

**AN ENTRY FOR THE GUNNING MEMORIAL (VICTORIA JUBILEE) FUND PRIZE
IN PHYSIOLOGY (BELL PRIZE) FOR 1988**

by R.F. Price



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INTRODUCTION

In this dissertation, I am going to describe some of the research which I have carried out under the supervision of Dr. M. B. Dutia in the Department of Physiology since 1985 for the degree of Ph.D. My principal interest has been in the muscle stretch receptor known as the muscle spindle, the main aim of this work being the identification and characterisation of muscle spindle afferents from "b2c spindle units". In the first part of this thesis, I hope to provide a sufficiently detailed synopsis of more than a century of study of the muscle spindle to allow a reader unacquainted with this field to appreciate what is meant by "b2c spindle unit", and the importance of being able to tell that one is recording from one. However, the quantity of relevant literature is far too great for me to adopt a rigorous approach to its exposition here. Instead, I have opted to present a "potted history" of the most relevant material without providing detailed references. The interested reader might consider studying some recent reviews by Matthews (1972,1981), Boyd (1981) and Hulliger (1984) for a more complete picture of the field. The remainder of this dissertation contains a description of my experimental work which can conveniently be presented in two parts. In the first, I shall describe experiments performed on a neck extensor muscle, biventer cervicis, in which a form of "intermediate" spindle afferent behaviour attributed to afferents from "b2c spindle units" was first described. In the second, I shall describe a series of confirmatory experiments which were carried out in various of the hindlimb muscles.

Part I: HISTORICAL OVERVIEW

A. The "classical" view of spindle structure and innervation.

The muscle spindle was first described by the anatomist Kuhne in the 1860s in the course of studies on the microscopic structure of mammalian skeletal muscles. In addition to large numbers of skeletal muscle fibres (EXTRAFUSAL MUSCLE FIBRES) arranged in bundles separated by blood vessels and nerves, he saw occasional small structures about 150 um in diameter at their widest point, and tapering towards the ends; these he named muscle spindles after their shape. At their widest (mid)point, spindles consisted of a space containing what appeared to be a number of small muscle fibres, surrounded by a dense connective tissue sheath and receiving a number of nerve fibres. Suggestions as to what the spindles might be ranged from foci of formation of new bundles of extrafusal muscle fibres to foci of inflammation induced by muscle trauma. It was not until better histological techniques became available (particularly gold and silver based stains which show up nerve fibres especially well), that it was realised that the innervation of the muscle fibres in muscle spindles (the INTRAFUSAL MUSCLE FIBRES) was unusually rich and complex, leading to the suggestion that the muscle spindle might be a sensory structure providing the central nervous system with information about the state of the muscle in which it lay.

In contrast to the extrafusal muscle fibres which receive one axon each which contacts them at a single point along their length ("plate ending"), the intrafusal fibres are invariably innervated by more than one

axon, the exact number often being hard to determine due to an extremely complicated terminal branching pattern of the axons. Nevertheless, elegant studies by Ruffini and others revealed that three broad types of nerve ending can be distinguished in a muscle spindle. At its midpoint, each muscle spindle receives a single, large diameter (12-20 μm) myelinated axon which pierces the connective tissue capsule to enter the fluid-filled space surrounding the intrafusal muscle fibres. Here it branches several times, a branch running to each of the intrafusal fibres on the surface of which it forms a spiral ending. This ending is called the PRIMARY ending, and by extension the axon running to it is a PRIMARY axon. In addition, a variable number (0-5) of smaller myelinated axons (6-12 μm diameter) enter the spindle near the primary axon. These branch somewhat less profusely to end on some (but not necessarily all cf. the primary) of the intrafusal muscle fibres next to the primary ending. The terminals of these axons were originally described as "flower spray" endings, since a number of fine filaments were seen to run in several directions over the surface of the intrafusal fibres, but it is now known that the majority of these axons form spiral terminals similar to those of the primary ending though less well defined. Not surprisingly, perhaps, these endings are called SECONDARY endings and the axons which form them secondary axons. Finally, a variable number of very fine myelinated axons (2-8 μm) enter the spindle closer to the ends of the intrafusal fibres, where they terminate as "plate" endings similar to those on extrafusal muscle fibres. On anatomical grounds, Ruffini felt that all these axons were sensory, although the plate endings did have some features in common with the motor end-plates of extrafusal fibres. The "classical picture" of the spindle which emerged from this work is illustrated in Fig. 1 (a).

It was Sherrington in 1894 who first showed that the muscle spindle was indeed a sensory structure. It was known that sensory axons running towards the cord (AFFERENT axons) entered the dorsal roots to reach it, whereas motor axons leaving the cord (EFFERENT axons) did so through the ventral roots. Sherrington therefore exposed and cut the lumbosacral ventral roots and allowed the animal to recover. Several months later, he examined both the peripheral nerves and the muscles of the affected limb histologically. Many axons in the nerves were seen to have degenerated, as had the axons innervating the extrafusal muscle fibres, which had wasted considerably in the interim. The surprising result at the time was that the majority of the nerves to the intrafusal fibres of the muscle spindle were unaffected, proving beyond doubt that the muscle spindle was a sensory structure. Sherrington felt that any degeneration of nerves to spindles in his work was due to damage of the dorsal roots during the initial surgery, but subsequent experiments in the 1920s revealed that if all the dorsal roots were sectioned, some (motor) axons still survived in the spindle. In particular, dorsal root destruction resulted in the degeneration of primary and secondary axons, but not of the axons which formed plate endings. Alternatively, sectioning of the ventral roots resulted in the degeneration of the plate endings only; cutting both dorsal and ventral roots caused all the axons to degenerate. Thus, Ruffini's earlier conclusion that primary and secondary endings were sensory was confirmed, but the small axons forming plate endings were shown to be motor. Due to the small size of the intrafusal fibres and their scarcity relative to extrafusal fibres, it was felt to be rather unlikely that their activation via the motor axons could cause significant tension development in the muscle, and instead it was proposed that the fusimotor supply might modify the sensory information passed by the spindle to the central nervous system. Although Sherrington did show that afferent activity from muscle spindles had a profound influence on the behaviour of the parent muscle, being responsible, for example, for the

stretch reflex whereby a stretched muscle reflexly contracts in order to regain its original length, the exact nature of the information the spindles provided and how this was influenced by the fusimotor system could not be explored, due mainly to the primitive state of electronics at the time. It was not until the invention of the string galvanometer, and after it the cathode ray oscilloscope and high-gain, low-drain amplifiers that any further progress was made.

B. Early electrophysiological studies of spindle afferents.

The first electrophysiological studies of muscle spindle afferents were performed in 1933 by B.H.C. Matthews in the cat. His approach was to peel a fine strand off the nerve to the soleus muscle and to place it over a pair of fine wires connected to amplifiers. Although the finest strand that one can peel off in this way contains hundreds of axons, many of these will be motor axons and will not be active since they are no longer connected to the spinal cord. Of the remaining afferent axons, many will have been damaged or destroyed during splitting, so that, with luck, only one or two afferents will be active, allowing the experimenter to study the behaviour of a single afferent from the muscle. Matthews was able to identify three main types of afferent in the soleus muscle nerve. The first, which he called type A, usually discharged impulses even when the muscle was slack; the smallest of muscle stretches dramatically increased their firing rate, particularly during the period of stretching, and when the muscle length was held constant after a stretch, their firing rate decreased appreciably over the next few seconds. In response to contraction of the parent muscle, type A afferents fell silent, resuming their spontaneous discharge after the contraction. For a stretch of fixed size, type A afferents discharged at higher frequencies as the speed of stretching increased, showing them to be capable of detecting velocity of muscle stretch as well as absolute muscle length. Type B afferents were usually silent at short muscle lengths, but could be made to discharge nerve impulses by stretching the muscle taut or by making the muscle contract. The third type of afferent, type C, was quite rare and irrelevant to this thesis. Type A and B responses were exactly what was expected of muscle spindle afferents and Golgi Tendon Organs afferents respectively on the basis of the following theoretical considerations. Consider the schematic diagram of a muscle in Fig. 2; the muscle spindle can be seen to be mechanically "in parallel" with the extrafusal fibres, that is if the extrafusal fibres are stretched, so will the intrafusal fibres be, whereas if the extrafusal fibres contract and shorten, the intrafusal fibres of the muscle spindle will also shorten. Golgi Tendon Organs (or GTOs) are sensory endings which lie on the junction of the extrafusal fibres with the tendon of a muscle. They are mechanically "in series" with the extrafusal fibres of a muscle (Fig. 2) and so will be stretched by stretching the muscle or by contraction of the extrafusal fibres. The firing rate of a spindle afferent would therefore be expected to increase after muscle stretch but to fall during muscle contraction...type A..., whereas that of GTOs should increase under both circumstances...type B. This method of distinguishing GTO afferents from spindle afferents is still standard.

Subsequently, Matthews divided his type A afferents into two groups (designated A1 and A2) on the basis of their responses to muscle stretch. He suggested that these groups might correspond to primary and secondary axons, but his classification has not stood the test of time. The ability to state whether one has recorded from a primary or a secondary axon is clearly of tantamount importance to an understanding of spindle function,

since these afferents are known to innervate different parts of the spindle and so might be expected to behave differently. A more reliable indicator of axon type became available a few years later, namely the axonal conduction velocity. Erlanger and Gasser (1937) showed that axons of different sizes conducted nerve impulses at different velocities, so that if a nerve containing axons of a range of diameters was stimulated at one end, and electrical potential was recorded from the nerve at the other end, then the potential was seen to vary with time after the stimulus, forming a series of waves of potential change as impulses conducted in progressively slower axons reached the recording electrodes. Larger axons were shown to conduct action potentials more rapidly than smaller ones, and, by 1939, Hursh had produced conversion factors for the cat which allowed one to calculate the likely conduction velocity of an axon of known size (or vice versa). It was well known that primary axons were generally larger than secondaries, with, at least in hindlimb muscle nerves, relatively little overlap between their diameter ranges, and it was a simple matter to calculate the range of possible conduction velocities of primary and secondary afferents. Provided only that a sufficiently long conduction distance was available to allow accurate timing of delays and therefore measurement of conduction velocity, an experimenter ought to have been able to state reasonably accurately whether he had been recording from a primary or a secondary ending. It took a surprisingly long time for this technique to be applied; in 1951 Hunt & Kuffler made the conceptually simple step from recording from spindle afferents in muscle nerves to recording from them in dorsal roots, the latter technique not only being technically easier since there is less connective tissue to hamper splitting in dorsal roots than there is in peripheral nerves, but also offering a conduction distance of about 15 cm from the muscle nerve to the recording point over which to time the action potential. Even then, another 3 years passed before Hunt used conduction velocity measurements to distinguish primary and secondary spindle afferents, primaries conducting at more than 72 m/sec and secondaries at less than 72 m/sec. In practice, uncertainties about the diameters of axons in the dorsal roots compared to their diameters at the spindle, and about the exactitude of Hursh's conversion factor resulted in the adoption of the following criteria: primaries conduct at above 80 m/sec, secondaries at below 60 m/sec; afferents conducting at between 60 and 80 m/sec were unclassified and were often excluded from consideration in order avoid confusion.

C. Early electrophysiological work on the gamma fusimotor system

When the axons supplying the intrafusal plate endings described by Ruffini were eventually proved to be motor axons, it was tacitly assumed that they must be branches of the main motor axons to the extrafusal muscle fibres, as was the case in amphibia and reptiles. In the 1930s, however, the possibility that they might constitute a separate motor outflow from the spinal cord began to be considered. In particular, it was suggested that the many small diameter motor axons seen in ventral roots might terminate in the periphery as the motor axons to spindles. The first evidence in favour of this concept was provided by Leksell who developed a preparation in which only action potentials in the small motor fibres to a muscle were allowed to reach it. Recording afferent activity from the muscle nerve, he found that stimulation of the small fibres, which produced no muscle tension, resulted in a substantial increase in the amount of afferent discharge coming from the muscle. It was Leksell who coined the term GAMMA which is so frequently used in conjunction with the

term fusimotor fibre (see heading of this section) to describe the motor axons to a spindle. As described above, Erlanger & Gasser recorded a series of waves of change in electrical potential at one end of a nerve at various times after stimulating the other end. They introduced the practice of assigning letters of the Greek alphabet to these waves, and Leksell simply used the next available letter, gamma, to describe the slowly conducted wave recorded from active small motor fibres. Hunt & Kuffler, who introduced the technique of recording from afferents in dorsal roots also stimulated single gamma fusimotor axons in ventral roots and were able to perform a refined version of Leksell's experiment. Their basic conclusions were the same, namely that the gamma axons constituted a motor supply to the spindle which was, at least physically, independent of the main motor supply to the extrafusal fibres, and that the effect of activity in this motor supply was to increase the discharge frequency of spindle afferents. In addition, their technique revealed that a particular gamma axon increased the discharge of several spindle afferents, and that a particular afferent was influenced by several gamma axons, i.e. that there is considerable convergence and divergence in the motor innervation of muscle spindles.

(For the sake of completeness, I should perhaps state that it is now known that some of the motor axons to muscle spindles are indeed branches of the main motor axons to extrafusal fibres. This form of innervation is called beta-fusimotor innervation. Only about 50% of spindles receive beta-fusimotor axons, and in a given spindle, beta axons are usually outnumbered at least 2:1 by gamma axons.)

D. The "intermediate" picture of the spindle.

Although it had long been realised that the intrafusal fibres varied in size, it was not until the end of the 1950s that intrafusal muscle fibres were subdivided into two groups. The first, called NUCLEAR BAG fibres, had relatively large diameters (e.g. 20-25 μm in the soleus muscle), were relatively long, usually extending well beyond the capsule at either end of the spindle and characteristically had agglomerations (bags) of nuclei in their central regions underlying the primary sensory ending, a cross section at this point often showing the profiles of several nuclei. The NUCLEAR CHAIN fibres, on the other hand, were smaller (e.g. 8-18 μm in soleus) and shorter, rarely stretching beyond the spindle capsule. The nuclei in their central regions formed a single file (chain), so that only one nuclear profile was seen in a particular cross section of the primary sensory area. A muscle spindle typically possesses two bag fibres and four or five chain fibres. Subsequently, many other light- and electron-microscopic differences between bag and chain fibres have been described, and it is now possible to categorize an intrafusal fibre during a physiological experiment (see description of visualised spindle experiments below). Having discovered that two types of intrafusal fibre existed, it was only natural to enquire whether the sensory and motor axons reaching the spindle might not be distributed differently to them. Considering the sensory innervation first, it was soon found that Ruffini's original description of the primary ending as innervating all the intrafusal fibres in a spindle was correct; the description was merely brought up to date by stating that the primary ending innervated both nuclear bag fibres and nuclear chain fibres. It was equally quickly discovered that the secondary ending was preferentially distributed to the nuclear chain fibres, on which it formed annulospiral endings, with some additional "spray endings" on bag fibres. As for the gamma fusimotor axons, Ruffini's original finding was extended by the description of

"trail endings" in addition to the "plate endings" he had seen. Trail endings are diffuse networks of fine terinal filaments which run considerable distances along the intrafusal fibres and contact them at several points. Boyd felt that gamma plate endings were restricted to nuclear bag fibres, whilst trail endings were restricted to chain fibres. Whilst this hypothesis was controversial at the time and eventually had to be abandoned (see below) it became enshrined in what Matthews has called the New Anatomy of the Spindle which is illustrated in Fig. 1 (b).

At about the same time as the New Anatomy was elaborated, Cooper was performing the first experiments aimed at finding differences in behaviour of primary and secondary spindle afferents. Having identified primary and secondary afferents on the basis of conduction velocity, she stretched the muscle with a ramp-and-hold waveform (see Fig. 3) and observed the effect this had on the firing rate of the afferents. Her finding, which has been confirmed many times since in a variety of preparations and in several species, was that, whilst both types of afferent discharged tonically at rest and at the new length, only the primaries showed a dramatic burst of firing during the ramp stretch to frequencies substantially greater than those which were reached when the muscle was held at the longer length (Fig. 3). A measure of this behaviour was soon introduced by Matthews, namely the DYNAMIC INDEX, which is the difference in firing rate at the very end of the ramp stretch and that 0.5 seconds later. The dynamic index of an afferent is proportional to the amplitude of muscle stretch, and, for a given amplitude, to the velocity of stretch; under a particular set of conditions, the dynamic index of primary afferents is generally about 2-5 times that of secondaries. Another measure of afferent response to ramp stretch introduced at about this time was the LENGTH SENSITIVITY. As illustrated in Fig. 3, this is the increase in firing rate produced by a stretch divided by the amplitude of the stretch. The values of length sensitivity obtained for primaries and secondaries were very similar at around 4 impulses/second/mm stretch. Thus it became clear that the spindle is capable of providing the CNS with two separate and slightly different signals of muscle status, one being proportional to both muscle length and rate of change of length (primaries), the other just to length (secondaries). Since then, muscles have been stretched in other ways (e.g. sinusoidally, with triangular waveforms, with vibration), all of which show that the primary afferents are more sensitive to the dynamic components of stretch than are the secondaries.

The New Anatomy of the Spindle described above led to the expectation that two types of gamma fusimotor axon might exist, and that they might differ in their effects on primary and secondary afferents if their distribution to the two types of intrafusal fibre was indeed segregated. It was Matthews who first confirmed this expectation by showing that stimulation of one class of gamma axon resulted in a large increase in the dynamic index of primary afferents in response to a ramp stretch with only a small increase in their firing rate at the resting length, whereas stimulation of the other class of gamma resulted in an increase in their firing rate at rest, but actually reduced the response to stretch (Fig. 4). These two classes of gamma axon were called DYNAMIC GAMMAS and STATIC GAMMAS respectively. A particular gamma axon proved true to type in several spindles, that is if it was classified as a dynamic gamma on the basis of its effects on one primary afferent, it would also be classified as a dynamic gamma if any of the other primary afferents it influenced were found. Dynamic gammas were rarely seen to affect the discharge of secondary afferents, whereas static gammas caused substantial increases in their firing rate and it was therefore concluded that static gammas must innervate intrafusal fibres common to both primary and secondary

afferents...i.e. chain fibres (Fig. 1 (b)), and that dynamic gammas must innervate only bag fibres. Their varied effects on primary and secondary afferents could then be attributed to different functional characteristics of bag and chain fibres.

E. The current view of spindle structure and innervation.

By the mid 1960s, a satisfying level of understanding of spindle structure and function seemed to have been achieved. This illusion was short-lived, however, mainly due to the anatomical work of Barker and his collaborators, who found that gamma trail endings were not restricted to chain fibres as Boyd had suggested. The gamma plate endings, on the other hand, did appear to be restricted to the bag fibres. Barker's view of spindle morphology at that time is illustrated in Fig. 1 (c)). One implication of his results was that the mechanisms of static and dynamic fusimotor action might be quite unrelated to the morphological differences between bag and chain intrafusal fibres. In support of this possibility was the observation that all the intrafusal fibres in rabbit spindles looked like cat bag fibres, yet quite typical dynamic and static fusimotor action on spindle afferents could be observed. The field was thrown into a temporary state of confusion until enzyme histochemists subdivided the nuclear bag fibres into bag1 and bag2 types on the basis of their myosin ATPase profiles. The hypothesis that dynamic and static gammas innervated different intrafusal fibres and thereby produced different effects on spindle afferents could now be reinstated by proposing that dynamic gammas innervated one of the bag fibres, whilst static gammas innervated the other type of bag fibre and the chain fibres. Evidence that this was the case accumulated over a number of years, not without some heated debates on the way, finally resulting in the current picture of spindle structure and innervation. As illustrated in Fig. 1 (e), it is now believed that the bag1 fibre is exclusively innervated by dynamic gammas (which form plate endings on it), leading to its alternative name of dynamic nuclear bag fibre (or DNB), and that the dynamic gammas almost never innervate the other intrafusal fibres. The bag2 fibre (static nuclear bag fibre or SNB) and chain fibres receive trail endings from static gammas. As for the sensory innervation, the main concepts from earlier descriptions still apply, namely that primary afferents form sensory terminals on all the intrafusal fibres, whereas secondaries mainly end on chain fibres. It has been realised that the incidence of secondary endings with collateral terminals on one or other of the bag fibres is rather higher than had been thought, with, depending on the muscle under investigation, up to 80% of secondaries having bag2 terminals, and up to 35% bag1 terminals. The significance of these collateral terminals is uncertain, however, since the area of contact with the bag fibres is usually less than 10% of that with chain fibres.

Recently, Boyd has suggested that two types of static gamma can be recognised physiologically (see below for a description of the technique used in his experiments); the first innervates the bag2 fibre in every spindle to which it runs. In addition, it may, in some spindles, innervate some of the chain fibres. Such axons are called "bag2 static gammas". "Chain static gammas" innervate several of the chain fibres in every spindle to which they run; in addition, they may occasionally innervate the bag2 fibre. Other physiological and anatomical evidence supports this division, but the concept is too new to have been exhaustively tested.

F. Properties of the intrafusal fibres.

In this section, I shall summarise the results of Boyd et.al., Barker et.al. and Emonet-Denand et.al. which have accrued since 1976. One of the techniques with which they were obtained merits description, if only to illustrate the difficulty of this kind of work, and the persistence and dexterity required for it. The initial steps in the technique are conventional, consisting of isolating single afferents from the muscle under study in filaments of the dorsal roots, as well as single gammas in filaments of the ventral roots. Next, an afferent axon is recorded from while the gammas are stimulated in turn in order to determine whether any of them innervate its spindle. This step is repeated until all the afferents have been screened; one which was influenced by several of the gammas is selected, and the muscle is manipulated in order to localise the parent spindle. The experimenter then carefully peels away strips of the extrafusal muscle fibres under microscopic viewing, until he can see the spindle itself. The central portion of the spindle which carries the sensory terminals is exposed, along with as much of the more peripheral parts of the spindle as is possible without damaging its nerve and blood supplies. The result of all this effort is that the experimenter can see the intrafusal fibres and sensory endings of a living spindle at rest and, with appropriate precautions, during muscle stretching and/or during stimulation of gamma motor axons whilst simultaneously recording from one of its afferents in the dorsal roots. It was with this elegant and powerful technique, for example, that it was shown that only the bag1 fibre contracts when dynamic gamma axons are stimulated and that static gamma axons may be divisible into two classes. Further refinements include introducing microelectrodes into visualised intrafusal fibres in order to study their electrical properties.

