THE USE OF SUCCINYLCHOLINE IN THE IDENTIFICATION AND CHARACTERISATION OF AFFERENT AXONS FROM TANDEM MUSCLE SPINDLES

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ABSTRACT

Classically, muscle spindles are described as single encapsulated sensory receptors which lie in the belly of most skeletal muscles. They contain three types of specialised intrafusal muscle fibre to which sensory and motor innervation is distributed in a characteristic fashion; sensory innervation is of two main types - primary and secondary - which differ in their distribution to the intrafusal muscle fibres. The study of these spindles has led to a high level of understanding of the relationship between structure and function in the muscle spindle. However, in recent years, a variant of the classical muscle spindle, the **tandem spindle**, has become a focus of attention since some of its encapsulations have been found to contain only two of the three types of intrafusal fibre seen in classical spindles. This has led to considerable speculation as to the functional properties of the primary afferents innervating these encapsulations. Previous attempts to examine such afferents have been unsuccessful, due to the absence of a reliable means of differentiating primary afferents from single and tandem muscle spindles. The drug succinylcholine (SCh) is known to produce contracture in two of the three classes of intrafusal muscle fibre whilst paralysing the third. Since the effects on afferent stretch sensitivity of contraction in each class of intrafusal muscle fibre are known, SCh can be used to assess the pattern of intrafusal termination of afferents studied electrophysiologically. This approach has previously been used to differentiate primary afferents from secondaries when alternative means were not applicable, but the aim of the present experiments has been to extend this work to allow the differentiation of primary afferents from classical spindle encapsulations (which have the full complement of intrafusal muscle fibres) and those from tandem encapsulations which lack a bag_1 fibre which would otherwise be activated by SCh.

Three sets of experiments were performed. In the first, afferents from the cat neck extensor muscle biventer cervicis were studied, since this muscle is known to be particularly rich in tandem spindles. Four types of afferent response to

intraarterial infusions of SCh were identified, three corresponding to the behaviour previously reported for hindlimb muscle spindle afferents. The fourth had not previously been seen, and showed features which indicated that the afferents activated by SCh in this way were probably the b_2c primary afferents arising in tandem muscle spindles. Other evidence in the form of the passive properties of these afferents was adduced to support this diagnosis. In the second series of experiments using the medial gastrocnemius muscle of the hindlimb, four patterns of SCh activation were again seen for muscle spindle afferents; these were all very similar to the patterns seen for biventer afferents, including that presumed to belong to b_2c primary afferents from tandem spindles. Additional evidence that b_2c primary afferents from tandem muscle spindles had been correctly identified came from the measurement of their conduction velocities, which indicated that the majority of presumed b_2c primary afferents from tandem muscle spindles had conduction velocities which overlapped with those of slower b_1b_2c primary afferents from classical encapsulations, as was expected on histological grounds. In the final series of experiments, the spindles of origin of afferents from the tenuissimus muscle which had been studied electrophysiologically were located in the muscle, excised and studied histologically in serial transverse sections. Seven afferents were diagnosed by SCh as primaries arising in classical muscle spindles, and the histological reconstruction indicated that their parent spindles indeed contained all three intrafusal muscle fibre types. In contrast, the single afferent diagnosed by SCh as a b_2c primary arising in a tandem muscle spindle was subsequently found to innervate an encapsulation of a tandem spindle containing only two types of intrafusal muscle fibre. The histological evidence thus supports the SCh-based classification of primary afferents, and in particular indicates that b_2c primary afferents from tandem muscle spindles can be readily identified during electrophysiological experiments.

Contrary to expectations, the functional characteristics of b_2c primary afferents from tandem spindles were not markedly different from those of classical

primary afferents. In particular, b_2c primary afferents from tandem muscle spindles were no less sensitive to dynamic components of a muscle stretch than were primary afferents from classical spindles, which suggests that, at least in the de-efferented muscle spindle, the intrafusal bag_1 fibre, which is absent from tandem spindles, cannot be the sole determinant of dynamic sensitivity as was classically described. Dynamic sensitivity in the de-efferented spindle may instead result from electrical adaptation in the sensory spirals of primary endings, whether or not they terminate upon a bag_1 fibre, though in the presence of fusimotor activity changes in the properties of bag_1 fibres certainly become predominant and give rise to the high dynamic sensitivity seen for b_1b_2c primary afferents from classical muscle spindles but not for b_2c primary afferents from tandem spindles (this is the basis of the SCh test). The functional characteristics of all four types of afferent identified by SCh were similar in the biventer and medial gastrocnemius experiments, and were also comparable to previously reported values. The findings of the present work indicate that a functional rôle for b_2c primary afferents from tandem spindles should not be sought at the receptor level. Rather, the information they provide to the CNS must be examined in the context of natural movements occurring in the presence of fusimotor activity which brings out differences in behaviour of b_1b_2c and b_2c primary afferents. The way is open for others to investigate this topic, since b_2c primary afferents can now be readily identified.

SUMMARY

(1). Changes in the responses to ramp-and-hold muscle stretching of muscle spindle afferents during exposure to the drug succinylcholine (SCh) have been examined in neck and hindlimb muscles of the anaesthetised cat. These changes were examined in the context of the known effects on intrafusal muscle fibres of cholinomimetic drugs (Gladden 1976; Boyd 1985a) and of the known effects of mechanical activity in the various intrafusal fibres on the discharge of primary and secondary spindle afferents (e.g. Boyd, Gladden, McWilliam & Ward 1977; Boyd 1981b, 1986). The intrafusal distribution of the afferents' sensory terminals could then be deduced, a facility which was used to particular effect to identify "b_2c primary afferents" from units of tandem muscle spindles.

(2). The majority of afferents were further investigated with a battery of tests, each of which had previously been employed by other workers to characterise muscle spindle afferents. The parameters measured for each afferent were: the coefficient of variation of its resting discharge, its sensitivity to sinusoidal muscle stretching and its sensitivity to longitudinal vibration of the muscle. In some cases, dynamic indices in response to standard ramp stretches were also measured. Two useful comparisons could then be made, namely that between the properties of neck and hindlimb muscle spindle afferents and that between the properties of b_2c primary and other afferents.

(3). In the initial series of experiments on the biventer cervicis muscle of the neck, four patterns of afferent response to infusions of SCh were described. About 1/3 of the afferents were rapidly and powerfully excited by SCh in two phases, showing large increases in their discharge rate between stretches ("biassing") in Phase I, followed by 2-3 fold increases in their dynamic indices in Phase II of activation. Generally, their position sensitivities were reduced by 20-80% over the course of the infusion. These changes were interpreted as reflecting the effects upon a primary afferent of combined contracture of the nuclear bag_2 fibre (producing biassing and reduced position sensitivity; Boyd 1981b, 1986) and the nuclear bag_1 fibre (increasing

dynamic index), and such afferents were considered to be "b_1b_2c primaries".

(4). A further 1/3 of the biventer afferents were gradually and weakly activated by SCh infusion, showing only a small degree of biassing, with either unchanged or slightly reduced dynamic indices and length sensitivities. Since other afferents in the same experiment were powerfully excited by the same dose of SCh, this response to SCh indicated that nuclear bag fibres did not substantially influence the discharge of these afferents, and they were therefore identified as secondary afferents with sensory terminals mainly on nuclear chain fibres in the juxta-equatorial region; soleus spindle afferents classified by conduction velocity as secondaries were activated by SCh in a very similar manner (Dutia 1980).

(5). A single afferent was encountered which behaved initially like the presumed secondary afferents during SCh infusion. However, this afferent subsequently exhibited a substantial increase in its length sensitivity which was typical neither of b_1b_2c primary nor of secondary afferents. Instead, the response of this unit to ramp stretching when fully activated was very similar to that of a small number of "truly intermediate" afferents described by Dutia (1980) in the course of similar experiments on the soleus muscle. The behaviour of this afferent and its passive properties both indicated that it was likely to be a secondary afferent, possibly with a significant input from the static bag_2 fibre to its discharge.

(6). The remainder of the biventer afferents, over 1/3 of the total sample, exhibited a different and previously unobserved pattern of excitation by SCh. In these afferents, a large and rapid biassing of the discharge similar in magnitude and time course to that of b_1b_2c primary afferents was accompanied by an increase in the variability of the afferent discharge, and by a substantial reduction in the length sensitivity, sometimes to zero; the dynamic index was never increased, rather it was usually nearly abolished. These changes were interpreted as the effects on a primary afferent of contraction of the static nuclear bag fibre alone (Boyd 1981b;

Boyd 1986); the absence of a bag_1 fibre influence on the discharge of these presumed primary afferents was taken to mean that the parent spindles lacked a bag_1 fibre, since other afferents in the same experiment were readily identified as b_1b_2c primaries. The afferents activated by SCh in this manner were therefore presumed to be b_2c primary afferents.

(7). In a previous study of the activation of b_1b_2c primary afferents by SCh (Dutia 1980) it was reported that activation proceeded in three clearly distinguishable phases, whereas in the present experiments only two phases were identified. In particular, in the present experiments there was no late increase in afferent length sensitivity (Phase III of Dutia 1980) which Dutia had attributed to the delayed contracture of the less SCh-sensitive bag_2 fibre; this could possibly have been interpreted as evidence that the bag_2 fibre had not been recruited by the standard SCh infusion rate of 100 μg/kg/min used in the present experiments. However, the use of larger doses of SCh (up to 500 μg/kg/min) failed to reveal Phase III-like behaviour in any b_1b_2c primary afferent, despite activation of some to the point where they exhibited depolarisation block (Kidd & Vaillant 1974). In a different approach to this problem, the dose of SCh was increased in steps from sub-threshold levels to a dose of around 200 μg/kg/min in an attempt to identify the separate recruitment of the bag_1 and bag_2 fibres. On a number of occasions, events reflecting such separate recruitments were indeed seen, and indicated, surprisingly (c.f. Gladden 1976), that the SCh sensitivity of the bag_2 fibre in neck muscle spindles may actually be somewhat greater than that of the bag_1 fibre. The less sensitive bag_1 fibre was generally recruited by infusions of around 60 μg/kg/min, indicating that the standard 100 μg/kg/min dose of SCh would have been sufficient to fully activate both types of nuclear bag fibre.

(8). The values of the passive properties of biventer spindle afferents studied in the present experiments revealed a number of points. Firstly, the range of values

observed for each of the parameters was very similar to that reported by others for hindlimb muscle spindle afferents; these findings complement those of Richmond & Abrahams (1979a) who studied a different set of parameters of neck spindle afferent sensitivity. Secondly, for each parameter of spindle afferent sensitivity studied, the values were found to form a smooth continuum, with no evidence of clustering of values which could be used *a priori* to differentiate the various types of spindle afferent axon; attempts to reveal some such clustering by plotting all three parameters against each other in a three-axis plot were unrewarding. However, when this three-axis plot was reexamined whilst taking into account the pattern of SCh activation of each afferent, clustering did become apparent; this is analogous to considering a similar continuum of values in a hindlimb experiment and breaking it down by taking into account afferent conduction velocities (e.g. Matthews 1963). Thus, the presumed secondary afferents were grouped in one quadrant of the plot with low coefficients of variation of their resting discharge, low sensitivities to sinusoidal muscle stretch and low sensitivities to muscle vibration, all of which were in keeping with the values reported for their hindlimb counterparts. The b_1b_2c primary afferents formed a diffuse population with higher values for all the parameters, similar to those reported for hindlimb primary afferents. Finally the properties of b_2c primary afferents spanned a very similar range to those of b_1b_2c primary afferents, which explains the inability of Banks, Ellaway & Scott (1980) to differentiate b_1b_2c and b_2c primary afferents from peroneal muscles on the basis of their mechanical sensitivities alone.

(9). In similar experiments performed on the medial gastrocnemius muscle, four patterns of activation by SCh were again seen and were interpreted as above. Secondary and b_2c primary afferents behaved exactly as in biventer, though b_2c primaries were much rarer in the medial gastrocnemius experiments. Rather more secondary afferents with apparent bag fibre contact were encountered in the medial gastrocnemius than in biventer and of twelve, eleven were diagnosed as having a

substantial collateral termination upon the dynamic bag_1 fibre. The remaining afferent behaved like that seen in biventer and may have had collaterals on the static bag_2 fibre.

The majority of the medial gastrocnemius b_1b_2c primary afferents were activated in two Phases, as were the biventer b_1b_2c primaries. In Phase I, afferent discharge was biassed by SCh infusion and in Phase II it showed the very large increase in dynamic index which characterises b_1b_2c primary afferents; changes in position sensitivity, however, varied considerably from only small increases to very large increases typical of Phase III as defined by Dutia (1980) in a few instances. None of the afferents, however, showed the reduction in position sensitivity which was typical of b_1b_2c primary afferents from biventer. In only 2 afferents was there a clear Phase III of activation during which the position sensitivity of the afferent increased appreciably. This variability was found within as well as between experiments, and persisted even when higher than standard doses of SCh were used, suggesting that it was not caused by inadequate access of the drug to some spindles. Instead, it may reflect differences between Ia afferents in the mode of interaction of the signals generated in the Ia terminal branches supplying the dynamic and static nuclear bag fibres to determine the final primary afferent discharge.

(10). In the experiments using medial gastrocnemius, it was possible to measure the afferent conduction velocity in addition to the other afferent properties measured during the biventer experiments. This in turn allowed the classification of afferents on the basis of conduction velocity to be compared to the classification of the same population on the basis of response to SCh. The majority of afferents classified as secondary by their response to SCh infusion would also have been so classified by virtue of a conduction velocity below 60 m/sec and, as in the biventer experiments, these afferents had sensory properties in accord with previous reports for hindlimb secondary afferents. Similarly, the vast majority of b_1b_2c primary afferents had, as expected, conduction velocities greater than 80 m/sec,

with appropriate sensory properties. About half of the secondary afferents with bag fibre contact identified by SCh conducted impulses at less than 60 m/sec, and most of the rest at 60-80m/sec; in addition, their sensory properties were closer to those of other secondary afferents than to those of b_1b_2c primary afferents, both these observations supporting their identification by SCh. Finally, over half of the b_2c primary afferents conducted at over 80 m/sec and another quarter at 60-80 m/sec; this range of conduction velocities matches very well with the range expected of b_2c primary afferents on the basis of their known afferent diameters. The sensory properties of presumed b_2c primary afferents tended to overlap with those of b_1b_2c primary afferents more than with those of secondary afferents, again lending support to their identification by SCh. The caution exhibited in the past with regard to the classification of afferents conducting at 60-80 m/sec (see Matthews 1972) was shown to be amply justified, since all four classes of afferent which could be identified by SCh infusion were equally represented in this subpopulation of the sample.

(11). In a final series of experiments performed on the tenuissimus muscle, an attempt was made to demonstrate histologically that b_2c primary afferents indeed originate in spindle units containing only one nuclear bag fibre. The location in the muscle of the capsule of origin of selected spindle afferents was identified electrophysiologically and marked, and a block of muscle containing the parent spindle was subsequently excised, sectioned and stained with Haematoxylin & Eosin (H&E); muscle spindles are readily identified in H&E stained sections, and the intrafusal fibres can be differentiated into bag and chain types on the basis of fibre diameter and nuclear arrangement in their equatorial regions. In this way, the parent spindle of one afferent, characterised by its pattern of SCh excitation as a b_2c primary afferent, was shown to contain only one nuclear bag fibre; this bag fibre was followed in serial sections into a second spindle unit at the far end of the excised block of tissue, suggesting that the parent one-bag spindle was part of a

tandem linkage. Although enzyme histochemistry was not performed to confirm it, this single-bag spindle was almost certainly a b_2c spindle unit; b_1c single-bag spindles are exceedingly rare, and none has ever been reported to make up part of a tandem spindle (Bakker & Richmond 1981; Banks, Barker & Stacey 1982; Kucera 1982b; Richmond, Bakker, Bakker & Stacey 1986). Seven b_1b_2c primary afferents were also traced to their parent spindles which all proved to contain at least two nuclear bag fibres.

(12). The similarity between the sensory properties of b_2c primary afferents and those of b_1b_2c primary afferents in both neck and hindlimb muscles was a somewhat surprising finding, since the absence of a bag_1 fibre influence on the discharge of b_2c primary afferents might have been expected to demonstrably reduce their dynamic sensitivity compared to that of b_1b_2c primaries; indeed candidate b_2c primary afferents have been selected on this basis in the past (e.g. Richmond & Abrahams 1979a). This finding indicates that it is an oversimplification to ascribe the dynamic sensitivity of spindle primary afferents to the visco-elastic properties of the bag_1 fibre alone, at least in the de-efferented spindle. Instead, it must be assumed that in this situation either the bag_2 fibre, or chain fibres, or both, have significant viscosities to confer some dynamic sensitivity on Ia afferents, or else that some electrical property of the sensory terminals on these fibres can confer dynamic sensitivity. In the presence of dynamic fusimotor drive, the situation is altered by the well-documented enhancement of "creep" in the bag_1 fibre which presumably confers far greater dynamic sensitivity on Ia afferents than that due to these postulated alternative mechanisms. Given the similarity between the resting sensory properties of b_1b_2c and b_2c primary afferents, it appears that a functional role for b_2c primary afferents must be sought in the context of ongoing fusimotor activity. What this role might be is still a matter for debate, but the way is now free for further investigation of this topic.

ADDITIONAL PAPERS

Price RF & Dutia MB (1987a). Properties of cat neck muscle spindle afferents and their activation by succinylcholine (SCh). Neuroscience Letters S29: 122.

Price RF & Dutia MB (1987b). Properties of cat neck muscle spindles and their excitation by succinylcholine. Experimental Brain Research 68: 619-630.

Price RF & Dutia MB (1989a). Physiological properties of tandem muscle spindles in neck and hindlimb muscles. Progress in Brain Research 80: 47-56.

Price RF & Dutia MB (1989b). Functional characterisation of "intermediate" sensory endings in cat muscle spindles. Neuroscience Letters, In the press.

Dutia MB & Price RF (1990). Response to stretching of identified b_2c spindle afferents in the anaesthetised cat. J. Physiol. 420: 101P.

INTRODUCTION

The muscle spindle, a complex stretch receptor found in varying numbers in almost all mammalian skeletal muscles, has been the subject of intensive study for over a century. With the exception of the vestibulo-cochlear apparatus, it differs from most mechanoreceptors in that its sensitivity can be controlled by the central nervous system, a facility which doubtless contributes to its structural complexity. In spite of this complexity, understanding of the detailed functional properties of muscle spindle afferent axons and of the intrafusal mechanisms underlying these properties has proceeded apace over the past 30 years. In reaching the current position, however, some aspects of spindle anatomy and physiology have necessarily been less well studied. In particular, the vast majority of workers over the past century have studied cat hindlimb muscle spindles; when, more recently, a few groups have looked at spindles in muscles of other body regions such as the jaw, the neck and the tail, they have discovered substantial differences in spindle structure and innervation from which it has become apparent that not all aspects of hindlimb muscle spindle functioning will necessarily apply elsewhere. One such finding is that complex spindle aggregations are very much more common in axial muscles than they are in most hindlimb muscles, such that in some muscles the "typical" single encapsulated sensory ending of hindlimb muscles is in the minority (see e.g. Bakker & Richmond 1981). Of especial interest in this respect is the structure named the "tandem spindle" by Cooper & Daniel (1956).

The muscle spindle consists of a bundle of specialised intrafusal muscle fibres invested in the middle by a connective tissue capsule which is pierced by motor and sensory axons running to innervate the intrafusal fibres. Although the majority of hindlimb muscle spindles contain only one encapsulation, it has long been recognised that a small number of spindles exist with two or more capsules spaced out along a single intrafusal bundle, with some of the intrafusal fibres being common to both. This structure is the tandem spindle. Because their incidence is

generally rather low, tandem spindles have not received much attention in the past. However, in recent years there has been considerable interest in the tandem spindle since it has been shown that at least one of the linked capsules of a tandem spindle, namely the "b_2c spindle unit" (Banks, Barker & Stacey 1982), consistently lacks one of the three types of intrafusal muscle fibre namely the dynamic nuclear bag fibre or bag_1 fibre (Bakker & Richmond 1981; Kucera 1982b; Banks, Barker & Stacey 1982). This finding has led to considerable speculation as to the functional properties of the Ia afferent from b_2c spindle units, but attempts to study these afferents electrophysiologically have so far been unsuccessful due to the absence of a reliable means of identifying them during an experiment. The primary aim of the present work has therefore been to develop a means of identifying b_2c primary afferents, initially in dorsal neck extensor muscles since they are particularly rich in tandem spindles. Once this was achieved, the functional properties of b_2c primary afferents were open to study and could be compared to those of single spindles in a number of muscles. A secondary aim of this work has been to determine to what extent the functional characteristics of de-efferented neck muscle spindle afferents resemble those previously reported for hindlimb spindle afferents.

The work described in this thesis was carried out by myself, under the supervision of Dr. M. B. Dutia in the Department of Physiology, University of Edinburgh between October 1985 and October 1988. Some of the results reported here have been published (Price & Dutia 1987a,b; Price & Dutia 1989a,b).

R. Price

LITERATURE REVIEW, PART 1

The structure and function of typical mammalian hindlimb muscle spindles.

In recent years, there have been many excellent reviews of the progress made in understanding the muscle spindle (e.g. Matthews 1964,1972,1981a,b; Barker 1974; Boyd 1981a,c; Boyd & Smith 1984; Hulliger 1984). Of the various structures employed by these reviewers in detailing the complex interactions between anatomical and physiological studies, I find the most readily understandable to be that of Matthews (1981a) and have therefore adopted it here. Matthews divides the evolution of our knowledge of the spindle into three eras, namely Landmarks (1860-1960), Advances (1960-1970) and Consolidation (1970-1980); these will be considered in turn, although within each section findings will not necessarily be considered in chronological order, and I have extended the Consolidation era up to the present day.

LANDMARKS (1860-1960).

According to Ruffini (1898), Weismann (1861) was the first to notice the presence in frog skeletal muscles of scattered bundles of striated muscle fibres which were much thinner than those around them; that these "Weismann bundles", or intrafusal bundles as they are now called, were extensively innervated was first confirmed independently in the early 1860s by Kölliker in the frog and Kuhne in mammals. Kölliker called these structures "muscle buds", since he felt that they were sites of production of additional muscle fibres for the main muscle. However, other roles had been attributed to them (see Huber & DeWitt 1897), and Kuhne, not wanting to embrace any in particular, simply named them "muscle spindles" after their appearance in teased preparations. Despite later attempts by Ruffini (1898) to rename them "neuromuscular spindles" in order to emphasise the presence of both nervous and muscular elements, it is the nomenclature of Kuhne which has survived into the present except in French laboratories where they are called "fuseaux neuromusculaires".

By the time Huber & DeWitt (1897) and Ruffini (1898) wrote on the

subject, many of the gross morphological features of muscle spindles described in the current literature had been elucidated. Thus, it was appreciated that the intrafusal fibres were of various lengths (though only one type was recognised), the longest being many millimetres in length, that at one or more points along the length of the intrafusal bundle there was an increase in its diameter which was caused by the acquisition of a multilamellate connective tissue capsule enclosing a fluid space in which the bundle of intrafusal muscle fibres lay, and that this encapsulated zone was associated with an exceedingly rich innervation of the intrafusal fibres at this point. Some details of this innervation had been unravelled by Ruffini in the early 1890s using cat hindlimb muscles stained with gold chloride; he was able to define three categories of nerve ending in the spindle, namely the primary, secondary and plate endings, which differed in their point of innervation of the intrafusal bundle, their detailed structure and the size of the axon which supplied them. The primary ending , which was supplied by a large, myelinated axon, was found in every spindle (hence the term primary) and was applied to the intrafusal muscle fibres in the form of rather regular annular or spiral terminals near their midpoint ("equatorial region" of Sherrington 1894). The secondary ending was not found in every spindle (and hence seemed of secondary importance); where present, it was supplied by a somewhat thinner myelinated axon than that supplying the primary ending, and invested the intrafusal bundle to one side of the primary ending with terminals of a more irregular morphology, consisting of sprays of C and S shaped claspers. The plate endings were supplied by yet thinner myelinated axons and were situated towards the end of the fluid space; the majority had a form similar to that of the neuromuscular junction on the extrafusal muscle fibres, though differences in their detailed structure were described which precluded their immediate identification as motor axons; Ruffini also described a web-like ending of some of these fine myelinated axons. Muscle spindles were described as receiving simple, intermediate or complex innervation on the basis of the number of secondary endings present in them, simple spindles

receiving none, intermediate spindles one and complex spindles two or more; complex spindles were the commonest, followed by simple spindles with intermediate spindles being the rarest.

On morphological grounds, Ruffini inclined to the view that all three types of ending were sensory in nature, and this impression was apparently confirmed by Sherrington (1894) in the course of degeneration experiments in cat and monkey. He showed that after section of the ventral roots, which caused extensive degeneration of the motor nerves to extrafusal fibres and of the extrafusal fibres themselves, there was very little degeneration of the nerves to muscle spindles, thus identifying them as sensory axons; any minor degeneration observed in his preparations was thought to have been caused by damage to the dorsal root ganglia during section of the ventral roots, rather than by the degeneration of a motor supply to the spindle, since the intrafusal muscle fibres evinced no degenerative changes. However, a very brief report by Onanoff (1890) of comprehensive degeneration experiments in the dog suggested that there was indeed a motor supply to the muscle spindle in addition to the sensory innervation, as had earlier been suggested by Kerschner (1888); he found, in contrast to Sherrington, that section of ventral roots alone resulted in the degeneration of some of the nerves to spindles and that some axons survived ablation of the dorsal root ganglia. The work of Onanoff appears to have been overlooked at the time, and Kerschner's idea that plate endings had a motor function was treated rather disparagingly (see e.g. Ruffini 1898).

It was not until the turn of this century that the existence of a motor innervation of muscle spindles was proven for the lizard (Cipollone 1898) by the fact that branches of axons which innervated the extrafusal muscle fibres could be traced to terminations on the polar regions of intrafusal fibres. Firm proof that mammalian muscle spindles also received motor innervation had to wait until comprehensive

degeneration experiments as performed by Onanoff were repeated in the 1920s by various workers (Boeke 1927; Hinsey 1927; Hines & Tower 1928) who showed that plate endings survived ablation of the dorsal root ganglia and lumbar sympathetic chain but degenerated after section of the ventral roots. At this time, Hinsey (1927) also noted that ablation of the dorsal root ganglia caused both primary and secondary endings to atrophy, thus confirming both to have a sensory role; the muscle spindle was thus established as a complex sensory organ consisting of one type of muscle fibre with two types of sensory innervation and a motor supply.

