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SKELETO-FUSIMOTOR FIBRES IN THE RABBIT

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SUMMARY

1. In rabbits, repetitive stimulation of single motor axons to lumbrical muscles elicits both a contraction of extrafusal muscle fibres and an increase in the discharge frequency of spindle primary endings.

2. This activation of the sensory endings can be attributed to the contraction of intrafusal muscle fibres because it persists after the contraction of extrafusal muscle fibres has been suppressed by selective curarization of their neuromuscular junctions.

3. In non-curarized preparations the frequency of most of the afferent discharges continues to increase when rates of stimulation of motor fibres exceed the tetanic fusion frequency of the extrafusal muscle fibres.

4. The effect of repetitive stimulation of motor fibres that supply extrafusal and intrafusal muscle fibres was studied on the responses of primary endings to phasic stretch. Of twelve fibres, eight were found to exert a dynamic effect and four a static one.

5. Selective curarization of the extrafusal neuromuscular junctions does not modify the nature of the static and dynamic effects.

INTRODUCTION

The presence in rabbits of motor axons which branch to supply intrafusal and extrafusal muscle fibres, also called skeleto-fusimotor or β motor fibres, was recently demonstrated in histological preparations by Adal & Barker (1965) (see also Barker, 1968).

The aim of the experiments reported here was to provide physiological confirmation for the existence of such motor axons and to determine their action on primary spindle endings at constant muscle length and during phasic changes of length.

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A preliminary communication of this work has been published (Emonet-Dénand, Jankowska & Laporte, 1969).

METHODS

The experiments were made on adult rabbits weighing 3–3·5 kg which were anaesthetized with pentobarbitone sodium, 20–25 mg/kg, given intravenously (Nembutal, Abbot Laboratories). During the early stages of the dissection (laminectomy and removal of the spinal cord below L 4) a temporary deeper anaesthesia was obtained by giving the animal a mixture of of 1–2 % halothane–oxygen. Additional small doses of Nembutal diluted to 2 mg/ml. in a solution of gelatin (Plasmagel, Bellon Laboratories) were injected whenever necessary.

The muscles used for these experiments were the lumbrical muscles of the hind foot. They were chosen because Adal & Barker (1965) have described motor axons to these muscles with branches running to extrafusal and to intrafusal muscle fibres, and because lumbrical muscles are supplied by very few nerve fibres. With such small muscles it is much easier than in large muscles to identify β motor fibres because single motor fibres have to be tested only on 2–4 spindles. This advantage compensates for the failures which occur when some nerve fibres are damaged during the preparation of single fibres.

The three lumbrical muscles of the rabbit hind foot correspond to the deep lumbrical muscles of the cat. They are situated between the terminal tendons of the flexor longus digitorum muscle and originate from the distal part of its expanded tendon.

Most experiments were done on the medial or first lumbrical as its motor nerve was readily accessible and could be freed over several millimeters from other terminal branches of the plantaris nerve. Occasionally the third lumbrical was used.

The preparation was similar to that described by Bessou, Emonet-Dénand & Laporte (1965) in cats but with some alterations. The lumbo-sacral vertebral column was fixed by two clamps, one on the bodies of L 3–L 4 lumbar vertebrae, the other on the coccygeal vertebrae when it was suspected that fixing the coxal bone with lateral pins was responsible for blocking the conduction in L 7 spinal nerves. The spinal cord below L 4 was removed to provide more room for preparing single fibres. The knee, the leg and the foot which was fully extended with its lower aspect upward, were immersed in a vessel filled with Locke solution which was maintained at a constant temperature of 37° C.

The part of the flexor longus digitorum common tendon on which the lumbrical muscle is inserted was isolated from the rest of the tendon. It was then fixed to a rigid metal bar and displaced rostrally by 1–2 mm so as to avoid damage to the blood vessels supplying the lumbrical muscle when the muscle was stretched by 2–3 mm in the opposite direction.