(1) The bag1 fibre

If a ramp stretch is applied to a muscle spindle that has been visualised as described above, a property specific to the bag1 fibre comes to light, namely that of "intrafusal creep". During a ramp muscle stretch, the sensory spirals on the bag1 fibre are pulled apart by an amount which depends on the amplitude of stretch. The striking observation is that, when the muscle is now held at the longer length, the sensory spirals on the bag1 fibre move back together over the next few seconds. Correspondingly, the firing rate of a primary afferent increases during a stretch, but decreases substantially in the first few seconds after the end of the stretch phase. If a dynamic gamma axon is stimulated with the muscle at the resting length, there is only minor shortening of the ends of the bag1 fibre, producing a small opening of the sensory spirals and hence a small increase in the basal firing rate. If, however, the dynamic gamma is stimulated during a ramp stretch, the initial opening of the sensory spirals in response to the same stretch as before is greater, as is the degree of intrafusal creep after the stretch and the dynamic index. Thus it is the mechanical property of intrafusal creep in the bag1 fibre which underlies the dynamic index of primary afferents, rather than some form of adaptation in the electrical properties of the primary axon itself. It appears that, during a stretch of the bag1 fibre, its ends are substantially stiffer than the central parts, so that more of the stretching is taken up by the central regions, thus opening up the sensory spirals. When the muscle is held stretched, the stiffness of the ends decreases with time, and some of the energy stored in the stretched central areas is transferred to the ends, stretching them and shortening the middle, thus allowing the sensory spirals to move together. Presumably, these stiffness changes are enhanced by stimulation of dynamic fusimotor axons; the exact intrafusal mechanisms giving rise to these

stiffness changes are currently being investigated.

Contraction of the bag1 fibre generally has little effect on secondary afferents since these innervate mainly the chain fibres, but occasionally it can produce a clear increase in dynamic index, though of much lesser degree than that seen for primaries.

(2) The bag2 fibre.

The bag2 fibre does not show intrafusal creep. When it is stretched, the sensory spirals on it are opened up, but then stay open for as long as the muscle is held stretched. In response to stimulation of a static gamma which innervates the bag2, there is a substantial shortening of the ends of the bag2 fibre, with concomitant separation of the sensory spirals. Again, this persists for as long as the static gamma is active, leading to a maintained biasing (increase) of the discharge rate of afferents with terminals on this fibre. The degree of biasing is almost directly proportional to the degree of opening of the sensory spirals. This biasing is accompanied by a reduction in dynamic index of primary axons (since the large afferent signal consequent upon the bag2 contraction is more important than that of the unstimulated bag1 fibre in determining the final afferent rate). The effects of bag2 fibre contraction on afferent length sensitivity are variable; often there is little or no effect, the rest of the time, a decrease.

The effects of bag2 fibre contraction on secondary afferents are variable; secondaries which do not innervate the bag2 fibre are obviously unaffected by bag2 fibre contraction. Those with bag2 terminals respond to contraction of the bag2 fibre in much the same way as primaries (i.e. biasing +/- decrease in length sensitivity) although less dramatically since the terminal contact area of secondaries with the bag2 fibre is usually less than 10% of their total intrafusal contact area.

(3) The chain fibres.

The chain fibres also show no signs of intrafusal creep. Instead, their characteristic mechanical property is a high (relative to the bag fibres) tetanic fusion frequency. The tetanic fusion frequency is that frequency of motor axon stimulation at which the individual twitches of a muscle fibre cease to be distinguishable, the mechanical response consisting instead of a smooth, continuous contraction. For chain fibres, the tetanic fusion frequency is around 75-100 impulses/second which means that under normal physiological conditions, static gamma activity is likely to produce oscillatory contractions of the chain fibres. Under these conditions, the central region of the chain fibres is subjected to repeated small stretches, which briefly open up the sensory spirals of the primary axon. At sufficiently high frequencies of stimulation of a static gamma axon which innervates the chain fibres, the discharge rate of the primary afferent is found to be the same as the rate of stimulation of the static gamma since each impulse in the gamma axon produces a twitch of the chain fibres and this results in a single impulse passing up the afferent axon. The discharge of the primary axon is said to be "driven" by the gamma axon. When a primary is being driven in this way, it becomes rather insensitive to muscle stretch; small stretches may not change the firing rate at all, and larger stretches often only increase the firing rate when the muscle is lengthening, especially if several static gammas are active at once. Thus, unfused contractions of the chain fibres appear to "clamp" the discharge rate of primaries at some value, at the expense of their

ability to signal changes of length to the CNS. Although this seems a strange thing to do, it may be important in maintaining a degree of reflex excitation of the homonymous alpha motoneurons, particularly during muscle shortening when primaries fall completely silent in the absence of activity in the static gammas.

The effects of chain fibre contraction on secondary afferents are quite different, consisting of a large biasing of their discharge rate and a substantial increase in their length sensitivity. Again, these effects are particularly marked if several static gammas are active at once; the presence/absence of concomitant bag2 fibre contraction appears to be irrelevant. When chain fibres are active, therefore, the secondary endings signal muscle length particularly well, in marked contrast to the primaries. The different effects of chain fibre contraction on primary and secondary afferents are probably related to the different positions of their sensory terminals on the chain fibres, those of the secondaries overlying areas of the chain fibres which are contractile whereas those of the primary overlie an area rich in nuclei and poor in contractile proteins.

The chain fibres are felt to function as a single entity, since the stimulation of a single static gamma almost always results in the contraction of several chain fibres together, and since there is a high incidence of "sensory cross-terminals" between the chain fibres, that is that unlike the spiral endings around the bag fibres, those around individual chain fibres are connected together by common axons and so are in electrical continuity. It is clear that, whilst the resting properties of the bag2 fibre and chain fibres are similar, their properties when they have been activated by static gammas are quite different. For a given stretch of the muscle, spindle afferent discharge will vary not only with the degree of activity in the static gamma axons, but also with the type of static gamma that is active, i.e. bag2 static gamma versus chain static gammas provided the two types can be activated independently by the CNS. Such considerations may help to explain why nature has provided two peripheral targets for static gammas whereas there is only one for dynamic gammas.

G. The "b2c encapsulation".

Up to now, I have refrained from discussing the variations in spindle morphology which have been described almost from the start. Of these, by far the commonest is the "tandem spindle". As illustrated in Fig. 5, tandem spindles consist of two or more capsules connected in series with each other by one of the bag fibres. The shared bag fibre has been shown histochemically to be the bag2 fibre. Each spindle encapsulation therefore contains at least a bag2 fibre; the number of additional intrafusal fibres varies between the encapsulations. At least one of them has a bag1 fibre and several chain fibres; typically, it receives one primary axon, one or more secondaries and several gamma axons and appears to be identical to typical single encapsulations. Such encapsulations are called b1b2c encapsulations after their intrafusal fibre complement. The other type of encapsulation contains the shared bag2 fibre and a few chain fibres, but no bag1 fibre and is therefore called the b2c encapsulation. It also receives a primary axon in the usual position on the intrafusal fibres; there is usually no secondary ending, although occasionally there may be one. Only one or two gamma motor axons innervate it, and their endings are exclusively of the trail type which is compatible with their being static gammas. Tandem spindle almost always contain at least one b2c encapsulation, the commonest form being {1 x b1b2c + 1 x b2c}.

Although b2c encapsulations are much less common than the b1b2c type, their incidence is not negligible. In most cat hindlimb muscles, the primary afferents from b2c encapsulations usually make up less than 10% of the total; however, in some muscles, especially in the neck, this may rise to 25% or more. The "b2c afferent", as I shall call the primary afferent from b2c encapsulations from now on, is particularly interesting for a number of reasons. Firstly, since it has no bag1 fibre, an examination of its properties could provide corroborative evidence as to the importance of the bag1 fibre in determining the dynamic sensitivity of primary endings. b2c afferents might be expected to have smaller dynamic indices than primaries from b1b2c encapsulations (I shall call these b1b2c primaries) and more like those of secondaries, but to have other features more in common with b1b2c primaries than secondaries by virtue of the position of their terminals on the intrafusal fibres. Secondly, b2c afferents provide another experimental model in which to investigate the interaction of bag2 and chain fibres in determining the discharge of primary afferents when static gammas are activated. Thirdly, because b2c afferents have no bag1 fibre, they will not be influenced by dynamic gamma activity; thus if one were to record from b2c afferents during voluntary movements, one ought to be able to deduce the degree of static gamma activity during the movement from changes in the behaviour of the afferent without the potential confusion of a concomitantly varying dynamic input from a bag1 fibre. A final point of interest is the matter of the central connectivity of b2c afferents. Are they "wired up" in the spinal cord in the same way as typical primary endings? The answer to this question may shed new light on the uses to which the central nervous system puts incoming information about the state of the muscle.

Given that it might be interesting to study b2c afferents, why has this not yet been done? The answer to this is that nobody has yet been able to tell when they were recording from a b2c afferent. Whilst primary and secondary afferents can generally be differentiated on the basis of their conduction velocity (see above), there is no absolute dividing line. Even if one could neglect the uncertainties involved in measuring the conduction distance during an experiment, and in the conversion factor between axon size and conduction velocity, it is clear from anatomical studies that the ranges of diameters measured at the spindle for primary and secondary axons overlap to a degree which depends upon the muscle of origin. The diameters of b2c afferents lie at the lower end of the primary range and overlap the upper end of the secondary range. Consequently, an axon with a conduction velocity in the 60-80 m/sec range (diameter 10-13 μ m) is equally likely to be a b1b2c primary, a secondary or a b2c afferent. Similar considerations of overlapping ranges apply to all the other commonly used differentiating tests (e.g. dynamic index, sensitivity to vibration etc.). For this reason, two attempts to study b2c afferents in the past decade have failed. A different approach might be to do experiments on visualised spindles (see above), in which one would isolate spindles at random until one chanced upon a b2c encapsulation which would then be examined in detail; this would have to be done a number of times to build up an adequate sample of b2c afferents. At a recent meeting in Glasgow, the late professor Boyd, who was one of the originators of the visualised spindle preparation, reported the effects of static gamma stimulation on the response to stretch of a primary afferent (identified by its conduction velocity) whose spindle of origin was later found to be a b2c encapsulation. Although he was not actually looking for b2c afferents, he had been doing such experiments for at least a decade, and would surely have reported the behaviour of other b2c afferents had he found any. Whilst such an approach is feasible, it suffers from the

serious disadvantages of daunting technique and excessive expense in terms of time and animals. On the other hand, it has the distinct advantage that one could be certain of recording from a b2c afferent.

There is, however, no need to resort to such a difficult approach. A simpler solution to the problem would be to record from spindle afferents in the standard manner and then to induce the bag fibres to contract. A primary afferent from a b1b2c encapsulation should show a marked rise in dynamic index due to bag1 fibre contraction; a b2c afferent would not show this. Instead, as the reader could deduce by examining section F. above, such afferents would be expected to show a large increase in firing rate, possibly with a reduction in length sensitivity. Secondary endings would be expected to be either unaffected by bag fibre contraction, or to show small changes in dynamic index and/or length sensitivity if they have substantial bag fibre contacts. It only remains to decide how to activate the bag fibres selectively; obviously, the bag1 fibre could be activated by dynamic gamma stimulation. The bag2 fibre is more of a problem, since the static gammas which make it contract often also innervate some of the chain fibres. Luckily, however, nature comes to our aid. It was discovered in 1976 that gamma motor axons use the same neuromuscular transmitter as is used by the alpha motor axons to the extrafusal fibres, namely acetylcholine (ACh), since dripping this chemical onto a visualised spindle caused the intrafusal fibres to contract. More useful than ACh, though, is a synthetic analogue called succinyl choline (SCh); this drug, which is used as a neuromuscular blocking agent clinically, binds to the receptors for ACh and activates them, causing the extrafusal muscle fibres to be depolarised. Initially, this results in twitching of the muscle, but SCh is not catabolised at the end plate and so produces a maintained depolarisation of the muscle fibres which paralyses them. The interesting point is that SCh affects the intrafusal fibres differently. The chain fibres behave very like extrafusal fibres, i.e. they twitch initially but rapidly become paralysed and slack. The bag fibres must be exposed to a somewhat higher dose of the drug before they contract, but unlike the chain fibres, they are not subsequently paralysed; instead, their contraction is strong and sustained as long as the level of SCh remains above threshold. SCh therefore presents us with a way of selectively activating the bag fibres which are present in a spindle. This is exactly what we require, and should in principle enable the identification of b2c afferents. SCh has been used in this way before to characterise the responses of hindlimb primary and secondary afferents, but at that time b2c afferents were not the subject of much curiosity and no attempt was made to identify such afferents. It was the principle aim of this work to reapply this technique, using it specifically to search for b2c afferents.

Part II. EXPERIMENTS ON A NECK EXTENSOR MUSCLE OF THE CAT

I. EXPERIMENTAL RATIONALE

As mentioned above, the incidence of b2c encapsulations varies between muscles, and is particularly high in the neck extensor muscles. In addition, the neck muscles contain very large numbers of muscle spindles, and on both counts it seemed sensible to attempt to identify b2c afferents in these muscles first. We chose to use the powerful neck extensor muscle biventer cervicis since it is well-defined, easy to expose and easy to stretch repeatably. In a previous study of afferents from the biventer cervicis muscle, Richmond & Abrahams performed a cervical laminectomy to

expose the C2-C4 dorsal roots which they then split repeatedly until filaments containing single afferents from biventer cervicis were obtained. When investigating muscle spindle afferent properties, it is essential that the motor supply to the spindles be controlled; generally, this is most readily achieved by dividing the appropriate spinal ventral roots and reducing the efferent activity to zero, but in the cervical region, the roots are so short and the cord itself so large relative to the spinal canal that it is not always possible to see the ventral roots clearly enough to ensure that they are all cut (and that none of the blood vessels which run with them is cut) and physical de-efferentation is therefore not feasible. Richmond & Abrahams circumvented this problem by infusing Gallamine intravenously in order to postsynaptically block cholinergic neuromuscular transmission both at the extrafusal endplates and at the intrafusal terminals of fusimotor axons, effectively chemically de-efferenting the muscle spindles. Unfortunately, a similar approach could not be used in the experiments reported here, since the response to SCh was to be a critical test used to differentiate between b1b2c primary and b2c afferents and Gallamine would interfere with this test. We therefore decided to revert to the technique used by B.H.C. Matthews in the 1930s (see above), namely that of cutting the muscle nerve itself and splitting the peripheral stump until filaments containing single afferents were obtained. This is substantially more difficult than splitting dorsal roots, due to the far larger connective tissue content of peripheral nerve compared to dorsal roots, but it does at least ensure total spindle de-efferentation and permits the intrafusal fibres to be activated by SCh infusion.

The biventer muscle has an unusual compartmentalised structure, as do several of the dorsal neck extensor muscles. Four or five blocks of extrafusal muscle fibres are linked in series with each other, separated by transverse, full-thickness, tendinous inscriptions at approximately equal intervals (about 1.5 cm). Each of the "compartments" thus delimited contains large numbers of muscle stretch receptors and receives a separate muscle nerve from one of the branches of the C2-C4 dorsal rami. The compartmental nerves may be expected to carry afferents from the corresponding muscle compartment, although initially the possibility of afferent axons crossing a tendinous inscription to run in an adjacent compartmental nerve was not ruled out. Similarly, fusimotor innervation of biventer spindles was expected to reach them through the corresponding compartmental nerve, with the possibility of axons arriving from other nerves not being neglected. In view of the requirement for complete de-efferentation of spindles in these experiments, the approach was to cut all the compartmental nerves.

Finally, it is known that intra-arterial infusions of SCh are more likely to fully activate hindlimb muscle spindles than intravenous ones, so we decided to use intra-arterial infusions to activate spindles from biventer in these experiments. The blood supply of biventer cervicis in the cat is mainly via small blood vessels entering it with its muscle nerves; it is impossible to cannulate these vessels for close-arterial drug infusion and even if it were, doing so would impede subsequent splitting of the muscle nerves. Instead, infusions were made into the main trunk supply, which in the cat is an anastomosis between the occipital artery and the deep cervical artery. The former arises from the the ipsilateral common carotid artery (along with other muscular branches), the latter from a branch of the ipsilateral subclavian artery; if the right biventer muscle is used, as was done here, both these main vessels are accessible to infusion via a cannula placed in the left common carotid artery.

II. SURGICAL PROCEDURES

In the experiments described in this part of the thesis, a total of 21 cats was used over a period of ten months. Each cat was starved for 24 hours prior to surgery. Anaesthesia was induced in all cats by inhalation of an O₂/N₂O/Halothane mixture, initially by placing the animal in a box and allowing the gaseous mixture to enter, and subsequently via a face mask during the early surgery. The last part of the surgery, and the subsequent experimental manipulations were then carried out under pentobarbitone anaesthesia (SAGATAL 45 mg/kg body weight, supplemented as required and administered via a femoral venous cannula [see below]).

The anaesthetised animal was placed on its back on an electrically heated blanket, the ventral surface of the neck was shaved and a midline incision was made through the skin from the larynx to the sternum. The underlying muscles were cleared from the skin by blunt dissection and separated down the midline to expose the trachea into which was inserted an appropriately sized plastic T-piece. At this point, the anaesthetic mixture was connected directly to one side-arm of the T-piece and its flow rate and percentage Halothane content were suitably adjusted to maintain deep anaesthesia. In the same incision, the left common carotid artery was located along with the Vagus nerve and internal jugular vein, separated from them and cleared of connective tissue. Two loops of suture were passed around it, the more cranial of which was tied tightly whilst a loose loop was made in the more caudal. The vessel was clamped at its point of exit from the thorax, and a small hole was made in its wall between the threads. A nylon cannula of an appropriate size and filled with heparinised saline (5,000 units/l) was inserted into the opened artery, the loose loop was tightened around the cannula so as to hold it firmly but still allow it to be advanced as required, the clamp was removed and the cannula was pushed 2-3 cm further into the vessel before the caudal ligature was made fast. The cranial ligature was also tied around the cannula for extra security, and the ventral neck incision was sewn shut.

Next, an incision over the left groin was made to expose the femoral blood vessels and nerve. A nylon cannula was placed in the femoral vein and was subsequently used to administer the pentobarbitone used to maintain anaesthesia during the experiment. The skin incision was sewn shut and the animal was turned to lie on its belly. The Halothane anaesthesia was withdrawn, and the animal was gradually weaned onto barbiturate anaesthesia.

The animal was placed in a head holder consisting of a bite bar placed in its mouth and an adjustable clamp which was screwed down onto the frontal bones. The head was held so as to straighten out the neck and separate the scapulae in the midline, and a midline skin incision was made from the top of the skull to a point level with the angles of the scapulae and opened out laterally by blunt dissection. Throughout the procedures to be described below, the exposed muscles were kept moist with warm physiological saline. This incision exposed muscles of the ear and the trapezius muscles which insert onto the lambdoidal ridge of the occipital bone at the back of the skull. A hole was made in the ear muscles towards their anterior edge allowing access to the tissue plane between them and the temporalis muscle deep to them. Blunt dissection caudally in this plane cleared the posterior edge of these muscles from trapezius and the lambdoidal ridge and allowed them to be removed. A small hole was then made

in one of the trapezius muscles near the midpoint of the lambdoidal ridge, allowing access to the plane between it and the splenius muscle deep to it. Trapezius was cleared from splenius by blunt dissection, cut away from the lambdoidal ridge and separated from the contralateral muscle in the midline as far caudally as the incision allowed. It was then reflected laterally, and the trapezius muscle of the opposite side of the neck was prepared in the same way. An identical process to that described for trapezius was then used to reflect the splenii, thus exposing the biventer cervicis muscles which are easily recognised by the dense connective tissue bands which run diagonally across them (see e.g.). The two biventer muscles were carefully separated from each other in the midline, and from the neighbouring complexus and obliquus capitis muscles, and were removed from the lambdoidal ridge, either by cutting them away with scissors, or by using a pair of bone rongeurs to remove a slither of bone with the muscle attachment. They were separated from each other down the midline as carefully, and over as long a length, as possible, and a length of stout suture was stitched to the cut tendon of the right hand biventer muscle for subsequent attachment to an electromechanical muscle stretcher.

A wire frame was sewn to the margins of the skin incision to fashion a pool, after which a small incision was made over the fourth or fifth thoracic vertebra, exposing its spinous process which was cleared of tissue to permit the attachment of a vertebral clamp. This served to stabilise the lower cervical and upper thoracic (C7 - T2) vertebrae from which slips of the biventer muscle take origin. The animal was then transferred to a brass frame to which the head holder, skin pool and vertebral clamp were all attached at suitable heights and inclinations to keep the neck straight and approximately horizontal whilst allowing access for subsequent procedures. The pool was then filled with paraffin oil which was maintained at 32-35 degrees centigrade by radiant heat from a lamp.