This "classical picture" of the spindle (Matthews 1964) persisted nearly unchanged for the next 30 years, the only additions made to it being those of Barker (1948). In a study of rabbit spindles, he confirmed most of the findings of Ruffini with one exception: where Ruffini (1898) felt that "One can say that exceptional forms of primary endings approach somewhat the secondary type, while exceptional forms of secondary ending distantly recall here and there the type of the primary ending", Barker found much less difference between the two, both having a mixture of annulospiral and C or S shaped clasping terminals. Nevertheless, the other differences between primary and secondary endings were confirmed; thus the afferent supplying a primary ending (the primary afferent) was thicker than that to the secondary ending (secondary afferent), being 8-12 μm in diameter compared to 6-9 μm and the secondary ending lay to one side of the primary. The terms "myotube" and "nuclear-bag" were introduced to describe the characteristic arrangement of nuclei in the intrafusal fibres underlying the sensory endings. A 300 μm length under the primary ending contained a dense aggregation of spherical nuclei which occupied most of the fibre cross-section leaving only a narrow rim of cytoplasm; this nuclear-bag region was poorly striated and therefore held to be non-contractile. In contrast to this, the myotube regions to either side of the nuclear-bag region, on which secondary endings lay if present, contained a central

chain of oval nuclei surrounded by cytoplasm and were clearly striated and so presumably contractile.

The first physiological study of the muscle spindle was carried out by Matthews (1933) during the ascendancy of the classical picture of the spindle. Using newly developed techniques for recording the electrical activity of nerve fibres, he studied the discharges of single afferent axons isolated in fine filaments of various cat hindlimb muscle nerves and found two main types of afferent, A and B. Type B afferents behaved as though they were connected in series with extrafusal fibres for their discharge was increased both by muscle twitches and by sufficiently large passive stretches of the muscle, whereas type A afferents behaved as though arranged in parallel with extrafusal fibres, their discharge falling during muscle twitch but being increased by stretch. The type A pattern of responsiveness was that predicted for muscle spindle afferents, whereas the type B pattern was that expected of Golgi tendon organs on theoretical grounds (Fulton & Pi-Suner 1928); this remains the only means of differentiating muscle spindle and tendon organ afferents. Having identified muscle spindle afferents in this way, Matthews went on to show that they were extremely sensitive to muscle stretch and could be made to fire tonically by elongating the muscle until the passive tension in it was just a few grammes weight; furthermore, he established that spindle afferents were sensitive to both absolute muscle length and to the rate of change of length, the former since the steady-state discharge of afferents increased with increasing length, the latter because the discharge of afferents during a period of stretching often overshot the steady-state rate when the muscle was held at the new length.

Naturally, Matthews attempted to subdivide his type A afferents into two groups, one corresponding to Ruffini's primary afferents, the other to the secondaries; his approach was to examine the effects on afferent discharge of short trains of stimuli applied to the muscle nerve at an intensity slightly greater than that required to produce a maximal twitch of the muscle, the idea being that

this would recruit thin motor axons running to the intrafusal muscle fibres as well as the alpha motor axons to the extrafusal fibres. It had not, at that time, been confirmed that the thin myelinated fusimotor axons seen entering the spindle were also thin in the muscle nerve (an alpha motor fibre could have subdivided many times, narrowing at each division, in order to produce these fine terminal branches), but the experiment nevertheless revealed two subtypes of afferent; A1 units were silenced during the train of stimuli as they had been during the single twitches, but A2 units were made to fire at a higher rate despite the accompanying muscle contraction. Since primary endings were known to lie on poorly striated, and therefore presumably non-contractile, nuclear-bag regions, it was supposed that they would be stretched by activity in the adjacent contractile regions when fusimotor axons were excited, and A2 units were therefore identified as primary afferents; the converse argument was applied to A1 units which were identified as secondary afferents. The effect of activity in the fusimotor axons was thus shown to be to increase the firing rate of spindle afferents, even in the face of muscle shortening. Although the A1/A2 classification scheme later foundered when compared to the more accurate scheme based on afferent conduction velocity (Harvey & Matthews 1961a; see below), Matthews' work nevertheless paved the way for much subsequent study.

When mammalian fusimotor innervation was proven to exist, it appears to have been tacitly assumed that the origin of the fusimotor axons was, as in reptiles and amphibia, in side branches of axons to extrafusal muscle fibres. The suggestion by Langley (1922), that the bimodality of myelinated fibre diameters he observed in ventral roots might be due to the existence of two populations of motor axon, large ones to extrafusal muscle fibres, small ones to plate terminals on intrafual fibres, was not greatly favoured, despite the evidence of Matthews (1933) that at least some of the fusimotor axons in a muscle nerve were indeed thinner than the motor fibres to extrafusal muscle. That Langley had been correct was first

strongly suggested by the work of Leksell (1945) and then put beyond doubt by Kuffler, Hunt & Quilliam (1951). Leksell stimulated the nerve to the gastrocnemius muscle whilst recording the electrical activity of whole ventral roots and identified a small peak in the compound action potential which was present only when the intensity of the stimulus was several times higher than that required to produce maximal contraction of the muscle. Following Erlanger & Gasser (1937), who had called the peak in the compound action potential produced by activity in the large motor fibres to extrafusal muscle fibres the alpha wave, Leksell labelled this small wave the gamma wave, and the axons producing it the gamma motor axons; the gamma wave occurred at a longer latency from the stimulus than did the alpha wave, indicating that the gamma axons conducted impulses more slowly than alpha axons and were therefore thinner than them. He went on to develop a method of producing a confirmed selective block of the conduction of action potentials in large axons by applying pressure to the sciatic nerve and then showed that activity set up in gamma motor fibres by stimulation of ventral roots and conducted past the region of block produced an increase in the massed afferent activity recorded from the gastrocnemius muscle nerve but did not generate measurable tension in the muscle.

A few years later, Kuffler, Hunt & Quilliam (1951) introduced the now standard spinal root single fibre technique and used it to confirm and extend Leksell's findings. Fine filaments were teased from both dorsal and ventral roots until each contained only one axon connected to the muscle under study. Spindle and tendon organ afferents were identified in the standard manner (Matthews 1933), and gamma axons by their high electrical threshold and low conduction velocity (15-55 m/sec). Single gamma axons were shown to increase the discharge rate of single spindle afferents but not to affect the discharge of Golgi tendon organs, nor to develop any tension in the muscle. Subsequently this powerful technique was used to show that a single gamma axon could excite several different spindle

afferents, and likewise that a single spindle afferent could be excited by several (2-5) separate gamma axons (Hunt & Kuffler 1951).

The final contribution in the landmark era was the recognition that afferent axons could be categorised on the basis of their conduction velocities (Merton 1953; Hunt 1954). It had been known since the time of Sherrington (1894) that the diameters of muscle afferent axons covered a wide range from about 1.5 - 20 μm but it was not until much later that fibre diameter spectra were published which contained several peaks and troughs suggesting the presence of distinct populations of afferents (Eccles & Sherrington 1930; LLoyd & Chang 1948). Speculation that the different populations of afferents might subserve different roles was reinforced by Lloyd (1943) who used graded electrical stimulation of muscle nerves to recruit progressively smaller afferents and showed that different sized afferents produced different reflex effects when active. He recognised four groups of afferents, and, on somewhat arbitrary grounds, related each to one of the peaks in nerve fibre diameter spectra; Group I afferents were defined as having axonal diameters of 12-20 μm, Group II as 6-12 μm, Group III as 2-6 μm and Group IV as <2 μm.

In order to examine the functional characteristics of different sized afferent axons in more detail, some means of identifying them during physiological experiments was required. This was provided by the work of Erlanger & Gasser (1937) who demonstrated that the electrical threshold and conduction velocities of axons depended upon their diameters, with large axons having a low threshold and a high conduction velocity and vice versa; not long afterwards, Hursh (1939) found empirically that the conversion factor relating axonal diameter to conduction velocity was around 6 in the cat, that is that if one multiplied an axon's diameter in micrometres by 6 one would obtain its conduction velocity in metres per second and vice versa. In principle, therefore, by 1940 physiologists were in a

position to identify primary and secondary muscle spindle afferents on the basis of their conduction velocities, for the two classes of spindle afferent were known to differ in diameter (measured at the spindle but presumed to apply in the muscle nerve too) with primary afferents falling into Group I and secondary afferents into Group II.

In practice, this discriminant was not applied until the dorsal root single fibre technique for recording muscle spindle afferents was introduced in 1951 by Kuffler, Hunt & Quilliam; the preparation of single unit filaments in muscle nerves suffers from the disadvantage that only a short length of nerve is available over which to measure the conduction delay of impulses travelling in the filament, and this delay will therefore be very short; in a dorsal root recording arrangement the distance is around 15 centimetres for hindlimb muscles and conduction delays become measurable. Using this new technique, Hunt (1954) measured the conduction velocities of 625 muscle mechanoreceptors identified as spindle or tendon organ afferents in the standard manner (Matthews 1933) and showed the distribution of conduction velocities of muscle spindle afferents to be bimodal, with the trough between the two populations lying at about 72 m/sec which corresponds to a diameter of about 12 μm; afferents conducting at over 72 m/sec were identified as spindle primary afferents and those conducting at less than 72 m/sec as secondary afferents. The dividing line of 72 m/sec was recognised by Hunt not to be absolute, but it has sometimes been taken to be; the validity of this division will be considered in more depth below (Literature Review, Part 2, p. 75). Although he succeeded in distinguishing primary and secondary afferents, Hunt could not find any significant differences in their functional characteristics, bar a difference in their threshold to passive stretch which was subsequently found not to be universally applicable (Matthews 1972).

ADVANCES (1960-1970).

With hindsight, it is apparent that Barker, in his 1948 studies of spindle anatomy, was unfortunate in his choice of experimental animal, for the rabbit does not show some of the features which initiated this era (Barker & Hunt 1964). Instead, it was towards the end of the 1950s and in cat and human studies that it began to be appreciated that intrafusal fibres, which had long been known to be morphologically rather heterogeneous, actually existed as two populations. Thus, Cooper & Daniel (1956) found that in human spindles nuclear bags were large and prominent in some intrafusal fibres but absent in others, and Boyd (1956) observed that each spindle in the cat tenuissimus muscle contained some large diameter fibres which extended from one end of the spindle to the other, as well as a number of thinner fibres which were about half as long. By 1962, when Boyd published his findings in detail, the consensus of opinion was that, in most muscles, two types of intrafusal fibre existed, namely the nuclear bag fibres and nuclear chain fibres (Swett & Eldred 1960; Barker & Ip 1961; Boyd 1962; Bridgman, Eldred & Eldred 1960; Cooper & Daniel 1963). Nuclear bag fibres were characterised by the presence in their equatorial regions of a nuclear-bag as defined by Barker (1948), whereas in nuclear chain fibres the nuclei were arranged in a single-file chain. Nuclear bag fibres were generally of larger diameter than chain fibres (up to 30 μm cf. 18 μm) and were longer, extending beyond the ends of the capsule whereas chain fibres generally lay within the capsule; there was some debate as to the extent of overlap between these features of the two classes of fibre, and Barker & Gidumal (1961) felt that they could recognise a third, intermediate type of fibre, but subsequent work favoured a dichotomy and revealed further light- and electron-microscopic differences between bag and chain fibres (see e.g. Matthews 1972). Having described two types of intrafusal fibre, the distribution to them of the two types of sensory axon was examined. Here, there was early agreement (Barker 1962; Boyd 1962; Cooper & Daniel 1963) that the primary ending consisted of spiral

terminals distributed to every one of the bag and chain fibres in a spindle whereas the secondary sensory terminals mainly invested the chain fibres, with occasional branches contacting the myotube regions of bag fibres.

At about the same time as the two types of intrafusal fibre were being described, evidence was accumulating which pointed to the fact that the motor innervation of the spindle was of two types. Firstly, it had been known for some years (Hunt 1952) that the neurotransmitter at fusimotor terminals was probably acetylcholine; histochemists studying the distribution within the spindle of the enzyme acetylcholinesterase now discovered that it was very widespread, covering most of the intracapsular parts of the intrafusal fibres including the juxta-equatorial regions often invested with secondary sensory endings (Coers & Durand 1956) and that two forms of localisation could be observed, namely plate-like deposits in the polar parts of the intrafusal bundle which corresponded to the location of the plate endings described by Ruffini (1898), and a new, diffuse distribution in the juxta-equatorial regions (Hess 1961). Correspondingly, degeneration studies combined with classical staining techniques soon confirmed the presence of a diffuse motor ending, the "γ2 network", in the vicinity of secondary endings (Boyd 1962; Cooper & Daniel 1963) which had presumably been overlooked before or misinterpreted as part of the secondary ending. Finally, there was evidence that two types of fusimotor fibre could be distinguished by their diameter at the point of entry into the spindle, γ1 fibres being thicker (3 μm) than γ2 fibres (1 μm) (Boyd 1962; Barker 1962).

The first functional dichotomy to be confirmed was one that Matthews had sought in the 1930s and which had been suspected to exist since the time of Ruffini (1898), namely a difference in the functional characteristics of primary and secondary afferents. Categorising soleus spindle afferents as primary or secondary on the basis of their conduction velocities, Cooper (1959,1961) examined their responses to a stretch of the muscle and found that the discharge of primary afferents was very rapid during the stretch and markedly overshot the rate which

was subsequently sustained at the new length, i.e. that the primary afferent was sensitive to the dynamic components of muscle stretch; the new observation was that secondary afferents were much less dynamically sensitive, their discharge during a stretch hardly exceeding that maintained after it. These experiments were performed on decerebrate cats in which fusimotor activity was known to exist and in which intrafusal fibres were therefore active; the same findings were subsequently made in de-efferented preparations (Lundberg & Winsbury 1960; Harvey & Matthews 1961b; Matthews 1963), as well as in response to a variety of forms of muscle stretch including muscle taps (Lundberg & Winsbury 1960; Bessou & Laporte 1962) small-amplitude vibration of the muscle (Bianconi & Van der Meulen 1961; Brown, Engberg & Matthews 1967a) ramp stretches (Bessou & Laporte 1962; Matthews 1963) and sinusoidal muscle stretches (Bessou & Laporte 1962; Lennerstrand 1968a; Matthews & Stein 1969a). Thus, the velocity sensitivity attributed by Matthews (1933) to all spindle afferents was now seen to be a specific property of primary afferents; both primary and secondary afferents were sensitive to absolute muscle length, and apparently to a similar degree, for the slopes of the relationships between their discharge frequencies and muscle length were similar (Harvey & Matthews 1961b; Jansen & Matthews 1962b; Bessou & Laporte 1962).

When they were first studied physiologically, gamma fusimotor axons were considered to be of one type only, since fusimotor axons all had a similar excitatory effect on both primary and secondary afferents (Hunt 1954). However, as soon as it became apparent that two types of motor terminal existed in spindles, a corresponding duality of fusimotor action was sought. Initially, Bessou, Emonet-Dénand & Laporte (1962) extended the experiments of Hunt (1954) by studying the effect on primary and secondary afferents of stimulation of gamma axons of widely ranging conduction velocity; their hypothesis was that axons which were thick at the spindle (γ1) might be so all the way to the ventral roots and would so conduct impulses relatively rapidly, so that fast gamma axons might be equated

with a γ1 plate termination and slow ones with a γ2 network termination. In the event, they were unable to find any consistent differences between the actions of fast and slow gamma axons, and subsequent histological work showed that there was no difference between the diameters of γ1 and γ2 axons in the intramuscular nerve bundles anyway (Boyd & Davey 1962; Adal & Barker 1965).

The breakthrough came when Matthews and his co-workers stimulated gamma fusimotor axons during ramp stretching of the muscle and studied the behaviour of primary afferents (Matthews 1962; Crowe & Matthews 1964a,b). The ''dynamic index'', which was defined as the difference between the firing rate of an afferent at the end of a ramp stretch and that 0.5 seconds later, was introduced as a convenient (but arbitrary) measure of the dynamic sensitivity of spindle afferents (Crowe & Matthews 1964a); two types of gamma axon were then readily identified, ''dynamic gamma axons'' being those which increased the dynamic index of primary afferents, whilst ''static gamma axons'' reduced the dynamic index. Other differences in the action of dynamic and static gamma axons were also described; thus activity in static gamma axons prevented primary afferents from falling silent during muscle shortening which they routinely did in both the de-efferented state and during dynamic gamma axon stimulation. Finally, static gamma axons quite often drove the discharge of primary afferents 1:1, that is that for every impulse in a static gamma axon, one impulse was evoked in the primary afferent; driving by dynamic gamma axons was never observed. That this duality of action was due to properties inherent to the fusimotor axons, rather than to differences between individual primary endings was shown by the facts that: (i) primary afferents could be influenced by several different gamma axons, some of which had a dynamic, others a static action; and (ii) when the action of an individual gamma axon on the discharge of several different primary afferents from the same muscle was studied, it was always found to be the same (Crowe & Matthews 1964b; Brown, Crowe & Matthews 1965). In agreement with the anatomical findings, the

conduction velocities of dynamic and static gamma axons overlapped extensively, although the slowest axons tended to have a static action (Brown, Crowe & Matthews (1965).

Another fundamental difference between the action of dynamic and static gamma axons came to light a little later, namely that static gamma axons influenced both primary and secondary afferent discharge, whereas dynamic gamma axons very rarely affected the discharge of secondary afferents (Appelberg, Bessou & Laporte 1966; Brown, Engberg & Matthews 1967b). This finding had some bearing on a controversy which was in full swing at the time, and which had to do with the intrafusal distribution of the two types of motor terminal. Boyd (1962) favoured a selective distribution of motor innervation, with γ1 axons running to form γ1 plate endings on bag fibres exclusively, whilst γ2 axons terminated as γ2 networks on chain fibres only. The functional duality of fusimotor action strongly supported this interpretation; the prevailing model of the intrafusal mechanisms underlying the functional properties of spindle afferents was that mechanical properties of the intrafusal fibres varied along their lengths in a manner related to the degree of striation, so that sensory terminals in different positions "saw" a different transformation of the muscle stretch (Matthews 1964). Specifically, the velocity sensitivity of the primary ending over and above the length sensitivity it shared with the secondary ending was explained by postulating a lower viscosity of the nuclear-bag regions of bag fibres compared with their other regions due to the paucity of myofibrils in this area; dynamic fusimotor action was then most simply explained by proposing that it caused contraction of the polar parts of bag fibres, enhancing the difference between their viscosity and that of the nuclear-bag regions, and, since only primary afferents ended on nuclear-bag regions, dynamic fusimotor action was restricted to primary afferents. Static fusimotor action was supposed to be effected by contraction of chain fibres, which could therefore influence both primary and secondary afferents.

Dynamic gamma axons were therefore equated with Boyd's γ1 axons and static gamma axons with γ2 axons (Matthews 1962; Brown, Crowe & Matthews 1965).

In favour of this simple picture of the spindle were a number of other physiological observations. Firstly, there were three lines of evidence that the contractility of intrafusal fibres varied considerably: (i) single shocks to individual gamma axons sometimes produced appreciable excitation of primary afferents and sometimes did not; when they did, repeated stimulation of the same axon produced 1:1 driving of the afferent discharge up to very high rates of stimulation (Kuffler, Hunt & Quilliam 1951); subsequently only static gamma axons were shown to produce driving (Crowe & Matthews 1964a); (ii) extracellular recording of the electrical activity produced in intrafusal muscle fibres by stimulation of single gamma axons (Eyzaguirre 1960) indicated that in some cases it was propagated (complex, polyphasic potential) as is typical of mammalian fast-twitch muscle fibres, whereas in others it was non-propagated (monophasic, with polarity reversal when recording electrodes were moved) and suggestive of non-twitch intrafusal contraction; subsequently, static gamma axons were shown to produce propagated potentials, and dynamic gamma axons non-propagated potentials (Bessou & Laporte 1965); (iii) the ''frequencygram'' developed by Bessou, Laporte & Pages (1968); this consisted of the superimposition in successive sweeps of the beam of a storage oscilloscope of the instantaneous firing rate of a primary afferent when gamma axons were stimulated. At low stimulation rates, the frequencygram consisted of a series of waves of increased discharge rate, each corresponding to a stimulus applied to the gamma axon and presumably caused by the transient contraction of intrafusal fibres; the significant feature of these frequencygrams was that when static gamma axons were stimulated, the waves could often be distinguished for stimulation rates well over 100/sec indicating a rapidly-contracting effector, whereas for dynamic gamma axons the waves were distinguishable only at the lowest stimulation rates,

indicating a slowly-contracting effector.

Secondly, evidence that the mechanical properties of bag fibres were responsible for the velocity sensitivity of primary afferents came from experiments on visualised muscle spindles; this preparation, which was pioneered by Boyd (1958), was obtained by carefully paring away extrafusal fibres until the capsule of a spindle and its intrafusal bundle could be seen. The technique was now applied in rats (Smith 1966) and cats (Boyd 1966) to show that, when a muscle spindle was suddenly stretched, the chain fibres did not move once the new length had been attained, whereas, over the course of about half a second, the bag fibres moved back towards the spindle equator. Although the amplitude of this "creep" (Smith 1966) was small (about 3% of the applied 40 μm stretch) it was highly significant, for it was in a direction which caused the primary ending to shorten, and which presumably decreased its level of excitation; furthermore, the time course of the creep was shown to be similar to that of the adaptation of the primary afferent discharge after a stretch (Boyd & Ward 1969), and the mechanical adaptation was therefore assumed to underlie the electrical adaptation. Finally, the correspondence between non-twitch intrafusal fibres and bag fibres, and between twitch fibres and chain fibres, was established by Boyd (1966) who showed that stimulation of single gamma axons could produce either vigorous twitch contraction of chain fibres or slower, smaller movements of bag fibres which were probably caused by focal contraction in more polar parts of the fibre. Boyd later found that almost 90% of fusimotor axons produced activity in either bag or chain fibres selectively (Boyd 1971).

The simple picture of the spindle, appealing though it was, was vigorously disagreed with by Barker and his co-workers (Barker 1962; Barker & Ip 1965; Barker, Stacey & Adal 1970) on two main grounds. Most importantly, using an improved silver-impregnation method which they developed, they

repeatedly found, unlike Boyd, that individual motor axons could be seen to innervate both bag and chain fibres in the same spindle, and that, in general, bag and chain fibres were equally likely to receive γ1 plate and γ2 network endings (or "trail endings" as γ2 networks were more accurately termed by Barker & Ip 1965). They also cited the situation in the rabbit as evidence against the simple model of the spindle; Barker & Hunt (1964) showed that many hindlimb muscle spindles in this species contain only nuclear bag fibres, and that, in the few forelimb muscle spindles in which chain fibres were also found, these were in the minority (c.f. cat spindles) and constituted "something of an optional extra" (Matthews 1972) element. Yet primary and secondary afferents from rabbit muscle spindles behaved much as they did in the cat, and dynamic and static fusimotor axons could be distinguished in the standard manner (Emonet-Dénand, Laporte & Pages 1966), all of which seemed to militate against the acceptance of the concept that bag and chain fibres had significantly different mechanical properties and that these underlay the functional differences between primary and secondary afferents. However, as was pointed out by Matthews (1972), the second argument is based upon the unwarranted assumption that the mechanical properties of an intrafusal fibre are dictated by its particular pattern of equatorial nucleation; subsequently, it proved possible to identify several types of rabbit intrafusal fibre with presumably different contractile properties on the basis of ultrastructural and histochemical criteria, thus bringing rabbits back into line with other Mammalia and removing one of Barker's objections to the simple model of the spindle (Spiro & Beilin 1969; Corvaja 1970).

Matters threatened to become even more complicated when skeleto-fusimotor, or beta, axons, which terminated on both extrafusal and intrafusal muscle fibres, were shown to exist in mammals in addition to the two types of gamma axon. For about thirty years after its first identification, the motor innervation of muscle spindles was thought to derive from side branches of alpha motor axons as

had been seen in lizard muscles (Cipollone 1897); after the work of Leksell (1945) and Kuffler, Hunt & Quilliam (1951), however, the received wisdom was that gamma axons were the source of motor axons to spindles, and the possibility that alpha axons might branch to innervate spindles was not often contemplated. There was some anatomical evidence that this might occur (Boyd 1959; Cooper & Daniel 1963), but the first really firm evidence came from physiological experiments in a cat lumbrical muscle, during which it was observed that stimulation of single motor axons to extrafusal muscle fibres produced excitation of some spindle afferents, and that: (i) as the rate of stimulation was increased, the strength of extrafusal contraction reached a plateau at about 50 Impulses/sec, whereas the excitation of spindle afferents continued to increase with increasing stimulation rate up to 200 Impulses/sec and (ii) when the animal had received a dose of curare just adequate to block extrafusal contraction, stimulation of the axon continued to excite spindle afferents (Bessou, Emonet-Dénand & Laporte 1965); all the beta axons encountered were shown to have a dynamic action on primary afferents. Subsequently, Adal & Barker (1965) traced individual fusimotor axons in teased preparations of the same small muscle and demonstrated anatomically that mixed intrafusal/extrafusal innervation existed.

These findings had various implications, of which one was that beta axons might be the source of intrafusal plate endings (since they had this form of ending on extrafusal fibres), and that gamma axons might form only trail terminals, a concept which would have completely undermined the simple picture of the spindle. This possibility was made very unlikely by the fact that beta innervation was not found in many spindles whereas all spindles received plate motor endings, but was only completely eliminated when Barker, Stacey & Adal (1970) distinguished three, not two, types of motor termination in cat spindles mainly on the basis of their pre-junctional features under the light microscope; they named them p1 plates, p2 plates and trail endings and subsequently demonstrated (Barker, Emonet-Dénand,

Laporte & Stacey 1980) that p1 plates were formed by the intrafusal branches of beta axons, p2 plates by dynamic gamma axons and trail endings by static gamma axons. Both p2 plate and trail endings were found in all spindles, whereas p1 plates were found in varying numbers of spindles depending on the muscle under study. On the other hand, a different problem arose, since p1 plate terminals were described on both bag and chain fibres, yet beta axons apparently exerted an exclusively dynamic action on primary afferents and so would have been expected to innervate bag fibres only in Matthews' model of the spindle.