The tendon of the lumbrical muscle was attached to an isometric myograph mounted on a puller (see Emonet-Dénand & Laporte, 1969, for the description of the puller). The extremity of the myograph was fixed rigidly when the muscle was submitted to ramp stretches.

Single afferent fibres were prepared from L 7 to S 1 dorsal roots. They were considered as being connected to primary endings when on phasic muscle stretch the endings discharged in a manner similar to that of cat spindle endings connected to Group I fibres. However the conduction velocity of these fibres was relatively slow, between 65 and 50 m/sec. One of the fibres connected to such a phasic ending was conducting at only 40 m/sec.

The discharges of the endings were recorded either in the conventional way or with an instantaneous frequency-meter.

Single motor fibres were prepared from S 1 to S 2 ventral roots. Their action potentials were led from thin filaments after stimulation of the nerve twig supplying the lumbrical muscle. To avoid spread of current to cut motor branches to interosseus muscles the twig to the lumbrical muscles was separated over several millimetres from other nerve branches and the cathode of stimulation was placed over the point of entry of the nerve into the muscle, the anode being on the belly of the muscle.

Selective curarization of the extrafusal neuromuscular junction was achieved by the cumulative action of gallamine triethiodide (Flaxedil) and of repetitive stimulation of motor axons at 150–300/sec. Small quantities of Flaxedil were injected intravenously, at 1 min intervals; total doses varied between 0·7 mg and 2 mg/kg.

RESULTS

Evidence for the existence of skeleto-fusimotor fibres in lumbrical muscles

The criteria for the identification of skeleto-fusimotor fibres were those used in similar experiments on cats (Bessou *et al.* 1965). It was considered that a motor axon innervated both intrafusal and extrafusal muscle fibres if on repetitive stimulation it elicited an increase in the discharge frequency of primary endings which could not be attributed to mechanical actions, such as stray pull or compression, exerted on the sensory endings by the contraction of extrafusal muscle fibres. This increase in the discharge was not related to the tension developed by extrafusal muscle fibres. It continued to increase for rates of stimulation higher than the frequency for which maximal tension was reached and it persisted after the contraction of extrafusal muscle fibres was suppressed by selective curarization of their neuromuscular junctions (see Methods).

A typical example of the action exerted by a skeleto-fusimotor fibre is illustrated by Figs. 1 to 3.

Fig. 1 shows that repetitive stimulation of a single motor fibre produced both the contraction of a motor unit and a discharge of a spindle ending which was previously inactive. At stimulus frequencies of 9/sec and 28/sec (records 4 and 5) an afferent impulse was discharged after each stimulus. At 100/sec, this being a rate of stimulation for which the tetanic contraction was maximal (record 6), the ending discharged more rapidly. After a further increase of the rate of stimulation (record 7) the tension developed by extrafusal muscle fibres did not change, while the frequency of discharge of the ending markedly increased.

Fig. 2 illustrates the absence of any relation between the tension developed by the motor unit and the increase of discharge frequency of the sensory ending. For the three rates of stimulation (100, 150 and 200/sec) which produced the same maximal tetanic tension the frequency of discharge of the ending (measured during the last tenth of a second of the stimulation period) was respectively 68, 90, 100/sec.

Fig. 3 shows the effect elicited by stimulation of the same motor fibre after an intravenous injection of Flaxedil. At a certain level of curarization, transmission failed in the neuromuscular junctions of the extrafusal muscle fibres shortly after the beginning of a period of repetitive stimulation at 300/sec. The contraction of the extrafusal muscle fibres was completely suppressed during the remaining period of stimulation, whereas the sensory ending continued to discharge as long as the stimulation lasted. It may be noted that the contraction of the extrafusal muscle fibres must have limited the activation of the primary ending because immediately