Under an operating microscope, the right biventer muscle was reflected laterally to expose its ventral aspect on which the C2-C4 compartmental nerves could be seen. These were cleared of connective tissue and followed towards the midline before being sectioned as close as possible to their emergence through the paravertebral muscles. Taking care not to damage either, the C2 and C3 nerves were then cleared from the network of blood vessels running to the biventer muscle with them and from the overlying complexus muscle; nerve branches to complexus, which also run in these dorsal rami, were cut wherever possible, but sectioning of all branches was often difficult to achieve for the C3 nerve due to the criss-crossing of blood vessels in this area. Finally, the biventer muscle was folded back to the midline and the prepared segmental nerves were drawn from under its medial edge and laid on the paravertebral muscles. In this way, it was usually possible to produce a 1-2 cm length of the biventer nerves for splitting.

III. ELECTROPHYSIOLOGICAL METHODS

(a) Apparatus

The apparatus used in this series of experiments is illustrated in the block diagram of Fig. 6. Afferent action potentials were recorded from filaments of the biventer muscle nerves with bipolar silver wire hook electrodes connected via a high-input-impedance probe head to an ac preamplifier. Signals were usually amplified 1,000 times, bandpass filtered (500 - 5,000 Hz) and then ac amplified a further 20 times. The

amplified action potentials were displayed on one beam of a four beam storage oscilloscope. They were also led to a spike trigger module which produced a pulse at its output for each input action potential when gating thresholds were appropriately set. These pulses were passed to an audio amplifier so that the unit could be listened to during an experiment, to a Cromemco Systems Three microcomputer for on-line analysis of the resting discharge (see below) and to an instantaneous ratemeter. The ratemeter output, a voltage proportional to the reciprocal of the interval between a particular action potential and the one preceeding it, was displayed on another channel of the Tektronix oscilloscope and was used both to assess the reliability of the triggering and to estimate the response of a unit to muscle stretching during the experiment.

The arrangements made for muscle stretching are also illustrated in Fig. 6. A Ling Dynamic Systems V406 electromechanical puller and an associated power amplifier were used. The puller had been slightly modified by the attachment to its armature of a rod aligned with the axis of armature movement. The biventer muscle was fixed by a stout thread to a side arm mounted on this rod at right angles to it. The rod, which was made of two lengths of perspex with a 1 cm long iron lug sandwiched between them, ran through the cylindrical coils of an LVDT length transducer which was attached to the body of the puller. Movements of the iron lug, reflecting movements of the rod and side arm and therefore changes in muscle length, produced changes in the magnitude of an output dc voltage from the LVDT. This length signal was amplified and fed to a third channel on the Tektronix oscilloscope. The muscle puller was driven by setting the gain on its power amplifier to a fixed value and changing the frequency and/or amplitude of an externally derived command signal. Sinusoidal commands were provided by a signal generator whilst ramp-and-hold commands were generated by the Cromemco microcomputer via an 8-bit digital-to-analogue converter. The two command signals, together with synchronising trigger pulses at the start of each cycle of stretching, were routed through a switching and attenuation circuit to permit selection of one or other command and also to allow variation of the command amplitude passed on to the power amplifier. The whole arrangement was calibrated periodically by observing armature movement microscopically and monitoring LVDT length signal on the oscilloscope during sinusoidal driving at 1 Hz. During an experiment, a desired amplitude of muscle stretch was achieved by setting the attenuation to a level which produced the appropriate LVDT signal. A shortcoming of this stretching system was that it was not part of a closed-loop servo system. As a result, its dynamic performance suffered so that ramp-and-hold stretches were often slightly curvilinear; in addition, as the frequency of a sinusoidal command signal was increased, the amplitude of muscle stretch became progressively smaller for a given attenuation setting. Both problems were inescapable with the apparatus available, but were at least constant throughout the series of experiments.

The amplified action potentials, muscle length signal and synchronising trigger pulses were recorded along with voice commentary on a four-channel FM tape recorder for subsequent analysis.

(Placement of stimulating electrodes

As described above, it is routine when studying muscle stretch receptors to differentiate muscle spindles from Golgi tendon organs on the basis of their response to contraction of the parent muscle. Initially, therefore, attempts were made to study biventer afferent responses to muscle twitch.

It soon became apparent, however, that afferent responses to muscle twitch could not be used to distinguish between spindles and tendon organs in biventer (see Results), and no arrangements for muscle stimulation were made thereafter. In most of the experiments in which biventer contractions were evoked, bipolar silver ball electrodes were sutured to the dorsal surface of each of the four most cranial muscle compartments to allow them to be activated directly. The electrodes were fashioned from 100 μ m diameter insulated silver wire, two strands of which were braided together to within 2 cm of their ends. The insulation was burnt off a short length of the free ends of the wires which were then allowed to melt into small (approx. 1 mm diameter) balls. Appropriate connectors were soldered to one end of the wires for connection to isolated stimulators which delivered stimulating pulses of 1 ms duration at a voltage about five times the threshold required to produce discernible twitching. One silver ball of each pair was stitched to the medial edge of biventer near the front of a compartment; the other was stitched to the lateral edge near the back of the compartment. It was hoped that this diagonal arrangement would activate the majority of the extrafusal fibres in that compartment with minimal activation of fibres in the adjacent compartments. In two experiments, an alternative arrangement was used. Bipolar silver hook electrodes were used to stimulate segmental nerves other than the one from which afferents were being isolated, thereby ensuring activation of all the extrafusal fibres innervated by them. Stimulating pulses were 0.1 ms in duration, and the voltage was adjusted to be twice the voltage which just produced discernible twitching in the compartment. The remaining compartment was activated with silver ball electrodes as described above.

A second standard technique is to measure the conduction velocity of the afferents. As described above, this is easy in the hindlimb since there is a considerable distance between the muscle nerve and a dorsal root filament over which to time conduction. In the present experiments, the total length of nerve available was under 2 cm, and it was not feasible to measure conduction velocity.)

(b) Preparations for recording

The thread attached to the biventer tendon was tied to the puller in such a way as to stretch the muscle to approximately its in vivo length of 7 cm. In order for a stretch of biventer to clear the skull, the puller had to be mounted above and to the right of the animal's head; with this arrangement, the long axis of biventer was about 15 degrees above and 20 degrees to the right of its in vivo position. Next, a small perspex platform was introduced into the pool and one of the prepared biventer nerves was placed on it. A pair of fibre optic light guides was used to illuminate the nerve and, under high magnification, any remaining fat and connective tissue were cleaned away using fine instruments. The cut end of the muscle nerve was then held in one pair of watchmaker's forceps and the epineurium was peeled off it by alternately cutting it with fine scissors and peeling it back with a second pair of watchmaker's forceps. Great care had to be taken not to produce a constricting ring of connective tissue further along the nerve as this would have resulted in pressure block of

axons passing through it. It was usually possible to desheath about 0.5 - 1 cm of the nerve in this way, the main limitation being the disappearance of the nerve under the medial edge of the biventer muscle. The desheathed length of nerve was then repeatedly subdivided and placed on the recording electrodes, until a filament containing a single tonically active unit with approximately constant spike amplitude and duration was obtained. Occasionally, two units were studied together if it proved possible to reliably discriminate them with the spike trigger module.

IV. EXPERIMENTAL PROTOCOL

When a single-unit filament had been obtained, it was first tested by stretching the biventer muscle manually and if it did not respond with an increase in its discharge rate it was suspected to originate in the complexus muscle and was discarded. Similarly, if the discharge rate fell upon biventer stretch the unit was discarded. In some of the experiments, the response of the unit to contractions of various muscle compartments was determined at this point.

All other units were then subjected to the following tests in the order listed:

(1) Determination of the coefficient of variation of the resting discharge. The muscle was left at the resting length while the discharge of a unit was recorded. The output pulses of the spike trigger module were passed to the Cromemco microcomputer which timed and stored the intervals between successive pulses (i.e. the inter-spike intervals); up to 512 intervals were timed, after which the mean interval and the standard deviation of the intervals were calculated. The coefficient of variation was calculated as {standard deviation / mean}.

(2) 1 Hz sinusoidal muscle stretching at various amplitudes, of which one was usually 1 mm. Fifty to sixty cycles of stretching were recorded on tape for off-line analysis.

(3) Sinusoidal vibration. Starting at a frequency of 10 Hz, and an amplitude of about 120 μ m, the frequency of the sinusoidal command signal to the muscle stretcher was slowly increased whilst triggering from the afferent under study and displaying its instantaneous firing frequency. As the frequency of stretching was increased, the afferent discharged once per cycle of stretch (1:1 driving) until a critical frequency was reached beyond which it failed to discharge every time the muscle was stretched. This failure appeared as a banding in the display of instantaneous discharge rate, and the stretch frequency at which this failure first occurred was noted.

(4) Repeated ramp and hold muscle stretching during exposure to SCh delivered intra-arterially by an infusion pump. Afferent firing was recorded continually on tape before, during and after exposure to the drug. The total duration of recording was about twenty minutes - 1 minute of control, usually about 5 minutes of drug exposure and about 15 minutes of recovery. At least a further half hour of recovery time was always allowed before another exposure to SCh, and in practice substantially longer than this was required before another single unit was found.

The experiment was continued until both the C2 and C3 muscle nerves had been thoroughly investigated. This usually took about 12 hours and gave an average yield of 5 units per experiment.

V. DATA ANALYSIS

In this section, I describe the analysis of those tests which did not yield a result during the experiment, namely the sinusoidal stretching test and the SCh exposure test.

Sinusoidal sensitivity

The sensitivity of a unit to sinusoidal stretching was determined by playing back the afferent responses recorded during the experiment to the Cromemco microcomputer which averaged them and fitted a 1 Hz sinewave to the average. Fig. 7 illustrates the method, which consists of dividing each cycle of stretching into 24 "bins" of equal duration and entering into each bin the number of afferent spikes which occurred during the corresponding part of the stretch cycle. Usually about 50 cycles of stretching were averaged to produce a "cycle histogram" as shown in Fig. 7. A variety of techniques exists for fitting a 1 Hz sine wave to such histograms, of which we used a variant of standard Fourier analysis which permits one to fit to a cycle histogram containing empty bins (afferent silenced for part of the cycle). This is desirable for full comparison of units only some of which are silenced, if one assumes that some aspect of the transduction processes at all spindles would be truly sinusoidally modulated but that those units which fall silent do so since they are unable to use negative firing frequencies to indicate their excitability - i.e. empty bins represent an undefined firing frequency rather than a true zero rate. Where appropriate, interactive software was used to delimit a silent period in a histogram, a Fast Fourier Transform was then performed to calculate the amplitude, the dc level and the phase relative to muscle length of the best fitting 1 Hz sine, and the resultant amplitude value was adjusted by an amount which depended on the number of zero bins in the histogram.

For the majority of afferents, the amplitude of the fitted sine was the same as the sensitivity of the afferent (units: Impulses/sec/mm stretch) since the stretch amplitude to which they had been exposed was exactly 1 mm. In some of the early experiments, however, units were not subjected to stretches of exactly 1 mm; straightforward division of the fitted sine amplitude by the stretch amplitude to obtain a sensitivity would not yield a meaningful value, since this calculation assumes a linear relationship between spindle afferent discharge and muscle stretch amplitude, whereas the actual relationship is known to be distinctly non-linear. Instead, a 1 mm value was obtained either by linear interpolation between two values obtained with stretch amplitudes straddling 1 mm, or else by extrapolation to 1 mm from the data for several stretch amplitudes greater than 1mm.

Suxamethonium exposure

For each unit successfully activated by SCh, a printout of the muscle length signal and afferent instantaneous rate during the exposure was obtained on a two channel chart recorder. This was checked to see that triggering of the unit was consistent over the entire recording, in which case the afferent action potentials, length signal and trigger pulses were played back to the Cromemco microcomputer for digitisation. The length signal was digitised (8-bit analogue-to-digital) at 142 Hz, whilst the interspike intervals were timed to a resolution of 70 μ sec. The digitised

data were stored in floppy-disc files and were subsequently mainly used to produce the figures in this dissertation and elsewhere. Initially, attempts were made to automate the measurement of such values as the dynamic index of an afferent, but the results proved too prone to corruption by mis-triggers and other "glitches" which the human operator would ignore and so the main analysis of patterns of activation of units by SCh was qualitative and was done using the chart recorder output.

VI. RESULTS

A total of 112 afferents from biventer were studied in this series. All were tonically active at the in situ "resting length" and were excited by stretching the biventer muscle. As intimated above, however, the standard means of differentiating between spindle afferents and those from Golgi tendon organs, namely response to muscle contraction, could not be used to confirm that all 112 afferents were from muscle spindles. An example of the problem encountered is shown in Fig. 8. The particular arrangement in this experiment is represented schematically at the top where the first three compartments of biventer, delineated by dotted lines, are shown. The afferent action potentials shown in the lower panels were recorded from a filament of the C3 nerve, stimulating electrodes were on the second and third muscle compartments and the sensory ending under investigation was presumed to lie in the second compartment. In response to contraction of the second compartment (stimulus is the large spike with the dot below it) the afferent firing rate was transiently decreased, both when a single stimulus was applied (panel A) and during a train of 8 stimuli at 27 Hz (panel B). In a hindlimb muscle, this form of response would be diagnostic of a muscle spindle afferent. However, if the neighbouring third compartment was stimulated, the firing rate of the same afferent now increased, both in response to a single stimulus (panel C) and to a train of stimuli (panel D), a response normally diagnostic of a Golgi tendon organ afferent. Co-contraction of several compartments produced variable effects, presumably reflecting a complex interaction between the tensions produced in the several active compartments and the particular stretch receptor under study. As a result, this test was abandoned after the first few experiments.

Activation by SCh.

Of the 112 afferents encountered, 72 were successfully activated by SCh and studied in detail. In the majority of the experiments, the dose of SCh used was 100 $\mu\text{g/kg}$ body weight/min, since this was the dose used in similar experiments in the hindlimb. Fig. 3 shows the parameters of the afferent response to ramp stretch which were studied here. The INITIAL DISCHARGE is the firing rate of the afferent immediately before the application of a ramp-and-hold stretch, the DYNAMIC RESPONSE is the peak firing rate reached just before the end of the stretching phase and the POSITION RESPONSE is the firing rate 0.5 seconds after the end of the muscle stretch. From these values, two other parameters of the afferent response can be calculated, namely the dynamic index [=dynamic response - position response] and the length sensitivity [=position response - initial discharge]. Changes in one or more of these parameters during SCh infusion were used to classify spindle afferents.

22 of the 72 afferents activated by SCh showed small, slow increases in their initial discharge rate, accompanied by increases of about the same size in their dynamic response and position response as illustrated

in Fig. 9 A,B. The result of these changes was that the afferent firing rate was biased to somewhat higher values, but the basic form of the response to ramp stretches was largely unaltered, a pattern of activation very similar to that shown by secondary afferents from hindlimb muscles. When the infusion was switched off, these changes reversed over a period of 5 to 10 minutes, and subsequent activations of the same unit resulted in qualitatively very similar effects. Also illustrated in Fig. 9 are an example of a cycle histogram of the response of one of these afferents to 1 Hz, 1 mm sinusoidal stretching of biventer (panel C) and a histogram of the coefficients of variation of all 22 (panel D). The coefficients of variation of these afferents were generally less than 0.035, their sensitivities to sinusoidal muscle stretch ranged from 1 to 24 Impulses/sec/mm, and in response to small-amplitude muscle vibration they were rarely driven 1:1 at frequencies much above their resting discharge rate (40 Impulses/sec or less). Both the pattern of activation of these units, and their passive properties are very similar to those reported for spindle secondaries from hindlimb muscles. These characteristics are summarised in Fig. 12 in which these afferents, presumably spindle secondaries, are shown as open squares.

The remaining 50 afferents were rapidly and dramatically excited by SCh infusion in contrast to the 22 afferents just described, and two clearly different patterns of activation by SCh could be discerned. The initial discharge of all 50 afferents was substantially increased to between 80 and 150 Impulses/sec, and this was accompanied by a marked increase in the variability of the discharge and a reduction in the length sensitivity, sometimes to zero. In 25 of the afferents, no further changes in afferent response occurred, even if the infusion was continued for several more minutes; this pattern of SCh activation is illustrated in Fig. 10 A,B. In addition to these changes, the remaining 25 afferents showed a considerable increase in their dynamic response which reached peak frequencies of up to 250 Impulses/sec and resulted in a substantial increase in dynamic index over the control value. An example of this pattern of activation is illustrated in Fig. 11 A,B. The other properties of these 50 afferents spanned very similar ranges as is illustrated in Fig. 12 by the intermingling of the triangles and filled circles. Their coefficients of variation ranged from 0.03 to 0.12, their sinusoidal sensitivities from 25 to 75 Impulses/sec/mm, and they were driven 1:1 by vibration at 40-220 Hz. These passive properties are comparable to those reported for hindlimb primary afferents.

The increase in dynamic sensitivity of the 25 units which showed it was similar to, though less marked than, that shown by primary afferents from the soleus muscle when activated by SCh. As in the hindlimb, this effect is presumably caused by contraction of the bag1 fibre when the levels of SCh rise above threshold. These 25 afferents (filled circles in Fig. 12) were therefore presumed to be primaries from b1b2c encapsulations in biventer. The remaining 25 afferents had passive properties similar to those of the b1b2c primaries, but their SCh activations were clearly different, showing features of a strong bag2 fibre input without any bag1 fibre input. These afferents (triangles in Fig. 12) were presumed to be the primary afferents from b2c encapsulations...the elusive b2c afferents. However, the details of the SCh activation of presumed b1b2c primaries in biventer were slightly different from those of soleus primaries, leaving this identification open to doubt. The important difference was that soleus b1b2c primary afferents were activated in three distinct phases as illustrated in Fig. 13. Phase I consisted of a small biasing similar to the entire activation of secondary afferents and was followed by an increase in the dynamic response of the primary (Phase II) similar to that

seen in biventer. However, nearly all of the soleus primaries went on to develop a substantial increase in position response which resulted in an increase in length sensitivity (Phase III). This was interpreted as the effect of later contraction of the bag2 fibre, since there was evidence that the bag2 fibre was less sensitive to SCh than the bag1 fibre. It was therefore possible that, in the biventer experiments, the concentration of SCh at the spindles was too low to activate the bag2 fibre; this would lead to the absence of a Phase III in the activation of blb2c primary afferents, and the response of the 25 presumed b2c afferents might be interpreted in terms of an unusually large Phase I. Although this interpretation was felt to be unlikely to be true due to the size and rapid time course of the activations observed, a number of experiments were performed using larger doses of SCh (200-500 $\mu\text{g/kg/min}$). Typically, afferents were activated more rapidly by the higher dose of SCh, but showed no further effects on their sensitivity to stretching than those seen during infusion at 100 $\mu\text{g/kg/min}$. If the higher infusion rate was continued for some minutes, however, the response to stretch of the afferent became erratic; initially it became unable to fire at the high frequencies reached during the dynamic response, and subsequently it fell completely silent during muscle stretch (see Fig. 14). This type of behaviour is characteristic of a condition known as depolarisation block and would almost certainly have been seen in soleus had such high doses been used. It is therefore unlikely that the absence of Phase III in these experiments was due to incomplete activation of the intrafusal fibres.

A second possible explanation for the absence of a Phase III of activation in neck primaries could be that the sensitivities to SCh of bag1 and bag2 fibres in neck muscles are about equal and substantially greater than in the hindlimb. In this case a 100 $\mu\text{g/kg/min}$ infusion of SCh might cause both bag fibres to contract together powerfully and might produce a different pattern of activation of blb2c primaries than that seen in the hindlimb. A final set of 3 experiments was therefore carried out in order to investigate this possibility; we selected probable primary afferents on the basis of their passive properties (see Fig. 12) and exposed them to increasing doses of SCh (from about 20 to about 200 $\mu\text{g/kg/min}$) by increasing the pump rate appropriately. The very lowest doses had no noticeable effect on afferent response, but when the dose reached 30-40 $\mu\text{g/kg/min}$ the units began to be activated. This threshold value is not markedly lower than that in the hindlimb. The subsequent behaviour of two units from these experiments is illustrated in Figs. 15 and 16. In both cases, there was a sudden, transient episode of excitation consisting of a biasing of the afferent discharge, an increase in discharge variability and a reduction in dynamic index and length sensitivity (Fig. 15 A, Fig. 16 A), the afferent response at this point being a scaled down version of that of the presumed b2c afferents at full activation. Subsequent events fell into two classes. In some cases, as illustrated in Fig. 15 B (open arrow), there was a second, more powerful transient excitation but on this occasion there was a clear increase in dynamic response about 10 seconds after the start of the event (Fig. 15 B, filled arrow). This second excitation also waned, but a residual dynamic sensitivity remained after the biasing had gone. The SCh dose was increased to 60 $\mu\text{g/kg/min}$ at this time, and shortly afterwards the unit was powerfully activated to its maximal level of excitation; increasing the dose to 120 $\mu\text{g/kg/min}$ produced no further changes in the afferent responses to stretch, which remained similar to those of the 25 presumed blb2c primary afferents in the earlier experiments (compare Fig. 15 C with Fig. 11). This type of behaviour was seen on several occasions in different animals and appears to indicate that in biventer the bag2 fibre is actually more sensitive than the bag1 fibre (cf. hindlimb where the inverse applies).

On a number of other occasions, afferents behaved as shown in Fig. 16. Here, the first transient excitation was followed by a similar but larger excitation, but there was no sign of an increase in dynamic response (Fig. 16 B). Shortly afterwards, there was a powerful excitation to a maximal level, still with no increase in dynamic sensitivity (Fig. 16 C). Increasing the SCh dose to 200 $\mu\text{g/kg/min}$ produced no further changes in the afferents response (not illustrated). Such units behaved in all respects like the 25 presumed b2c afferents in the earlier experiments (compare Fig. 16 C with Fig. 10).