Barker continued to stress the fact that plate and trail endings could be found on both bag and chain fibres, although he conceded that plate endings were somewhat commoner on bag fibres and trail endings on chain fibres. His viewpoint was finally, and most elegantly, confirmed by the experiments of Barker, Emonet-Dénand, Laporte, Proske & Stacey (1973). These workers reduced the motor innervation of the cat tenuissimus muscle to a single gamma axon by sectioning all the ventral roots supplying the muscle apart from a filament containing a single, slowly-conducting axon running to the muscle; they then allowed about ten days for the sectioned axons to degenerate, after which primary afferents from the muscle were isolated in the dorsal roots, the remaining gamma axon was classified in the standard manner (Crowe & Matthews 1964a) and silver-stained muscle spindles teased from the muscle were examined with the light microscope. All the axons studied were static gamma axons and it transpired that their terminals were regularly distributed to both bag and chain fibres in the same spindle, and that when, in some spindles, innervation was restricted to only one type of intrafusal fibre, either bag or chain fibres could be the recipient. A further finding was that static gamma axons invariably formed trail endings, implying that dynamic gamma axons formed p2 plate terminals.

Thus, despite extensive physiological support for the picture of the spindle promulgated by Boyd (1962) and Matthews (1964), incontrovertible

histological evidence showed it to be incorrect, and this era closed in some confusion.

CONSOLIDATION (1970-1988).

At various points in the 1960s, the possibility had been raised that more than two types of intrafusal fibre might exist. At the outset, Barker & Gidumal (1961) had felt that in about a third of cat hindlimb muscle spindles, intrafusal fibres morphologically intermediate between bag and chain fibres could be identified, but most workers favoured a dichotomy. Subsequently, Yellin (1969) stained rat intrafusal fibres for the enzymes phosphorylase and succinate dehydrogenase, and felt that at least four types of fibre could be differentiated, and, on a combination of histochemical and ultrastructural criteria, Barker & Stacey (1970) identified three types of intrafusal fibre in the rabbit. The Consolidation era opened with the establishment of a subdivision of cat and monkey nuclear bag fibres into two types by Ovalle & Smith (1972); staining for myosin ATPase, these authors showed that bag_1 fibres were stained after acid pre-incubation but not after alkaline pre-incubation, whereas bag_2 fibres were stained after both acid and alkaline pre-incubation. This subdivision was rapidly confirmed in other species (e.g. rabbit, Banks & James 1974; human, Gladden 1974), though initially a variety of histochemical techniques was used and there was some uncertainty as to the correspondence between the fibre types described by various groups. This uncertainty was resolved by Banks, Harker & Stacey (1977), and authors subsequently adopted the terminology of Ovalle & Smith (1972). Other morphological differences between the two bag fibres were then described, including the facts that bag_2 fibres generally have a higher content of glycogen than bag_1 fibres (Banks, Harker & Stacey 1977) and have more elastic fibres associated with them in their polar regions than bag_1 fibres (Gladden 1976); under the electron microscope, chain fibres show a distinct M line in the middle of their sarcomeres

throughout the fibre, and the bag_2 fibre in all parts except the equatorial region, but the bag_1 only has an M line in its most polar parts (Banks, Harker & Stacey 1977). Typically, a cat muscle spindle contains one of each type of bag fibre and 4-6 chain fibres, though some spindles may contain many more intrafusal fibres, especially in spindles of non-hindlimb muscles (Literature Review, Part 2, p. 66 et seq.) and in other species such as man.

The full import of this subdivision was not immediately appreciated, but by about 1974 it began to be realised that the simple picture of the spindle could be made to fit with both the histological and the physiological findings with a single modification which was entirely in keeping with the original concept and with the new enzyme histochemical findings. This was that the two types of bag fibre might have different mechanical properties, one of them possessing the visco-elastic properties required to provide the primary afferent with its dynamic sensitivity, the other having properties commensurate with a contribution to static sensitivity. Primary afferents would have terminals on all three classes of intrafusal fibre, whereas secondary afferents would not have terminals on the "dynamic bag fibre", and a selective distribution of motor innervation could be reinstated by postulating that the dynamic bag fibre received dynamic fusimotor innervation exclusively, whilst the static bag fibre and the chain fibres received only static fusimotor innervation.

Support for this idea was very soon forthcoming, mainly thanks to the skillful application of the isolated spindle technique, technical advances in which meant that spindles could be visualised whilst still fully connected to their nerve and vascular supplies; it was then possible to isolate fusimotor axons in the ventral roots, observe their effects on afferent discharge and observe and film their effects on the various intrafusal muscle fibres. Thus it was shown at about the same time by Bessou & Pages (1975) and by Boyd, Gladden, McWilliam & Ward (1977) that dynamic gamma and beta axons invariably elicited contraction of one of the bag

fibres in a spindle, that static gamma axons could cause contraction of either chain fibres or bag fibres or both in different spindles and, significantly, that the bag fibre activated by static gamma axons was always different from that activated by dynamic gamma or beta axons innervating the same spindle. Boyd's group was so sure of this separate innervation that they introduced the terms dynamic and static bag fibre to stress it. The relationship between the dynamic and static bag fibres and the histochemical types of Ovalle & Smith (1972) was determined by Boyd, Gladden, McWilliam & Ward (1977) by injecting the identified dynamic bag fibre with a dye and then examining the spindle histologically; on all five occasions on which this was achieved, it was shown that the marked dynamic bag fibre had few elastic fibres associated with it and was therefore the bag_1 fibre, whilst the static bag fibre was the same as the bag_2 fibre.

For a while, it appeared that a whole new controversy was about to start, for a different anatomico-physiological technique was producing results which disagreed with some of those obtained from isolated spindle experiments. This was the glycogen depletion method (Edström & Kugelberg 1968), which was first applied to intrafusal fibres by Brown & Butler (1973). The technique involves the protracted stimulation of single fusimotor axons, often with periods of restricted blood supply in order to encourage anaerobic metabolism in the contracting intrafusal fibres; under these conditions, the glycogen content of intrafusal fibres is reduced, and this is revealed histochemically by weak or absent PAS staining. Working before the subdivision of bag fibres had been firmly established, Brown & Butler (1973) showed that dynamic gamma axons invariably depleted bag fibres whereas static gamma axons could deplete bag fibres, chain fibres or both; later they showed that only one of the bag fibres in a spindle was depleted by static gamma action and that this was generally the larger of the two present in most spindles (Brown & Butler 1975), which probably identified it as the bag_2 fibre. On the other hand Barker's group, whilst agreeing that dynamic gamma axons almost invariably

depleted just the bag_1 fibre, felt that static gamma axons were non-selectively distributed, this term now being used to indicate that both types of bag fibre were innervated by static gamma axons. Thus, most static gamma axons depleted both bag and chain fibres (Barker, Emonet-Dénand, Harker, Jami & Laporte 1976), but the bag fibre involved was equally likely to be the bag_1 or the bag_2 fibre (Barker, Emonet-Dénand, Harker, Jami & Laporte 1976; Emonet-Dénand, Jami, Laporte & Tankov 1980). At about this time, the clear division of fusimotor action into two categories was critically reassessed by one of its original exponents (Emonet-Dénand, Laporte, Matthews & Petit 1977), and six classes of gamma fusimotor action were identified, ranging from classical dynamic action (Category I) to classical static action (Category VI), the intermediate categories producing fusimotor effects which could be mimicked by some degree of admixture of pure static and pure dynamic action; a possible interpretation of these results was that it indicated the presence of some non-selective innervation of intrafusal fibres, thus supporting the glycogen depletion findings.

The distribution of beta fusimotor innervation was also studied with the glycogen depletion method at this time, and turned out, happily, not to be a cause of controversy, as dynamic beta axons depleted only the bag_1 fibre (Barker, Emonet-Dénand, Harker, Jami & Laporte 1977), whilst other beta axons, which were later shown to have a static fusimotor action (Jami, Lan-Couton, Malmgren & Petit 1979), depleted the longest chain fibre in some spindles (Harker, Jami, Laporte & Petit 1977). The bone of contention was, therefore, the question of static gamma innervation of the bag_1 fibre. On the one hand, it was suggested that static-gamma-evoked glycogen depletion of the bag_1 fibre was an artefact caused by some form of indirect coupling between contractile activity in the bag_2 and chain fibres and metabolic activation of the bag_1 fibre, and on the other hand it was suggested that slow, weak contraction of the bag_1 fibre elicited by static gamma axons might be overlooked in isolated spindle experiments. Clearly, one of the techniques had to be

misleading one of the groups, and the verdict soon came down in favour of the isolated spindle experiments.

The clinching experiments were twofold; firstly, Barker and his collaborators (Barker, Bessou, Jankowska, Pages & Stacey 1978; Banks, Barker, Bessou, Pages & Stacey 1978) inserted microelectrodes into visualised intrafusal fibres and recorded their electrical activity during stimulation of static or dynamic gamma axons; they then injected a fluorescent dye into the fibre and examined the spindle histologically. Of thirteen fibres synaptically activated by static gamma axons, all were either chain or bag_2 fibres and seven of nine fibres activated by dynamic gamma axons were bag_1 fibres. A surprising finding, and one of uncertain significance, was that one chain fibre and one bag_2 fibre were activated by dynamic gamma axons; co-innervation of the bag_1 fibre and one of the chain fibres in the same spindle has since been seen in around 10% of tenuissimus spindles (Kucera 1984; Boyd & Gladden 1985), but co-innervation of the bag_1 and bag_2 fibres by the same motor axon is very rare. The second line of evidence favouring selective innervation of the bag_1 fibre has come from exacting histological work which involved the reconstruction of the fusimotor innervation of spindles from serial transverse sections and teased, silver-impregnated spindles and showed that the presence of trail endings on, and hence static gamma innervation of, bag_1 fibres is very rare (Banks, Barker & Stacey 1981; Banks 1981). Various suggestions have been advanced as to why the glycogen depletion method produced artefacts in the bag_1 fibre (see Hulliger 1984).

The distribution of sensory terminals to the intrafusal fibres was also reexamined by the anatomists after the bag fibres were subtyped. As expected, it was found that primary endings always had terminals on every intrafusal fibre present in a spindle (Banks, Barker & Stacey 1979). This finding was later extended with the observations that about 35% of the total area of contact between a primary

axon and intrafusal fibres lies on the bag_1 fibre, 25% on the bag_2 fibre, and the rest distributed between chain fibres, and that the bag_1 fibre is supplied by a separate first-order branch of the Ia axon in 73% of tenuissimus spindles (Banks, Barker & Stacey 1982). It was also confirmed that secondary endings lie mainly on the chain fibres, although there was a position-dependant variation in the degree of collateral termination upon bag fibres, such that S1 secondaries (Boyd 1962) which are adjacent to the primary ending "almost invariably also innervate both bag fibres" (Banks, Barker & Stacey 1981) whereas secondary endings situated further from the primary had less bag fibre contact, losing first the bag_1 contact and then the bag_2 contact. A quantitative study (Banks, Barker & Stacey 1982) later produced the surprising finding that 68% of secondary endings (mainly S1) contacted both bag_1 and bag_2 fibres as well as chain fibres, and that another 27% contacted one type of bag fibre, usually the bag_2, as well as chain fibres; only 25% of secondary endings conform to the classical view that secondary terminals are restricted to chain fibres. However, the functional significance of this collateral innervation is still uncertain; even in S1 secondary endings, at least 75% of the contact area is on chain fibres, and this proportion will be higher for other secondary endings.

It will be appreciated that the histological findings broadly support the revised simple view of the spindle (p. 42), and functional studies provided extensive further support. Various groups (Bessou & Pages, 1975; Boyd 1976a,b; Boyd, Gladden, McWilliam & Ward 1977), using the isolated spindle technique, showed the several intrafusal fibres to have quite different mechanical properties. Thus, activity in the appropriate gamma axons induces twitch-like responses throughout a chain fibre pole, and as the stimulation rate is increased chain fibres continue to show oscillatory tetanic contractions up to stimulation rates of 150 Hz. Both the bag fibres contract more slowly than chain fibres and show only focal contraction; the bag_2 fibre contracts somewhat faster than the bag_1 fibre and

produces a much greater extension of the sensory terminals applied to it. The gradation of contraction speed and strength from the fastest chain fibres to slowest bag_1 fibres matches the histochemical and ultrastructural variations between the fibres which show chain fibres to be rather similar to extrafusal twitch fibres, the bag_1 to be similar to tonic extrafusal fibres, and the bag_2 fibre to be intermediate between these extremes (Banks, Harker & Stacey 1977; Barker, Banks, Harker, Milburn & Stacey 1976). Also in line with these findings is the observation that chain fibres show propagated action potentials on stimulation of their motor innervation (as do extrafusal twitch fibres), whilst bag fibres show non-propagated potentials (Banks, Barker, Bessou, Pages & Stacey 1978; Barker, Bessou, Jankowska, Pages & Stacey 1978).

The bag_1 fibre was shown to have a unique mechanical property, namely a time-dependant change in the stiffness of its polar regions after a stretch; this property was revealed in the phenomenon of creep which had been seen a decade earlier (p. 37) but which was now localised to the bag_1 fibre. After the dynamic phase of a ramp stretch of the spindle, the poles of the bag_1 fibre gradually yielded and were extended by potential energy stored in the stretched equatorial region; as a result, the primary sensory terminals on the equator of the bag_1 fibre became less extended over the course of a few seconds after the stretch. The amplitude of the creep could be quite small in the absence of fusimotor activity, but when dynamic gamma or beta axons were stimulated, it increased dramatically; fusimotor stimulation could also produce quite anomalous behaviour in the bag_1 fibre, for it was not uncommon to observe sarcomere convergence at a point on the bag_1 fibre which was subsequently shown not to have any motor terminals near it (Boyd, Gladden, McWilliam & Ward 1977; Banks, Barker, Bessou, Pages & Stacey 1978). This phenomenon could not have been caused by the spread of an action potential from motor terminals into uninnervated extracapsular regions of the bag_1 fibre, for intracellular recording in this region has revealed only

electrotonically decaying potentials (Gladden 1981); furthermore, it was not an artefact since it can be mimicked by injection of depolarising current into the bag_1 fibre via a microelectrode (Hunt & Wilkinson 1985). Instead, it was suggested that the contractile properties of the bag_1 fibre might vary along its length , mirroring known ultrastructural variations such as the presence/absence of an M line in sarcomeres under the electron microscope (Banks, Harker & Stacey 1977) and the degree of development of the sarcotubular system (Adal 1985), such that in its extracapsular regions activation would produce sarcomere shortening whereas in the intracapsular regions cross-bridges would form and increase the stiffness of the fibre without appreciable sarcomere shortening (Matthews 1981a). Depolarisation of the intracapsular regions of the fibre underlying the motor terminals might then not produce visible shortening in this part of the fibre, whilst the electrotonically decaying potential might still sufficiently depolarise the extracapsular portion of the fibre to produce sarcomere shortening. The idea that activation of the bag_1 fibre may involve an increase in its stiffness rather than overt shortening is supported by the observation of Emonet-Dénand & Laporte (1981) who showed that activation of dynamic gamma axons with either single shocks or brief trains had no appreciable effect on the firing rate of primary afferents at constant muscle length, but that if the same stimulus was applied during a stretch of the muscle there was a clear, transient increase in afferent firing, the magnitude of this effect increasing markedly with increasing stretch velocity.

Current thinking has it that the stiffness changes in the bag_1 fibre are caused by stretch activation, that is that when the bag_1 fibre is stretched, some mechanism causes an increase in cross-bridge attachment, and that dynamic fusimotor action enhances stretch activation. The mechanism initially proposed was that stretch caused permeability changes in the bag_1 fibre membrane, leading to depolarisation and activation (Boyd 1976a), but this has now been supplanted by the idea that it is the rupture of cross-bridges formed before the stretch which

provokes the increase in stiffness (Gladden 1986). The evidence for this latter mechanism derives from the observations that: (i) in visualised spindles, stretch activation can be seen as stretch-evoked sarcomere shortening in the bag_1 fibre which is accompanied by an increase in stiffness of the fibre, but only when the bag_1 fibre has been stretched by more than about 3% of its initial length (Poppele & Quick 1981); dynamic fusimotor stimulation produces a greatly enhanced stiffness of the bag_1 fibre near the motor terminals, such that a stretch fails to extend sarcomeres in this region (Poppele 1985). In the preparation used by Poppele, the intrafusal fibres were clamped extracapsularly by chucks attached to a puller and so might have been damaged; however, in intact isolated spindles, Boyd, Gladden & Ward (1987) observed a movement of the bag_1 fibre towards its poles during a stretch which exceeded that of the other intrafusal fibres, suggesting that stretch activation of the bag_1 fibre was also occurring in this preparation; (ii) repetitive sinusoidal stretching of the peroneus brevis muscle with amplitudes likely to have stretched spindles by about 0.5% did not produce glycogen depletion in the bag_1 fibre (Emonet-Dénand, Jami, Laporte & Tankov 1980), whereas bursts of rapid stretching of lumbrical spindles by about 10% did produce glycogen depletion in the bag_1 fibres (Laporte, Emonet-Dénand & Hunt 1985). The figure of 3% probably represents the extension of the bag_1 fibre needed to rupture stable cross-bridges, producing stretch activation which, since it is an active process, depletes glycogen stores. On the other hand, Dickson, Gladden, Halliday & Ward (1989) have recently reported that preliminary experiments, during which isolated spindles were filmed under stroboscopic illumination as they were stretched so that the movement of individual sarcomeres could be seen, have so far failed to reveal stretch-evoked shortening (i.e. stretch activation) of the bag_1 fibre; it should be pointed out, however, that the absence of stretch-evoked lengthening of sarcomeres is also evidence of stretch activation.

The relationship between the mechanical characteristics of intrafusal

fibres, their activation by fusimotor axons and the response of spindle afferents to large ramp muscle stretches has been examined in great detail by Boyd and his collaborators in recent years (Boyd 1981b, 1986; Boyd, Murphy & Moss 1985; Boyd, Murphy & Mann 1985; Boyd, Sutherland & Ward 1985). Their findings will be summarised briefly, dealing with the action on afferent sensitivity of one fibre type at a time; in all cases, the results were obtained from experiments in which it was visually confirmed that fusimotor stimulation was producing contraction in only one fibre type, though the effects on afferent discharge of co-activation of bag_2 and chain fibres by non-specific static gamma axons were also examined.

The bag_1 fibre.

With the muscle held at a fixed length, sustained stimulation of dynamic gamma or beta axons produces a transient increase in primary afferent discharge which then decays towards the control value but generally does not reach it; the residual biassing is usually quite small (about 20 Imp/sec), probably because the bag_1 fibre does not shorten appreciably when activated and so does not greatly stretch its complement of sensory terminals. When the muscle length is ramped up to a new value, the classical dynamic action, an increase in dynamic index (Crowe & Matthews 1964a) is immediately apparent. However, at moderate velocities of stretching it is also apparent that there are several phases to the primary afferent response, namely the fast and slow rise phases which are followed by the fast and slow fall phases, which were not classically described. There is some variability between primary afferents in the way these phases of the ramp response are altered by dynamic fusimotor action; generally, the slopes of the slow rise and decay phases are clearly increased, and sometimes the velocity sensitivity of these phases are also increased. In contrast, dynamic fusimotor effects on the fast rise and fall phases are much less marked. The net effect of these changes is to increase the classical measure of dynamic sensitivity, the dynamic index.

The enhancement of the slow rise and fall phases is explained by the related phenomena of stretch activation and creep which have already been described. During a stretch, stretch activation increases the stiffness of the polar regions of the bag_1 fibre, transmitting a greater fraction of the lengthening to the sensory spirals and so increasing the peak discharge frequency achieved during the stretch and therefore increasing the length sensitivity of the afferent measured under dynamic conditions (slope of slow rise phase); after the stretch, enhanced creep in the bag_1 fibre causes a greater reduction of discharge from the peak value, and hence the greater slope of the slow fall phase. Despite this enhanced slow fall phase, the difference between the discharge of the afferent before the stretch was applied and that a few seconds after the end of the ramp stretch, which is a measure of the position sensitivity of the afferent, may be somewhat increased by dynamic fusimotor action especially at higher velocities of stretching, reflecting a greater increase in the slow rise phase than in the slow fall phase of the afferent response. The fast fall phase of the response, which can contribute the major part of the dynamic index measurement in the de-efferented primary afferent, is very dependent upon the velocity of stretching but is not greatly affected by dynamic fusimotor action; both it and the fast rise phase are probably the result of ionic rather than mechanical adaptation (Boyd 1981c, 1985b).

Classically, dynamic fusimotor action is said to only rarely affect secondary afferent discharge (p. 35), but more recent studies have demonstrated that, whilst some secondary afferents are unaffected by contraction of the bag_1 fibre, others show an increase of variable magnitude in the dynamic length sensitivity (slope of slow rise phase) and its velocity sensitivity, as well as an increase in the position sensitivity of the afferent. Presumably, those secondary afferents which are unaffected by bag_1 fibre contraction do not terminate significantly upon the bag_1 fibre, whilst the others are affected by the same mechanisms as for primary afferents, though in proportion to their contact area on the bag_1 fibre which is known to vary (Banks, Barker & Stacey 1982). Boyd (1985a) states that 1 in 10 secondary afferents shows a

moderate increase in dynamic sensitivity as a result of bag_1 fibre contraction, a fraction rather lower than that expected on the basis of the anatomical finding of Banks, Barker & Stacey (1982) that up to 68% of secondary afferents have some bag_1 fibre contact. In contrast, bag_1 fibre contraction has a small biassing action on approximately half of secondary afferents, which is more in keeping with histological understanding of the frequency of bag_1 fibre contacts in secondary afferents; this latter observation suggests that the extent of bag_1 fibre contact of many secondary afferents is insufficient to produce noticeable changes in afferent stretch sensitivity, and this would fit with the observation that even S1 secondaries which have definite bag_1 fibre contacts generally have 75% or more of their total terminal contact area on the chain fibres in the spindle (Banks, Barker & Stacey 1982).

The bag_2 fibre.

Contraction of the bag_2 fibre with the muscle held at a fixed length produces a substantial biassing of primary afferent discharge which is directly related to the degree of extension of the primary sensory spirals on the bag_2 fibre produced by shortening in the capsular sleeve region. The biassing may be up to 150 Imp/sec in amplitude, though it decays with time after the onset of fusimotor stimulation; this is probably a result of transmission failure at the fusimotor terminals during high-frequency stimulation, since adaptation of this sort is not seen when SCh (an acetylcholine analogue which acts at fusimotor terminals) is used to activate the bag_2 fibre (Literature Review, Part 3, p. 86; also present experiments, p. 124). A feature of this biassing action, which is not stressed by Boyd's group but which is of relevance to the work reported here, is that it is generally accompanied by substantial irregularity in the afferent discharge which, judging from the published examples, is usually at least double its control value. When the muscle is ramp stretched, the afferent response superimposed on this high level of discharge has greatly reduced fast rise and fast fall components. The effect on the slow rise phase of the

response appears to be quite variable: in an early report, Boyd (1981b) showed examples in which it was clearly greatly reduced, yet in later work (Boyd, Murphy & Moss 1985) the data suggest that the slow rise phase is minimally affected by bag_2 fibre contraction; examination of the figures in this latter work, however, suggests that the measurements were performed after adaptation of the on-response to gamma axon stimulation, whereas in the earlier work they were made when the biassing was still very substantial. It will be argued below that the observation appropriate to this work is that the slow rise phase of the response is substantially decreased by bag_2 fibre contraction which produces substantial biassing. The slow fall phase is generally unaffected by bag_2 fibre contraction.

The net effect of these changes is that the primary afferent discharge is strongly biassed, irregular, and shows a greatly attenuated dynamic and static sensitivity to length changes. These effects probably occur because the bag_2 fibre shortens so much that it takes up nearly all the free play in the primary sensory spirals distributed to it, so that a subsequent stretch is unable to produce much further separation of the terminals (Boyd 1981c).

The effect of bag_2 fibre contraction on secondary afferent discharge depends, as for bag_1 fibre effects, upon the extent of secondary termination upon it, and the data of Banks, Barker & Stacey (1982) suggest that at least 80% of secondary afferents have some contact with the bag_2 fibre. Correspondingly, the majority of a fairly small population of secondary afferents have been reported to be influenced by bag_2 fibre contraction, although the effects are rather unremarkable. At a fixed muscle length, there is generally some biassing of secondary afferent discharge, usually by 10-25 Imp/sec, though occasionally by up to 40 Imp/sec; the slow rise and fall phases are generally unchanged, though they may sometimes be slightly increased, and the fast rise and fall phases are usually decreased. Since all phases of the response to ramp stretching of secondary afferents are smaller than their counterparts in the primary afferents, the small changes in them provoked by bag_2 fibre action

usually do not markedly alter the stretch sensitivity of secondary afferents; generally, the dynamic index is somewhat decreased by bag_2 fibre action, but changes in position sensitivity are as likely to be small decreases as increases.

The chain fibres.

Contraction of chain fibres, whether produced by static gamma axons or static beta axons (Jami, Petit & Scott 1985), generally drives primary afferent discharge to some degree. This is presumably due to repeated stretching and releasing of the sensory terminals by unfused contractions in chain fibres. At intermediate muscle lengths, driving is commonly 1:1 for stimulation rates up to about 75 Hz, then falls to 1:2 or 1:3 for higher frequencies of gamma axon stimulation, and at stimulation rates greater than 100 Hz, primary afferent discharge often becomes totally irregular. In the case of tenuissimus primary afferents, driving of the afferent rate by chain fibre contractions often completely abolishes the response to ramp stretches of moderate velocity, though faster stretches may produce a burst of discharge during the dynamic phase which "breaks through" the driving; even this can be abolished by simultaneously stimulating several static gamma axons innervating chain fibres. Effectively, therefore, chain fibre action makes the primary afferent discharge completely insensitive to muscle length changes, whether lengthening or shortening.