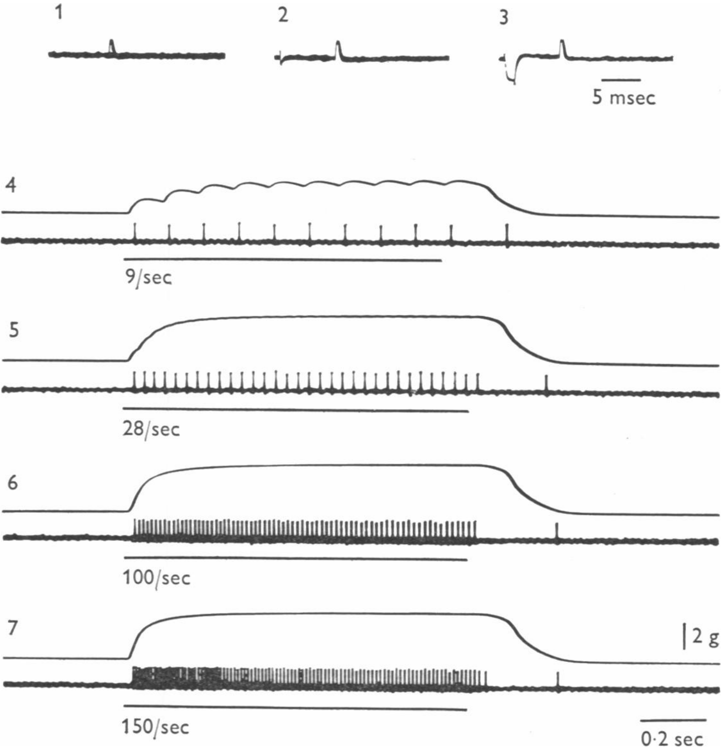

Fig. 1. Discharges of a primary ending produced by repetitive stimulation of a single motor axon at various frequencies. Third lumbrical muscle. 1–3: action potential of the motor fibre recorded in a S II ventral root filament after stimulation of the nerve twig to the third lumbrical muscle: superimposed records. The strength and the duration of the stimulus was increased to obtain successively records 1 to 3. Conduction velocity of the motor fibre: 43 m/sec. 4 to 7: *upper traces*, contraction of the motor unit; isometric recording; *lower traces*, discharge of a spindle afferent fibre recorded in S I dorsal root filament. Conduction velocity of the afferent fibre: 53 m/sec.

after the contraction disappeared the rate of discharge of the ending increased. The progressive diminution observed in the latter part of the stimulation period was probably due to a slight action of Flaxedil on the intrafusal neuromuscular junctions themselves.

There is no doubt that the sensory ending on which these observations were made belonged to a spindle because when stretch of the muscle was increased, the ending had a resting discharge which paused during the

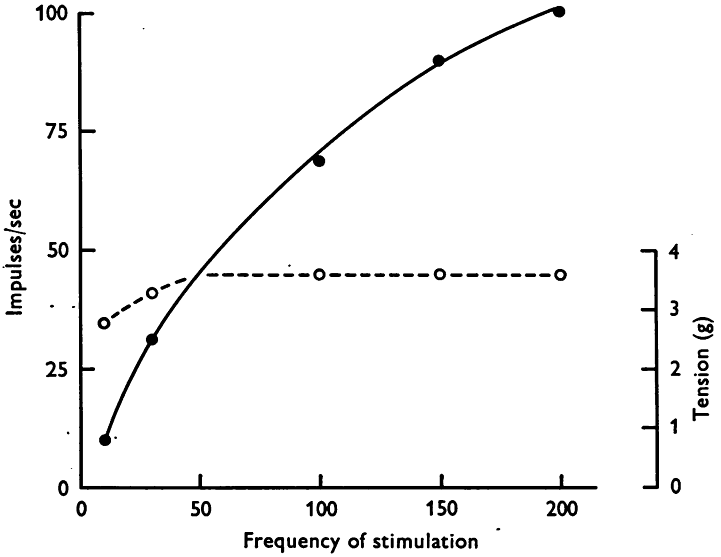

Fig. 2. Comparison of the frequency of discharge of a primary ending (filled circles) and of the tension developed by a motor unit (open circles) for various frequencies of stimulation of a single motor fibre. Same spindle as in Fig. 1.