Only one afferent was encountered in these experiments whose excitation by SCh did not fit into one of the three classes described above. The activation of this unit, which is illustrated in Fig. 17, consisted of a gradual biasing of the afferent rate, similar to that seen for the presumed secondary afferents but of substantially greater magnitude. This biasing was accompanied by a somewhat increased discharge variability, but the increase was not as great as that shown by either presumed blb2c or presumed b2c primary afferents (compare Fig. 17 with e.g. Figs. 15,16). Finally, the length sensitivity of this afferent was substantially increased, unlike that of presumed blb2c and b2c primaries. Its resting characteristics were similar to those of presumed secondaries, and it appears in Fig. 12 as a filled square. The behaviour of this afferent was similar to that of a small number of "truly intermediate" afferents which have been described in the hindlimb and which will be considered in the discussion.

VII. DISCUSSION

The interpretation of the results described above does not follow a conventional course since, for reasons which have been covered, two standard measurements were not available to us in the biventer preparation. Firstly, afferent conduction velocities could not be measured and most commonly used criterion for classifying afferents as primaries or secondaries was lacking. Secondly, the response of biventer afferents to muscle contraction could not be usefully measured. In the absence of these two tests, the evidence that the large majority of the 112 afferents studied here were indeed muscle spindle afferents rather than tendon organ afferents is the following:

(1) All the units were spontaneously active at the resting muscle length, when the passive tension in the muscle was low. Only a small minority of Golgi tendon organ afferents are active under similar circumstances in the hindlimb.

(2) The majority of the afferents were acutely sensitive to small stretches (see vibration responses) which Golgi tendon organs are not.

(3) Many of the afferents were subsequently activated by SCh in a manner quite atypical of Golgi tendon organs which are either not affected by SCh infusion or only weakly so. However, the activation of some GTOs is quite similar to that of spindle secondaries, and the possibility cannot be excluded that some of the presumed secondary afferents were in fact tendon organ afferents. Had it been possible to measure afferent conduction velocity, this possibility could have been excluded since GTOs conduct much more rapidly than do secondary afferents. On the whole, it is felt

that the degree of contamination of this sample by tendon organ afferents is likely to be small, and that any such contamination would not adversely effect the main conclusions drawn from this work.

As far as the inability to measure conduction velocity is concerned, it transpires that the conduction velocities measured by others for biventer afferents are substantially lower than those seen in the hindlimb, and that the range of values does not show two separate peaks for the primary and secondary afferents, so that in biventer conduction velocity probably could not be used to distinguish the two main afferent classes anyway. A similar situation pertains in some other muscles (eg. extraocular and masticatory muscles), and people studying the spindle afferents from these muscles have also classified them on the basis of the effects of SCh on their responses to stretch. This classification is based upon the work of Dutia in the late 1970s who showed that secondary afferents are characterised by a weak biasing of their discharge rate with roughly parallel increases in dynamic and position response similar to that illustrated in Fig. 9 A,B above, whereas the cardinal feature of SCh activation of primaries (which is tacitly taken to mean b1b2c type primaries) is an increase in their dynamic response and dynamic index superimposed on a substantial biasing of the initial discharge (see Fig. 11). The pattern of activation of secondaries is explained by the fact that the majority of their sensory termination is on nuclear chain fibres which are paralysed by SCh (see Historical Overview, Section G). The moderate biasing is probably caused by depolarisation of the sensory terminals by K⁺ ions which are released by the paralysed extrafusal fibres and probably also by the paralysed chain fibres. In contrast, the primaries terminate extensively on the two bag fibres and are therefore influenced by their sustained contraction during SCh infusion. The contraction of the bag1 fibre results in a large increase in dynamic sensitivity, and that of the bag2 fibre in the large biasing. On the basis of the similarity between their patterns of activation by SCh and those just described, we have classified 22 biventer afferents as secondaries (Fig. 12, open squares) and 25 as b1b2c primaries (Fig. 12, filled circles). In support of these classifications is the fact that the passive properties of the biventer secondaries were very similar to those of hindlimb secondaries, and likewise for the b1b2c primaries.

The remainig 26 afferents were not placed in either of these categories. Two morphologies could underly their different behaviour, namely the b2c spindle unit (see Historical Overviews, Section G) and those secondaries which make substantial contact with nuclear bag fibres. Correspondingly two types of behaviour were seen as illustrated in Figs. 13,20 . One afferent had many features in common with the 22 secondaries we studied but showed signs of a significant influence from the bag2 fibre in that its length sensitivity was increased by SCh infusion. Similar afferents have been found in hindlimb experiments where they were called "truly intermediate"; they were thought to be secondaries with significant bag fibre contacts, and the similarity of the passive properties of this single unit to those of the 22 secondaries support that interpretation.

The final 25 afferents were presumed to be the b2c primary afferents that we had set out to record from for the following reasons. Firstly, their activation by SCh is entirely compatible with a strong, isolated bag2 input and is exactly what one would predict for b2c afferents (see Historical Overview, Section F,G). This pattern of SCh activation has not been seen before, but the experiments with higher SCh doses than the standard 100 µg/kg/min make it rather unlikely that it could be an artefact due to incomplete bag fibre activation. The experiments using

gradually increasing doses of SCh revealed transient biasing events for both presumed b1b2c and presumed b2c afferents, whereas only presumed b1b2c afferents showed subsequent increases in dynamic response, and these were often sustained after the biasing waned (e.g. Fig. 16 B). Presumably the transient biasing is due to threshold level contractions and relaxations of the bag2 fibre and any subsequent increase in dynamic response is caused by bag1 fibre contraction. Secondly, the passive properties of these units were similar to those of primaries in a variety of muscles, and b2c afferents are defined histologically as primaries. Finally, large numbers of these afferents were seen and the biventer muscle is known to be particularly rich in b2c spindle units; in this sample, about half of the presumed primary afferents were classified as b2c type, the corresponding figure from histological work being between 25% and 35%. The technical problems of these experiments, in particular the large amount of connective tissue in muscle nerves which makes splitting a rather damaging procedure, mean that this sample is probably not truly random and no great store can be laid by these numbers, but the only real candidate for the termination of this proportion of primary-like but non-b1b2c afferents is the b2c spindle unit.

The conclusion is, therefore, that b2c afferents can be readily identified by changes in their response to ramp-and-hold stretching during SCh infusion. Interestingly, it appears that b2c afferents do not represent a different class of muscle receptor, since their passive properties are completely indistinguishable from those of b1b2c primaries despite the absence of the bag1 fibre. The only functional difference between b2c and b1b2c primary afferents is that the latter are controlled by both the static and the dynamic gamma and beta fusimotor systems, whereas the former are only innervated by static gammas. It is clear that a particular pattern of fusimotor output from the spinal cord would have substantially different effects on b1b2c and b2c afferents, but how this is used to advantage remains to be seen.

The compartmental nature of biventer.

The excitation of presumed spindle afferents by contraction of biventer (Fig. 8) was initially quite a surprise, but, upon closer examination, this result is perfectly explicable. The serial compartmental structure of the biventer muscle has been described above, but I did not elaborate on the arrangement of the extrafusal muscle fibres. It has been shown that each compartment contains its own separately innervated population of extrafusal muscle fibres which extend between two adjacent tendinous inscriptions but do not penetrate them. The extrafusal fibres of each compartment are therefore mechanically in series with all the stretch receptors in the other muscle compartments, both tendon organs and muscle spindles. The argument presented in section B of the historical overview can now be reapplied and shows that a biventer muscle spindle should respond to contraction of its own compartment with the usual reduction in firing rate, whereas the contraction of any other compartment(s) should cause an increase in firing rate; this is exactly what we saw in these experiments. The effect of combined contractions of the spindle's parent compartment and one or more of the in-series compartments is difficult to predict since it depends upon the relative strengths of contraction in the various compartments. If the parent compartment produces more tension than the rest, then the spindle will be unloaded and its firing rate will fall; conversely, if the other compartments contract more powerfully than the parent compartment, the spindle firing rate will increase. The perceptive reader may feel that a spindle afferent ought still to be distinguishable

from a tendon organ afferent since the discharge of the latter should be increased by the contraction of any muscle compartment(s) since it is mechanically in-series with ALL of the extrafusal fibres, whereas there should be one compartment whose extrafusal fibres are in parallel with a spindle afferent and whose contraction produces a fall in its firing rate. However, two factors still conspire to make this test unreliable. Firstly, if only one compartment is made to contract, the inactive compartments are slack and behave like floppy springs. The result is that the active compartment shortens substantially without a dramatic increase in overall muscle tension, and since it is muscle tension which tendon organs are sensitive to, there may not necessarily be an increase in tendon organ afferent discharge rate. Secondly, in these experiments one would always have to activate at least one of the muscle compartments using surface muscle electrodes, namely the compartment whose muscle nerve was being split to find afferents. There is no guarantee that the compartment would be completely activated, and it has been shown in hindlimb muscles that if only part of a muscle is activated, some tendon organs show a reduction in firing rate as the muscle twists and makes some regions slacker than they were at rest. In the light of these considerations, there did not seem to be much point in routinely examining biventer afferent responses to muscle contraction.

Although the compartmentalised structure of biventer was somewhat of a nuisance in these experiments, it did nevertheless lead on to a number of interesting theoretical considerations. For example, an analogous situation could be deemed to exist in the hindlimb in that muscle spindles of one muscle are mechanically in series with the extrafusal fibres of antagonist muscle(s) and so would be excited by their contraction, as in Fig. 8, panels C,D. What is unusual about biventer is that this excitatory arrangement exists within a single muscle which acts as a synergistic whole to produce useful tension at its ends. In the hindlimb if the muscle spindles in one muscle are stretched by contraction of its antagonist, a number of reflex pathways are activated, including the monosynaptic stretch reflex in which the spindle activity excites the motoneurons of its parent muscle which makes it contract and counteract the stretch produced by the antagonist, and the Ia reciprocal inhibition reflex in which the same spindle activity acts via an interneuron to inhibit the activity of the antagonist muscle. Do the same reflex connections exist at an inter-compartmental level in multi-compartmental muscles, so that the individual compartments are effectively each others' antagonists, or are there different patterns of central connectivity from those seen in "single compartment" muscles? Along the same lines, if one compartment contracts more strongly than a neighbouring one, is the resultant rise in spindle and tendon organ afferent activity from the neighbouring compartment used to equalise the tension production in the individual compartments, or is there some advantage in being able to leave it unbalanced? Also, if muscle spindles can behave in a similar way to tendon organs when single compartments contract, why bother with tendon organs? Finally, how do the forces produced by single muscle compartments summate to produce tension at the two ends of the muscle? I have only attempted to study the last of these problems, and technical difficulties soon persuaded me to continue with the mainstream of this work, but these questions are probably worthy of a more concerted effort in the future. Their answers may help us to decide whether these multi-compartmental muscles are simply developmental anomalies, or whether they subserve some special purpose.

Part III. CONFIRMATORY EXPERIMENTS PERFORMED ON HINDLIMB MUSCLES

Having developed a means of identifying b2c afferents in the course of the biventer experiments, we decided to apply it to hindlimb spindles for three reasons. Firstly, it is clear from histological investigations that b2c spindle units do exist in hindlimb muscles, but previous attempts to identify their afferents have failed. Secondly, we would have the opportunity to perform dorsal root afferent recordings and so would be able to measure afferent conduction velocities and examine the conduction velocity spectrum of b2c afferents which we were unable to do in the biventer experiments. Finally, there is a hindlimb muscle, the tenuissimus, which has features which provide us with a unique opportunity to histologically confirm that any b2c afferents we record from actually derive from b2c spindle units. The hindlimb experiments were performed in two stages; initially we looked for b2c afferents from the medial gastrocnemius muscle, since this muscle is reported to contain the highest proportion of b2c spindle units of all the hindlimb muscles, a proportion nearly as high as that of biventer. Subsequently we went on to look for b2c afferents from tenuissimus.

A. MEDIAL GASTROCNEMIUS EXPERIMENTS

METHODS

Surgical procedures

A total of 10 animals was used in this series. They were anaesthetised in the same way as in the biventer experiments, with the minor modification that intravenous barbiturate was administered via a cannula in one of the external jugular veins. Cannulae were placed in the trachea and in the left femoral artery in the standard manner. The femoral arterial cannula was advanced centrally until its tip was adjudged to lie in the vicinity of the aortic bifurcation since it was to be used to infuse SCH into the right hand femoral artery in order to activate spindles in the right hand medial gastrocnemius muscle.

Preparation of the right hindlimb started with the exposure and division of the femoral and obturator nerves on the anterior aspect of the thigh in order to reduce the amount of extraneous neural traffic reaching the spinal cord from irrelevant muscles, skin, joints etcetera. Similarly, the branches of the sciatic nerve running to innervate the hamstring muscles were found and cut, as were as many as possible of the branches innervating the area of the tail. An incision was then made over the back of the calf to expose the triceps surae muscle group (medial and lateral gastrocnemii and soleus); the nerves to the lateral gastrocnemius and soleus were cut, as was the common peroneal nerve which innervates all the other muscles and the skin of the lower hindlimb. The upshot of all this denervation was that only afferent axons from the medial gastrocnemius muscle would be active in the lumbosacral dorsal roots from which we were proposing to record, greatly simplifying their identification and isolation.

Next, the medial gastrocnemius tendon was separated from those of the lateral gastrocnemius and soleus muscles and stitched to a length of stout thread for subsequent attachment to an electromechanical puller as in the biventer experiments. The medial gastrocnemius muscle was separated from its neighbours sufficiently to allow it to be well stretched by the puller. Dessication was prevented by stitching a wire frame to the margins of the skin incision to fashion a pool and filling it with paraffin oil

maintained at 30-35 degrees centigrade by radiant heat from a lamp.

Finally, an incision was made in the midline of the back from about the third lumbar vertebra to the level of the second sacral spine in order to perform a laminectomy to expose the cauda equina. The erector spinae and paravertebral muscles were removed in order to expose the spines and laminae of the lumbosacral vertebrae as clearly as possible. These were then carefully removed with bone rongeurs to expose the dura mater investing the spinal cord itself, generally from L4 to S1, and a second pool was made over the spinal incision and filled with warm paraffin oil. The animal was then transferred to a brass frame as before and held in a position in which the spinal pool was horizontal and at a level allowing easy access to the spinal cord. The right hindlimb was clamped roughly horizontally with hip pins under the iliac crests and two clamps, one holding the thigh, the other the foot. Fibre optic light guides were used to illuminate both the pools, and under microscopic guidance the nerve branches to medial gastrocnemius were looped over a bipolar silver wire electrode for subsequent stimulation. A hole was then made in the dura at L4 and this hole was extended caudally as far as possible, exposing the cord itself and the cauda equina. Using fine instruments, the cut edge of the dura was drawn to one side in order to expose the points at which the dorsal and ventral roots exited to the periphery; the L7 (cats have seven lumbar vertebrae cf. humans' five) exit level was identified by its position (approximately level with the tops of the iliac crests) and by the fact that it is the largest of the roots in this area. The L5-S1 dorsal roots were then traced back to their point of entry into the cord and cut as close to this as possible, and the corresponding ventral roots were divided. The majority of the gamma axons to the gastrocnemius exit in the L6, L7 and S1 roots, so the cutting of these ventral roots should have completely de-efferented the spindles in gastrocnemius. Correspondingly, the afferent axons running from gastrocnemius to the cord are accessible in the L6-S1 dorsal roots.

Electrophysiological procedures.

The recording and muscle stretching systems were identical to those used for the biventer experiments except that in these experiments a pair of identical recording channels was available so that two afferents could be simultaneously studied. Basically, afferent action potentials were recorded with bipolar hook electrodes, amplified, filtered, displayed on an oscilloscope and recorded on an FM tape for subsequent analysis. The muscle was stretched as before and the muscle length signal was displayed and recorded as before (Fig. 6). Generally, the L7 and S1 dorsal roots were repeatedly subdivided to find medial gastrocnemius afferents; this was helped by stimulating the medial gastrocnemius muscle nerves once per second and looking for filaments of the dorsal roots which contained evoked action potentials with a constant waveform and latency, since such activity could only be caused by the conduction of an action potential along a gastrocnemius afferent into the filament in question. Such filaments were then split until they contained a single, tonically active afferent from medial gastrocnemius. Spindle afferents were identified by the reduction in their firing rate during contraction of the medial gastrocnemius in the standard manner and the delay between stimulation of the muscle nerve and the arrival of the afferent action potential at the dorsal root was recorded for each unit. Units were further studied with the same battery of tests as in the biventer experiments (see above). The dose of SCh used was the usual 100 $\mu\text{g/kg/min}$ in most of the experiments, but in three it was around 180, 200 and 220 $\mu\text{g/kg/min}$. addition. At the

end of the experiment, the sciatic nerve was exposed over its entire length and the distance between the points of stimulation and recording was measured. The conduction velocity of the afferents could then be calculated as {distance/time taken}. These experiments continued for the same length of time as the biventer experiments (i.e. twelve or more hours), but the yield of afferents was about twice as great due to the relative simplicity of subdividing dorsal roots.

RESULTS AND DISCUSSION

A total of 88 medial gastrocnemius afferents was studied in this series, of which 86 could be categorized by the effects on them of SCh infusion. Four classes of SCh activation were seen, three of which were very similar to those described above for biventer afferents. Thus, 11 afferents underwent the small, gradual biasing of their discharge typical of secondary endings (Fig. 18 panel A), 53 afferents showed large increases in dynamic sensitivity typical of b1b2c primaries (Fig. 18 panel B). When fully activated by SCh, the discharge of b1b2c primaries from medial gastrocnemius reached substantially higher frequencies than those from biventer (dynamic response of 350 Impulses/sec or more, cf. 250 Imp/sec or less); correspondingly, gastrocnemius b1b2c primaries showed much larger increases in their dynamic index than those from biventer. The basic form and timecourse of the activation of b1b2c primaries in the two muscles was otherwise very similar. Interestingly, the majority of the b1b2c primaries from medial gastrocnemius did not show a Phase III of activation (e.g. Fig. 18, panel B) even when SCh doses of more than 200 µg/kg/min were used. This is in agreement with the results from biventer, but disagrees with those from soleus. A further 12 afferents behaved like the b2c afferents described above (Fig. 18 panel C), and the final group of 10 afferents showed intermediate patterns of activation. In 9 cases, this consisted of a gradual biasing of the discharge like that of secondaries, though of greater magnitude, and a small but definite increase in the dynamic index like that of b1b2c primaries but of a far lesser magnitude (Fig. 18, panel D). A single afferent behaved like that illustrated in Fig. 17, i.e. like a secondary but with a clear increase in its position sensitivity. The interpretation of the first three patterns of SCh activation is the same as for biventer, i.e. that they represent the activations of afferents from secondary, b1b2c primary and b2c primary endings respectively. The remaining 10 afferents, which in keeping with earlier work should be called "truly intermediate" afferents, are interpreted as being secondaries with significant areas of contact with the bag1 (9) or the bag2 (1) fibre. Nearly all the "truly intermediate" afferents studied in the medial gastrocnemius nearly all showed a bag1 input whereas nearly all those seen in the original soleus experiments, and the only one seen in the biventer experiments showed a bag2 input. Medial gastrocnemius is a relatively fast muscle in which it might make sense to have an enhanced dynamic sensitivity in spindle afferents, whereas both soleus and biventer are slow, postural muscles in which an enhanced length sensitivity might be appropriate; such fine tuning of spindle afferent characteristics has been postulated to occur in other muscles but has yet to be confirmed. It is quite possible that the apparent difference in relative frequency of the two types of "truly intermediate" afferent is an artefact caused by sampling errors in the various experiments.

In support of the conclusions based on the effects of SCh can now be adduced information about the axonal conduction velocities of the

afferents, as illustrated in Fig. 19. In this figure, I have included lines which delimit three ranges of conduction velocity, one from 0-60 m/sec, one from 60-80 m/sec and the last from 80-140 m/sec. As was described in the Historical Overview, it is generally accepted that an afferent conducting at 60 m/sec or less is a secondary, and that one conducting at more than 80 m/sec is a primary, but that, because the axon diameters of the biggest secondaries and the smallest primaries overlap, an afferent conducting at 60-80 m/sec could equally well be a primary or a secondary. Considering first the afferents "diagnosed" as secondaries on the basis of their activation by SCh, it can be seen from the two graphs of Fig. 19, in which these afferents appear as open diamonds, that the majority of them had conduction velocities clearly in the secondary range and all but one of the rest were in the uncertain 60-80 m/sec range; furthermore, the coefficients of variation and sensitivities to vibration of these afferents were similar to those reported for secondaries elsewhere, so that all three results lead to the conclusion that these afferents were secondaries. Similarly, those 56 afferents diagnosed as b1b2c primaries by SCh infusion almost all had conduction velocities in the primary range (i.e. above 80 m/sec), with only three in the 60-80 m/sec range, and had coefficients of variation and sensitivities to vibration similar to those described for primaries by others (Fig. 19 crosses). Turning next to the b2c afferents, these can be seen to lie in several of the quadrants delimited on the graphs; over half of them clearly had conduction velocities in the primary range and so would have been called primaries on this the most common basis for typing afferents during experiments (Fig. 19, filled triangles). Another quarter of the afferents had conduction velocities in the 60-80 m/sec range and the remainder had secondary conduction velocities. These results reinforce our conclusion that afferents with this pattern of activation must be b2c primaries, since the axon diameters of b2c primaries are known to overlap the bottom end of the range for b1b2c primaries and so would be expected to conduct at velocities overlapping the bottom end of the range for b1b2c primaries. The values for coefficient of variation and sensitivity to vibration of b2c afferents were quite widely spread out, but they tended to overlap more with those of b1b2c primaries than with those of secondaries (compare Fig. 19, diamonds, filled triangles and crosses). An analogous argument to that applied to b2c afferents suggests that the "truly intermediate" afferents seen in these experiments were indeed secondaries with bag1 contacts, since half of them had secondary conduction velocities and most of the rest conducted at 60-80 m/sec (Fig. 19, filled squares); furthermore, their coefficients of variation and sensitivities to driving were more like those of secondaries than those of primaries (compare Fig. 19, diamonds, filled squares and crosses).