The position with regard to chain fibre action on secondary afferents is more equivocal, partly as a result of some recent ultrastructural investigations of fusimotor distribution within the spindle which will be discussed shortly. I shall present here the view which was held in 1981, just before this ultrastructural work was started, since the more recent work is still incomplete and not universally accepted. Chain fibre action on secondary endings is very powerful, as would be expected from the distribution of secondary terminals (see page 45). At a fixed muscle length, contraction of that pole of chain fibres on which the secondary

ending lies produces biassing of the secondary afferent discharge by 70-100 Imp/sec, though as with bag_2-evoked biassing of primary afferent discharge, this declines with time after the onset of stimulation. The response to a ramp stretch is quite variable, some secondary afferents showing a substantial increase in dynamic length sensitivity (slope of slow rise phase), and consequently an increase in static length sensitivity since chain fibres do not show creep after a stretch; other secondary afferents show no increase in length sensitivity, or even a slight reduction. Where present, increased dynamic length sensitivity is presumably caused by enhanced stiffness of polar parts of chain fibres transmitting more of an applied stretch to the secondary sensory terminals; this effect ought not to be velocity-sensitive since the chain fibre does not show stretch activation, but this matter has not yet been investigated.

It is thus apparent that each intrafusal fibre has characteristic properties which produce certain features of the afferent response, presumably by generating an electrical signal in the sensory terminals applied to the fibre. A question which has not yet been addressed is that of the manner in which signals simultaneously generated in sensory terminals on a number of intrafusal fibres are "mixed" for transmission to the CNS along a single axon. Two possibilities were put forward by Crowe & Matthews (1964a,b), namely that each spiral could provide a non-propagated generator potential which influenced a single pacemaker site, presumably central to the point where the afferent branched to supply intrafusal fibres, or alternatively that each sensory spiral might have its own pacemaker site. In the first scheme, the instantaneous afferent rate would be determined by the summed contributions from all generator potentials, whereas in the second, the individual impulse trains would interact at branch points, the train with the highest instantaneous rate being the major determinant of the final impulse train.

Experimental investigation of this matter involves the simultaneous excitation of two sensory spirals whilst examining primary afferent discharge.

This has been attempted by co-stimulation of a static and a dynamic gamma axon, although not with the isolated spindle technique, so that the exact nature of the intrafusal activity evoked by the gamma axons was not known (Lennerstrand 1968b; Schafer 1974; Cheney & Preston 1976b; Hulliger, Matthews & Noth 1977b,c; Hulliger & Noth 1979). The findings are in favour of a model with several independent pacemakers, the main line of evidence for this being the occurrence of "occlusion" (Crowe & Matthews 1964b); in this phenomenon, the effect of combined dynamic and static fusimotor stimulation on the response to some form of stretch (ramps, Crowe & Matthews 1964b; triangular waveform, Lennerstrand 1968b; sinusoidal stretch, Lennerstrand 1968b; Hulliger, Matthews & Noth 1977b,c; Hulliger & Noth 1979) is less than the sum of the two effects acting separately; occlusion can be partial or complete. For example, Hulliger, Matthews & Noth (1977b,c) showed that for small amplitudes of sinusoidal stretching, static fusimotor action almost completely occluded dynamic action, that as the amplitude of stretching increased the occlusion became less complete as the dynamic contribution increased, and that, for large stretches, there was generally summation of static and dynamic contributions at the peak of a stretch, but complete occlusion of the dynamic action by the static in the trough of the response.

Whilst occlusion could have a mechanical origin, for example if one intrafusal fibre were partially unloaded by simultaneous contraction of another in parallel with it, the favoured view is that it is caused by switching between pacemakers as one becomes more dominant. Hulliger & Noth (1979) found that when static fusimotor action and the amplitude of length changes were kept constant, but the degree of dynamic fusimotor action at the trough of a sinusoidal stretching cycle was independently varied by modulating the rate of dynamic gamma axon stimulation, occlusion of the dynamic action by the static action always occurred when the absolute firing rate during static stimulation alone exceeded that during dynamic stimulation alone, despite the fact that the pattern and degree of dynamic

action could be presumed to take up any slack induced by mechanical unloading. In addition, occlusion of dynamic action by static action was demonstrated at fixed muscle length by the fact that inter-spike interval histograms of Ia discharge constructed during separate dynamic and static fusimotor action were often quite different, and that the histogram obtained during combined dynamic and static fusimotor action was either not different from that obtained during static action alone, or else showed some slight summation of the dynamic and static actions. In order to explain complete static occlusion of dynamic action in the trough of a sinusoidal response, but only partial occlusion of the static by the dynamic response at the peak of the stretch, Hulliger & Noth (1979) invoked a modification of the multiple-pacemaker scheme, suggesting that each sensory terminal might have an associated pacemaker, but that these several pacemakers were serially linked to another situated at some point central to the first-order branch of the Ia axon; during trough occlusion, pacemaker switching from a predominantly dynamic (presumably bag_1) pacemaker to a predominantly static one would occur, whereas during peak summation the pacemakers associated with the sensory spirals would be temporarily overridden, the central pacemaker becoming dominant and summating generator potentials electrotonically conducted to it from the sensory spirals along the terminal branches. A similar model has been proposed for frog muscle spindles which are more amenable to direct electrical study (Ito & Komatsu 1976; Brokensha & Westbury 1978).

More recently, Dutia (1980), in experiments to be described below (p. 86), has found evidence of switching between pacemakers associated with the bag_1 and bag_2 fibres after a ramp stretch when both bag fibres had been powerfully activated by the drug succinylcholine. Quick, Kennedy & Poppele (1980) have found histological evidence, based upon staining for ferricyanide ions, that multiple spike-initiation sites exist in cat Ia axons at their terminal heminodes, and Quick (1986) has shown that, in isolated spindles in which the intrafusal fibres have been

crushed and allowed to degenerate, the inter-spike intervals of Ia axons show clustering about 2 or more favoured values which he interpreted as the contributions of several spike-initiation sites. Boyd (1986) has stated that the effect of non-selective static gamma axons on Ia discharge depends upon the relative strength of inputs from bag_2 and chain fibres, such that the Ia discharge is indistinguishable from that which would be evoked if just the more powerfully affected fibre type were active; Boyd has also argued in favour of pacemaker switching between the bag_1 and bag_2 Ia terminals when a spindle containing an active bag_2 fibre is ramp-stretched (Boyd 1985b, his p. 139). The ability of chain fibre contraction to drive Ia discharge and completely occlude the dynamic response of the primary afferent to a ramp stretch can be taken to show the existence of pacemaker dominance, a prerequisite for pacemaker switching. Finally, Auriemma & Moore (1985) have applied pseudo-random length changes to a muscle while recording primary afferent discharge rate; in one run, the spindle was de-efferented, whilst in the other a static gamma axon was stimulated at a rate determined by a Geiger-counter (i.e. at random). In both cases, spike-triggered length averages revealed the presence in the afferent train of stretch-evoked spikes, but in the latter case there is an additional set of gamma-stimulation-evoked spikes in the afferent train, suggesting that two pacemakers were generating independent trains which then mixed to determine the final Ia discharge.

These various findings all favour models of the primary afferent which invoke several independent pacemakers which interact to determine the final afferent discharge. The possibility that a similar situation may also apply in secondary afferents does not seem to have been studied to date.

VIEWS ON THE EXISTENCE OF SUBTYPES OF STATIC GAMMA AXON.

In a 1981 review (Boyd 1981c), Boyd felt able to say that "The controversy about spindle motor innervation is thus resolved.". Unfortunately, as in fact discovered by Boyd's own group, this statement may have been premature. The presence of two effectors for static fusimotor action, the bag_2 and chain fibres, has been a puzzle for some time, for whilst they clearly have very different properties (see p. 50 et seq.), extensive non-selective innervation of them by static gamma axons would seem to preclude the central nervous system from making use of these differences. It has therefore been suggested that two types of static gamma axon might exist, which by their distribution to the two static effectors might permit the CNS to make use of their different properties (e.g. Boyd, Gladden & Ward 1983). Indirect support for this concept had come from the repeated observation that static gamma action was rather variable (Crowe & Matthews 1964a,b; Brown, Crowe & Matthews 1965; Lennerstrand & Thoden 1968; Emonet-Dénand, Laporte, Matthews & Petit 1977), but the first direct evidence was provided by isolated spindle experiments (Gladden & McWilliam 1977; Gladden 1981) in which the contractile activity of intrafusal fibres was observed during stimulation or ablation of central nervous system structures. Some independence in the fusimotor activation of bag_2 and chain fibres was shown, for example, by the observation that contraction was more often elicited in the bag_2 fibre than in the chain fibres upon stimulating a restricted region around the cruciate sulcus of the parietal cortex. Recently, Wand & Scwharz (1985) have reported that injection of picrotoxin (a GABA antagonist) into the pars reticularis of the substantia nigra in ketamine-anaesthetised cats (in which there is tonic fusimotor activity) has effects on the sensitivity to sinusoidal muscle stretching of primary afferents which are consistent with the removal of a tonic static fusimotor action; in the same preparation, the sensitivity of secondary afferents is unaffected by picrotoxin, but when the ventral roots are divided, secondary afferents behave as though static

fusimotor drive had been removed. These findings have been interpreted as further evidence of the existence of two independently controllable classes of static fusimotor axon.

The principal support for the existence of two types of static gamma axon has come from complex experiments in which the action of single static gamma axons on several spindles has been visually confirmed; this work has repeatedly shown that some static gamma axons preferentially innervate the bag_2 fibre in every spindle which they run to (the "static bag gamma axons"), whereas others activate chain fibres in every spindle (the "static chain gamma axons"; Boyd, Gladden & Ward 1983; Boyd 1986). Nevertheless, non-selective innervation of bag_2 and chain fibres is unarguably a common occurrence (about 30% of cases, Boyd & Gladden 1985), so how can this be fitted with the idea of selective control of the two static effectors? The Glasgow group answers this in two ways; first, they have shown that the efficacy of non-selective static gamma axons is variable, so that one of the two static effectors is generally more powerfully activated than the other and is more important in determining afferent discharge. A more unusual proposal is that chain fibres might receive static gamma innervation from two types of axon, and that activity in these two types of axon might produce different contractile events in the chain fibres and so modulate the transmission of stretch along the activated fibres differently. This suggestion derives from the correlated study of fusimotor action on sensory endings and the electronmicroscopic reconstruction of the motor terminals which elicited it.

Under the electron microscope, Boyd's group have observed that all motor terminals on intrafusal fibres have a plate-like form, of which four types, m_a, m_b, m_c and m_d, are differentiated by the degree of indentation of the terminal into the intrafusal fibre, and by the amount of sub-junctional folding of the sarcolemma (Arbuthnott, Ballard, Boyd, Gladden & Sutherland 1982; Arbuthnott, Sutherland, Boyd & Gladden 1985). The various forms of ending are not present

on all intrafusal fibres; rather, the bag_1 fibre receives only m_b plate endings, and the bag_2 fibre only m_a plates, whereas chain fibres can receive m_a, m_c or m_d plates. Although chain fibres can receive three types of plate terminal, all the terminals formed on a given pole of a chain fibre are of the same type, though the opposite pole can receive terminals of another type. Many of the motor axons studied in these experiments had been classified physiologically, and foci of sarcomere convergence in the intrafusal fibres visualised during the experiment were used to localise the motor terminals; when this information was correlated with the ultrastructural findings, the following conclusion emerged: m_b plates, formed on the bag_1 fibre, are the terminals of dynamic gamma and beta axons; m_d plates formed on long chain fibres are the terminals of static beta axons; *ergo*, m_a and m_c plates are terminals of static gamma axons, and, since, with very few exceptions (Arbuthnott, Ballard, Boyd, Gladden & Sutherland 1982; Sutherland, Arbuthnott, Boyd & Gladden 1985), a particular gamma axon forms either m_a or m_c plates at all its terminals, at least in the single spindles thus far examined, two types of static gamma axon exist. The facts that only m_a plates are formed on the bag_2 fibre, and that m_c plates are only formed on chain fibres suggest that static bag gamma axons identified electrophysiologically form m_a plates and static chain gamma axons form m_c plates; interestingly, nearly all the published examples indicate that non-selective static gamma axons form m_a plates on both the bag_2 and chain fibres, suggesting that the form taken by terminals of a static gamma axon is not determined by the intrafusal fibres it contacts, but is rather an intrinsic property of that axon. In a more recent study (Gladden & Sutherland 1989) the terminals of 42 tenuissimus static gamma axons were reconstructed, and it was again found that a particular static gamma axon is true to type, forming only one type of terminal in all spindles it innervates.

The contractile activity elicited in chain fibres by static gamma axon stimulation has been seen to vary, in some cases being a diffuse contraction of the polar half of the fibres whereas in others there is a weaker contraction localised near

to the end of the chain fibres (Arbuthnott, Ballard, Boyd Gladden & Sutherland 1982; Boyd, Sutherland & Ward 1985), and there have been occasional reports that chain fibres can generate non-propagated rather than propagated potentials in response to stimulation of static gamma axons (Banks, Barker, Bessou, Pages & Stacey 1978; Gladden 1981). Recently, Hunt & Wilkinson (1985) showed that intracellular injection of depolarising current can also elicit either focal contraction or diffuse twitching of chain fibres, suggesting that the earlier observations were not caused by partial failure of neuromuscular transmission. The correlated ultrastructural studies suggest that the diffuse form of chain fibre contraction is centred on m_c plates, whereas m_a plates produce localised contraction as they do in the bag_2 fibre. These differences in contractility of chain fibre poles might be expected to vary the visco-elastic properties of the fibres differently and so to have different actions on sensory endings lying on the juxta-equatorial regions of the fibre. Evidence supporting this concept is fragmentary as yet. First, on one occasion, the terminals of a static chain gamma axon which increased the dynamic and static length sensitivity of a secondary afferent were shown to be m_a type (Boyd, Sutherland & Ward 1985). Local contraction of the ends of chain fibres, produced by m_a plates, might be expected to have such effects on secondary endings by increasing the stiffness of the end of the fibre and transmitting more of an applied stretch to the terminals situated juxta-equatorially (cf. p. 50). Second, two static chain gamma axons which produced 1:1 driving of the discharge of a primary afferent terminated in m_c plates (Boyd & Sutherland 1987); diffuse, non-fused contraction of a pole of a chain fibre might be expected to have this effect. Neither the effects of m_a-plate-evoked chain fibre activity on primary afferent sensitivity to stretch, nor the effects of m_c-plate-evoked chain fibre activity on secondary afferent sensitivity have yet been reported.

Studies of these proposed subtypes of static gamma axon are, however, still quite new, and too few correlations of terminal morphology with fusimotor

action have been made for the conclusions to be widely accepted, although the concept is teleologically very appealing. Moreover, this picture of intrafusal motor innervation does not currently sit well with alternatives (Banks, Barker & Stacey 1985; Kucera & Walro 1986). For example, unlike the Durham group (Banks, Barker & Stacey 1985), neither the Glasgow group (Arbuthnott, Ballard, Boyd, Gladden & Sutherland 1982), nor Kucera & Walro (1986) feel pre-junctional features such as the length of the pre-terminal axon to be of use in categorising motor axons. The Glasgow group do not define a trail ending as such, but axons which form multiple, spaced-out terminations on intrafusal fibres which would probably be defined as trail endings by Barker all form m_a plates; axons ending in m_c plates would probably not be called trail endings, yet Barker's group feel that static gamma axons all form trail endings. Similarly, Barker's group define p1 and p2 plate terminals on the bag_1 fibre, p1 plates being terminals of dynamic beta axons, p2 plates of dynamic gamma axons; Boyd's group define only m_b plates, these being formed by both dynamic beta and dynamic gamma axons. On the other hand, Banks, Barker & Stacey (1985) and Kucera & Walro (1986) both feel that the variation in electronmicroscopic structure of motor terminals is simply a function of their distance from the primary ending, rather than a specific feature of the axons. This matter has been discussed in depth by Boyd and Gladden (1985), where it was agreed that the groups would continue to differ; the Glasgow group has not yet published its findings in detail. Recently, the Durham group (Banks 1988) have moved some way towards a resolution of these difficulties by agreeing that, in a population of static gamma axons, the fastest conducting tend to innervate predominantly bag_2 fibres, the slowest chain fibres, and those of intermediate conduction velocity both bag_2 and chain fibres non-selectively. Although the issue of the existence of subtypes of static gamma axon is not resolved, I have introduced it here as it has implications which might affect the interpretation of the results to be reported below.

Very recently, Gladden & Sutherland (1989) have raised the possibility that static gamma axons might even be of 3 types. This suggestion, published in preliminary form, is based upon the detailed light and electron microscopic study of 42 static gamma axons to tenuissimus muscle spindles. One class of static gamma forms m_a terminals on the bag_2 fibre in every spindle it innervates, with only occasional m_a terminals on one or two chain fibres in some spindles; these axons are analogous to the static bag gamma axons of Boyd (1986). The second class of static gamma axon ends in m_c terminals on chain fibres only in all spindles innervated by it, and is analogous to the static chain gamma axons of Boyd (1986). The final class of static gamma axon forms m_a plate terminals on chain fibres in all the spindles it innervates, with m_a plate termination also on the bag_2 fibre in some spindles. This new model suggests that the high level of non-selectivity of innervation of bag_2 and chain fibres by static gamma axons in many spindles is only apparent, arising out of slightly less than perfect selectivity of two separate classes of static gamma axon which in the main preferentially innervate either chain or bag fibres with m_a plates; given the ability to independently control these two proposed classes of static gamma axon, the CNS may yet be able to selectively activate the two static effectors (chain and bag_2 fibres). A more detailed report of these findings is awaited with interest.

LITERATURE REVIEW, PART 2

The structure of muscle spindle complexes, especially the tandem muscle spindle.

Implicit in much of the physiological work reviewed above has been the assumption that all muscle spindles are basically identical, existing in the muscle as single encapsulated receptors with a single sensory zone and a predictable pattern of nerve supply. However, it was recognised very early on by histologists that considerable variation in the complexity of different spindles existed (e.g. p. 23) and that spindles did not always lie in isolation in the muscle. This observation has been extended in recent years, when the spindle content of muscles other than hindlimb muscles has been studied; this work has shown that the "model" single spindles of limb muscles are actually atypical in many axial muscles, in which spindles commonly occur in complex assemblages, often in restricted regions of a muscle. These assemblages have been given the generic name "conjunctive form" (Richmond & Abrahams 1975b), of which three types are recognised, namely paired linkages, parallel (or compound) linkages and tandem (or series) linkages:

(A) The simplest of the conjunctive forms is the paired linkage (Thompson 1970) in which two or more spindles lie in side-to-side or end-to-end contact with each other, bound together by perimysial connective tissue, but are at all times recognizably separate entities. These structures have been described in human (Cooper & Daniel 1963) and rat (Thompson 1970) muscles; in the cat, they are found in hindlimb muscles (Barker & Ip 1961) but are particularly common in neck muscles (Richmond & Abrahams 1975b,1979b).

(B) Parallel (compound) muscle spindles were first described by Sherrington (1894) in cat hindlimb muscles. In them, the capsules of several spindles, which are clearly separate in their peripheral parts, fuse to form a single capsule near their midpoints; nevertheless, the several intrafusal bundles maintain their individual identities, forming nuclear bags at different levels within the composite capsule, and apparently receiving separate primary afferent innervation (Richmond & Abrahams 1975b). Parallel spindles have been seen in human neck and lumbrical muscles (Cooper & Daniel 1963), frog fourth extensor digitorum

longus muscle (Barker, Cope & Ip 1960; Barker & Cope 1962), cat tail segmental muscles (Goldfinger & Fukami 1982) and, especially commonly, in rat tail segmental muscles (Thompson 1970) and perivertebral muscles of the cat neck (Richmond & Bakker 1982).

(C) The tandem (serial) linkage is one in which a single intrafusal bundle is invested with two to five capsules spaced out along its length, each of which is associated with a separate zone of innervation. It was so named by Cooper & Daniel in 1956 but had in fact been described long before. Barker & Ip (1961) have provided a brief review of the earliest descriptions of tandem spindles which they attribute to Cipollone in the lizard (1897) and Baum in the hedgehog (1900). Tandem spindles were probably first described even earlier than this, judging by the following line taken from Ruffini's classic paper on the cat spindle (in which he refers to his own work in the early 1890s):- "...along the organ occur one or more fusiform dilatations which ... coincide with regions of nerve-ending" (Ruffini 1898, p. 192). Huber & DeWitt (1897), who used the then newly developed methylene blue technique to stain axons and trace them in teased muscles, also described several zones of innervation spaced out along a single intrafusal bundle, and this in such casual fashion as to imply that the observation was not new to them.

Be this as it may, tandem spindles have been seen many times since; they are found in muscles of the frog (early literature cited in Barker & Ip 1961; Gray 1957; Barker & Cope 1962), of man (Cooper & Daniel 1956,1963), of the rat (Thompson 1970) and of the rabbit (Barker 1948). In the cat they are found in most hindlimb muscles; usually tandem spindles are said only to make up around 10% or less of the total spindle content of a hindlimb muscle (Swett & Eldred 1960; Barker & Ip 1961; Bridgman, Eldred & Eldred 1962; Banks, Barker & Stacey 1982; Kucera 1982b; Kucera & Walro 1987). However, it is technically difficult to identify all the tandem spindles in a muscle histologically, since the connections between individual

capsules are very tenuous and readily disrupted, and many of the earlier reports of tandem spindle frequency are probably underestimates. Very recent reassessment of the situation for the peroneus tertius muscle of the cat hindlimb has shown, for example, that as many as 67% of the spindles in this small muscle may form tandem linkages (Scott & Young 1987). It has, however, been recognised for about a decade that in other cat muscles, generally axial muscles and most notably the perivertebral and long extensor muscles of the neck, the incidence of tandem spindles is routinely high, being generally on the order of 35-50% of the total spindle content (Richmond & Abrahams 1975b,1979b; Bakker & Richmond 1981; Goldfinger & Fukami 1982; Richmond & Bakker 1982; Richmond, Bakker, Bakker & Stacey 1986).

A further level of complexity of spindle assembly is attained in the structure known as the "spindle complex" (Richmond & Abrahams 1975b) in which groups of spindles, often already grouped in one or more of the conjunctive forms described above, lie in close proximity to one another in a small region of the muscle. Such spindle complexes, which were first described in frog muscles where they are quite common (Gray 1957, Barker & Cope 1962), have only occasionally been seen in cat hindlimb muscles (Barker & Ip 1961), but have recently been reported to be quite numerous in perivertebral muscles of the cat neck in which complexes containing as many as 12 individual spindle units have been reported (Richmond & Abrahams 1975b, 1979b; Bakker & Richmond 1982; Richmond & Bakker 1982). Finally, the intimate end-to-end arrangement of a Golgi tendon organ and a muscle spindle as a "dyad", which is sometimes observed in hindlimb muscles (Marchand, Bridgman, Shumpert & Eldred 1971), is relatively commonplace in neck extensor muscles (e.g. Richmond & Bakker 1982); in biventer cervicis, for example, Golgi tendon organs are often placed serially between muscle spindles and tendinous inscriptions in the muscle, the spindle involved tends to be of the tandem type, and the capsule immediately adjacent to the Golgi tendon organ tends to be the b_2c spindle unit.

The tandem muscle spindle has been more extensively studied than the other conjunctive forms, and will be the subject of most discussion from here on. The first detailed analysis of tandem linkages in the cat was made by Barker & Ip in 1961 in the rectus femoris muscle; their investigations revealed one of the major features of tandem linkages, namely that the linked capsules of a tandem spindle are not all identical. Thus, in the commonest form of tandem linkage, the "double tandem" (Barker & Ip 1961), in which two capsules are linked by shared intrafusal fibres, one capsule was consistently found to be larger and longer than the other; each contained both nuclear bag and nuclear chain fibres, but the larger generally had more of each (e.g 3-6 bag fibres cf. 0-2).

In the early 1960s, the study of the physiological properties of spindle afferents, and of the intrafusal mechanisms underlying them, was in its infancy, and no particular significance was attached to these differences between linked capsules. In fact, little attention was paid to tandem spindles over the next decade, but in the late 1970s there was a resurgence of interest in them. By this time, two classes of nuclear bag fibre had been identified, and the contribution of their mechanical properties to the functional characteristics of primary afferent axons was becoming clear (Literature Review, Part 1, pp. 46 et seq.). In this atmosphere, the observation, made in neck tandem muscle spindles, that the smaller capsules often contained only one nuclear bag fibre, whereas the larger capsules always contained at least two (Richmond & Abrahams 1975b) took on a new significance, since the absence of either type of nuclear bag fibre might have been expected to significantly modify the response to stretch of primary sensory endings. The nature of the missing bag fibre was obviously of great interest, and it was soon shown by enzyme histochemical (Bakker & Richmond 1981; Kucera 1982b) and ultrastructural (Banks, Barker & Stacey 1982) techniques to be almost invariably the dynamic bag_1 fibre in both hindlimb and neck tandem spindles.

This finding has led to the adoption of the following nomenclature

when describing tandem spindles (Banks, Barker & Stacey 1982): any small capsules present, which contain only a static bag_2 fibre and a few chain fibres are called "b_2c spindle units", while the larger capsules containing both types of nuclear bag fibre are called "b_1b_2c spindle units". A double tandem spindle generally consists of a b_2c spindle unit and a b_1b_2c spindle unit, the static bag_2 fibre being continuous between the two capsules (Bakker & Richmond 1981; Kucera 1982); other combinations including b_1b_2c:b_1b_2c double tandems and various triple tandems such as b_1b_2c:b_2c:b_2c have also been described (Richmond, Bakker, Bakker & Stacey 1986).

Another difference between the intrafusal fibre complement of b_1b_2c and b_2c spindle units is in their chain fibre contents. The same range of chain fibre sizes and histochemical reactivities are found in b_1b_2c spindle units taking part in tandem linkages as are found in single spindles, including intermediate and long chain fibres (Kucera 1980,1982a); b_2c spindle units, however, are reported to contain either no long chain fibres (Richmond, Bakker, Bakker & Stacey 1986; Richmond, Bakker & Stacey 1988) or relatively few of them (Kucera & Walro 1987). Clearly, the b_2c spindle unit contains only a restricted subset of intrafusal muscle fibres, whereas the b_1b_2c spindle unit contains the full complement.