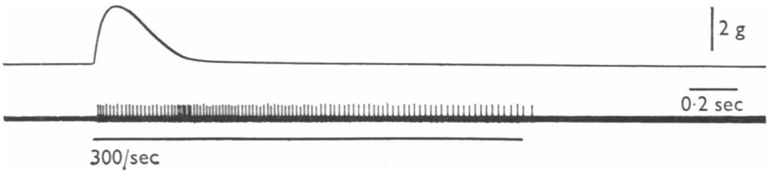

Fig. 3. Persistence of the activation of a primary ending elicited by repetitive stimulation of a single motor fibre after the development of a complete block of transmission at the neuromuscular junctions of extrafusal fibres produced by Flaxedil. Same spindle as in Figs. 1 and 2. In this experiment a total dose of Flaxedil, 2 mg/kg, was injected intravenously during a period of 5 min until a very small contraction of the extrafusal muscle fibres persisted at the beginning of a period of repetitive stimulation. This record was taken while the effect of Flaxedil was wearing off, approximately 30 min after the beginning of the injection of Flaxedil.

contraction of another motor unit. Furthermore, the persistence of the activation of the ending after the extrafusal junctions were blocked by Flaxedil rules out the possibility that this ending was a tendon organ.

Fig. 4 describes the action of a motor fibre which at first sight was not considered as being skeleto-fusimotor because the spindle ending paused when that fibre was stimulated at frequencies higher than 50/sec (see record 2). The two or three spikes which were elicited by each stimulus when the rate of stimulation was low (record 1) were attributed to re-extension of the spindle during the relaxation phase of each twitch. However, after an injection of Flaxedil which was given to study another motor fibre, a moderate but distinct increase in the frequency of discharge of this ending was produced by the repetitive stimulation of that motor fibre (record 3). This unexpected observation is interesting because it shows that some β fibres may have been overlooked, as no systematic attention was given to motor fibres whose repetitive stimulation at high frequency (50–200/sec) stopped the discharge of primary endings when the extrafusal junctions were not curarized. These β fibres probably exert a weak action on the spindles which is overcome by the unloading effect of the contraction of extrafusal muscle fibres.

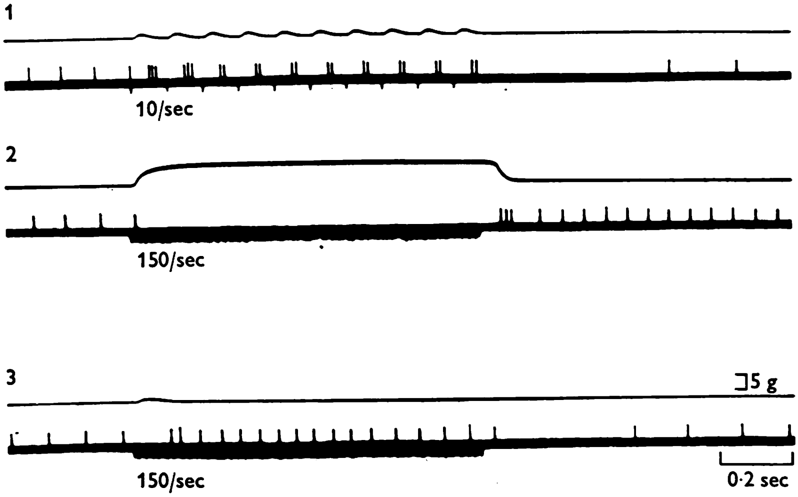

Fig. 4. Effects elicited on a primary ending by the repetitive stimulation of a single β fibre before and after curarization of the extrafusal neuromuscular junctions. Third lumbrical muscle. *Upper traces*: contraction of the motor unit, conduction velocity of the motor fibre was 41 m/sec. *Lower traces*: discharge of a spindle primary ending recorded in a S I dorsal root filament. 1–2: before curarization; 3: after Flaxedil (approximately 1 mg/kg).