There are two final points of interest to note from Fig. 19. Firstly, as has long been assumed, afferents conducting at 60-80 m/sec are indeed equally likely to be primary or secondary, and other standard tests such as measurement of coefficient of variation or sensitivity to vibration cannot be used to resolve the ambiguity reliably. The effects of SCh on the afferents can however achieve such a resolution and more, since one can also identify subtypes of primary/secondary. Secondly, whilst it is almost always true that an afferent conducting at more than 80 m/sec is a primary, this does NOT mean, as is often tacitly assumed, that the afferent in question is a b1b2c primary. From the data presented here, it would appear that only afferents conducting at above 100 m/sec can reliably be assumed to be b1b2c primaries without further investigation. This observation is of some importance to the interpretation of recent investigations of the role of the gamma fusimotor system in movements. In some of these experiments, the activity of the fusimotor system is not

directly observed, but rather deduced from the responses to stretching of spindle afferents. If, for example, a primary afferent is rapidly stretched to a new length in the course of a movement without showing an overshoot of its discharge rate at the end of the stretch (i.e. without there being a measurable dynamic index), then it is assumed that the dynamic gammas must have been silent during the movement whilst the static gammas were active. The reader will appreciate, however, that a b2c primary afferent would not be affected by activity in the dynamic gammas since it lacks the bag1 fibre which they innervate and probably about half of the b2c afferents would be classified as primaries during these experiments. Although b2c afferents from hindlimb muscles are quite rare, some of these experiments involve quite small sample sizes, and there is some potential for confusion which could easily be avoided if the experimentors used SCh infusion to confirm the presence of a bag1 fibre.

B. TENUISSIMUS EXPERIMENTS

Although at this stage we felt fairly certain that we had managed to identify b2c afferents both in the neck extensor muscles and in a hindlimb muscle, we felt that the series of experiments would be nicely rounded off by an attempt to show histologically that b2c afferents indeed derive from b2c spindle units. To do this, one would need to isolate spindle afferents until one chanced upon a b2c afferent and then localise the spindle in some way and remove the part of the muscle in which it lay for histological processing. The histological examination of this piece of muscle should reveal the presence of a spindle containing only one bag fibre and a number of chain fibres. Ideally, one would use enzyme histochemistry to prove that this single bag fibre was a bag2. Whilst this sounds straightforward, it would be nearly impossible in most muscles, since the spindles in a muscle tend to clump together in particular regions, so that there might be several spindles in the piece of tissue one isolates. Furthermore, exact localisation of the spindle in these muscles is exceedingly difficult. In this final series of experiments, we have turned to the tenuissimus muscle of the cat since its architecture avoids these problems. This long, thin straplike muscle is part of the hamstring group and runs from the hip to the back of the knee; its particularly useful feature is that its spindles line up along its length, so that it is unusual to find more than one spindle in a particular cross-section of the muscle.

METHODS

In this final series, we have so far used five cats, anaesthetised as in the two earlier series. A laminectomy was performed as described before to expose the L6-S1 dorsal roots under paraffin oil, and the muscle stretching and recording apparatus was identical to that used in the medial gastrocnemius experiments.

In the hindlimb, the femoral and obturator nerves and all the branches of the sciatic nerve bar that to tenuissimus were sectioned in order to reduce neural traffic in the dorsal roots. Tenuissimus was carefully separated from biceps femoris as far down towards the knee as possible where it was cut and attached with stout thread to the muscle puller. The tenuissimus nerve was placed over bipolar hook electrodes for

stimulation and the tenuissimus was stretched to about its in situ length. The animal was transferred to the brass frame as before, and the prepared hindlimb was fixed roughly horizontally. Previous (unpublished) work suggested that even intra-arterial infusions of SCH would be unlikely to fully activate tenuissimus spindles, so it was decided to apply SCH topically by dripping it onto the surface of the muscle over the region containing the spindle; it was hoped that since tenuissimus is very thin the drug would be capable of diffusing in to the spindle in sufficient quantities to activate the intrafusal fibres. As a result of this stratagem, we could not use the customary paraffin oil pool to prevent dessication; instead, we covered the majority of the exposed muscles with pieces of tissue paper soaked in physiological saline. Tenuissimus was kept moist during the isolation of single units by slackening the muscle until it lay in saline at the bottom of the leg incision, spraying saline onto it from an atomiser and covering it with a tent of moist tissues; during a recording session, the muscle was stretched out and periodically sprayed with saline to keep it moist, and during the administration of SCH, the saline in which the drug was dissolved served to keep it moist.

The spindle whose afferent was being recorded from was localised in the following way. The nerve to tenuissimus enters it about half way along its length where it divides into an ascending and a descending branch. These branches run towards the ends of the muscle, sending axons to innervate the line of spindles as they go. If a pair of silver ball electrodes placed across the muscle at right angles to its long axis, brief stimuli applied will electrically activate the axons in the intramuscular branch at that point. When a single unit filament was obtained in the dorsal roots, this pair of silver ball electrodes was moved along the length of the muscle, stimulating at each point. As illustrated in Fig. 20, stimulation of the intramuscular branch before the point where the axons to the spindle leave it produces an evoked action potential in the dorsal root filament, but this no longer happens when the electrodes have been moved beyond the spindle. If the stimuli are made very brief (e.g. 0.01 msec as here) and of just sufficient strength to reliably activate the intramuscular branch, then the position of the afferent termination can probably be located to within a few millimetres.

The experiment consisted of repeatedly isolating tenuissimus spindle afferents suspected to be primaries (on the basis of a short conduction delay from muscle nerve to dorsal root), locating them and activating them with SCH (100 - 250 µg/ml). The passive properties of the afferents were not routinely measured during these experiments. If a b2c afferent was found, its position was to be marked by placing microsutures through the muscle a known distance above and below the spindle. This procedure usually transected the intramuscular nerve branch, so that fewer afferents were subsequently found. Even if no b2c afferents were found, at least one b1b2c primary was to be marked per experiment. At the end of the experiment, the marked area(s) of muscle were removed and prepared for histological processing.

Histological processing.

Not having the appropriate expertise, enzyme histochemistry was decided against and instead routine Haematoxylin and Eosin (H & E) staining was used. This staining reveals the intrafusal fibres perfectly adequately, and the size of the fibres and arrangement of the nuclei in the central part of the spindle can be used to differentiate nuclear bag and nuclear chain fibres. If a spindle containing only one bag fibre were to be encountered, it would not be possible to say whether

this single bag fibre was a bag2 fibre. However, other workers have established that single bag spindle units are almost always b2c units.

The marked pieces of muscle were removed at the end of the experiment and allowed to relax in a moist atmosphere for about half an hour before being transferred to buffered formal saline for 48 hours. Fixed tissue samples were dehydrated and cleared in cedarwood oil for three days before embedding in paraffin wax. Serial 6 μ m thick transverse sections were cut right through the block, and every twentieth was mounted for staining. The sections were examined under a Nikon Optiphot microscope; any spindles seen were traced through as many serial sections as possible, making a note of the number of bag and chain fibres present, and photomicrographs were made of representative sections.

RESULTS

These experiments are still in progress, but the results so far are very encouraging. Seven presumed primary afferents have been activated with topical SCh and the activations seen have been very similar to those of medial gastrocnemius primaries. Six afferents showed an increase in their dynamic index typical of blb2c afferents and one showed typical b2c afferent behaviour. Of these, the b2c afferent and two of the blb2c afferents have been studied histologically. Fig. 21 correlates the histological and electrophysiological findings for the b2c afferent. The top panels (A,B) of this figure illustrate the SCh activation of this unit which can be seen to be similar to that of the other b2c afferents seen in the course of this work. Underneath are two photomicrographs of the spindle encapsulation which lay nearest to the afferent termination point identified by the electrical stimulation method during the experiment; as expected, a particular section of the muscle contained only one spindle. In the left hand photomicrograph (C), it can be seen that the spindle contains a single large (20 μ m diameter) intrafusal fibre and four smaller (12 μ m diameter) fibres. The right hand photomicrograph (D) shows a section through the central sensory area of this spindle in which the position of the large intrafusal fibre is occupied by a bag of three closely packed nuclei, whereas the smaller fibres contain single nuclei. The conclusion is that this spindle contained a single bag fibre, presumably a bag2, and four chain fibres, i.e. that it was a b2c spindle unit. The other end of the block of muscle was found to contain a two bag fibre encapsulation, presumably blb2c, and there was some evidence that the single bag spindle shared its bag fibre with this blb2c spindle unit, that is that the two units were linked in tandem. A similar correlation between morphology and electrophysiology is shown in Fig. 22. In this case, SCh activation of the afferent resulted in a clear rise in its dynamic index (A,B); correspondingly, the spindle found in its termination area contained two large intrafusal fibres and five smaller ones (C), and the large fibres contained bags of nuclei at the sensory part of the spindle whereas the small fibres showed only single nuclear profiles. This two-bag spindle was presumably a blb2c unit. The histology has only been completed for one other blb2c afferent and this one also ended in a two-bag spindle unit. Whilst it is not possible to prove that the b2c afferent whose behaviour is shown in the top panels of Fig. 21 actually innervated the single bag spindle, this conclusion fits most readily with all that has gone before.

FINAL CONCLUSION

The aim of these experiments has been to find a means of identifying the primary afferent axons from b2c spindle units (b2c afferents) of tandem muscle spindles in the cat. The drug succinylcholine has been used to activate the intrafusal bag fibres of muscle spindles in various muscles. In all the muscles studied, several patterns of afferent response to SCh have been seen, of which one is exactly that predicted for b2c afferents on the basis of the known properties of the bag fibres. Additional support for this identification comes from the fact that these afferents are often diagnosed as primaries on the basis of other tests including the axonal conduction velocity, the coefficient of variation of the resting discharge, the sensitivity to sinusoidal muscle stretch and the sensitivity to small-amplitude, high frequency vibration of the parent muscle, and all this in a number of different muscles. Preliminary histological evidence further supports this conclusion; only one b2c afferent has been found in tenuissimus to date, but the region of the muscle in which it terminated was shown to contain a b2c spindle unit. It is concluded that SCh activation is a simple and reliable means of identifying b2c afferents and that this test can be used to classify afferents which many, if not all, of the other commonly used tests would leave unclassified.

The passive properties of these b2c afferents do not appear to be markedly different from those of "normal" b1b2c primaries, thus ruling out the possibility that they represent a functionally different class of muscle receptor with significantly different transductive properties. This is a somewhat surprising result, since the absence of a bag1 fibre was expected to demonstrably reduce the dynamic sensitivity of these afferents compared to that of b1b2c primaries; however, under normal physiological conditions, such a difference might exist if there was tonic dynamic fusimotor activity. What the actual roles of b2c afferents in posture and locomotion might be is still a matter for debate, but the way is now open for further investigation.

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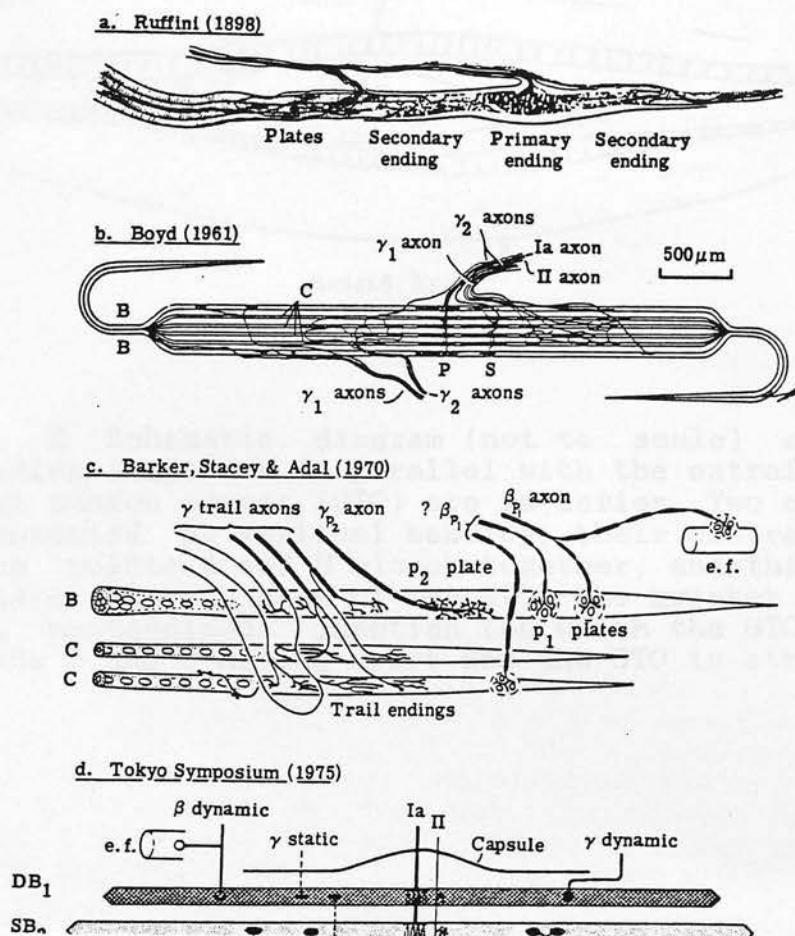
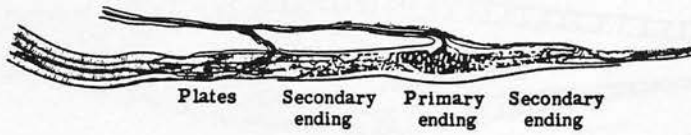
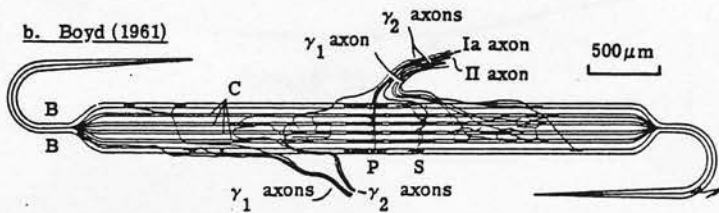


Fig. 1 A-E Stages in the understanding of muscle spindle innervation. **A** Ruffini (1898): primary ending, secondary ending and plate endings. Intrafusal fibres all the same. **B** Boyd (1961). Two types of intrafusal fibre, bags and chains (B,C in Figure). Primary innervates both; secondary mainly to the chains, with some sprays on the bag fibres. Two types of motor terminal: γ_1 plates and γ_2 trails; plates restricted to bag fibres, trails to chain fibres. This is the New Anatomy of the Spindle (see text). **C** Barker et.al. (1970). Sensory innervation as in B, but gamma fusimotor innervation is **non-selective**; both bag and chain fibres receive plate and trail endings. Note two types of plate ending p_1 and p_2 , since beta fusimotor innervation (see text) had been recognised. **D** Tokyo Symposium (1975). Two types of bag fibre recognised; bag1 fibre receives p_2 plates from dynamic gammas; bag2 fibre receives trailendings from static gammas, as do chain fibres; dotted lines: unagreed static gamma innervation of bag1 fibre. **E** London Symposium (1980). Current picture; bag1 fibre almost never receives static gamma/beta innervation and bag2/chains almost never receive dynamic gamma/beta innervation. Note: (i) Some static gamma innervate only the bag2, or only the chains (selective), whilst others innervate both (non-selective). (ii) Dotted lines for beta fusimotor innervation indicate that not all spindles receive beta axons.

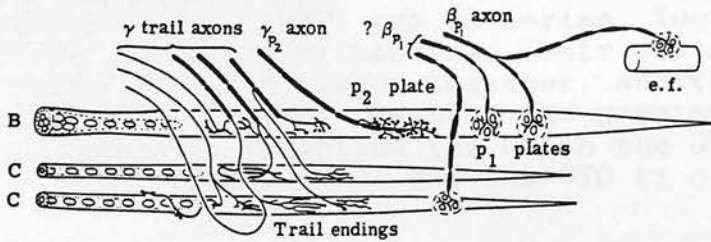
a. Ruffini (1898)



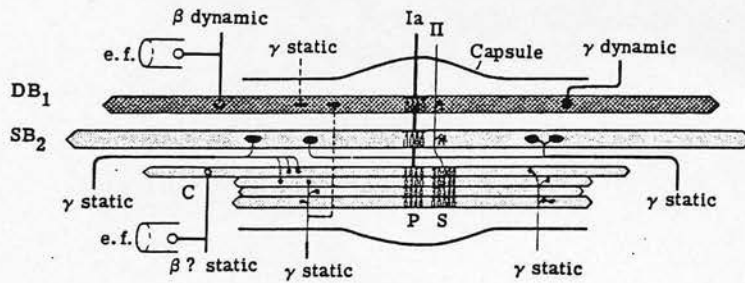
b. Boyd (1961)



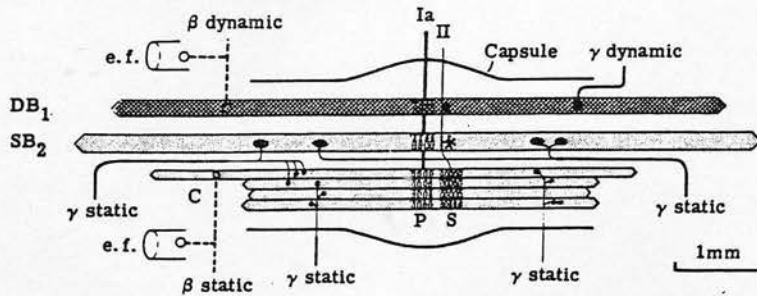
c. Barker, Stacey & Adal (1970)



d. Tokyo Symposium (1975)



e. London Symposium (1980)



— Extracapsular region — Capsular sleeve — Fluid space — Capsular sleeve — Extracapsular region —

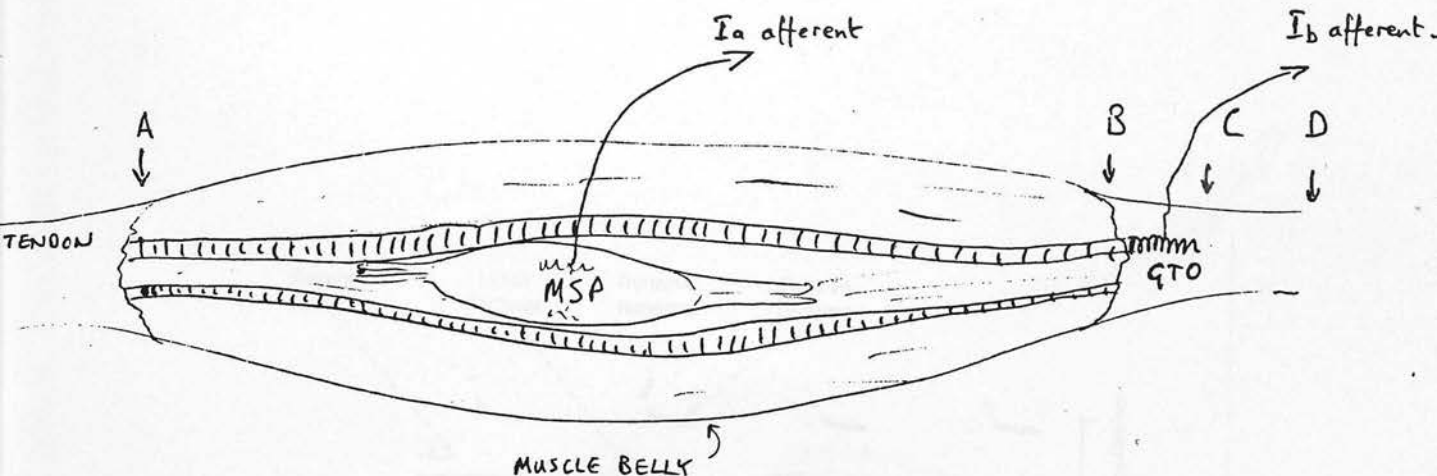


Fig. 2 Schematic diagram (not to scale) showing that muscle spindles (msp) are in parallel with the extrafusal fibres, whereas Golgi tendon organs (GTO) are in series. Two extrafusal fibres are represented by vertical banding; their contraction will tend to bring points A and B closer together, and this will shorten the spindle. In the case of the GTO, the greater stiffness of tendon than myotendinous junction (on which the GTO lies) results in points B and C moving apart and the GTO is stretched.

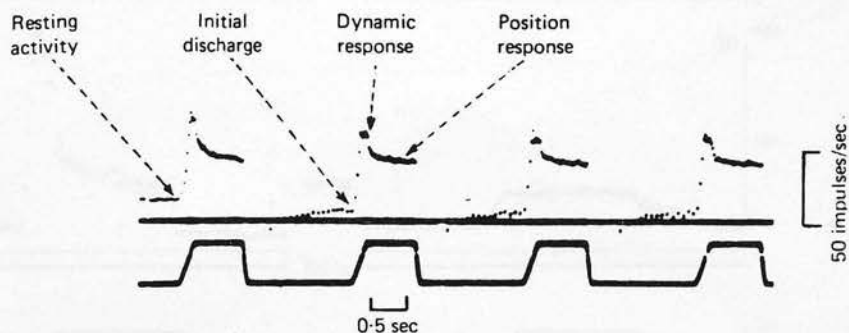


Fig. 3 An example of an afferent response to ramp muscle stretching, and the parameters of this response which have been studied (see text). Lower trace, length signal; upper trace, ratemeter output showing the discharge rate of the afferent against time. Each dot corresponds to the occurrence of a spike in the afferent signal, and the height of the dot above the baseline is inversely proportional to the time elapsed since the last spike. Before stretching, the afferent fired at the **resting** rate; when the sequence of ramps was applied, the discharge rate just before the next ramp stretch did not recover to the resting rate, so the **initial discharge** rate was measured.