The sensory innervation of b_1b_2c and b_2c spindle units has been examined in detail by several groups (Barker & Ip 1961; Banks, Barker & Stacey 1982; Richmond, Bakker, Bakker & Stacey 1986). It has repeatedly been found that b_1b_2c spindle units are apparently identical to conventional single spindles; they generally receive either intermediate or complex sensory innervation as defined by Ruffini (1898), with primary terminals of regular form (for definition, see Barker & Ip 1960) distributed to all intrafusal fibres in the encapsulation. In contrast, b_2c spindle units generally exhibit a simple innervation pattern, receiving only one large myelinated axon which has an irregular terminal

morphology, both under the definition of Barker & Cope (1960) and, in neck spindles (Richmond, Stacey, Bakker & Bakker 1985), in the sense that, in many cases, first-order branches of the axon are serially distributed to the intrafusal bundle, one branch running to innervate its nuclear bag, the other its myotube regions.

That this ending should nevertheless be considered to be a primary is attested to by the facts that: (a) the nucleation of the intrafusal fibres underlying the termination of the axon resembles that seen under primary axons in single spindles and b_1b_2c spindle units; (b) the relation of the ending to the capsule and fluid space is typical of primary innervation; and (c) in some b_2c spindle units (usually less than 10%; Richmond, Bakker, Bakker & Stacey 1986), the ending is accompanied by another which terminates to one side of it and resembles S1 secondary endings (Banks, Barker & Stacey 1982). In addition, sensory cross-terminals between endings on two intrafusal fibre types are rare, and non-myelinated pre-terminal axons are not formed from penultimate nodes of the axon (Kucera & Walro 1987), both these being features of primary rather than secondary endings (Banks, Barker & Stacey 1982). On the average, the diameter of the primary axon supplied to b_2c spindle units is less than that of the primary axon supplied to b_1b_2c spindle units (3.8 μm cf. 5 μm in neck muscle spindles, Richmond, Bakker, Bakker & Stacey 1986; 5.1 μm cf. 7.5 μm in hindlimb muscle spindles, Banks, Barker & Stacey 1982).

The existence of the primary ending in b_2c spindle units throws up a problem of nomenclature which is probably best addressed now in the interests of clarity later. This is that different authors mean slightly different things by the term primary axon; when it was first coined by Ruffini (1898), it was used to stress the difference between the large myelinated axon which has an annulospiral termination near the middle of ALL spindles and the additional myelinated axons which MAY be found (hence secondary) terminating to one side of it in some spindles, and this is still broadly the meaning attached by anatomists to the term. However,

electrophysiologists have slipped into the habit of calling an afferent axon primary if it has a certain conduction velocity, is sensitive to the dynamic component of a muscle stretch and/or can be activated by dynamic fusimotor axons, with the implicit assumption that the afferent in question has terminals on a dynamic nuclear bag_1 fibre in its spindle of origin. Clearly one cannot justify the use of the term primary in this latter sense to describe the afferent from the equatorial ending of a b_2c spindle unit which does not possess a dynamic bag_1 fibre; yet if one called it primary with the anatomical sense in mind, this might be expected to cause confusion should the afferent's properties ever become open to study. Very recently, Richmond appears to have decided to call this type of afferent the "b_2c afferent" (see Fig. 4.3, Richmond, Bakker & Stacey 1988), but there is a possible objection to this terminology too in that many secondary axons, especially those in the S1 and S2 positions adjacent to the primary ending (Boyd 1962), have terminals on the static bag_2 fibre as well as on chain fibres (Banks, Barker & Stacey 1982) and their afferents could therefore be described as b_2c afferents. In the light of these considerations, I have preferred to be completely explicit and have therefore called the equatorial afferents from b_2c spindle units "b_2c primary afferents" and those from b_1b_2c spindle units "b_1b_2c primary afferents".

The position with regard to the motor innervation of tandem spindles has been studied in detail by Richmond, Bakker, Bakker & Stacey (1986) and by Kucera & Walro (1987). Both groups agree that the b_1b_2c spindle units of tandem spindles are very similar to single spindles in their fusimotor innervation; they are supplied by many fusimotor axons (a mean of 4.6 per spindle pole, Richmond, Bakker, Bakker & Stacey 1986), both gamma and beta axons of both static and dynamic type as evinced by the presence of p1 and p2 plates as well as trail terminals on the intrafusal fibres. In contrast, b_2c spindle units receive relatively fewer fusimotor axons (a mean of 1.3 per spindle pole, Richmond, Bakker, Bakker & Stacey 1986), even when allowances are made for the absence of dynamic fusimotor

axons and the reduced number of intrafusal fibres in b_2c spindle units (Kucera & Walro 1987). In neck tandem spindles, motor terminals are exclusively of the trail form and are presumably all supplied by static gamma axons (Richmond, Bakker, Bakker & Stacey 1986), though in the hindlimb, there may also be occasional innervation by static beta axons which form plate terminals on long chain fibres (Kucera 1982b; Kucera & Walro 1987).

Both Richmond and Kucera find that the trail terminals in b_2c spindle units are usually distributed non-selectively to both the static bag_2 fibre and the chain fibres; occasionally, an axon supplies terminals only to the chain fibres in one pole, but there has only been one report of selective innervation of the bag_2 fibre, and this was an unusual case in that the axon in question had been physiologically identified as a dynamic gamma and had been shown to selectively innervate the bag_1 fibre in three other spindles (Boyd & Sutherland 1987). Whether such dynamic fusimotor innervation of b_2c spindle units is at all common has yet to be determined, although it might be expected not to be. In b_1b_2c spindle units, both non-selective and selective distributions of trail terminals are commonly seen. A systematic examination of the ultrastructure of the motor terminals in b_2c spindle units, as performed on single muscle spindles in hindlimb muscles by Arbuthnott, Ballard, Boyd, Gladden & Sutherland (1982) to distinguish two types of static gamma axon terminal morphology (Literature Review, Part 1, p. 60), has not yet been attempted. One b_2c spindle unit encountered by chance in the tenuissimus muscle has been studied in this way by Boyd & Sutherland (1987) who found it to contain m_a plates on the bag_2 fibre as expected; the type of terminal formed on chain fibres was not disclosed, but is a matter of some interest, for it might not only show that the fusimotor innervation of b_2c spindle units is even more restricted than is already appreciated if only one form of terminal were found, but it might also provide additional support for the contention by the Glasgow group that two or even three (Gladden & Sutherland 1989) types of static gamma axon exist.

The functional significance of the various conjunctive forms is still unclear. In the light of the current views on the genesis of spindle afferent responses (Literature Review, Part 1, pp. 50 et seq.), there is no particular reason to expect their afferents to have unusual properties, unless either the content of intrafusal fibres, or else the intrafusal distribution of innervation is unusual. The tandem muscle spindle fulfils both these requirements, but there is very little pertinent evidence for the parallel and paired conjunctive forms. Nevertheless, the close proximity of several spindle units in these conjunctive forms may have functional implications in terms of the central connectivity of their afferents, rather than of their intrinsic functional characteristics. For example, several groups have championed the idea that muscles are divided into a number of compartments, and that muscle spindles and Golgi tendon organs may be especially sensitive to mechanical perturbation of the compartment in which they lie, and may be especially powerfully connected to motor neurones in whose motor unit fields they are situated (see e.g. Binder & Stuart 1980); in such a system, spindle assemblages might have some special role. On the other hand, the possibility also exists that assemblages form simply because a muscle is overladen with spindles; perivertebral muscles of the cat neck, for example, may contain as many as 460 spindle units per gram of tissue (Bakker & Richmond 1982), and it seems unavoidable that these should pack close together in assemblages. These possibilities remain to be assessed.

Turning to tandem spindles, it seems extremely likely, given the degree of structural similarity between single spindles and the b_1b_2c spindle units of tandem spindles, that the functional properties of primary and secondary afferents from the latter will be very similar to those from the former. As for the b_2c spindle units, it would also seem unlikely that the properties of the occasional secondary afferent innervating them should be significantly different from those of secondaries innervating b_1b_2c spindle units and single spindles. The b_2c

primary afferent, on the other hand, has been suspected of having quite novel receptive properties thanks to its lack of a dynamic bag_1 fibre. In particular, several authors have suggested that b_2c primary afferents might correspond to "intermediate" afferents encountered during functional studies of the muscle spindle (e.g. Richmond & Abrahams 1979a; Inoue, Morimoto & Kawamura 1981).

Much of the experimental investigation of muscle spindle function since the early 1960s has been directed at establishing a functional dichotomy of spindle afferents to match the apparent morphological dichotomy of primary and secondary endings. Up to a point, this approach has been successful, but from the outset it has been appreciated that the division is not absolute, and that some "intermediate" afferents have properties which do not allow them to be placed into either the primary or secondary categories (e.g. Matthews 1963). There are, of course, as many different definitions of "intermediacy" as there are methods of categorising spindle afferents, for in most cases the concept arises from uncertainties in the positioning of diagnostic criteria in each system; the term has thus frequently been used as a euphemistic alternative to "unclassifiable".

The most widely used classification system is that based on conduction velocity which was introduced by Merton (1953) and Hunt (1954); in this case, as pointed out early on by Matthews (1963), the uncertainties arise not just because there is a degree of overlap between the diameters of primary and secondary afferent axons measured at the spindle, but also because it is by no means established that the diameter of an afferent remains the same all the way to the dorsal root, and because of uncertainties about the conversion factor between an afferent's diameter and its conduction velocity (Boyd & Davey 1968). As a result, the conduction velocity classification is now commonly used as follows: an afferent conducting at less than 60 m/sec is firmly diagnosed as being a secondary afferent, one conducting at over 80 m/sec as a primary afferent, and one conducting at 60-80

m/sec as an "intermediate" afferent (Matthews 1972). Strictly speaking, these values are only applicable in cat hindlimb muscles:- in other cat muscle nerves, the afferent diameter spectra may not be bimodal, so that it is impossible to identify a borderline between primary and secondary afferents (e.g. neck extensor muscles Richmond, Anstee, Sherwin & Abrahams 1976), and even sticking with hindlimb muscles, in other species the cut-off between primary and secondary afferent diameters may be differently positioned (e.g. baboon, Cheney & Preston 1976a; rat: Andrew, Leslie & Thompson 1973).

Using the classification by conduction velocity as a benchmark, a variety of functional characteristics of spindle afferents have been investigated with a view to using them to classify afferents. One of the commonest of these is the afferent sensitivity to dynamic components of muscle stretching, for it has repeatedly been shown that rapidly conducting (presumably primary) afferents are more dynamically sensitive than slowly conducting (presumably secondary) afferents to various patterns of muscle stretching (ramp stretch: Matthews 1963; small-amplitude vibration: Bianconi & van der Meulen 1963; Brown, Engberg & Matthews 1967a; Trott 1976; triangular stretch: Lennerstrand 1968a; sinusoidal stretch: Lennerstrand 1968a; Matthews & Stein 1969a; Cussons, Hulliger & Matthews 1977; Hulliger, Matthews & Noth 1977). However, there was always a degree of overlap between the characteristics of presumed primary and secondary afferents, so that some were classified as having "intermediate" properties. To complicate matters, an afferent classified as intermediate by its conduction velocity might often be reclassified as primary or secondary on the basis of its functional characteristics and vice versa; in fact it was quite unusual for both systems to categorise an afferent as intermediate (Matthews 1963).

Nevertheless, functional classification has proved useful in some circumstances; for example, muscle spindle afferents of cat jaw (Cody, Lee & Taylor 1972; Inoue, Morimoto & Kawamura 1982) and neck muscles (Richmond &

Abrahams 1979a; Chan, Kasper & Wilson 1987) and of rat tail muscles (Andrew, Leslie & Thompson 1973) cannot readily be classified on the basis of their conduction velocity, either because this cannot be measured for technical reasons, or else because the fibre diameter spectra of myelinated afferent axons from the muscle in question are not clearly bimodal (e.g. Richmond, Anstee, Sherwin & Abrahams 1976). In such cases, functional properties have been used to classify some of the afferents encountered as primary or secondary, but many others have had to be classified as intermediate; in one extreme study of this kind, the majority of afferents from sheep extra-ocular muscle spindles were classified as intermediate (Browne 1975).

Other afferent properties have been sought which might permit a more complete dichotomy. One such is the unitless measure of discharge variability, the coefficient of variation, first introduced by Matthews & Stein (1969b). This is defined as the standard deviation of the inter-spike intervals of a long train divided by the mean interval, and these authors showed that secondary afferents have values below 0.025 whereas primary afferents have values above 0.025; the histogram they published shows a remarkably small degree of overlap between the two populations, but afferents with intermediate conduction velocities had been excluded from their sample. If an alternative means of identifying primary and secondary afferents were used, so that afferents with intermediate conduction velocities could be studied, there would almost certainly turn out to be more overlap between their coefficients of variation. A recent variant of this approach has been to measure several aspects of afferent responsiveness and to plot these against each other; thus, Jami & Petit (1979), faced with a very extensive overlap in the dynamic indices of primary and secondary afferents from the peroneus tertius muscle calculated the ratio of the dynamic index to the static length sensitivity of the afferents and found that the separation between the classes improved, but was still not complete. Similar findings have also been reported by Wei, Kripke & Burgess (1986) when several indices of soleus and tibialis anterior spindle afferent responses to stretch, which had

been logarithmically transformed, were plotted against each other.

One problem inherent to all these attempts to classify spindle afferents on the basis of their passive properties is that they use as a benchmark a classification scheme, the afferent conduction velocity, which is itself not totally reliable. A more fundamental problem is that as the structure of the muscle spindle has been studied in greater detail with better techniques, there has been less and less reason to expect to find a dichotomy of afferent properties. Two recent findings are particularly important in this respect. Firstly, as was described above (Literature Review, Part 1, p. 46), it is now appreciated that the exact terminal distribution of secondary endings is very varied, ranging from restriction to chain fibres, to termination on all three types of intrafusal fibre; a range of stretch sensitivities is therefore to be expected of secondary afferents depending both upon the extent of their termination on the bag fibres, and upon the type of bag fibre contacted. Secondly, the recently identified b_2c primary afferent, which in whole-body terms must be far from a rarity, might be expected to have quite novel functional characteristics due to the absence of bag_1 fibre input to the discharge of an afferent which must otherwise be considered to be a primary afferent. It is hardly surprising to find that attempts to force afferents into a dichotomous classification scheme have met with only limited success.

A radically different approach to the functional classification of spindle afferents is required if "truly intermediate" and "unclassifiable" afferents are to be distinguished. Such a technique has in fact been available for over 30 years, although our understanding of intrafusal mechanisms and how these relate to afferent discharge has only been mature enough to allow it to be properly applied for the last decade. The basis of this technique is discussed in the final part of this Literature Review.

LITERATURE REVIEW, PART 3

Cholinergic drugs and the muscle spindle.

The action of cholinergic drugs on the muscle spindle was first investigated by Hunt in 1952, presumably as one aspect of the characterisation of gamma fusimotor axons with which he was involved at that time. He showed that the excitatory action of gamma fusimotor axons on cat soleus spindle afferents could be inhibited by the anti-cholinergic drug D-tubocurarine, enhanced by the cholinesterase inhibitor physostigmine and mimicked by IV injection of acetylcholine (ACh), this last effect being blocked by prior curarisation. From these findings, he concluded that neuromuscular transmission at the intrafusal motor terminals was cholinergic, as at the extrafusal neuromuscular junction, and that ACh affected spindle afferent discharge via contraction of intrafusal fibres and stretch of their sensory region.

Some of these findings were confirmed shortly afterwards by Granit, Skoglund & Thesleff (1953) who studied the effects on spindle afferent discharge of succinylcholine (SCh), a synthetic, slowly-metabolised analogue of ACh. They agreed with Hunt that D-tubocurarine could completely block the effect of gamma axons on spindle afferent discharge, and that injection of a cholinomimetic into the aortic bifurcation could excite spindle afferents. However, they also reported findings which they interpreted as evidence of a direct electrical effect of SCh on spindle sensory terminals. For this part of their work, filaments of ventral roots containing both alpha and gamma axons were stimulated with brief trains of shocks (5 impulses at 20 Hz); the stimulus intensity was gradually increased while the discharge of a spindle afferent was studied in a dorsal root filament. At low stimulus intensities, the spindle afferent discharge fell due to mechanical unloading by the extrafusal contraction, but at higher intensities, a burst of afferent impulses during the stimulation indicated that gamma action on spindle afferents was overcoming the mechanical unloading effect. When such a position was reached, small doses of D-tubocurarine were administered until the gamma evoked afferent burst had just been blocked; finally, an intra-arterial bolus injection of SCh was

administered, and in some cases, this injection of SCh was shown to be still capable of exciting the spindle afferent.

With hindsight, this can be seen to be rather flimsy evidence for a direct electrical action of SCh on sensory terminals for two reasons. Firstly, the gamma fusimotor action produced by brief, low-frequency trains of stimuli at the short muscle lengths implied by the low resting discharge rate of the afferents illustrated in the study of Granit, Skoglund & Thesleff (1953) would today be considered rather ineffectual (see e.g. Emonet-Dénand & Laporte 1981); presumably the dose of D-tubocurarine required to just block any weak effect would be rather lower than that required to completely block intrafusal neuromuscular transmission and the effect of SCh. Secondly, the gamma action must have been weak for a second reason, namely that only a few of the gamma axons to the spindle under study are likely to have been encountered in the ventral root filament which was stimulated. Nevertheless, the idea of a direct electrical action of SCh on spindle afferents entered the literature, and received support from the finding of Henatsch & Schulte (1958) that D-tubocurarine did not completely block the action of SCh on frog spindle afferent discharge. Interestingly, this supposed direct electrical action was not found to extend to Golgi tendon organ afferents, for these were not excited by SCh injections, even when the dose was several times that required to excite spindle afferents (Granit, Skoglund & Thesleff 1953). The concept of an direct action of SCh on muscle spindle afferent terminals continued to appear in the literature at least until 1969 (e.g. Kidd & Kucera 1969), but appears to have dropped out of favour more recently.

Support for the notion of a direct action of cholinergic drugs on sensory nerve terminals in general came from the work of Douglas and his collaborators; Douglas & Gray (1953) reported that close intra-arterial injection of ACh increased the discharge of cutaneous mechanoreceptors, and Douglas & Ritchie (1960) found that doses as low as 2 μg of ACh given intraarterially could elicit

trains of impulses in myelinated and unmyelinated cutaneous afferent axons. In both studies, there was no question of an effect mediated via contraction of either skeletal or smooth muscle, since the sensory receptors had no associated muscular elements and since various pharmacological pre-treatments had been used to block muscle contraction. In these experiments, a wide variety of sensory axons was excited by SCh, making the finding of Granit, Skoglund & Thesleff (1953) that Golgi tendon organs were insensitive to SCh somewhat surprising.

Brinling & Smith (1960) excited cat gastrocnemius spindle primary afferents with repeated injections of SCh into a jugular vein and found no evidence of tachyphyllaxis or cumulation in response to the repeated injections, provided that sufficient time elapsed between injections; depending upon the dose of SCh (12.5-800 μg/kg), this time ranged from 10 to 30 minutes. The effects of continuous IV infusions of SCh (200-400 μg/kg/min) were also studied; at the lower rates of infusion, the Ia firing rate was increased gradually to a plateau which was sustained as long as the infusion continued and declined gradually to the control rate when the infusion was stopped. Higher infusion rates produced an early overshoot in the afferent discharge rate which subsequently also settled to a plateau. Pretreatment with physostigmine enhanced the effect of a given dose of SCh, showing that SCh certainly had an action on primary afferents via the intrafusal fibres, whether or not a direct electrical action was also operative.

Smith & Eldred (1961) attempted to dissociate the possible indirect mechanical and direct electrical effects of SCh by stimulating gamma axons to cat gastrocnemius muscle spindles in the presence of ryanodine. This protocol produces irreversible contraction of extrafusal muscle fibres, and presumably also of intrafusal fibres, and was shown to reduce the effect on primary afferent discharge of bolus IV and IA injections of SCh. The incomplete blockade of SCh action was presumed to result from failure to stimulate all the gamma axons to the spindle, a

hypothesis which the authors attempted to verify by stimulating the entire tenuissimus muscle nerve in the presence of ryanodine. Whilst this did entirely block the effect of SCh on afferent discharge, the blood supply to the muscle had probably been severely restricted, so that the delivery of SCh was uncertain and may have been too low to elicit the direct effect.

Ottoson (1961) was the first to succeed in blocking the mechanical effects of SCh on spindle afferent discharge. In frog muscle spindles microdissected from the muscle, he cut or crushed the intrafusal fibres outwith the non-contractile sensory region. In this preparation, neither ACh nor SCh excited spindle afferents, whereas both had done so before the intrafusal fibres were damaged. Thus, in the frog, cholinergic drugs were shown to have a purely indirect action on spindle afferent axons.

Similar experiments to that of Ottoson not yet being feasible in cat studies, indirect approaches continued to be used. Fehr (1965) studied the action of SCh on primary and secondary afferents from the same tenuissimus muscle spindle and found that both were excited by the drug, but that the primary afferent was always excited more powerfully and at a shorter latency from an IV injection than the secondary afferent. He explained this finding by suggesting that both nuclear bag and nuclear chain fibres were made to contract by SCh but that the bag fibres contracted earlier, i.e. that they had a lower threshold; since primary afferent terminals were situated on both bag and chain fibres whereas those of secondary afferents were generally restricted to chain fibres, the action of SCh on primary afferent discharge was more rapid and more powerful than that on secondary afferent discharge. It was therefore felt to be unnecessary to invoke a direct electrical effect on sensory terminals to explain the action of cholinomimetics on cat spindle afferents, but there was no clear evidence that it did not exist.

An important advance was made by Rack & Westbury (1966) when

they studied the effects of SCh injections on soleus spindle afferent discharge during repeated ramp-and-hold muscle stretching. In these experiments, marked differences in the effects of SCh on primary and secondary afferent discharge were observed in addition to the different latency after injection and degree of biassing which had been seen by Fehr (1965). Thus, the dynamic sensitivity of primary afferents (assessed by the dynamic index) was greatly increased by the action of SCh, with a smaller, and rather variable, increase in the length sensitivity of the afferent, whereas the discharge of secondary afferents was simply biassed without appreciable change in either dynamic index or length sensitivity. The effects of SCh on primary afferent discharge increased with increasing dosage until the very high discharge rates achieved during stretching reached a limiting value which varied from axon to axon and was presumably set by the duration of an individual axon's refractory period. Rack & Westbury also showed the effect of SCh on primary afferents to be very similar to that produced by dynamic fusimotor stimulation; indeed the effect of low doses of SCh and weak dynamic fusimotor action on a primary afferent summated until the limiting discharge rate of the afferent was reached, leading them to conclude that the two effects were mediated by the same intrafusal mechanism. Their findings were interpreted in the light of the simple model of the spindle proposed by Matthews (1964; Literature Review, Part 1, p. 35); it was proposed that dynamic gamma axons controlled nuclear bag fibres, and that the contraction of these produced by SCh caused the increase in dynamic index of primary afferents, whereas the chain fibres were controlled by static gamma axons, and that their activation by SCh resulted in the biassing of both primary and secondary afferent discharge. Again, there was felt to be no need to invoke a direct electrical effect of SCh on sensory terminals, but there was no evidence that it did not exist.

Smith (1966) was the first to visually observe the effects of SCh on mammalian intrafusal fibres. Having isolated the sensory zone of rat muscle

spindles, he superfused the preparation with SCh and saw that some, but not all, of the intrafusal fibres contracted. In particular, "slow" intrafusal fibres, which were probably nuclear bag fibres, went into sustained contraction, or contracture, in the presence of SCh, whereas "fast" (chain) fibres not only did not contract in the presence of SCh, but also failed to contract in response to direct electrical stimulation. Thus, contrary to the suggestion of Fehr (1965) and Rack & Westbury (1966), the biassing of secondary afferent discharge by SCh could not have been caused by contraction of the nuclear chain fibres. Given the understanding at that time that secondary sensory terminals were almost exclusively distributed to chain fibres, this finding was taken as strong evidence of an indirect effect of SCh upon sensory terminals in cat muscle spindles. Although both Smith and Boyd (who performed similar experiments to Smith's at about this time but did not publish the results for many years - Boyd 1985a) were in an ideal position to repeat the experiments of Ottoson (1961) in mammals and thus settle this issue, they do not appear to have done so to date.

Gladden (1976) examined the effect of ACh on the intrafusal fibres of isolated cat muscle spindles. By this time, it had been realised that there were two types of nuclear bag fibre, and their various properties were a matter of great interest. In these experiments, the isolated spindle was shortened to a point at which the chain fibres and one of the two bag fibres became "kinked"; in this situation, isolated contraction of one intrafusal fibre type (detected by a reduction in the degree of kinking as slack was taken up) will not pull on adjacent fibres and give the false impression of contraction in those fibres also. When ACh (10^{-5} mg/ml) was added to the perfusate, one of the bag fibres, usually the less kinked one, was seen to contract in isolation, this contraction continuing as long as the ACh was present and reversing upon washing out the drug. At higher concentrations of ACh (10^{-4} mg/ml), the less kinked bag fibre again contracted, followed after a short delay by the more kinked bag fibre, and both remained contracted until the ACh was removed. Apart

from brief fasciculations when first exposed to ACh, the chain fibres were not seen to contract in these experiments and were insensitive to electrical stimulation after exposure to SCh, as Smith (1966) had earlier reported for rat spindles. These findings were shown shortly after also to apply to the effects of SCh on intrafusal fibres, although the results have only recently been published (Boyd 1985a).

Gladden correlated a number of other observations with those on intrafusal sensitivity to ACh, and showed that the more sensitive bag fibre was more slowly contracting than the less sensitive one, had fewer elastic fibres associated with it in its polar regions and had an enzyme histochemical profile characteristic of the bag_1 fibre of Ovalle & Smith (1972). She therefore concluded that chain fibres were completely paralysed by ACh, but that both types of bag fibre went into contracture, the bag_1 fibre having a lower threshold than the bag_2 fibre.

