, most of the identified βS axons displayed a high fatiguability of intrafusal action, which was never observed for βD axons (Emonet-Dénand & Laporte, 1974, 1975; Emonet-Dénand *et al.* 1975). Moreover, the 'unfused' frequencygrams of spindle afferent discharge obtained during high-frequency stimulation of β axons identified as static, suggest that these axons innervate intrafusal chain fibres that are effectors of static action.

Proportion of β axons in the motor supply to the peroneus tertius muscle

There are few quantitative studies of β innervation based on physiological identification tests. McWilliam (1975) studied two small muscles and found 11 % of β axons in the tenuissimus and 28 % in the abductor digiti quinti medius. In a relatively large muscle, the peroneus brevis, Emonet-Dénand & Laporte (1975) showed that 18 % of the axons supplying extrafusal muscle fibres were β axons, mostly dynamic (15·5 %). The most likely reason why they found so few βS axons is that, as stated by themselves, the method they used for demonstrating β axons is adequate essentially for β axons whose intrafusal neuromuscular junctions can resist high-frequency prolonged stimulation.

It was already known from histophysiological studies that βD axons innervate extrafusal motor units of the S type (Barker *et al.* 1977) and βS axons innervate FR units (Jami *et al.* 1979). The finding that some βS axons innervate extrafusal motor

units of the FF type could be expected since transmutations between FR and FF units have been considered to occur in normal muscle under given conditions of exercise or training (Burke & Edgerton, 1975).

The proportion of spindle poles receiving p_1 innervation in peroneus tertius muscle is 58% (Barker *et al.* 1970). Therefore, if p_1 plates are exclusively innervated by β axons, the proportion of spindles innervated by β axons should range between 58 and 100% whereas in the present study only 50% of peroneus tertius spindles were found to receive β innervation. On the other hand, the proportion of spindles innervated by βS axons is significantly higher (35% of primary and 45% of secondary endings) than the proportion of 27% found by Harker *et al.* (1977). These authors counted the spindles showing glycogen depletion after stimulation of a group of motor axons selected for conduction velocities above 90 m/sec. They did not stimulate axons with conduction velocities between 80 and 90 m/sec, and a notable proportion of βS axons are precisely found in this range (see Fig. 8).

In the 55–80 m/sec range of conduction velocities, more than one third (thirteen out of thirty-two) of the motor axons to peroneus tertius were β axons, mostly dynamic. This compares roughly with the findings of Emonet-Dénand & Laporte (1975) in peroneus brevis. It is therefore surprising that only 28% of peroneus tertius primary endings were found to be activated by βD axons, as against 72% in peroneus brevis. In fact, even if the results of experiments 6 and 7 are removed from the present sample, the proportion of βD-activated spindles comes out as only 39·5%, which gives similar proportions of peroneus tertius spindles innervated by βD and βS axons but leaves unexplained the difference between the two muscles.

The segregation of conduction velocities between βS and βD axons has no counterpart among fusimotor axons. The conduction velocities of static and dynamic γ axons are known to overlap, except in the range below 25 m/sec where only static axons have been found (Brown, Crowe & Matthews, 1965; Emonet-Dénand, Laporte & Pagès, 1966).

Dynamic β effects are likely to occur when slow-contracting motor units are activated, e.g. in maintenance of a posture or at the beginning of a movement, whereas static β effects should be related to the activation of fast-contracting motor units that are involved in rapid muscle shortening and develop large amounts of tension. Recent observations by Appenteng, Morimoto & Taylor (1980) indeed suggest that, in the cat jaw muscles, dynamic fusimotor action takes place when extrafusal contraction is minimal (i.e. presumably when low-threshold slow motor units are active) whereas static fusimotor action occurs in conjunction with active shortening of the muscle (i.e. when fast motor units are being recruited).

Activation of secondary endings by βD axons may be due either to the fact that a βD axon occasionally distributes a terminal branch to an intrafusal chain fibre in addition to its regular innervation of the bag_1 fibre (Barker *et al.* 1977) or to the fact that accessory terminal branches of secondary endings are often found lying on bag fibres as well as on chain fibres (Banks, Barker & Stacey, 1977). So far, there is no clue to decide between these two possibilities. Whatever may be the case, the activation of a secondary ending by a βD axon was never observed to elicit an increase in the dynamic sensitivity of this ending. Neither did it produce an important acceleration of the ending discharge (Fig. 9). These negative findings suggest that

although the activation of secondary endings by βD axons is not exceptional, its functional significance may be moderate.

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