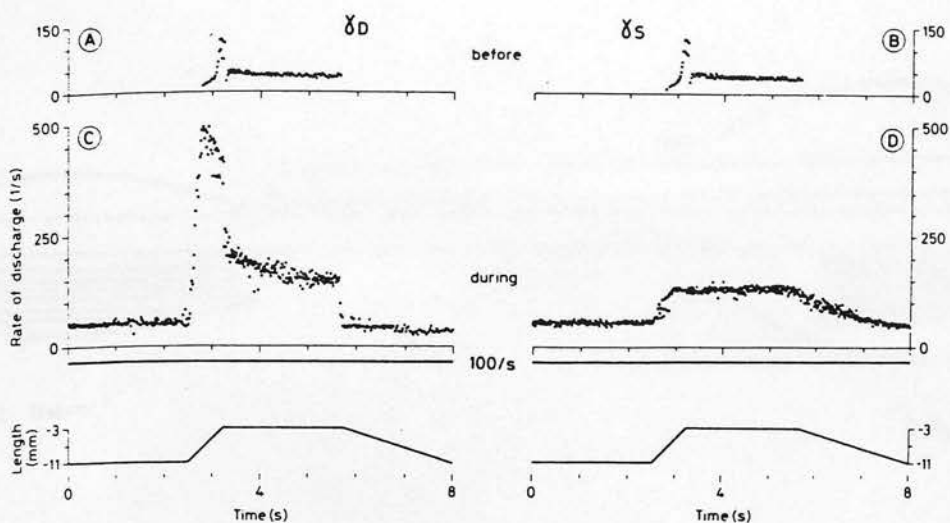


Fig. 4 A-D Classification of two types of gamma axon. **A,B** Control responses of a primary afferent to ramp stretching. **C** Response of the same spindle afferent to the same stretch when a dynamic gamma was stimulated. **D** Response of the same afferent to static gamma stimulation.

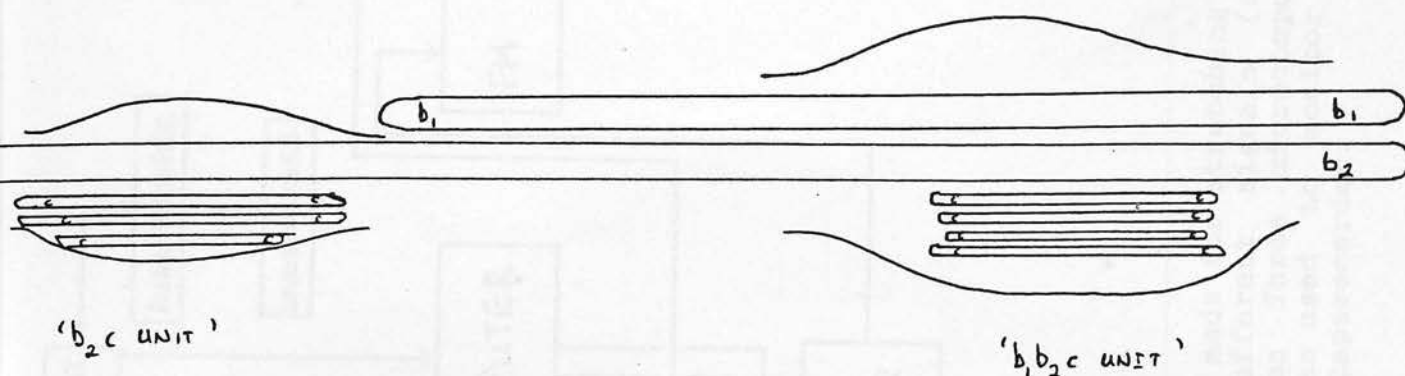


Fig.5 Diagram of the commonest form of tandem spindle. Two encapsulations are shown, linked by a common bag2 fibre. The capsule on the right contains a bag1 fibre, a bag2 fibre and a number of chain fibres...a b_1b_2c unit; it is basically identical to "normal" single spindles, except that it takes part in a tandem linkage. The capsule on the left contains only the shared bag2 fibre and a few chain fibres...a b_2c spindle unit.

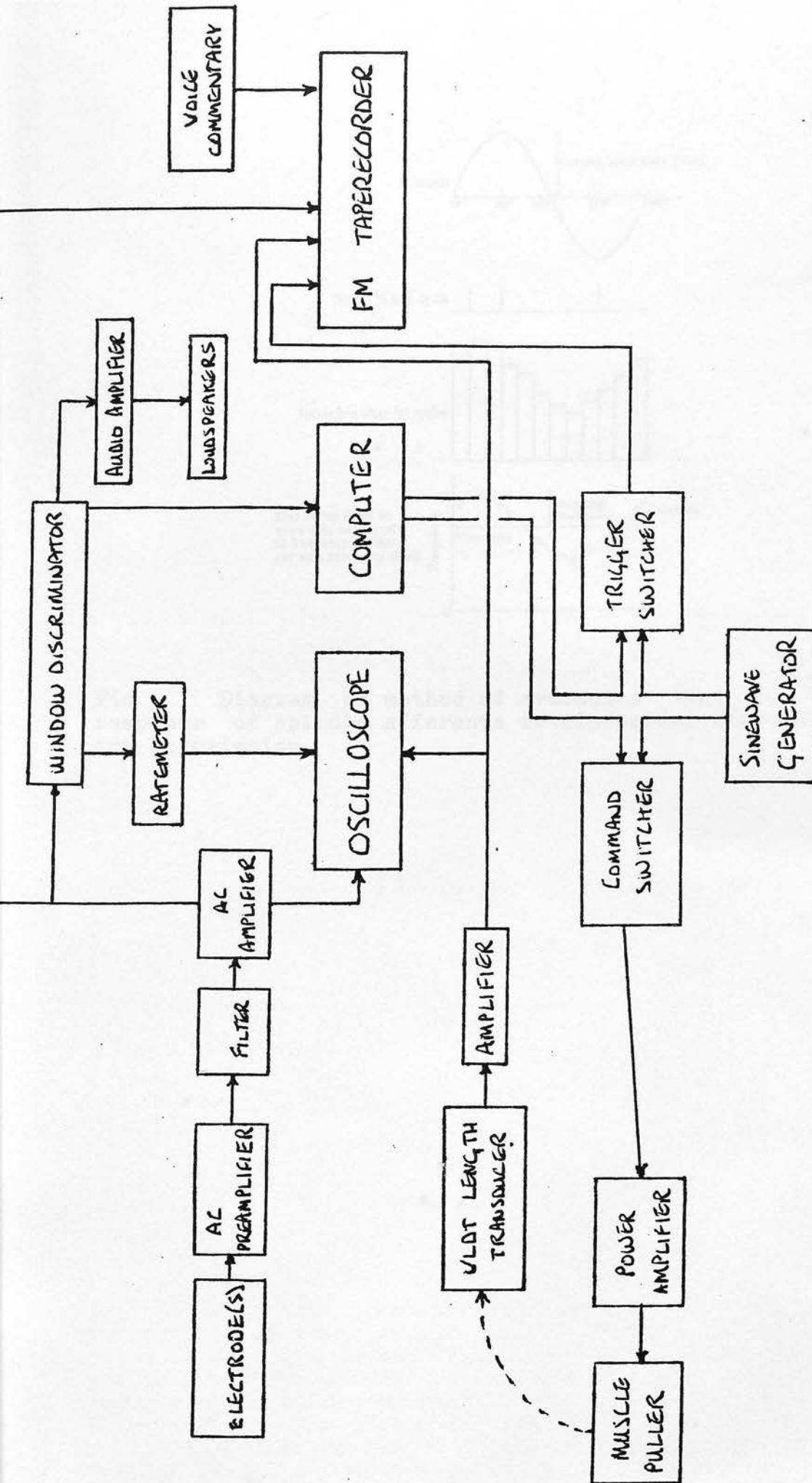


Fig. 6 Block diagram of the arrangements made for stretching the muscle (lower part) and for recording afferent signals (upper part). The computer was a Cromenco Systems Three microcomputer. Not illustrated is a set of oscilloscopes used to monitor the quality of the recordings made on the FM taperecorder.

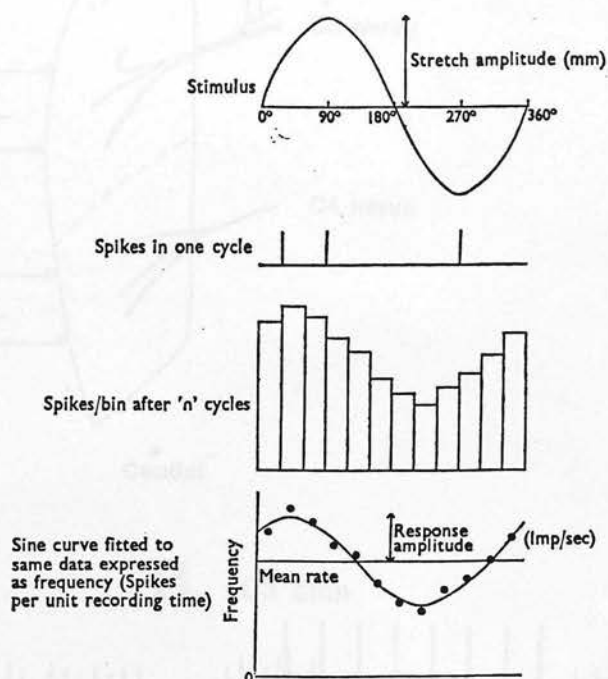


Fig. 7 Diagram of method of averaging used to determine the response of spindle afferents to sinusoidal stretching. See text for description.

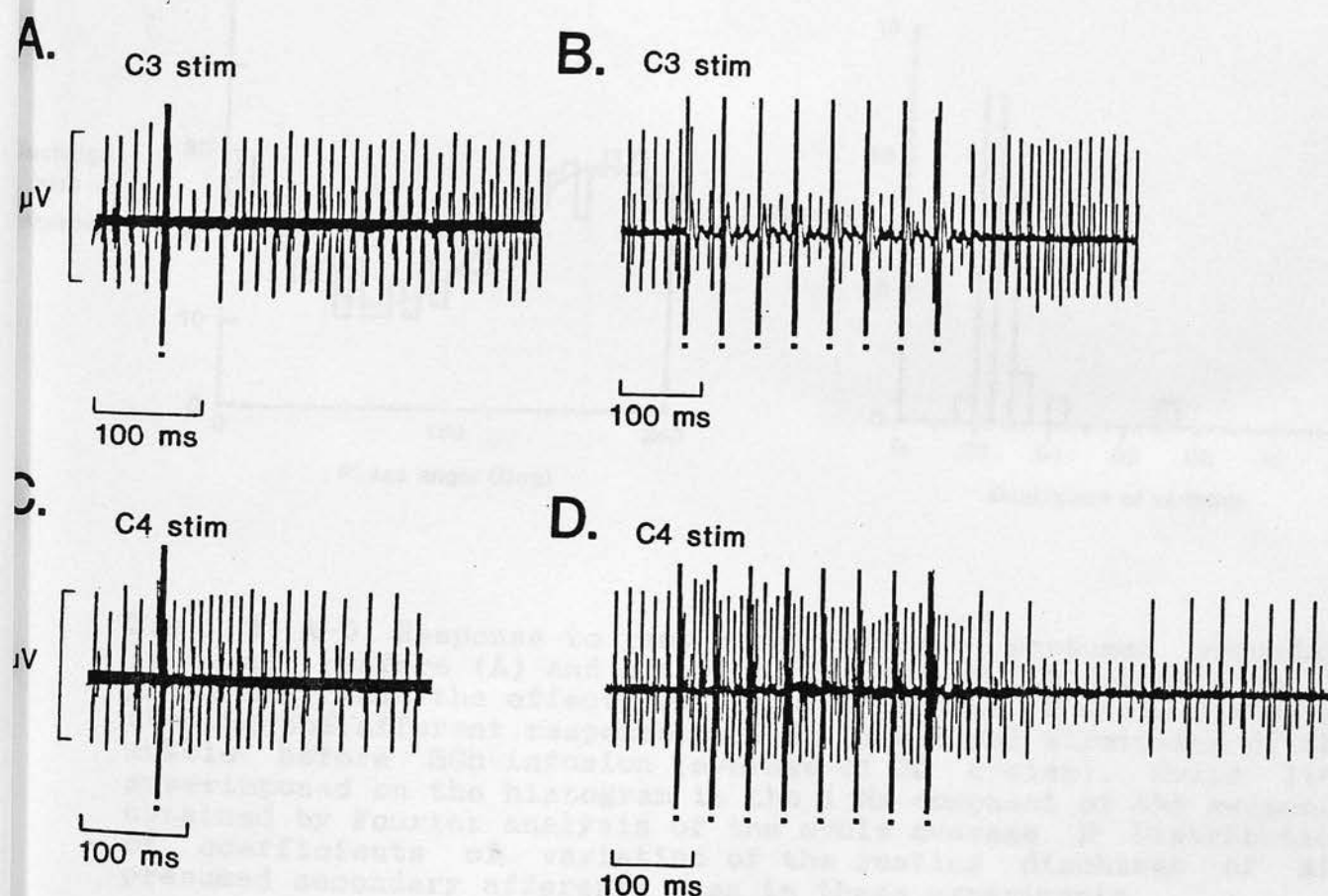
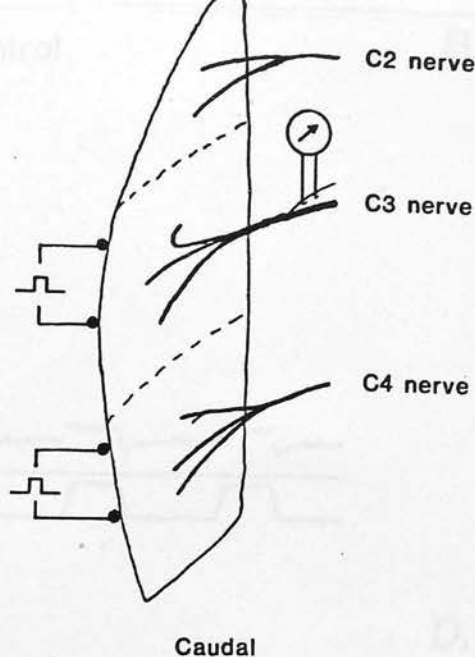


Fig. 8 A-D. Top: schematic representation of the first three compartments of biventer and their innervation by branches of the dorsal rami of the C2 and C3 spinal nerves. Broken lines indicate tendinous inscriptions delineating muscle compartments. **A,B** Response of a spindle afferent (larger unit) in a filament of the C3 nerve to a single stimulus (**A**) and a train of 8 stimuli at 27 Hz (**B**) applied to the C3 compartment. Note silencing of the resting discharge upon stimulation. **C,D** Excitation of the same spindle afferent by a single stimulus (**C**) and a train of 8 stimuli at 27 Hz (**D**) applied to the C4 compartment. Note increase in discharge frequency upon stimulation.

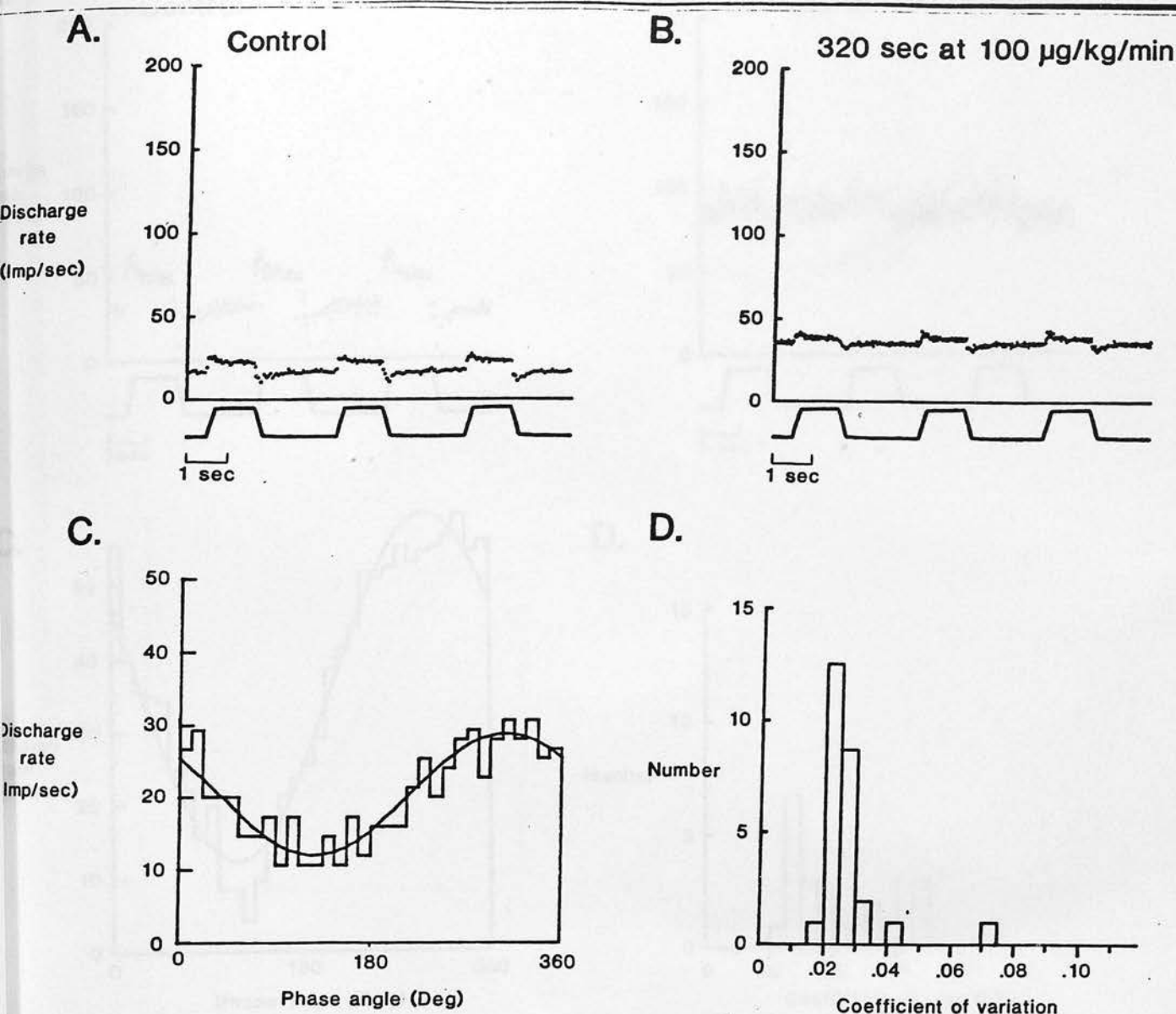


Fig. 9 A-D. Response to ramp stretches of a presumed secondary-afferent before (A) and after (B) 300 sec of SCH infusion at 100 $\mu\text{g/kg/min}$, when the effects of SCH were maximal. C Cycle histogram showing the afferent response to 1 Hz sinusoidal stretching of the muscle before SCH infusion (average of 30 cycles). Solid line superimposed on the histogram is the 1 Hz component of the response obtained by Fourier analysis of the cycle average. D Distribution of coefficients of variation of the resting discharge of all presumed secondary afferents seen in these experiments.

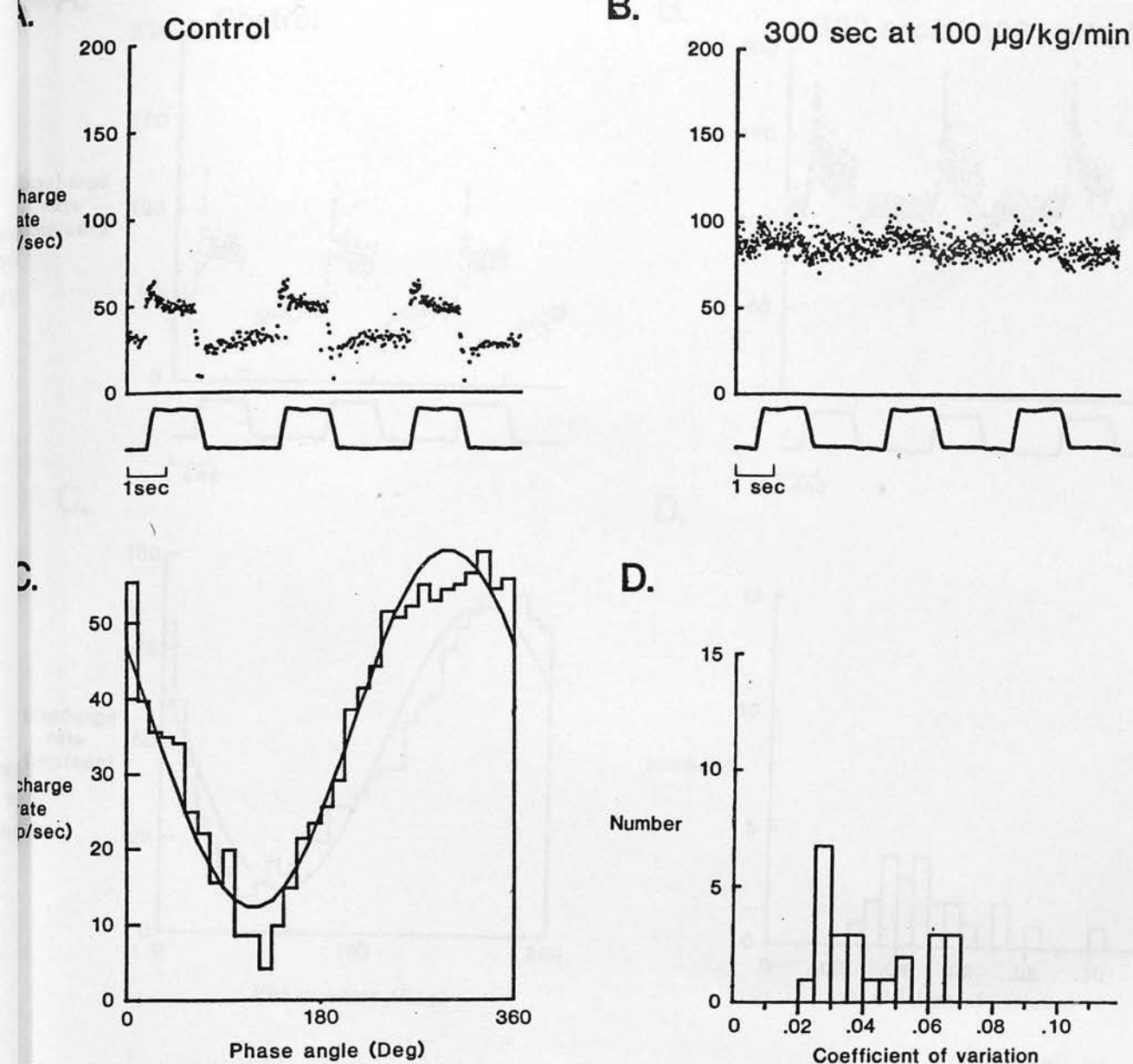


Fig. 10 A,B. Activation of a presumed b2c afferent by SCh infusion. C Cycle histogram of the afferent response to 1 Hz stretching (average of 50 cycles). D Distribution of coefficients of variation of presumed b2c afferents.