It soon became apparent that SCh might be a very useful experimental tool, since it permitted the activation of intrafusal bag fibres independently of chain fibres. This facility was especially useful when studying the actions of bag_2 fibre contraction on afferent sensitivity, since the only other means of inducing such activity (static fusimotor stimulation) very often also caused chain fibre contraction due to the frequency of non-selective static fusimotor innervation of the two types of intrafusal fibre (p. 40). The first to use SCh in this way was Dutia in a study of the effects of slow intravenous (IV) and intra-arterial (IA) infusions of SCh on soleus muscle spindle (Dutia 1978,1980) and Golgi tendon organ (Dutia 1978; Dutia & Ferrell 1980) afferent discharge during repeated ramp-and-hold muscle stretching. As had been observed by Rack & Westbury (1966), substantial differences between the responses of primary and secondary afferents to SCh infusion were seen. Thus, the discharge of secondary afferents (and of some low threshold Golgi tendon organ afferents) was simply biassed by a small amount, without notable changes in the dynamic index or length sensitivity. Primary afferents, on the other hand, were activated in three "Phases" by SCh; Phase I consisted of a small, gradual biassing

of the discharge similar to the entire activation of a secondary afferent. This was followed by Phase II during which the dynamic index of the primary afferent was dramatically increased, and finally, in 18 of 20 primary afferents, by Phase III during which the length sensitivity increased substantially.

Various interpretations of Phase I of activation of primary afferents and of the entire activation of secondary afferents and Golgi tendon organ afferents were considered by Dutia (1978). One was that, as discussed above, SCh might have a direct electrical effect on spindle afferents. However, in the absence of direct evidence for such an action, Dutia favoured an alternative explanation first put forward by Smith (1966), namely that potassium ions released from paralysed extrafusal fibres (Paton 1956), and possibly also from intrafusal fibres, especially paralysed chain fibres, might themselves depolarise sensory terminals. In favour of this mechanism were the following observations; firstly, Lippold, Nicholls & Redfearn (1960) reported that the discharge rate of cat tenuissimus spindle afferents in isolated spindle preparations was markedly increased by excess potassium in the perfusate. Secondly, Kidd & Vaillant (1974) showed in experiments on isolated rat muscle spindles that an increase in the concentration of potassium ions in the bathing medium from 5 mmol/l to 8 mmol/l produced a facilitation of the spindle afferent response to stretching, as was seen in Dutia's experiments. It is worth pointing out that an increase in the plasma concentration of potassium ions, which has been reported to follow IV injections of SCh (see Paton 1956), could also account for the "direct" electrical excitation on a variety of cutaneous afferent axons of cholinomimetics reported by Douglas and his collaborators (p. 81).

Phase II of the activations was interpreted as the effect upon primary afferent discharge of SCh-evoked contracture of the dynamic bag_1 fibre; the visualised spindle experiments of Boyd's group (Literature Review, Part 1, p. 47), which were

actually running contemporaneously with those of Dutia and in the same laboratory, allowed of no other interpretation of such profound increases in afferent dynamic index as Dutia routinely saw. The later changes of Phase III, were then naturally attributed to the effects of bag_2 fibre contracture on primary afferent sensitivity; these changes occurred only in primary afferent sensitivity, their timing of in relation to the start of a SCh infusion fitted with the reported lesser sensitivity of bag_2 fibres to the effects of SCh (Gladden 1976), and the increase in position sensitivity of the afferents also fitted with the understanding at that time of the action of the bag_2 fibre on primary afferent sensitivity.

The effects of SCh on afferent discharge could thus be used to reliably identify primary and secondary afferents during an electrophysiological experiment. In addition, Dutia (1980) was able to use responses to SCh infusion to attempt to differentiate "truly intermediate" and "unclassifiable" spindle afferents. The conduction velocity of all afferents was measured in his experiments, and a total of 14 had "intermediate" conduction velocities of 60-80 m/sec. When these 14 afferents were excited with SCh, 5 showed a pattern of activation typical of secondary spindle afferents and were reclassified as such; similarly, 4 afferents had patterns of excitation by SCh typical of primary afferents and were reclassified as such. The remaining 5 afferents were then diagnosed as "truly intermediate"; when activated by SCh, they behaved initially very much like secondary afferents, but subsequently underwent a significant increase in length sensitivity (4 afferents) or dynamic index (1 afferent). These delayed effects were all very small compared to similar effects seen in primary afferents, and were interpreted as evidence of bag_2 (4 afferents) or bag_1 (1 afferent) fibre influences on the discharge of secondary afferents, i.e. "truly intermediate" afferents were identified as secondary afferents with bag fibre terminals in addition to chain fibre terminals. At that time, such secondary afferents were felt to be quite rare, and the scarcity of "truly intermediate" afferents was expected.

As was discussed in Part 2 of this Literature Review, other workers had previously identified groups of afferent which were "intermediate" between typical primary and secondary afferents both in terms of their conduction velocity, and in terms of one or other of a variety of functional characteristics. The advance provided by the use of SCh-evoked changes in afferent behaviour as a means of reclassifying some "intermediate" afferents as primary or secondary is that it approaches as close to an anatomically-based reclassification as it is possible to get without isolating the spindle and visually confirming the identification. This has, of course, been done for one by the seminal work of Boyd and his collaborators which has been much referred to above. As a result, it is little more than a matter of pattern recognition to say whether an afferent has terminals on bag fibres, which fibres these are on and whether the ending is equatorial (primary) or juxta-equatorial (secondary). With identification at this level of detail possible, a diagnosis of "intermediate" need no longer mean "unclassified".

Since 1978, SCh has been used periodically by others to assist in the identification of muscle spindle afferents when other means were not sufficiently reliable (e.g. Prochazka & Wand 1980; Prochazka, Trend, Hulliger & Vincent 1989), but "intermediate" afferents, if even considered, have often remained effectively "unclassified" since the approach of Dutia (1980) was not applied (e.g. Inoue, Morimoto & Kawamura 1981). Other authors who have used SCh as an aid to classification of spindle afferents include Gregory & Proske (1987), who were trying to identify primary and secondary spindle afferents from kitten soleus muscles when these were too immature to have fully developed the functional characteristics of their adult analogues, and various groups who have used SCh as an alternative to fusimotor stimulation for producing bag fibre contraction selectively (Boyd 1985a; Dickson, Gladden, Halliday & Ward 1988). There does not appear to have been any attempt so far to use SCh in the manner introduced by Dutia in order to identify b_2c primary afferent axons, a task for which it seems eminently suitable. This has

been the main aim of the present experiments.

CHAPTER ONE

Experiments performed on the neck extensor muscle biventer cervicis.

1:1 EXPERIMENTAL RATIONALE.

As was described above (p. 67), the incidence of b_2c spindle units varies between muscles, but is particularly high in cat neck extensor muscles in which up to 50% of spindles occur in tandem linkages. Since each tandem linkage generally contains at least one b_2c capsule, at least 25% of some neck muscle primary afferents must come from single-bag spindle units, and the real percentage may in fact be higher, since tandem linkages in neck muscles often contain more than one b_2c capsule (Richmond, Bakker, Bakker & Stacey 1986). In addition, the neck muscles contain very large numbers of muscle spindles (Richmond & Abrahams 1975b), and it therefore seemed expedient to attempt to identify b_2c primary afferents in these muscles first. The biventer cervicis muscle was chosen since it is well-defined, readily exposed and easy to stretch repeatably along its natural axis of pull (the muscle is non-pennate: Richmond & Armstrong 1988) unlike some of the other long extensors of the neck.

In a previous study of afferents from the biventer cervicis muscle, Richmond & Abrahams (1979a) performed a cervical laminectomy to expose the C2-C4 dorsal roots which they subdivided repeatedly to obtain filaments containing only a single active afferent from biventer cervicis. When investigating muscle spindle afferent properties, it is clearly essential that the motor supply to the spindles be controlled; generally, this is most readily achieved by sectioning 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 vertebral 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 (Richmond & Abrahams 1979a; personal observations). Physical de-efferentation is therefore not feasible; instead, Richmond & Abrahams (1979a) opted to "chemically de-efferent" biventer spindles by infusing Gallamine intravenously in order to postsynaptically block cholinergic fusimotor neuromuscular transmission.

A similar approach was inappropriate in the present experiments, since the principle aim was to attempt to identify b_2c primary afferent axons on the basis of the effects on their properties of SCh infusions, and these effects would be interfered with by curariform drugs (Hunt 1952). Instead, it was decided to revert to the technique used by B.H.C. Matthews (1933), namely that of cutting the muscle nerve itself and splitting the peripheral stump until filaments containing single active afferents were obtained. Whilst this is substantially more difficult than splitting dorsal roots, due to the greater connective tissue content of peripheral nerves compared to dorsal roots, it ensures total spindle de-efferentation whilst still allowing activation of intrafusal fibres by SCh infusion.

The biventer cervicis muscle has an unusual compartmentalised structure, as do several of the dorsal neck extensor muscles (Richmond & Abrahams 1975a). Interposed between its origin and its insertion are four tendinous inscriptions which run diagonally across the full width and depth of the muscle and effectively divide it into five compartments linked serially to one another. Each compartment receives a separate muscle nerve from one of the C2-C5 dorsal rami (for arrangement of muscular branches, see Abrahams, Lynn & Richmond 1984, Plate 1), but the exact pattern of innervation of its intra/extrafusal muscle fibres was not known when the present experiments were started; whilst it seemed reasonable to expect that muscle spindles lying in a particular compartment would be innervated via the corresponding compartmental nerve, the possibility that motor axons might traverse a tendinous inscription to reach a spindle could not be ruled out. In view of the requirement for complete de-efferentation of spindles, it was therefore decided to locate and divide all the compartmental nerves.

Finally, while studying the effects of SCh infusions on soleus muscle spindle afferents, Dutia (1978) showed intra-arterial infusion to be the optimal route of administration of the drug (as assessed by the speed and extent of spindle afferent

excitation) and it was therefore adopted in the present experiments. The blood supply of biventer cervicis in the cat is via small vessels entering it with its muscle nerves and elsewhere (Reighard & Jennings 1935) which it would not be feasible to cannulate for close-arterial drug infusion. Instead, drug infusions were made into the main trunk supply, which in the cat is an anastomosis between the occipital artery and the deep cervical artery (Reighard & Jennings 1935). The former arises from the ipsilateral common carotid artery along with other muscular branches, the latter from a branch of the ipsilateral subclavian artery; the regional anatomy of the cat is such that, if the right biventer muscle is used, both these main vessels are accessible to infusion via a cannula placed in the left common carotid artery.

1:2.1 SURGICAL PROCEDURES.

In the experiments performed using the biventer muscle, a total of 21 cats weighing 1.9-5.2 kg was used. Animals were fasted overnight prior to surgery. Anaesthesia was induced in all cats by inhalation of an O_2/N_2O/Halothane mixture, initially by placing the animal in an airtight box and allowing the gaseous mixture to enter, and subsequently via a face mask during the early stages of surgery. The last part of the surgery, and the subsequent experimental manipulations were then carried out under pentobarbitone anaesthesia (SAGATAL, May & Baker; 45 mg/kg IV, supplemented as required).

The anaesthetised animal was placed on its back on an electrically heated blanket (CFP 8185), the ventral surface of the neck was shaved and a midline incision was made through the skin from the larynx to the sternum, and the exposed sternomastoid and sternothyroid muscles were cleared from the skin by blunt dissection and separated down the midline to expose the trachea. Using an aneurysm needle, a length of suture was passed behind the trachea and tied in a loose loop; the trachea was cut open between adjacent cartilagenous rings, and a plastic T-piece of an appropriate diameter was inserted into it and tied into position.

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 majority of the experiments, the left common carotid artery was located in the same incision, along with the vagus nerve and internal jugular vein, separated from them and cleared of connective tissue. In the first two experiments, an external jugular vein was exposed and cleaned. In either case, two loops of suture were passed around the vessel, 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 (Portex Ltd.) of an appropriate size and filled with heparinised saline (5,000 units/l) was inserted into the opened vessel, 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. This cannula was used to administer SCh.

Next, an incision over the left groin was made to expose the femoral blood vessels and nerve lying between the gracilis and sartorius muscles superficial to the adductors of the thigh. A nylon cannula was placed in the femoral vein and was used to administer the pentobarbitone anaesthetic during the remainder of the experiment. The skin incision was sewn shut, 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 then 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 holder was clamped so as to straighten out the neck and separate

the scapulae in the midline, and a midline skin incision was made from the vertex 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 normal saline. This incision exposed Mm. abductor auris longus & brevis and auricularis superior which partially overly the insertion of the trapezius muscle into the lamboidal ridge of the occipital bone at the back of the skull. A hole was made in auricularis superior towards its anterior edge allowing access to the tissue plane between it and the temporalis muscle deep to it. Blunt dissection caudally in this plane beneath the long and short abductors allowed these muscles to be removed and exposed the insertion of trapezius. A small hole was then made in one of the trapezius muscles near the midpoint of the lamboidal 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 lamboidal ridge and separated from the contralateral muscle in the midline as far caudally as the skin 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 procedure was 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. The greater occipital nerve, which runs with the C2 dorsal ramus branch to biventer on each side but pierces it and innervates the skin of the back of the neck (Abrahams, Lynn & Richmond 1984), was divided where it pierced the dorsal surface of biventer in order to reduce non-muscular neural traffic in the C2 biventer nerve.

The two biventer muscles were carefully separated from each other in the midline, and from the adjacent complexus and obliquus capitis muscles, and were removed from the lamboidal ridge, either by cutting them away with scissors, or by using a pair of bone rongeurs to remove a slither of occipital 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 (EPA 2, Davis & Geck) was stitched to the freed end of the right hand biventer muscle for subsequent attachment to an electromechanical muscle stretcher (Ling Dynamic Systems V406).

A wire frame was sewn to the margins of the dorsal neck 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 - T3) vertebrae from which slips of the biventer muscle take origin (Reighard & Jennings 1935). 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 filled with paraffin oil whose temperature was monitored by a thermoprobe (Clandon Scientific, YSI Tele-Thermometer) and maintained between 32 and 35 degrees centigrade by radiant heat from a lamp.

Under an operating microscope (Kyowa), the right biventer muscle was reflected laterally to expose its ventral aspect on which the C2-C5 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 perivertebral muscles. Taking care not to damage either, the C2 and both C3 nerve fascicles were then cleared from the network of blood vessels running to the biventer muscle with them and from the adjacent 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 bundles due to the crisscrossing 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 perivertebral muscles. In this way, it was usually possible to produce a 1-2 cm length of the biventer nerves for splitting.

Placement of stimulating electrodes.

As was described above (Literature Review, Part 1, p. 26), muscle spindle and Golgi tendon organ afferents are routinely identified electrophysiologically by their different responses to contraction of the muscle in which they lie. Initially, therefore, attempts were made to study biventer afferent responses to muscle twitch. It later became apparent, however, that afferent responses to muscle twitch could not be used to distinguish between muscle spindle and tendon organ afferents in biventer (Results, p. 109), 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 the muscle compartments to allow them to be activated directly. The electrodes were fashioned from 125 μm diameter Teflon insulated silver wire (Clark Electromedical) 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 balls about 1 mm in diameter. Appropriate connectors were soldered to the other end of the wires for connection to isolated stimulators (Digitimer Ltd., Model DS2) which delivered shocks of 0.1 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 compartmental 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 0.01 ms in duration, and the voltage was adjusted to be 2-3 times the voltage which just produced discernible twitching in the compartment. The remaining compartment was activated with silver ball electrodes as described above.

1:2.2. ELECTROPHYSIOLOGICAL METHODS.

(a) APPARATUS.

The apparatus used is illustrated in the block diagram of Fig. 1. 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 (Neurolog NL100) to an ac preamplifier (Neurolog NL104). Signals were usually amplified 1,000 times, bandpass filtered (500 - 5,000 Hz; Neurolog NL125) and then ac amplified a further 20 times (Neurolog NL105). The amplified action potentials were displayed on one beam of a four beam storage oscilloscope (Tektronix 5113). They were also led to a spike trigger module (Neurolog NL200) which produced a TTL pulse at its output for each input action potential when gating thresholds were appropriately set. These pulses were passed to an audio amplifier (Neurolog NL120) so that the afferent 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 (Neurolog NL 256). The ratemeter output, a voltage proportional to the reciprocal of the interval between a particular action potential and the one preceding it, was displayed on another channel of the Tektronix oscilloscope and was used both to assess the reliability of triggering by the spike trigger unit, 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. 1. A Ling Dynamic Systems V406 electromechanical puller and its associated

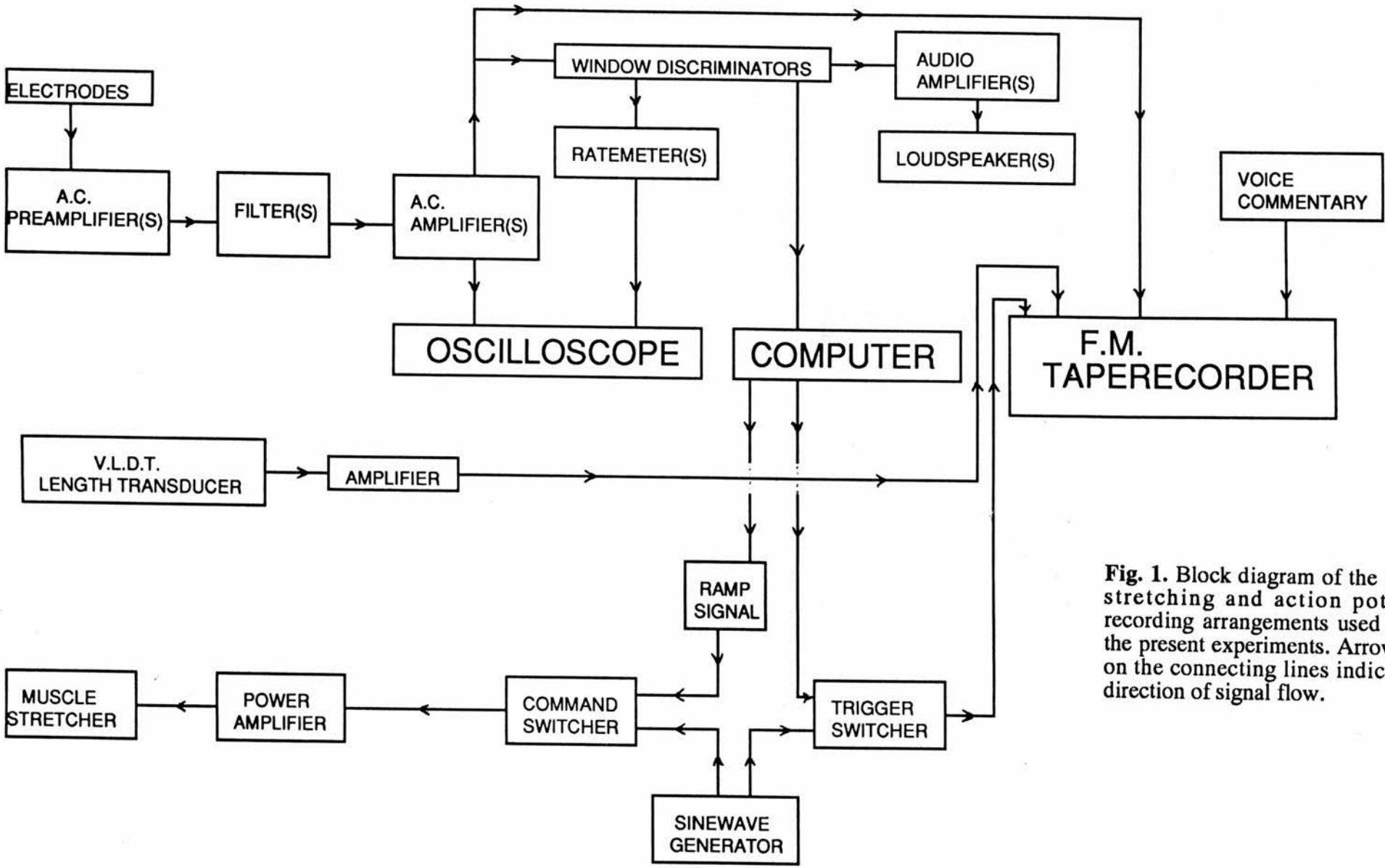


Fig. 1. Block diagram of the muscle stretching and action potential recording arrangements used during the present experiments. Arrowheads on the connecting lines indicate the direction of signal flow.

power amplifier (LDS PA 100) 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 (Penny & Giles LVDT D.S. 1317) 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 displayed on a third channel on the Tektronix oscilloscope. The muscle puller was driven by setting the gain on its power amplifier to a predetermined value and changing the frequency and/or amplitude of an externally derived command signal supplied to the power amplifier. Sinusoidal commands were provided by a signal generator (Feedback FG600) 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 the LVDT length signal 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 (Tandberg Instrumentation Recorder, Series 100) for subsequent analysis. Using a facility provided on the tape recorder, the signals recorded on each channel were immediately replayed onto a pair of two-channel oscilloscopes (Telequipment DM63) in order to assess the fidelity of the recording.

(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 (Richmond & Abrahams 1979a). 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. A small perspex platform was then introduced into the paraffin pool and illuminated by a pair of fibre optic light guides (FORT LUX 150) positioned above it. One of the prepared segmental muscle nerves was placed on the platform 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.

1:2.3. EXPERIMENTAL PROTOCOL.

When a suitable filament had been obtained, it was first tested by stretching the biventer muscle manually and if the afferent did not respond with an increase in its discharge rate it was suspected to originate in the complexus muscle and was discarded. In some of the experiments, the response of the afferent to contractions of various muscle compartments was determined at this point. All 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 the standard deviation divided by the mean.

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

(3) Exposure to sinusoidal vibration. Starting at a frequency of 10 Hz, and an amplitude of about 800 μm peak-to-peak, 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, afferents discharged once per cycle of stretching (1:1 driving) until a critical frequency was reached beyond which they

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 for each afferent.

(4) Exposure to repeated ramp and hold muscle stretching during exposure to SCh. In all but the first two experiments, the drug was infused intraarterially (Palmer Injection Apparatus F134). In the first two experiments, bolus injections of the drug were administered intravenously. In either case, the animal was artificially ventilated with a second Palmer pump (stroke volume and rate adjusted to be similar to the natural breathing pattern) when neuromuscular blockade set in. 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, 2 to 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 was attempted, and in practice substantially longer than this was required before another single unit was found. In all the experiments, an infusion rate of 100 μg/kg/min SCh was used, as has been the case in other series (Dutia 1980); in addition, in a number of experiments higher and/or lower infusion rates were also used which will be indicated as appropriate.

Experiments were continued until the C2 and C3 segmental muscle nerves had been thoroughly investigated. This usually took about 12 hours and gave an average yield of slightly under 5 units per experiment. At the end of the experiments, animals were despatched with an overdose of Sagatal.

Conduction velocity measurement.

The measurement of afferent conduction velocity, which is so routine in studies of hindlimb muscle spindle afferents, was not possible in these experiments for two reasons. Firstly, although the filaments of muscle nerve in which the afferents were studied contained only one electrically active axon, they must also

have contained many other axons capable of conducting evoked action potentials (e.g. some of the motor axons which would have been silent at rest since they were no longer connected to the spinal cord); the standard technique of stimulating the muscle nerve near the muscle and measuring the delay before an evoked action potential travelling in the axon under study arrives at the recording electrode could not, therefore, have been applied, since each stimulus would have produced a compound action potential at the recording electrode from which no meaningful latency would be measurable. Secondly, even the technique of spike-triggered averaging (Kirkwood & Sears 1975) could not have been used since the short length of muscle nerve available would have resulted in conduction latencies on the order of a single bin width with the electronic averaging modules available.

1:2.4. DATA ANALYSIS.

Most of the tests described above yielded a result at the time of the experiment. The two which did not were the sinusoidal stretching test and the SCh test. These were analysed off-line using a suite of programmes written by Dr. M. B. Dutia.

SINUSOIDAL SENSITIVITY.

The sensitivity of a unit to sinusoidal stretching was determined by constructing a cycle histogram of firing probability and using Fourier analysis to fit a 1 Hz sine to this histogram. Afferent spikes were replayed from tape through the spike trigger to the Cromemco microcomputer along with the muscle length signal and synchronising trigger pulses marking the start of each cycle of stretching. Each cycle was divided into 24 bins of equal duration; the number of afferent spikes which occurred during a particular interval was added to the contents of the corresponding bin . Usually, fifty cycles of stretching were averaged to produce the final histogram, to which a 1 Hz sine was fitted using a modification of standard

Fourier methods (Anderson, Blanks & Precht 1978) 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 process 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 (Hulliger, Matthews & Noth 1977a). Where appropriate, interactive software was used to place cursors to delimit a silent period in a histogram, a Fast Fourier Transform was used to calculate the amplitude, dc level and phase relative to muscle length of the best fitting 1 Hz sine, and the resultant amplitude value was multiplied by the adjustment parameter k calculated from the expression given below:

$$k = \{\pi + 2c + 2\mathrm{COS}(c)\mathrm{SIN}(c)\}/2\pi$$

where: $c = (1/2 - b)\pi$

and: b = the fraction of the stretch cycle for which the afferent is silenced.

For the majority of afferents, the amplitude of the fitted sine was equal to 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 actual stretch amplitude to obtain a value of sensitivity would not be justifiable, 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 (Matthews & Stein 1969a; Hulliger, Matthews & Noth 1977a). Instead, the fitted amplitude was adjusted to give a 1 mm value either by linear interpolation or by extrapolation in a manner described in detail in the Results (p. 138).

SUXAMETHONIUM EXPOSURE.