Action of skeleto-fusimotor fibres on the response of primary endings to phasic stretch

The nature of the action which skeleto-fusimotor fibres exert on the sensitivity of primary endings to phasic stretch was determined. The lumbrical muscles were stretched over 1–2·5 mm at constant velocities ranging between 8 and 15 mm/sec while single β fibres were repetitively stimulated. The modification of the discharge of the ending before, during and after the phasic stretch was observed before and after selective curarization of the extrafusal neuromuscular junctions.

Of twelve β fibres whose action could be completely analysed (out of a total of thirty identified β fibres), eight were found to be dynamic and four static, these terms being used in the sense defined by Matthews (1962) for fusimotor fibres. Selective curarization of the extrafusal junctions did not modify the nature of these effects which shows that they were due to the activation of intrafusal muscle fibres. However, the amplitude of these effects was sometimes reduced, probably because transmission in intrafusal synapses was slightly depressed for this level of curarization.

(*a*) *Dynamic skeleto-fusimotor fibres.* The actions exerted on a primary ending by a typical β dynamic fibre are illustrated by Fig. 5. The characteristic response of a primary ending to a ramp stretch is shown in record 1. When a β motor fibre was stimulated at 150/sec (record 2) the primary ending which had previously been inactive at this length began to discharge at a moderate rate before the muscle length was changed; during the ramp stretch it discharged at a much higher rate than during the corresponding period of record 1; the dynamic index, i.e. the difference between the frequency of firing of the ending at the end of the phasic stretch and the frequency at the final length measured 0·5 sec later (Crowe & Matthews, 1964) was notably increased.

Records 4 and 5 which were obtained after the transmission in extrafusal junctions was blocked by Flaxedil (see record 3) are essentially similar to records 1 and 2.

The conduction velocities of the β dynamic fibres were 70, 43, 42, 42, 35, 34, 28, 27 m/sec.

(*b*) *Static skeleto-fusimotor fibres.* Fig. 6 illustrates a typical static fibre. Record 1 is the response of the primary ending to a ramp stretch. Record 2 shows that the repetitive stimulation of this single β fibre produced a prominent increase in the rate of discharge of the ending before the onset of stretch. During the phasic stretch the rate further increased but there was no sudden drop in frequency at the end of the stretch and the dynamic index was slightly diminished.

As for the β dynamic fibre described in Fig. 5, curarization of the extrafusal junctions did not alter the effect of this static β fibre. Records 4 and 5, taken after curarization (see record 3), are comparable to records 1 and 2.

The conduction velocities of the β static fibres were: 50, 45, 42, 37 m/sec.

The observations illustrated by Figs. 5 and 6 were made from the same spindle, thus showing that a spindle can be activated by the two kinds of skeleto-fusimotor fibres.