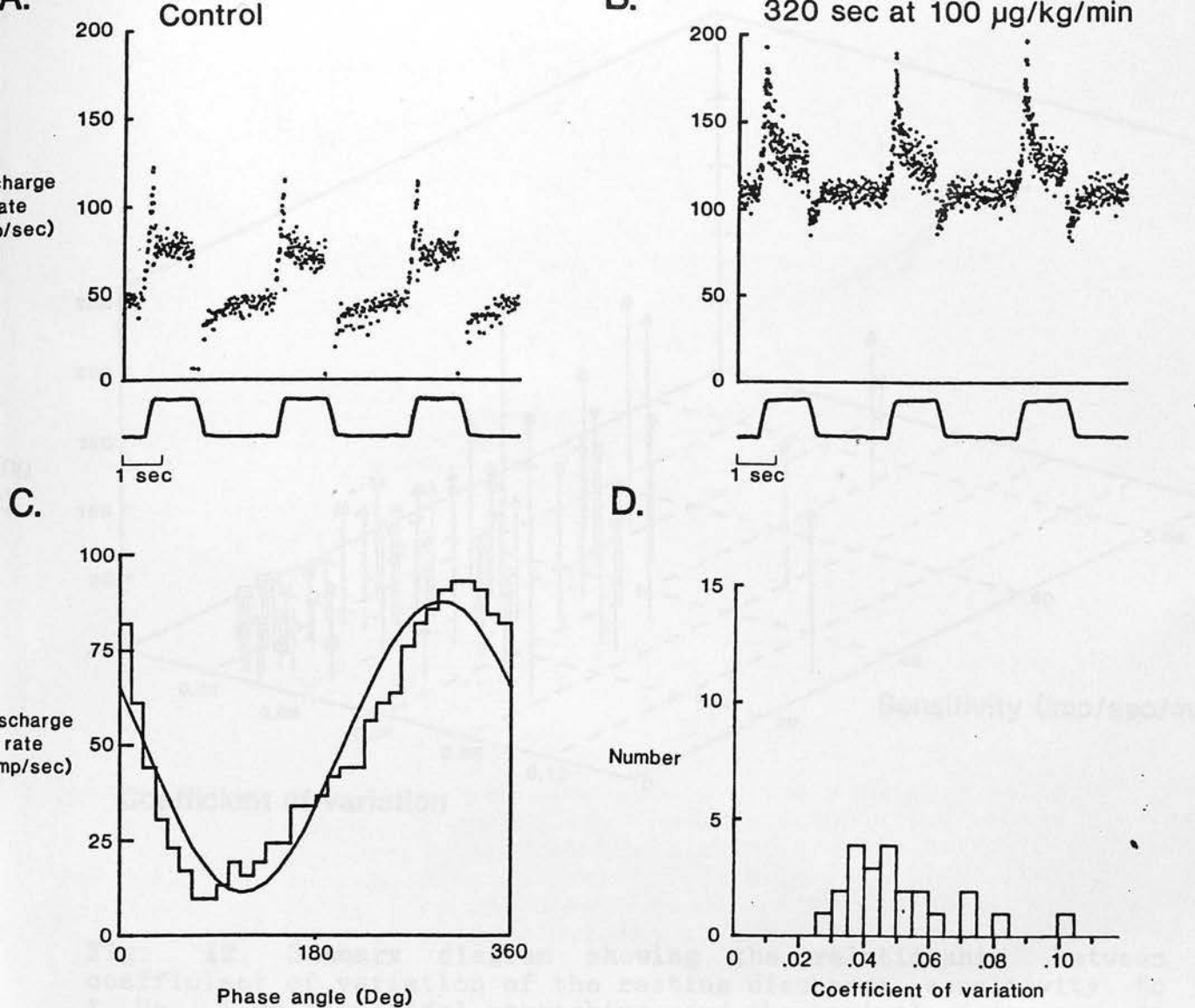


Fig. 11 A,B. Activation of a presumed blb2c afferent by SCh infusion. C Cycle histogram of the afferent response to 1 Hz stretching (average of 20 cycles). D Distribution of coefficients of variation of presumed blb2c afferents.

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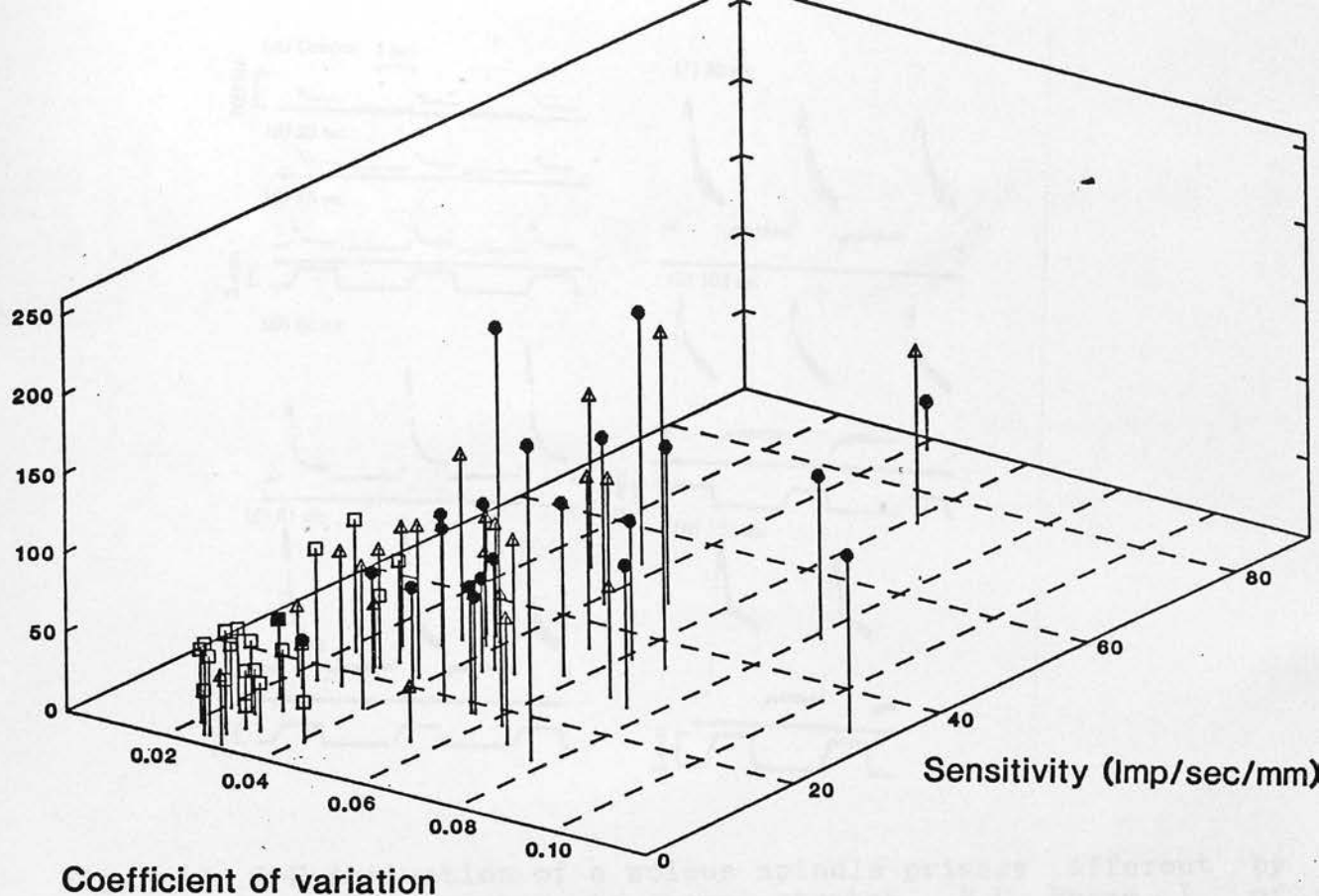


Fig. 12. Summary diagram showing the relationship between coefficient of variation of the resting discharge, sensitivity to 1 Hz, 1 mm sinusoidal stretching, and the maximal frequency to which afferents could be "driven" 1:1 by small-amplitude vibration, for 64 of the 77 biventer units studied in detail. Squares, secondary afferents; triangles, b2c afferents; filled circles, b1b2c primaries. Filled square "truly intermediate" afferent (see text).

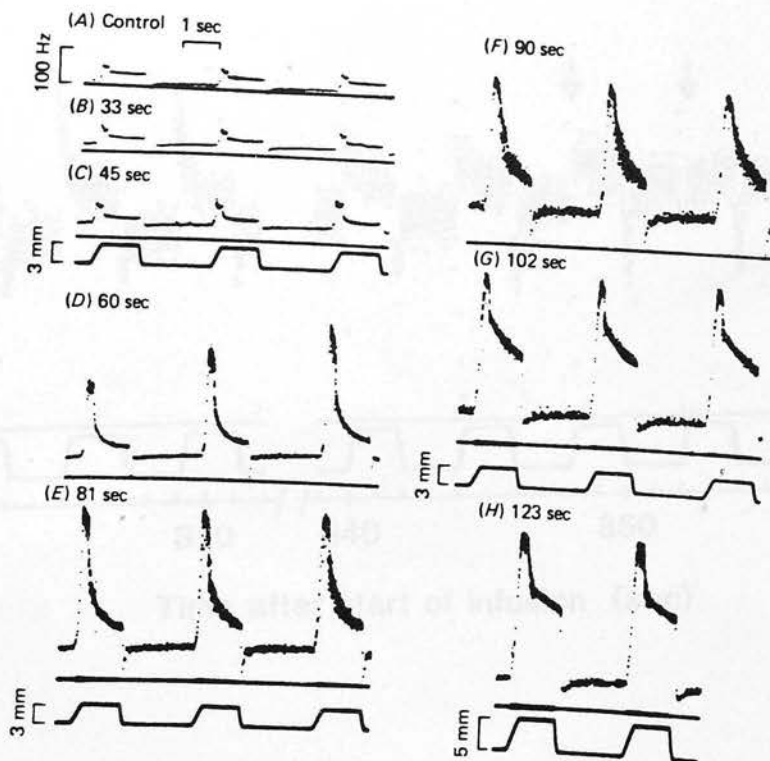


Fig. 13 A-H Activation of a soleus spindle primary afferent by SCh. A Control response to ramp stretch. B,C Phase I of activation, a small, gradual biasing of the discharge. D Onset of Phase II, a large rise in dynamic index. E Full-blown Phase II. F Onset of Phase III; discharge becoming more variable, and length sensitivity increasing. G,H Large increase in length sensitivity; full-blown Phase III.

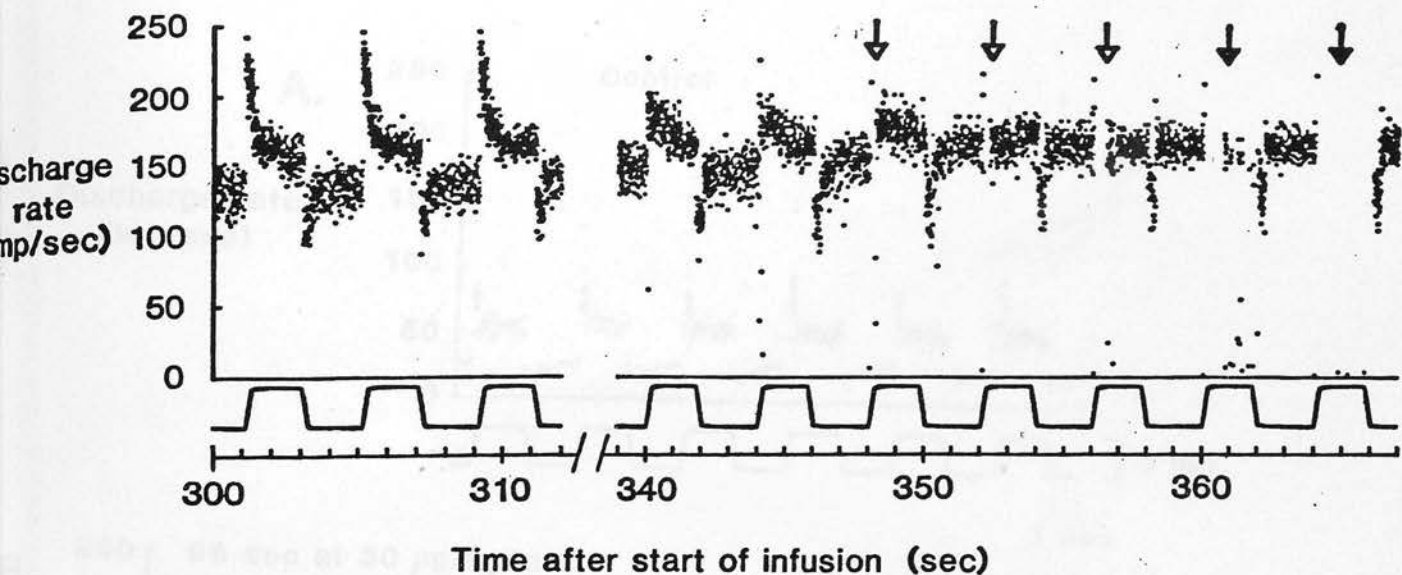


Fig. 14 Response of a presumed b1b2c afferent to a prolonged infusion of SCh at 200 $\mu\text{g/kg/min}$. First three stretches show the maximal activation of this afferent (cf. Fig. 11 B). The remaining stretches show the development of depolarising block (open arrows) which ultimately eliminates the response to stretch (filled arrows). Note absence of Phase III (cf. Fig. 13).

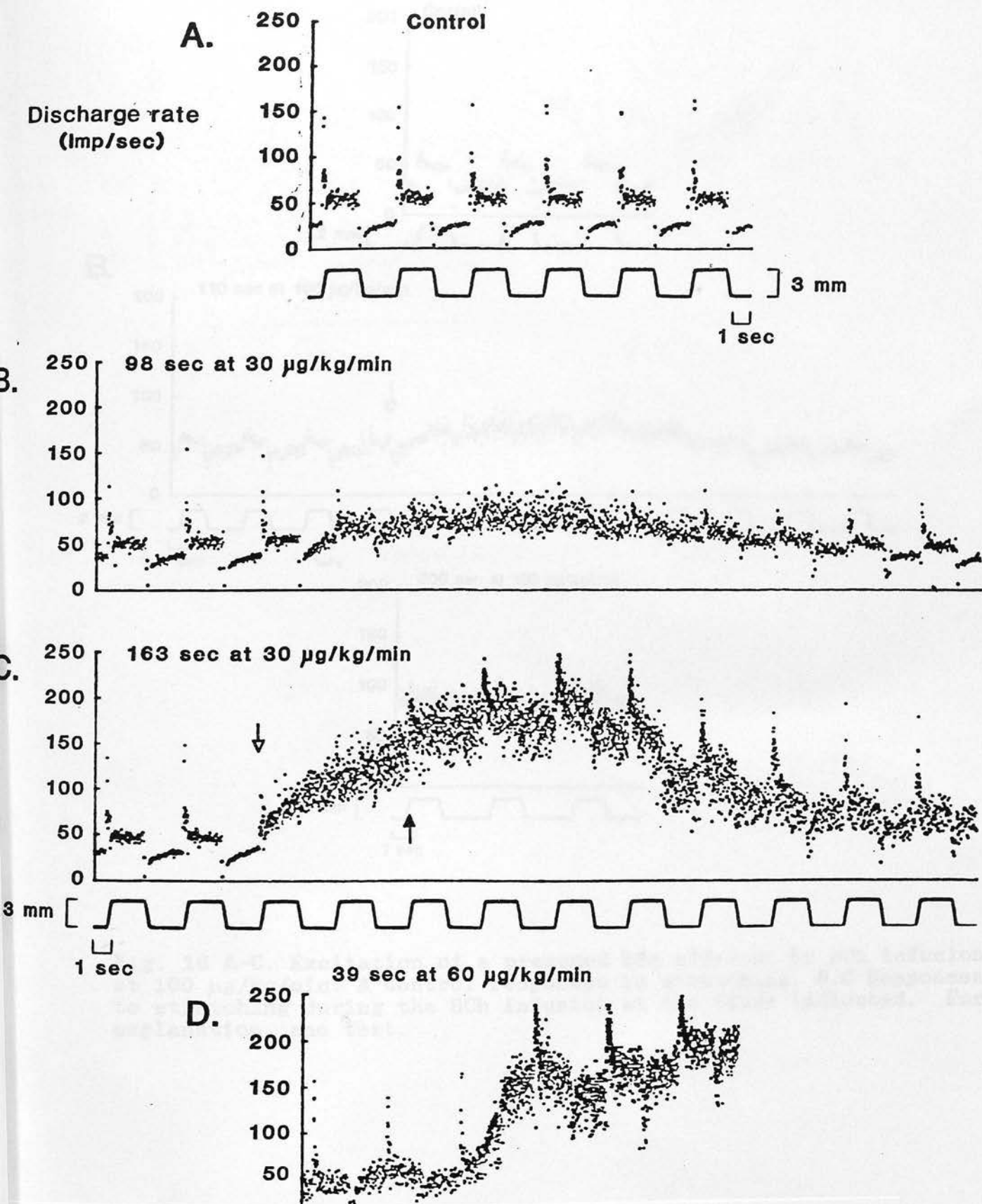


Fig. 15 A-D. Excitation of a presumed b1b2c afferent by SCh infusion at 30 $\mu\text{g/kg/min}$, subsequently stepped up to 60 $\mu\text{g/kg/min}$. **A** Control responses to stretching. **B-D** Responses to stretching at successive times during the SCh infusion as indicated. The last stretches in **D** show the maximally activated response. For explanation, see text.