For all units successfully activated by SCh, a printed copy of the muscle length signal and afferent discharge rate during exposure to the drug was obtained on an Electromed MX216 two channel chart recorder. This was checked to see that triggering of the unit was consistent throughout the recording, in which case the spikes were replayed through the spike trigger module to the Cromemco microcomputer, along with the length signal and trigger pulses. The length signal was digitised (8-bit A/D at a sampling rate of 142 Hz), whilst the interspike intervals were timed to a resolution of 70 μsec. The digitised data were written to floppy disc files and were subsequently mainly used to produce the figures in this thesis and elsewhere. The analysis of patterns of activation of units by SCh was qualitative and was based upon examination of the chart recorder output. Attempts were made to automate the measurement of the responses of afferents to repeated ramp-and-hold stretching, but the results proved rather prone to corruption by mis-triggers and other "glitches" which the human operator would ignore; nevertheless, the output of this programme provided a convenient means of assessing the time course of afferent activation by SCh infusion, and a number of the illustrations in this thesis derive from it. Interactive software permitted cursors to be placed by the operator at the beginning and end of the periods of ramp stretching, and measurements were then taken relative to these times. The afferent discharge rate before a stretch was found by averaging the inter-spike intervals of the action potentials which were recorded during a 100 msec time window leading up to the start of each stretch; similarly the discharge rate 0.5 sec after the end of the stretch was found by averaging inter-spike intervals over a 100 msec time window centred at 0.5 sec after the end of the stretch. Peak discharge rate was averaged over a 20 msec window leading up to the end of the stretch cycle; there was considerable variability in this measurement, since only a small number of action potentials occured during the 20 msec averaging window, and this was the main source of the "glitches" referred to above which limited the use of this programme.

CHAPTER ONE

RESULTS

1:3.1. THE SAMPLE.

A total of 112 afferent axons were isolated in the C2 and C3 muscle nerves to biventer cervicis in this series of experiments. Of these, 72 were studied in detail, and the remaining 40 units were partially studied. A problem encountered early in these experiments was that of differentiating muscle spindle afferent axons from others (i.e. Golgi tendon organs, free nerve endings etc.). As was intimated in the Methods section (p. 105), the usual means of differentiating afferents from Groups I-IV, namely conduction velocity, could not be applied for technical reasons. In addition, the differentiation between Gp. Ia (muscle spindle primary) and Gp. Ib (Golgi tendon organ) afferents on the basis of their responses to contraction of the parent muscle also proved inapplicable.

An example of the problem encountered is shown in Fig. 2. The particular stimulation and recording arrangements in this experiment are represented schematically at the top of Fig. 2, 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 running to the second compartment, stimulating electrodes were on the second and third muscle compartments and the sensory ending under investigation was presumed, but not known, to lie in the second compartment. In response to contraction of the second compartment, the firing rate of this afferent 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, of course, be diagnostic of a muscle spindle afferent. However, if the neighbouring third compartment was made to contract, 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. This ambiguous form of response to contraction of single compartments was seen both

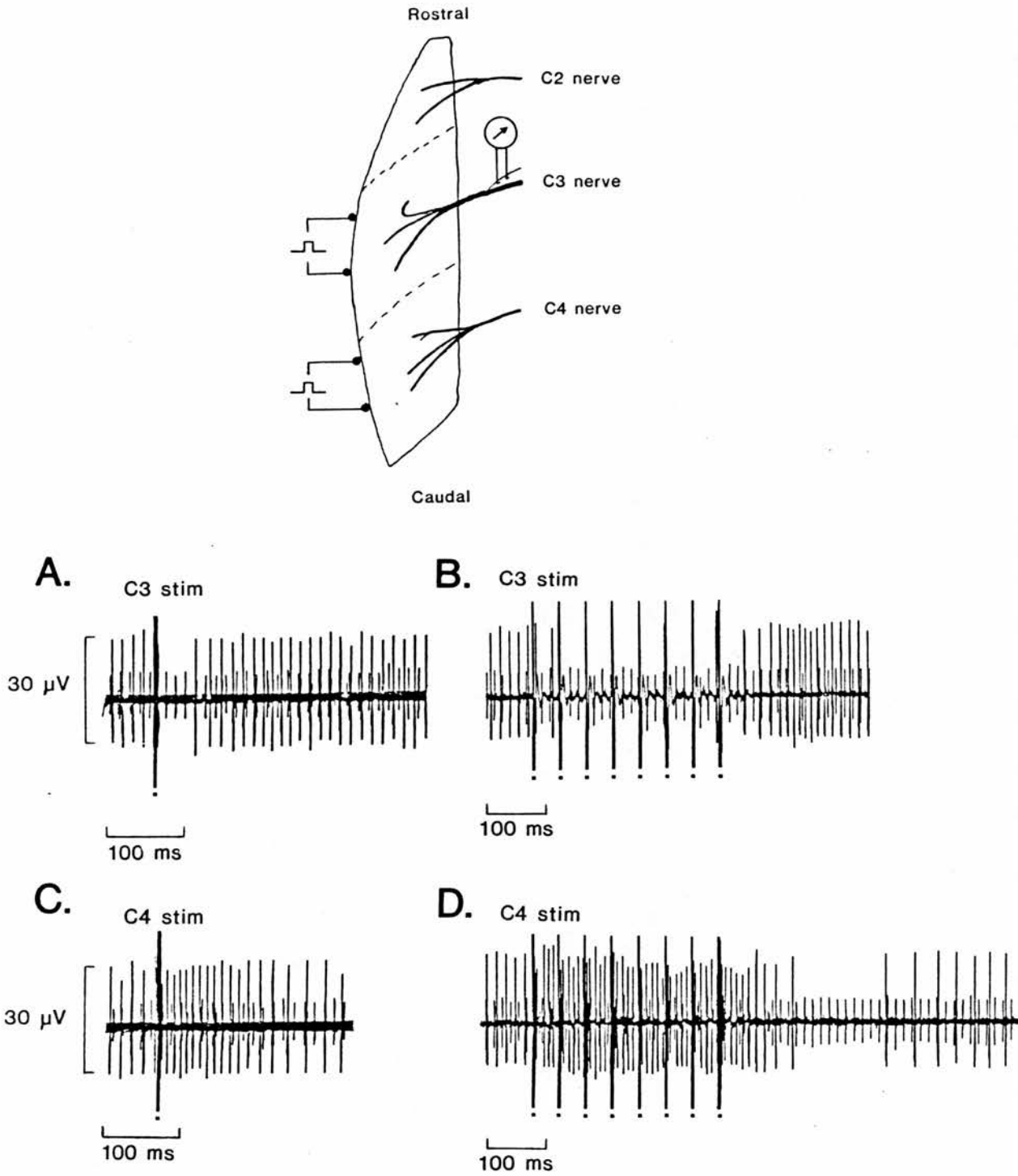


Fig. 2 A-D. *Top* Schematic representation of the three most cranial compartments of the biventer cervicis muscle and their innervation by branches of the dorsal rami of the C2-C4 cervical spinal nerves. Broken lines indicate tendinous inscriptions delineating the muscle compartments. **A, B** Response of a biventer afferent (large spike) 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 of the muscle. Note silencing of the resting discharge upon stimulation. **C,D** Excitation of the same afferent by a single stimulus (**C**) and by a train of 8 stimuli at 27 Hz (**D**) applied to the C4 compartment of the muscle. Note the increase in discharge frequency upon stimulation.

when the muscle was activated by surface electrodes, and, where this was possible, when it was activated via its segmental nerves. 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. Since it was not always possible to stimulate all the compartments of the muscle, and given the inherently ambiguous responses of biventer afferents to muscle contraction, this test was not used routinely after the first few experiments.

In the absence of the standard means of identifying muscle spindle afferents, the evidence that the afferents studied here did indeed derive from muscle spindles is as follows:

(i) In Fig. 3 is illustrated the passive length:tension characteristic of a cat biventer cervicis muscle which was determined during a different set of experiments (unpublished) with an arrangement identical to that used in the present series. It can be seen that at the resting muscle length, the passive tension in biventer is on the order of only 10 gm.weight. Nevertheless, the large majority of the afferents studied in this series discharged tonically at frequencies of 20 Imp/sec or more at the resting length.

(ii) Referring again to Fig. 3, it will be appreciated that a stretch of the biventer muscle by 3 mm from the resting length (the largest stretch used in this series) will only increase the passive tension in the muscle by about 5 gm.wt., yet all the afferents studied here were sensitive to such stretches, and many were sensitive to far smaller stretches of the muscle on the order of 50-100 μm.

(iii) As will be described in detail below, the majority of these afferents were subsequently powerfully excited by infusions of SCh (see e.g. p. 134).

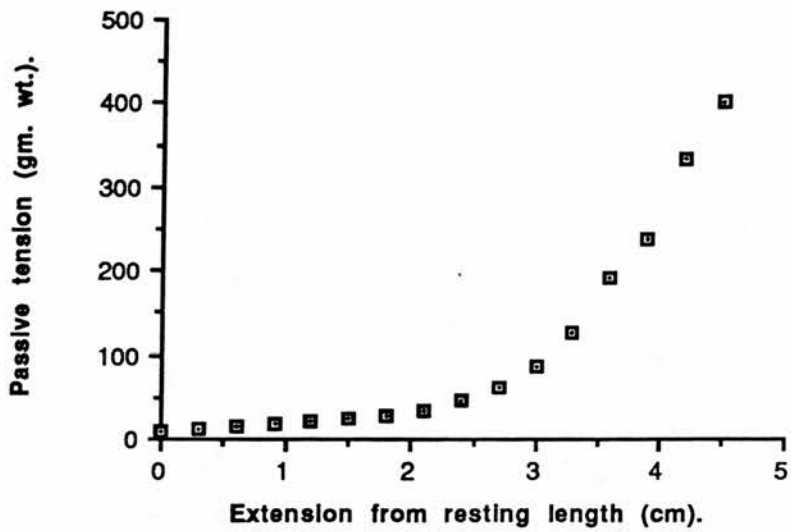


Fig. 3. Passive length:tension characteristic of a cat biventer muscle determined with a stretching arrangement very similar to that used in the present experiments. The resting length of the muscle was set at about its *in situ* value before detachment from the skull and is the set length which was used in the present experiments. Note the very small resting tension in the muscle, and the small effect of moderate length change upon the intramuscular tension.

None of these features is typically possessed by Golgi tendon organ afferents (Houk & Simon 1967; Stuart, Goslow, Mosher & Reinking 1970; Dutia 1978); however, a proportion of Golgi tendon organ afferents from hindlimb muscles are rather more sensitive than the majority, and are weakly activated by SCh (Dutia & Ferrell 1980), and so could, if they also exist in neck muscles, pollute one subset of the units which were studied here. This possibility and its likely significance to the findings of these experiments will be considered in the Discussion below.

1:3.2 ACTIVATION OF BIVENTER AFFERENTS BY SCh.

A total of 104 biventer afferents were studied before, during and after exposure to SCh whilst repeatedly stretching the muscle with a ramp-and-hold waveform. Generally the ramp waveform had a cycle time of about 4 seconds, with at least 1 second of rest between stretches; the amplitude and velocity of the stretch varied and will be indicated as appropriate, but in the large majority of cases, 3mm and 10 mm/sec respectively were used. Of the 104 afferents, the first 8 (2 experiments) were studied using intravenous bolus injections of SCh (1 - 2 mls of a 100 μg/ml solution), whilst the remainder were studied with intra-arterial infusions of SCh. All afferents were activated by infusions at 100 μg/kg/min in line with previous work (Dutia 1980), and some afferents were exposed to other infusion rates which will be indicated where appropriate. The analysis of the effects of SCh on biventer afferents was qualitative rather than quantitative; for the sake of simplicity, the data obtained with IV injections of SCh are not included here, although there were no apparent differences between the patterns of activation seen for these 8 afferents and those seen for the remaining 96 afferents excited via an intra-arterial infusion of SCh.

Following Dutia (1978), three parameters of the afferent response to ramp stretch were studied (Fig. 4). The **INITIAL DISCHARGE** is the firing rate of

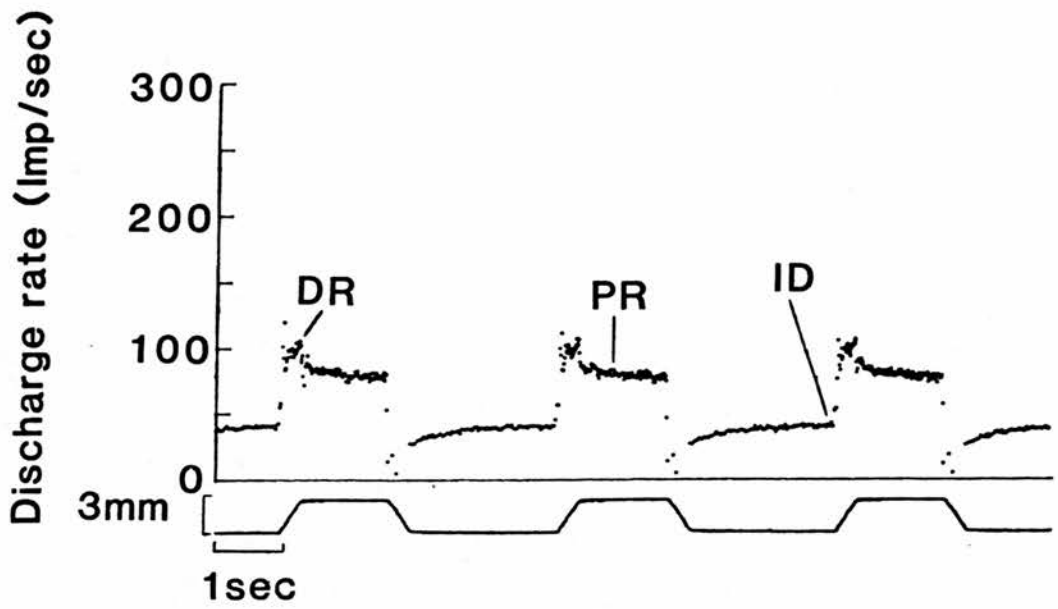


Fig. 4. Response of a muscle spindle afferent to ramp stretching (3 mm, 10 mm/sec), showing the three parameters of the afferent response routinely measured:- DR: dynamic response; PR: position response; ID: initial discharge. See text for more details.

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 (ignoring any initial burst) and the **POSITION RESPONSE** is the firing rate 0.5 seconds after the end of the muscle stretch. Note that this definition of dynamic response is not that used by Jansen & Matthews (1962) which has since been renamed the dynamic index (Crowe & Matthews 1964). The dynamic index can, however, be derived from these three parameters as {dynamic response - position response}; another useful parameter is the position sensitivity which can be found as {(position response - initial discharge)/ stretch amplitude}.

A. AFFERENT RESPONSES TO STANDARD 100 μg/kg/min INFUSIONS.

Changes in one or more of these parameters during a 100 μg/kg/min SCh infusion were used to identify four types of spindle afferent which will be considered in turn.

TYPE 1:

The first group of 28 afferents were activated by SCh in the manner illustrated in Figs. 5 and 6 for two afferents whose behaviour encompassed the extremes of response seen for type 1 afferents. In Fig. 5, A is illustrated the response of an afferent which had a rather low sensitivity to stretching of biventer in the control state; in this instance, the initial discharge of the afferent was 18 Imp/sec, the dynamic response was 28 Imp/sec, and the position response was 25 Imp/sec, from which it can be calculated that the dynamic index of this afferent was 3 Imp/sec, and its position sensitivity 2.3 Imp/sec/mm. An intra-arterial infusion of SCh was started at this point, and the time course of events thereafter is illustrated in Fig. 5, C.

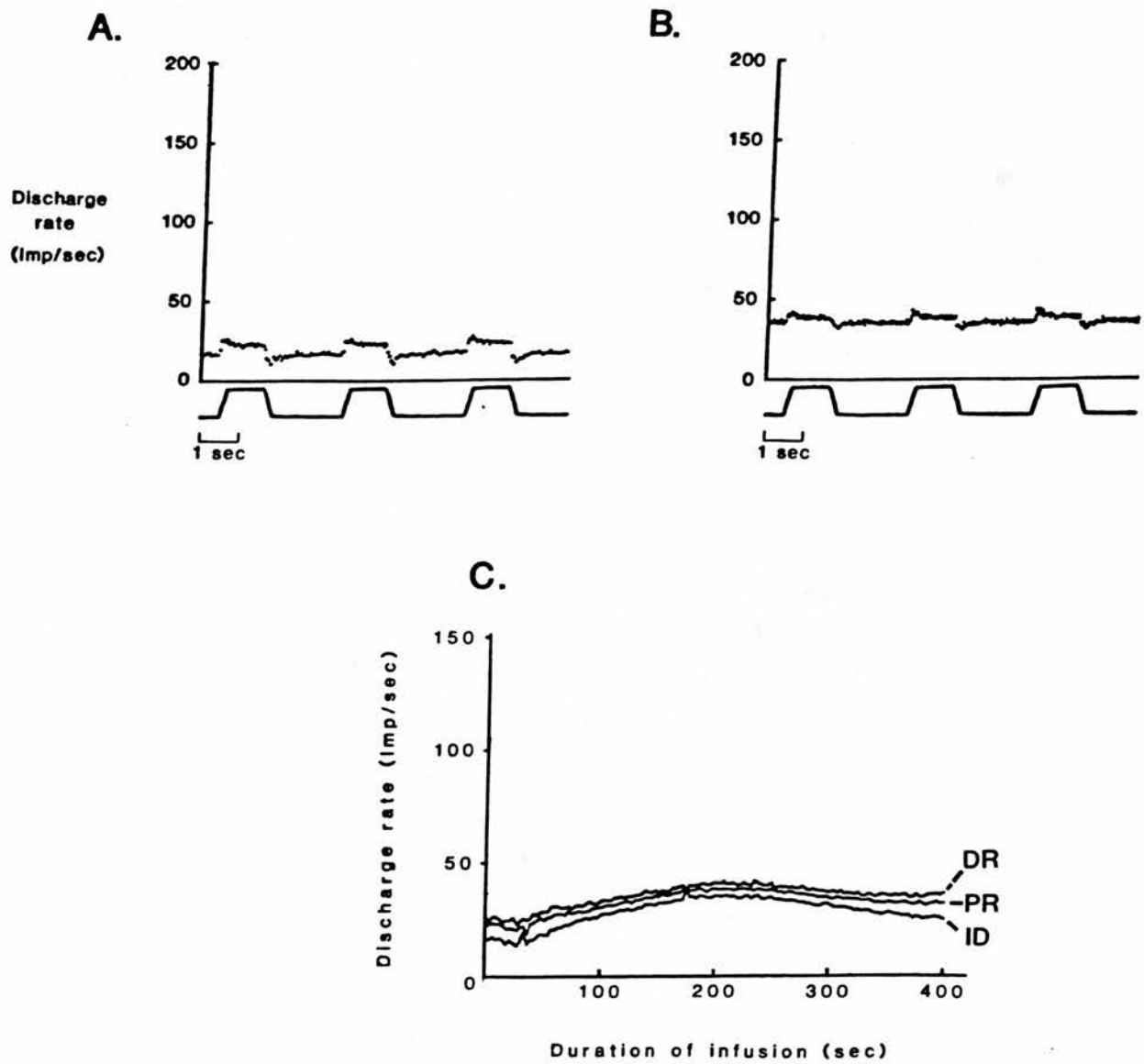


Fig. 5 A-C. The pattern of SCh activation of a biventer type 1 afferent. Response of this afferent to three ramp stretches (3 mm at 10 mm/sec) before (**A**) and after (**B**) exposure to an intra-arterial infusion of SCh at 100 μg/kg/min. The time course of the SCh activation of this afferent is shown in **C**; the responses in (**A**) were obtained at time zero, and those in (**B**) after 244 seconds of infusion. In **C**, the top line (DR) represents variation with time of the dynamic response of this afferent (see Fig. 4), the middle line (PR) variation with time of the position response, and the bottom line (ID) variation with time of the initial discharge; this convention will be used in all similar figures below. See text for further details.

About 35 seconds after starting the infusion, and shortly after a brief period of muscle fasciculation, the initial discharge rate of the afferent gradually began to increase; it continued to increase over the next 180 seconds, to reach a maximum of 38 Imp/sec after 220 seconds of SCh infusion, an increase of 20 Imp/sec over the control value. Over the same period, the dynamic response reached a maximum of 44 Imp/sec, and the position response a maximum of 42 Imp/sec (Fig. 5, B), such that the dynamic index of this afferent decreased marginally from 3 Imp/sec to 2 Imp/sec, and the position sensitivity by 55% from 2.3 Imp/sec/mm to 1 Imp/sec/mm; these values can be judged by eye from Fig. 5, C, for the dynamic index is reflected in the separation of the upper and middle lines, whilst the position sensitivity is a function of the separation of the middle and lower lines. The infusion was switched off after 250 seconds of infusion, and these changes in the discharge of the afferent all gradually reversed roughly in parallel so that there were no dramatic changes in the sensitivity of the afferent to dynamic and static components of the stretch. Full recovery to the control state for this afferent required about 5 minutes, though in general this time was proportional to the duration of the preceding SCh infusion.

The SCh activation of another type 1 afferent is illustrated in Fig. 6. This afferent had a more substantial, though still not large, control response to stretch (Fig. 6, A). In this case, about 50 seconds after the start of an IA infusion, the initial discharge began to rise from the control value of 35 Imp/sec, eventually reaching a peak value of 86 Imp/sec after 250 seconds of infusion (Fig. 6, C). Whilst this degree of biassing was considerably greater than that shown by the previous afferent (and in fact the majority of type 1 afferents), changes in the dynamic response and position response again closely mirrored those in the initial discharge (Fig. 6, C), so that the dynamic index of this afferent increased slightly from 11 Imp/sec to 14 Imp/sec, whilst the position sensitivity rose from 3.8 to 4.2 Imp/sec/mm over the same period. The infusion was switched off after 250 seconds

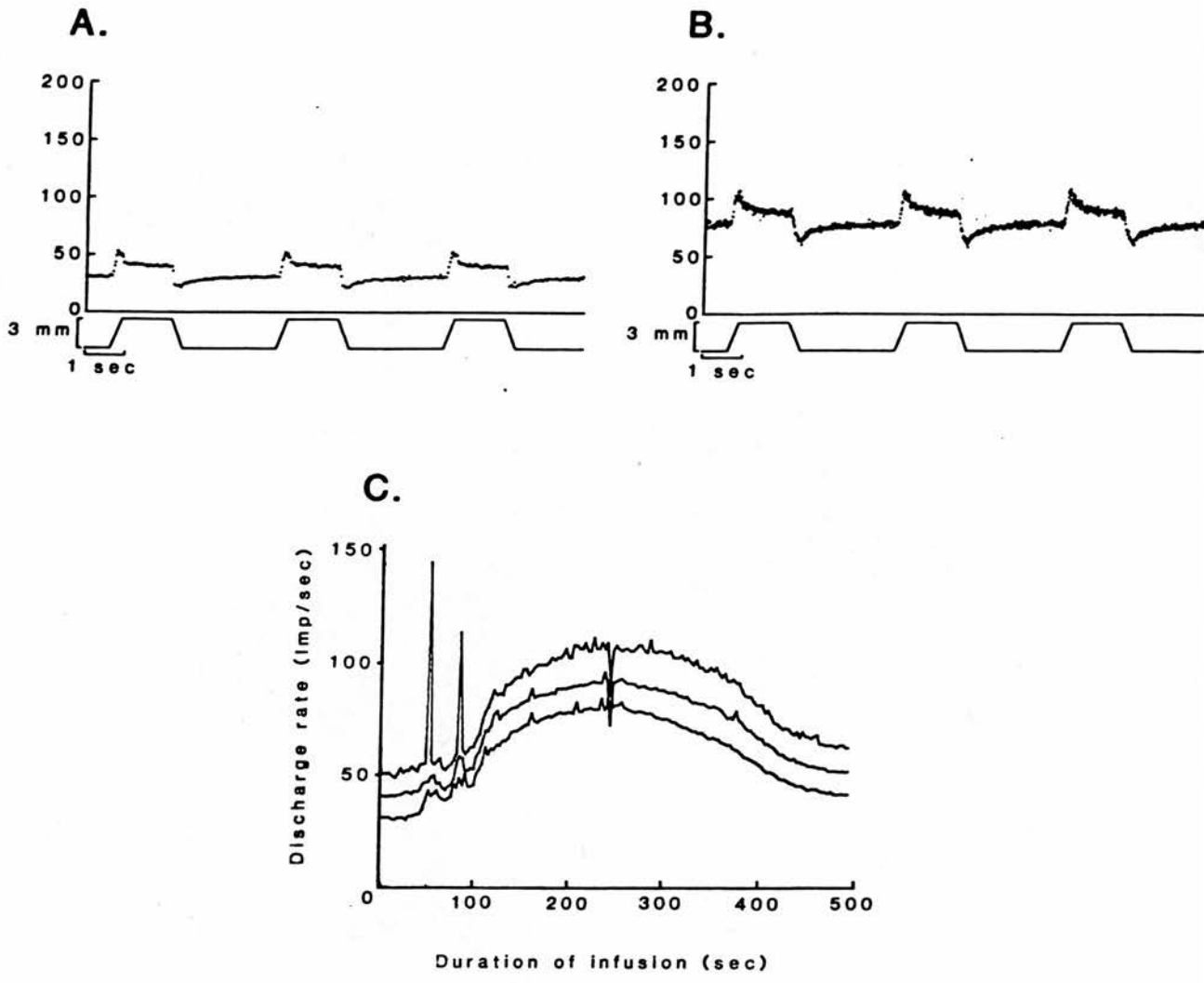


Fig. 6 A-C. The pattern of SCh activation of a different biventer type 1 afferent. Response of this afferent to three ramp stretches (3 mm at 10 mm/sec) before (**A**) and after 216 seconds (**B**) of exposure to a SCh infusion at 100 μg/kg/min. The time course of changes in dynamic reponse, position response and initial discharge are shown in **C** in the standard format. The large spikes in the dynamic response record at around 50 seconds were caused by fasciculation of the biventer muscle as extrafusal block developed, and that at 235 seconds was an artefact produced by switching off the infusion pump.

(the large spike in Fig. 6, C at this time represents a pump switching artefact), and as before these changes all reversed in parallel over the next few minutes.

The signature of this first class of afferent when exposed to SCh was, therefore, that the dynamic and position responses changed very much in parallel with the initial discharge over the course of an infusion so that, whilst the discharge rate between stretches was biassed to somewhat higher values by SCh, there was very little change in dynamic index or position sensitivity (Figs. 5 & 6, C), and the basic form of the response to a ramp stretch was barely altered (compare Figs. 5 & 6, A with B); in the large majority of cases (25 of 28), any small changes in stretch sensitivity involved a decrease when compared to the control value, and this was reflected in convergence of the three lines in illustrations such as Fig. 5, C. In the 3 cases in which either the dynamic index or the position sensitivity of the afferent increased, the increase was less than 30% of the control value and not felt to be of sufficient magnitude to warrant identification of these afferents as a distinct type.