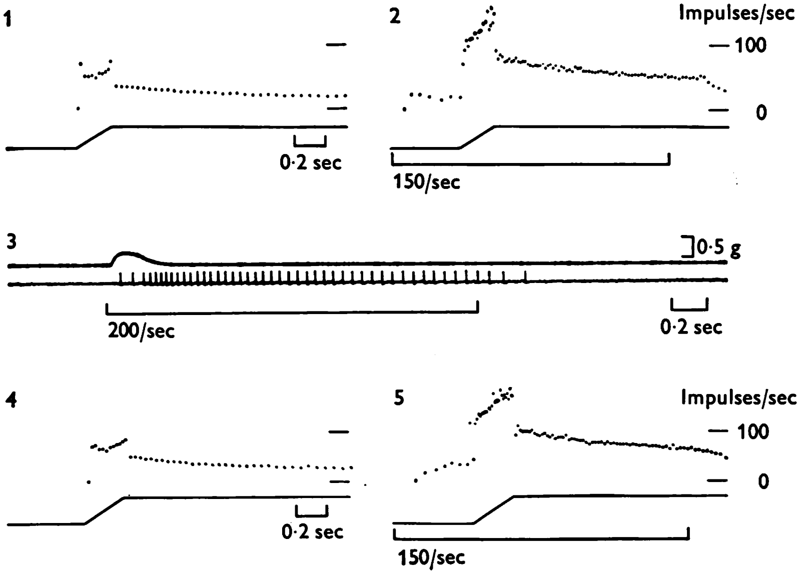

Fig. 5. Dynamic skeleto-fusimotor fibre. The discharges of a primary ending of the first lumbrical muscle were recorded with an instantaneous frequency-meter (upper trace of records 1–2, 4–5). The muscle was submitted to ramp stretches of 2 mm (lower traces of records 1–2, 4–5). 1–2: before curarization of the extrafusal neuromuscular junctions. 1: no stimulation; 2: stimulation of the motor fibre (conduction velocity: 28 m/sec) at 150/sec. 3: effects of repetitive stimulation of the same fibre after intravenous injection of Flaxedil (0·7 mg/kg). 4–5: records taken immediately after record 3; 4: no stimulation; 5: stimulation of the fibre at 150/sec.

DISCUSSION

The best physiological evidence for the existence of skeleto-fusimotor fibres in rabbits has been obtained because, as in cats (Granit, Homma & Matthews, 1959; Bessou *et al.* 1965; Carli, Diete-Spiff & Pompeiano, 1967) and in rats (Kidd, 1964) the junctions of the extrafusal muscle fibres are more easily curarized than the intrafusal junctions. A level of curarization can be obtained during which repetitive stimulation of single ventral root axons elicits extrafusal muscle fibre contraction only at the onset of the stimulation period, whereas it activates spindle endings as long as the stimulation lasts (see Bessou *et al.* 1965). In those conditions, it is evident that the activation of spindle endings cannot result from mechanical actions exerted on them by extrafusal muscle fibres since it persists when the contraction of these fibres has been eliminated.

Records such as the one illustrated by Fig. 3 are easy to interpret because they show the effects of stimulating single motor axons. The transient muscle contraction demonstrates that single axons supply extrafusal muscle fibres while the increase in the rate of discharge of spindle endings observed after the contraction of extrafusal muscle fibres has been abolished, proves they also supply intrafusal muscle fibres.

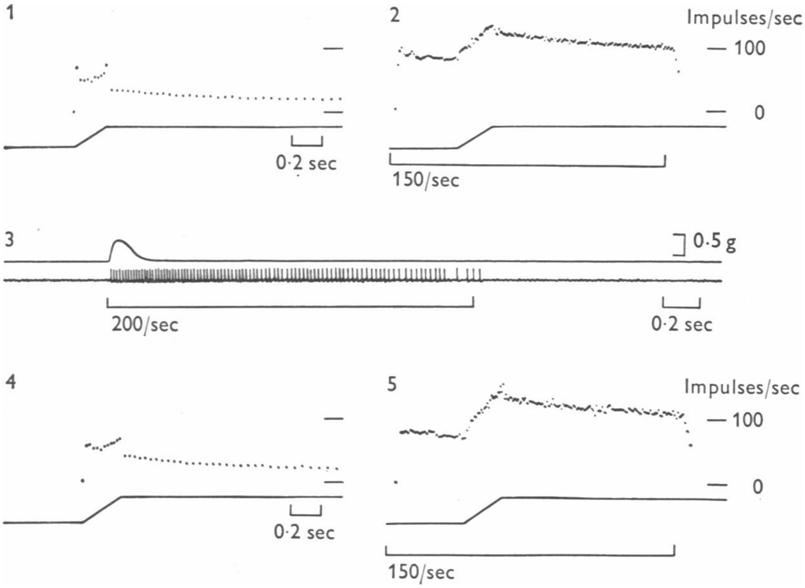

Fig. 6. Static skeleto-fusimotor fibre. Same spindle as in Fig. 5. 1–2: before curarization of the intrafusal neuromuscular junctions. 1: no stimulation; 2: stimulation of the motor fibre (conduction velocity: 37 m/sec) at 150/sec. 3: effects of repetitive stimulation of the fibre after intravenous injection of Flaxedil (0·7 mg/kg). 4–5: records taken immediately after record 3; 4: no stimulation; 5: stimulation of the fibre at 150/sec.