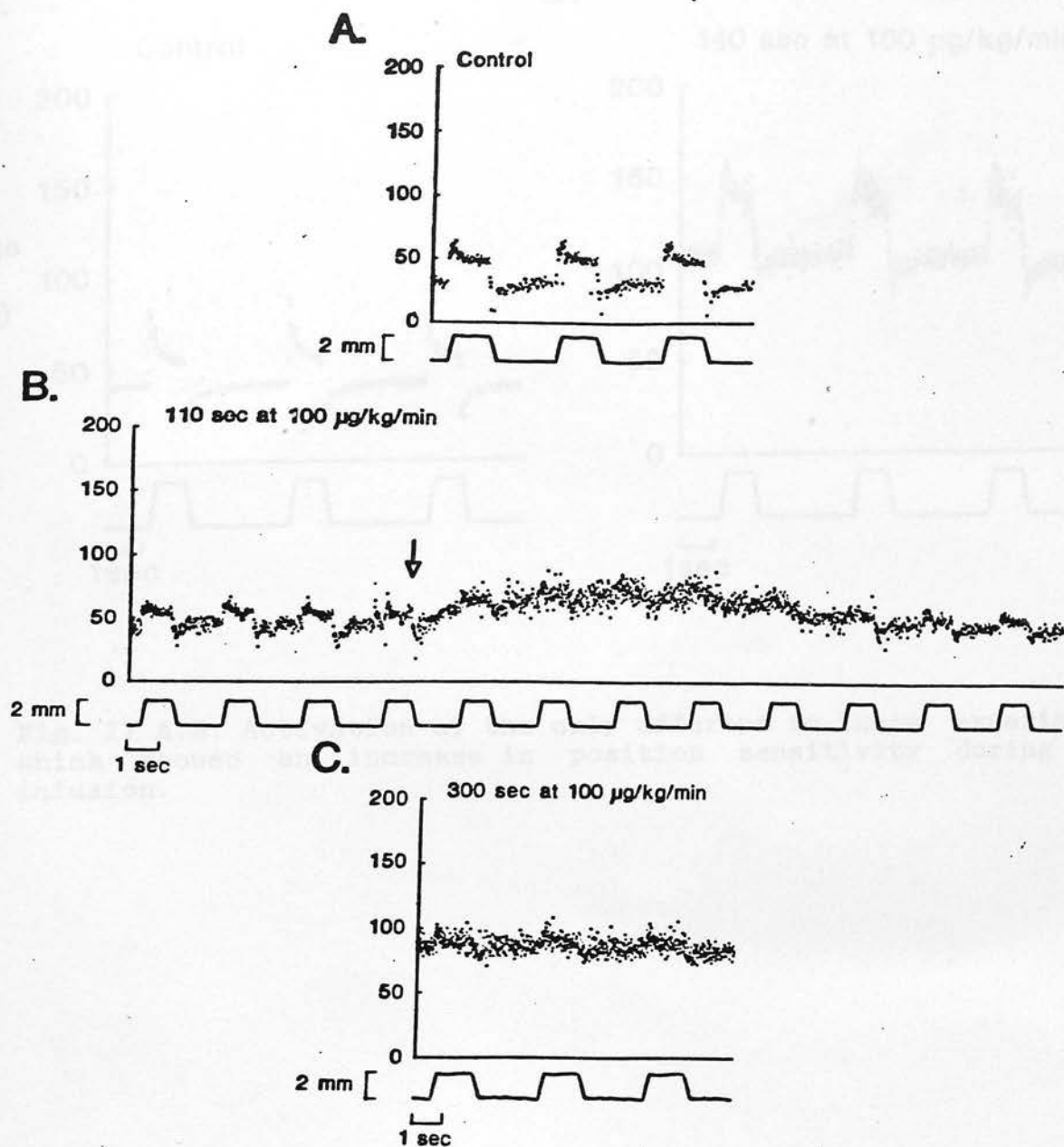
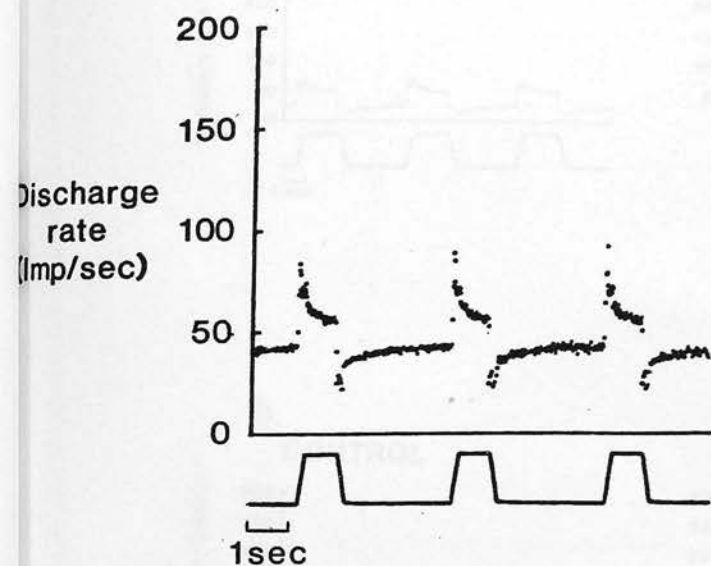


Fig. 16 A-C. Excitation of a presumed b2c afferent by SCh infusion at 100 $\mu\text{g/kg/min}$. **A** Control responses to stretching. **B,C** Responses to stretching during the SCh infusion at the times indicated. For explanation, see text.

A.

Control



B.

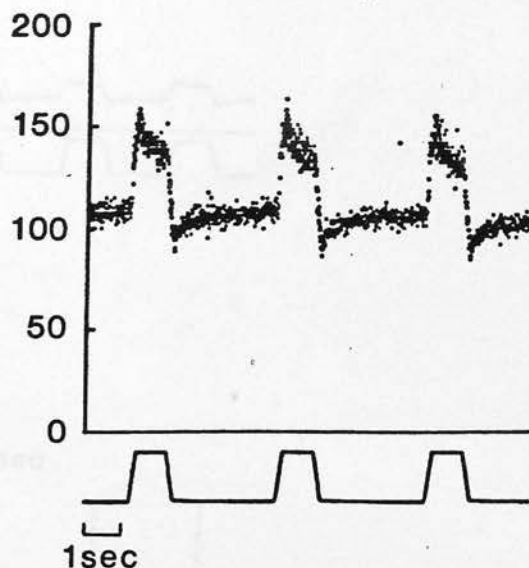
140 sec at 100 $\mu\text{g/kg/min}$ 

Fig. 17 A,B. Activation of the only afferent in these experiments which showed an increase in position sensitivity during SCh infusion.



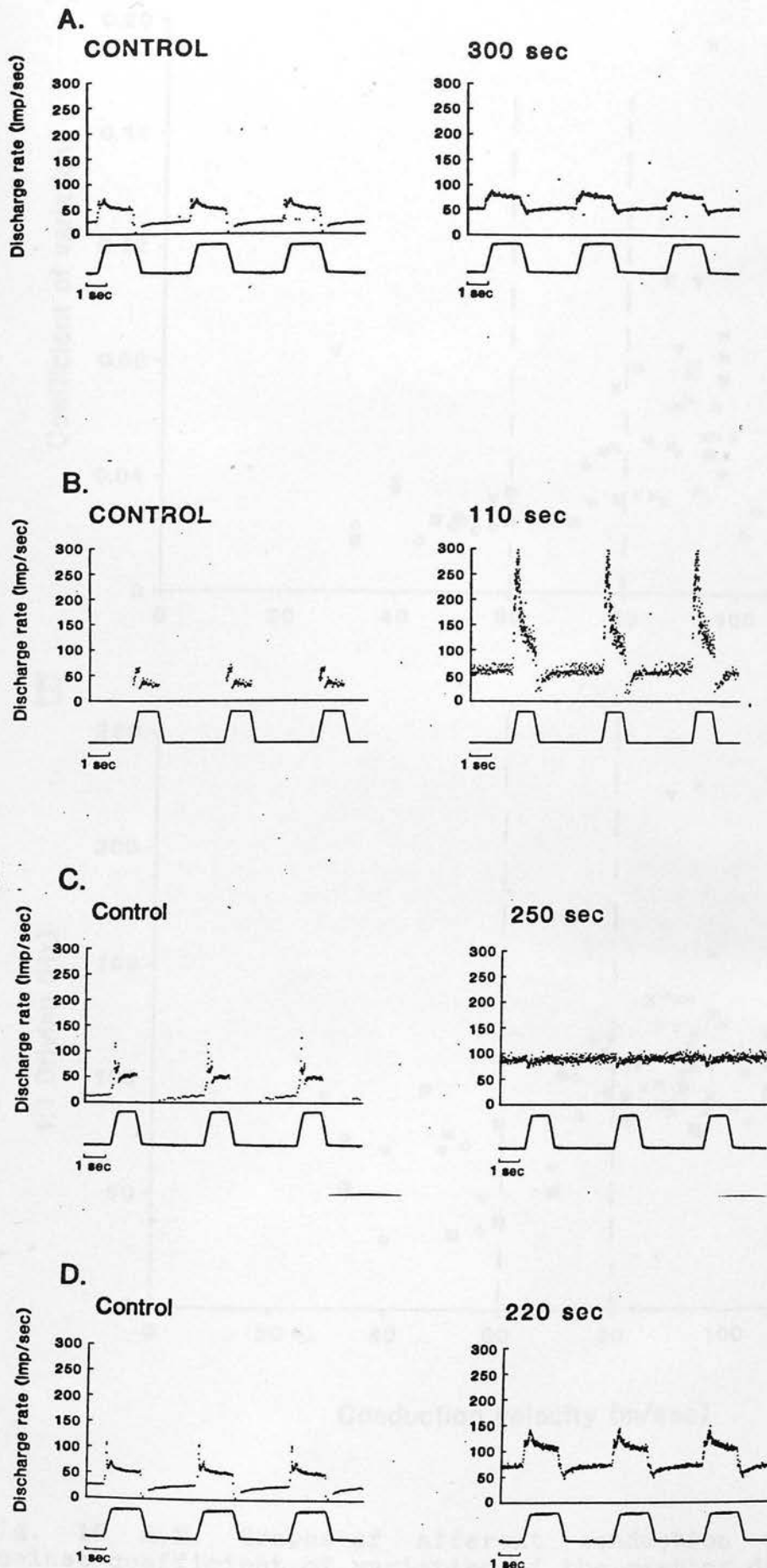


Fig. 18 A-D. Examples of the four patterns of activation by SCH infusion at 100 $\mu\text{g/kg/min}$ seen in the medial gastrocnemius experiments. **A** Activation of a secondary afferent. **B** Activation of a blb2c afferent. **C** Activation of a b2c afferent. **D** Activation of a "truly intermediate" afferent.

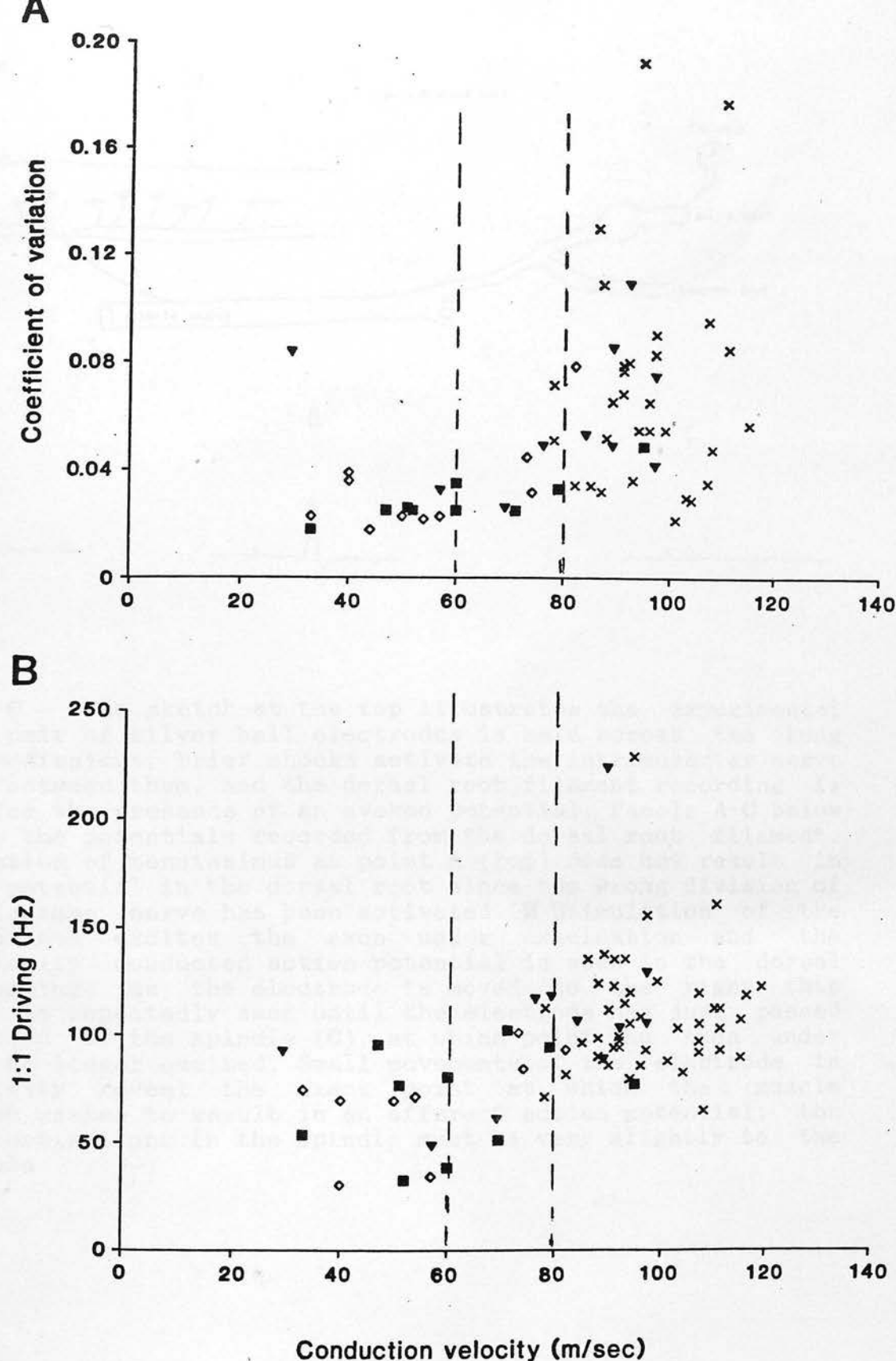


Fig. 19 A,B. Graphs of afferent conduction velocity plotted against coefficient of variation of the resting discharge (**A**) and frequency to which the afferent was driven 1:1 by small-amplitude vibration (**B**). Dotted lines delimit three ranges of conduction velocity (see text). Diamonds, secondaries; crosses, blb2c primaries; filled triangles, b2c afferents; filled squares, "truly intermediate" afferents.

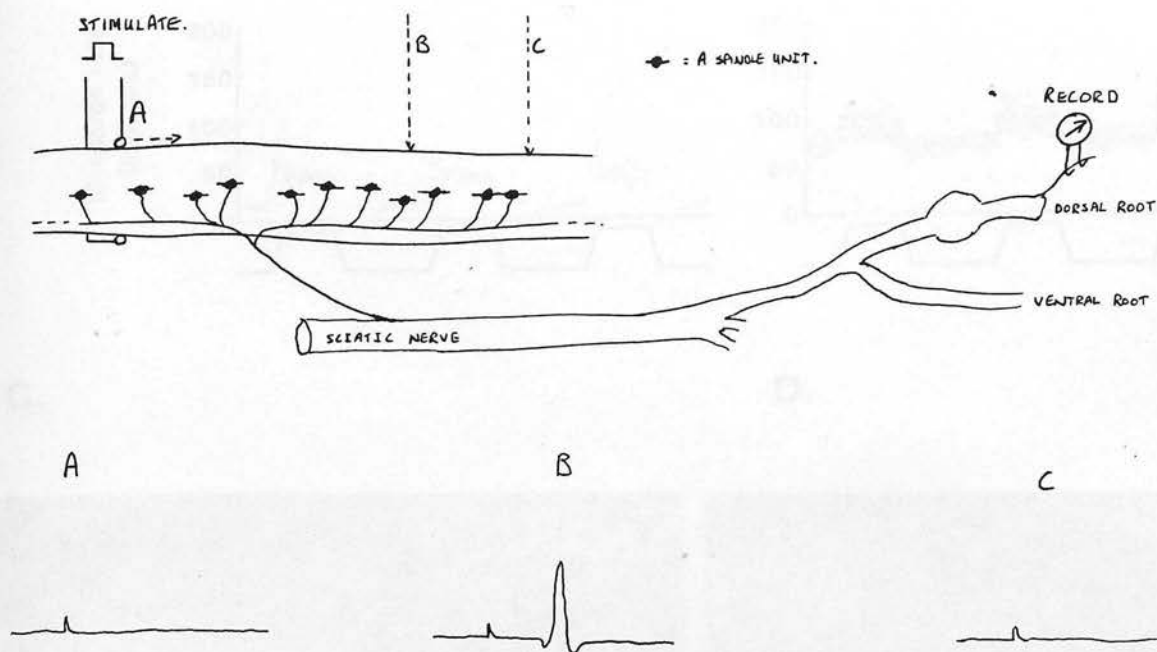
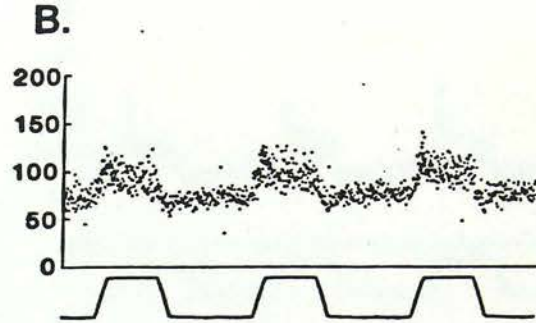
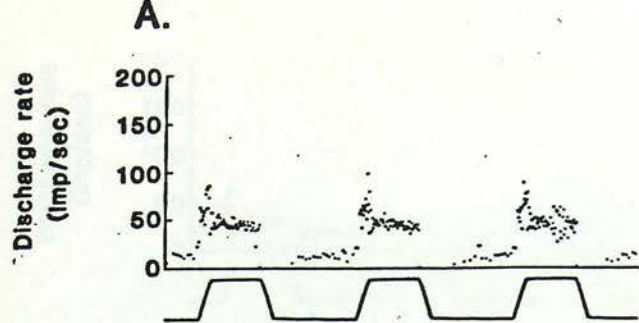


Fig. 20 A-C The sketch at the top illustrates the experimental setup. A pair of silver ball electrodes is held across the long axis of tenuissimus, brief shocks activate the intramuscular nerve branches between them, and the dorsal root filament recording is examined for the presence of an evoked potential. Panels A-C below illustrate the potentials recorded from the dorsal root filament. **A** Stimulation of tenuissimus at point A (top) does not result in an evoked potential in the dorsal root since the wrong division of the tenuissimus nerve has been activated. **B** Stimulation of the correct branch excites the axon under examination and the orthodromically conducted action potential is seen in the dorsal root recording; as the electrode is moved to the right this potential is repeatedly seen until the electrode has just passed the location of the spindle (**C**), at which point the axon under study is no longer excited. Small movements of the electrode in this vicinity reveal the exact point at which the muscle stimulation ceases to result in an afferent action potential; the afferent terminations in the spindle must be very slightly to the left of this.



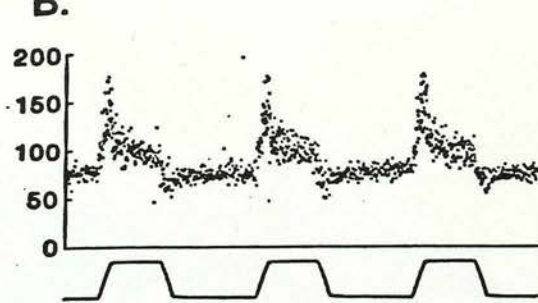
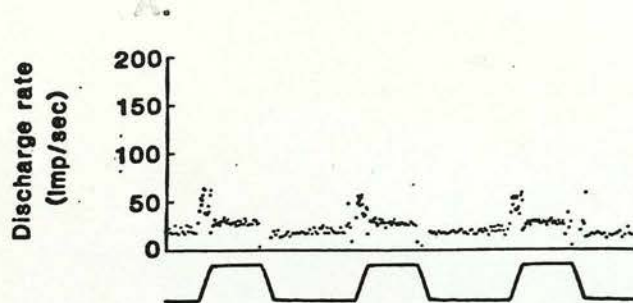
C.



D.



Fig. 21 A-D Correlated morphology and electrophysiology of a b2c unit. A,B Excitation of the afferent by topical SCh (100 $\mu\text{g/ml}$). C Transverse section of the muscle near the presumed termination point of the afferent; H&E stain showing one large and four small intrafusal muscle fibres. D 250 μm further along, at the equatorial sensory region of the spindle, one bag of three nuclei and four individual nuclei visible.



C.



D.

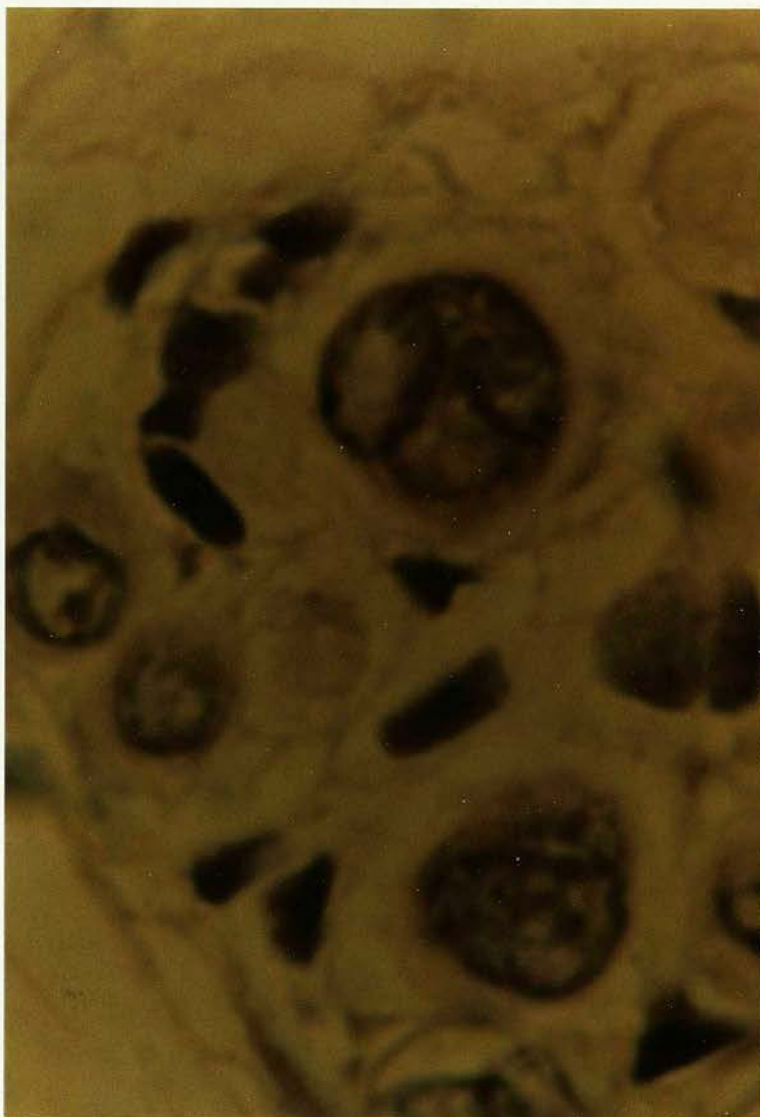


Fig. 22 A-D Correlated morphology and electrophysiology of a b1b2c unit. A,B Excitation of the afferent by topical SCh (100 $\mu\text{g}/\text{ml}$). C Transverse section of the muscle near the presumed termination of the afferent, showing two large and five small intrafusal fibres. D 360 μm further on, two bags of nuclei and a number of individual nuclei.