The spindle discharges which are due to direct action of extrafusal muscle fibre contraction on spindles disappear when the extrafusal junctions are blocked. Such discharges, which have also been observed in lumbrical cat's muscles (see Bessou *et al.* 1965), can often be recognized before curarization because their frequency does not augment when the rate of stimulation is increased beyond the tetanic fusion frequency.

Selective curarization of extrafusal junctions may disclose weak activation of spindle endings by β fibres (see Fig. 4) masked by the unloading action of contracting extrafusal muscle fibres.

The effects, static or dynamic, exerted by β fibres on the phasic responses of primary endings persist after curarization of the extrafusal junctions, which shows that these effects are the result of intrafusal muscle fibre activation.

We think that β fibres are more common in lumbrical muscles of rabbits than in cats because they were found in practically all experiments. A purely fusimotor supply to these spindles appears to be very limited or even lacking in some muscles as observed by Adal & Barker (1965). In our experiments the number of ventral root axons studied was too small to be certain that there were no purely fusimotor axons supplying this muscle, but it should be noted we have not observed γ axons and that two of the skeleto-fusimotor fibres had conduction velocities as low as 27–28 m/sec.

In mammals, physiological evidence for the existence of skeleto-fusimotor fibres has been obtained in cat deep lumbrical muscles (Bessou *et al.* 1965), in cat tibialis posterior muscle by Brown, Crowe & Matthews (1965) and in rat tail muscles by Kidd (1964, 1966). Whether or not it will be possible to demonstrate them in larger muscles is still uncertain because experimental conditions in such muscles are unfavourable.

In cats, all the β skeleto-fusimotor fibres so far analysed were shown to have a dynamic effect on the phasic response of primary endings (Bessou *et al.* 1965; Brown *et al.* 1965). In rabbits we found both dynamic and static β fibres, a situation which is comparable to that observed in frog spindles whose motor innervation is exclusively supplied by skeleto-fusimotor axons. Matthews & Westbury (1965) found that frog's slow axons have a dynamic effect and fast axons a static effect.

In previous work on rabbit fusimotor innervation (Emonet-Dénand, Laporte & Pagès, 1966; Emonet-Dénand & Laporte, 1969) it was assumed that all intrafusal muscle fibres were of the same type since Barker & Hunt (1964) had described typical rabbit spindles as composed of only nuclear bag fibres, although they reported the occurrence of spindles with both bag and chain fibres. The fact that rabbit spindles are supplied by both static and dynamic fusimotor axons was considered by us as one argument in favour of the hypothesis that functional differences between motor endings (plate endings and diffuse endings) were more important than differences between intrafusal muscle fibres to explain the static and dynamic effects. Recently, however, it has been shown by histochemical methods (Spiro & Beilin, 1969; N. James, personal communication; see also Ogata & Mori, 1962) and by electron microscope studies (Düring & Andres, 1969; D. Barker, C. R. Smith and M. Stacey, personal communication) that there is more than one type of intrafusal muscle fibres in the rabbit. It results from these observations that our argument founded on the presumed homogeneity of rabbit intrafusal muscle fibres cannot be maintained.

Before an interpretation of the action of static and dynamic β fibres can be proposed, it must be established whether all the motor endings of β axons on intrafusal muscle fibres of lumbricals have a similar morphology. If it were so, the existence of static and dynamic β fibres in the rabbit would constitute a good argument for attributing to different intrafusal muscle fibres the main role in determining the static and dynamic effects exerted by these motor fibres.

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Note added in proof

Since this paper went to press, H. Corvaja & O. Pompeiano have published an electron microscopic study of intrafusal muscle fibres in rabbit spindles (*Pflügers Arch. ges. Physiol.* 1970, **317**, 187–197). They describe two types of fibres which respectively resemble the nuclear bag and the nuclear chain fibres of cat spindles. The first type is made by myofibrils which have a thick Z line and lack a distinct M line. The second type is made by myofibrils which have a thin Z line and a prominent M line.

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