In marked contrast to type 1 afferents, afferents of all other types showed substantial changes in either dynamic or position sensitivity or both over the course of a SCh infusion.

TYPE 2:

Typical examples of the response to SCh of the 34 type 2 afferents identified are illustrated in Figs. 7 & 8. The afferent whose SCh activation is illustrated in Fig. 7 had, in the control condition, an initial discharge rate of 44 Imp/sec, a dynamic response of 100 Imp/sec and a position response of 74 Imp/sec (Fig. 7, A). Note that, although it is rather difficult to see this on the compressed timebase of Fig. 7, A, this afferent had quite a marked initial burst at the start of each stretch, so that the dynamic response was not as high as first examination might suggest. About 52 seconds after the start of the SCh infusion, the initial discharge of this afferent began to increase quite steeply, reaching a maximum of 106 Imp/sec about 120

seconds later (Fig. 7,C). Over the same period, the position response of the afferent increased to a lesser extent from 74 to 132 Imp/sec, so that the position sensitivity of the afferent fell by 60% from 10 Imp/sec/mm to 4 Imp/sec/mm. In the initial 44 seconds after the first signs of activation of this afferent, changes in its dynamic response closely paralleled those in its position response, such that the dynamic index was largely unaltered and remained between 80% and 100% of its control value (Fig. 7, C); later, around 100 seconds after the start of the infusion, the hallmark of the SCh activation of this class of afferent revealed itself as a substantial increase in the dynamic response, which in this case reached 180 Imp/sec. This increase in dynamic response heavily outweighed simultaneous increases in the position response which continued to change at much the same rate as it had done during the earlier stages of activation, so that the dynamic index of this afferent rose to a maximum value of 62 Imp/sec, an increase of 250% over the control (Fig. 7, B). There were no further changes in afferent discharge until the infusion was stopped after 215 seconds, upon which the response to stretch of the afferent returned towards its control state over a period of a few minutes; initially the dynamic response declined most rapidly, returning the dynamic index towards its control value. Later, the position response of the afferent began to fall more slowly than the initial discharge, so that the position sensitivity returned towards control values; finally the residual biassing of the discharge waned and the control condition was restored (Fig. 7, C).

Knowledge of an earlier study of the SCh activation of spindle afferents (Dutia 1980) somewhat coloured my analysis of these SCh activations, since in that work, afferents which showed large increases in dynamic index, such as this one, had been shown to be activated by SCh in several distinct phases. I therefore looked for such phases of activation in the present experiments. The first thing to note from examples like the activation illustrated in Fig. 7, C, is that it does not immediately

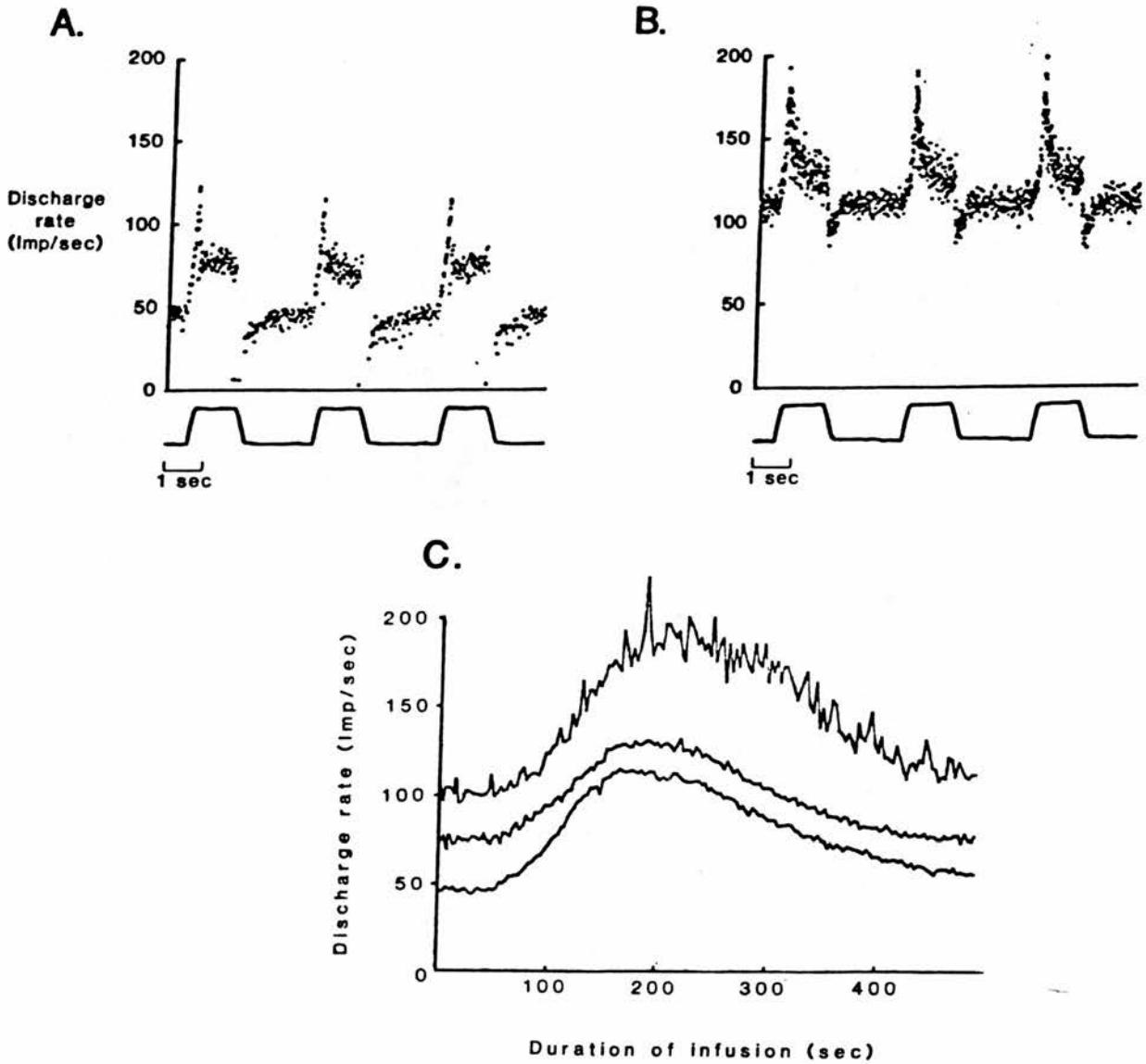


Fig. 7 A-C. The pattern of SCh activation of a biventer type 2 afferent. Response of this afferent to three ramp stretches (3 mm at 10 mm/sec) before (**A**) and after 196 seconds (**B**) of exposure to a SCh infusion at 100 μg/kg/min. The time course of the activation is shown in **C** in the standard format.

strike one that the afferent is being excited in several phases; this was the case for most of the type 2 afferents encountered. However, on close scrutiny of Fig. 7, C, the activation of this afferent can probably be split into two phases as follows: Phase I starts at the first sign of any change in afferent discharge, here at time 52 seconds, and following Dutia (1980) terminates when the dynamic response of the afferent begins to rise steeply; in the case of this afferent, there is a change in the slope of the dynamic response line (top line in Fig. 7, C) at 96 seconds after the start of the infusion. This was defined as the end of Phase I, which thus lasted 44 seconds, and the start of Phase II of activation. Note, however, that from the start of Phase II onwards, the changes in initial discharge, dynamic response and position response of this afferent all proceeded smoothly, with no further inflections suggestive of the onset of a third Phase of activation.

A second example of this pattern of SCh activation is illustrated in Fig. 8. In this case, the afferent had an initial discharge rate of 32 Imp/sec, a dynamic response of 74 Imp/sec (making allowance for the initial burst in its response to a ramp stretch) and a position response of 51 Imp/sec (Fig. 8, A). As before, SCh activation of this afferent proceeded in two phases, which in this case are rather more obvious than in the previous example; here, Phase I of activation started about 30 seconds into the infusion, at which time there was a rather abrupt biassing of the initial discharge by about 10 Imp/sec, accompanied by a reduction in position sensitivity from 6.5 Imp/sec/mm to 4.7 Imp/sec/mm and by an increase in the dynamic index from 23 Imp/sec to 29 Imp/sec (Fig. 8, C). Over the next 80 seconds of the infusion, the initial discharge continued to increase steadily to 50 Imp/sec, this change being paralleled by changes in the position response so that the position sensitivity remained fairly steady, though at a reduced level compared to the control. Over the same period, the dynamic response actually decreased slightly, so that the dynamic index of the afferent fell to approximately 75% of the control value. Phase II of activation then started after 145 seconds of infusion as indicated by the sudden increase in the slope of the dynamic

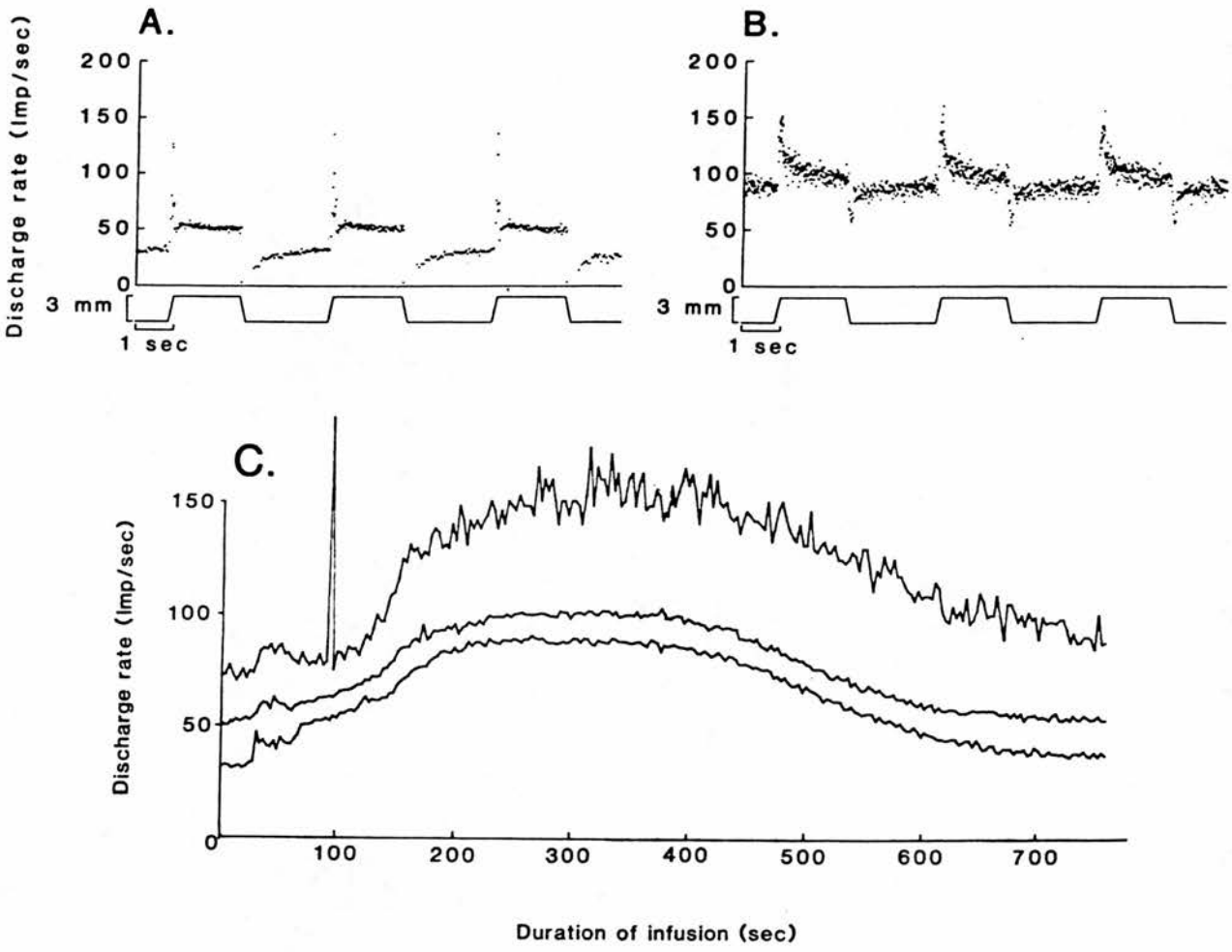


Fig. 8 A-C. The pattern of SCh activation of another biventer type 2 afferent. Response of this afferent before (**A**) and after 300 seconds (**B**) of SCh infusion at 100 μg/kg/min. The time course of the activation is shown in **C** in the standard format.

response line at this time; this increase in dynamic response was accompanied by somewhat more rapid biassing of the afferent discharge, such that, over the next 60 seconds or so, the initial discharge reached a maximum of 94 Imp/sec, this increase being closely paralleled by that in the position response so that the position sensitivity of the afferent remained at about 3 Imp/sec/mm, or 50% of the control value. On this occasion the dynamic response of the afferent increased to a peak value of around 150 Imp/sec, which resulted in a two-and-a-half fold rise in dynamic index to about 60 Imp/sec (Fig. 8, B). Again, there were no further changes in afferent sensitivity which might have been suggestive of a Phase III of activation, and the infusion was switched off after 330 seconds; thereafter, the three parameters of the afferent response returned gradually towards their control values over the next 400 seconds (Fig. 8, C), full recovery requiring a further five minutes beyond the time course illustrated in Fig. 8,C).

The action of SCh on this second class of afferent was thus, in two Phases of activation, to bias the discharge substantially, in all cases by at least 50 Imp/sec, and usually to instantaneous discharge rates of over 100 Imp/sec, to increase the dynamic index by a factor of 2-3, and to decrease the position sensitivity by 30-80%.

TYPE 3:

The third major group of afferents contained 33 units. Examples of the pattern of SCh activation exhibited by type 3 afferents are illustrated in Figs. 9-11. Considering Fig. 9 first, it can be seen that in the control condition, this afferent had an initial discharge rate of 30 Imp/sec, a dynamic response of 62 Imp/sec and a position response of 51 Imp/sec (Fig. 9, A). An infusion of SCh was then started, and the time course of subsequent events can be seen in Fig. 9, C. About 48 seconds after the start of the infusion, the initial discharge of the afferent began to rise steeply, accompanied by steep rises in the dynamic and position responses; over the course of eight

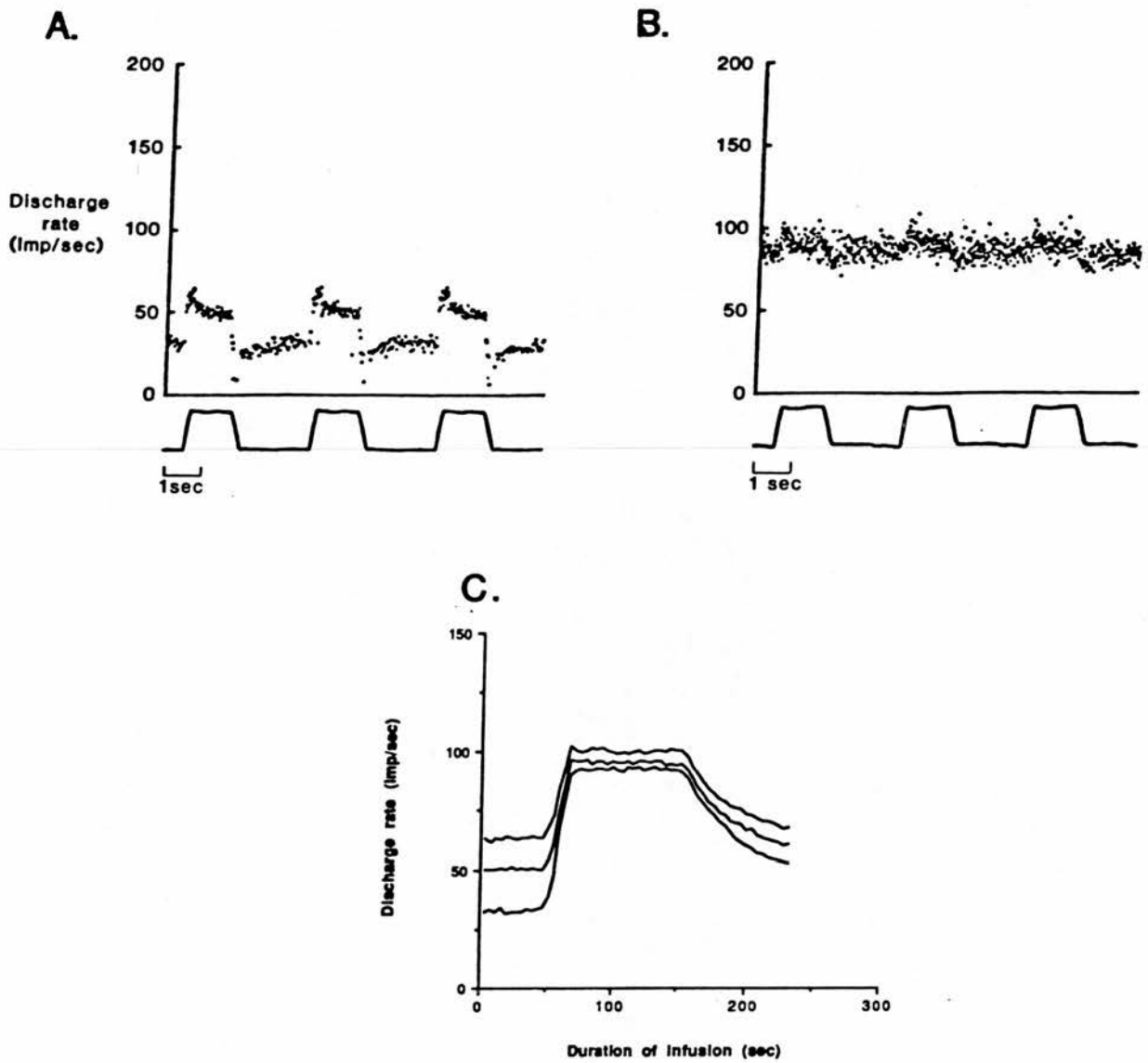


Fig. 9 A-C. The pattern of SCh activation of a biventer type 3 afferent. Response of this afferent to ramp stretching (3 mm, 10 mm/sec) before (**A**) and after 150 seconds (**B**) of exposure to SCh infusion at 100 μg/kg/min. The time course of this activation (**C**) is in the standard format, but in this case the data were measured by hand from a chartrecorder output.

stretch cycles, or 32 seconds, the initial discharge increased to 90 Imp/sec, and the discharge at this higher rate became very variable. Although both the dynamic and position responses increased over this period, they did not do so to nearly the same extent as did the initial discharge, so that the sensitivity of the afferent to muscle stretch was markedly attenuated over the course of the infusion (compare Fig. 9, A & B). The hallmark of afferents placed into this category was the dramatic reduction in position sensitivity which was superimposed upon this substantial and rapid biassing; in this instance, the position sensitivity of the afferent fell from 7 Imp/sec/mm in the control state to 1.5 Imp/sec/mm in the fully activated state. Characteristically, the dynamic index of the afferent also decreased over the course of the infusion, here from 11 Imp/sec to about 4 Imp/sec, though this last value was difficult to obtain due to the high degree of variability in the afferent discharge. As for the other classes of afferent described above, all these SCh-induced changes were readily reversible over the course of a few minutes after stopping the infusion, the exact time course depending upon the duration of the preceding infusion.

The SCh activation of another type 3 afferent is illustrated in Fig. 10. This afferent was rather more sensitive to muscle stretch in the control condition than the previous example of this type (compare Figs. 9, A and 10, A), having a position sensitivity of 13 Imp/sec/mm; the initial discharge was 30 Imp/sec, and the dynamic index about 10 Imp/sec. The time course of the SCh activation is shown in Fig. 10, C from which it can be seen that after 50 seconds the initial discharge of the afferent began to rise steeply; over the next 32 seconds, the initial discharge was biassed by 100 Imp/sec to 130 Imp/sec, this increase being accompanied by an increase in the discharge variability and by a 60% reduction in the position sensitivity to 5 Imp/sec/mm (Fig. 10, B). The infusion was switched off after 120 seconds, and the changes in stretch sensitivity of the afferent reversed smoothly over the course of the next 200 seconds (Fig. 10, C); full recovery required only a further 50 seconds, the relative rapidity of this afferent's recovery reflecting the short duration of the infusion

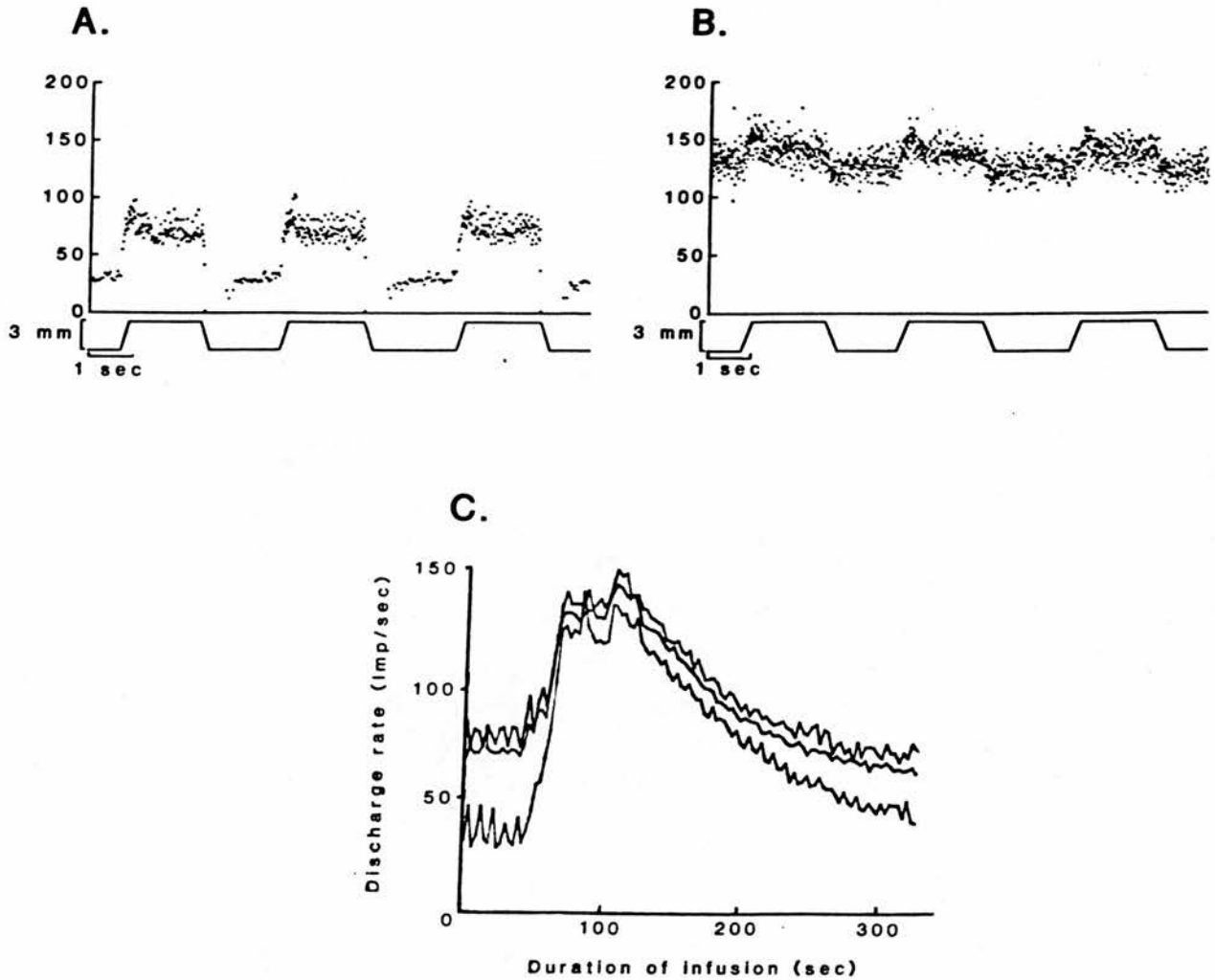


Fig. 10 A-C. The pattern of SCh activation of a different biventer type 3 afferent. Response of this afferent to ramp stretches (3 mm, 10 mm/sec) before (**A**) and after 100 seconds (**B**) of SCh infusion at 100 μg/kg/min. **C** Time course of SCh activation in the standard format.

compared to most of those illustrated above for type 1 and 2 afferents.

The final illustration of this type (Fig. 11) shows the pattern of activation of an afferent which was rather insensitive to stretching of biventer in the control condition (Fig. 11, A); in this instance, the dynamic index was 6 Imp/sec, the position sensitivity about 3 Imp/sec/mm and the initial discharge 32 Imp/sec. About 40 seconds after the start of this infusion, and after a brief period of muscle fasciculation, the initial discharge of this afferent rose steeply to around 98 Imp/sec over the course of 50 seconds. This biassing was accompanied by an increase in the discharge variability, by a reduction in position sensitivity of about 50% and by a reduction in dynamic index. When fully activated (Fig. 11, B), the afferent discharge was hardly affected by each muscle stretch and was of the same general form as that of the other afferents of this type (cf. Figs. 9 & 10, B). The infusion was switched off after 192 seconds; immediately following this, there was a transient further biassing of the discharge to around 120 Imp/sec, followed by a steady fall in all three parameters which returned to their control values over the next 215 seconds. Despite its unimpressive control responses, which were more reminiscent of type 1 afferents, this afferent is clearly identified as type 3 by its pattern of SCh activation.

The effect of SCh on this third class of afferent was thus to bias the afferent discharge rapidly and very substantially always by more than 50 Imp/sec, and usually to instantaneous frequencies of over 100 Imp/sec, whilst substantially reducing the position sensitivity and dynamic index and rendering the afferent rather insensitive to changes in muscle length.

TYPE 4:

The fourth class of afferent identified by SCh infusions contained only one member which could not be placed into any of the three major classes just described. The activation of this unit is illustrated in Fig. 12. In the control condition,

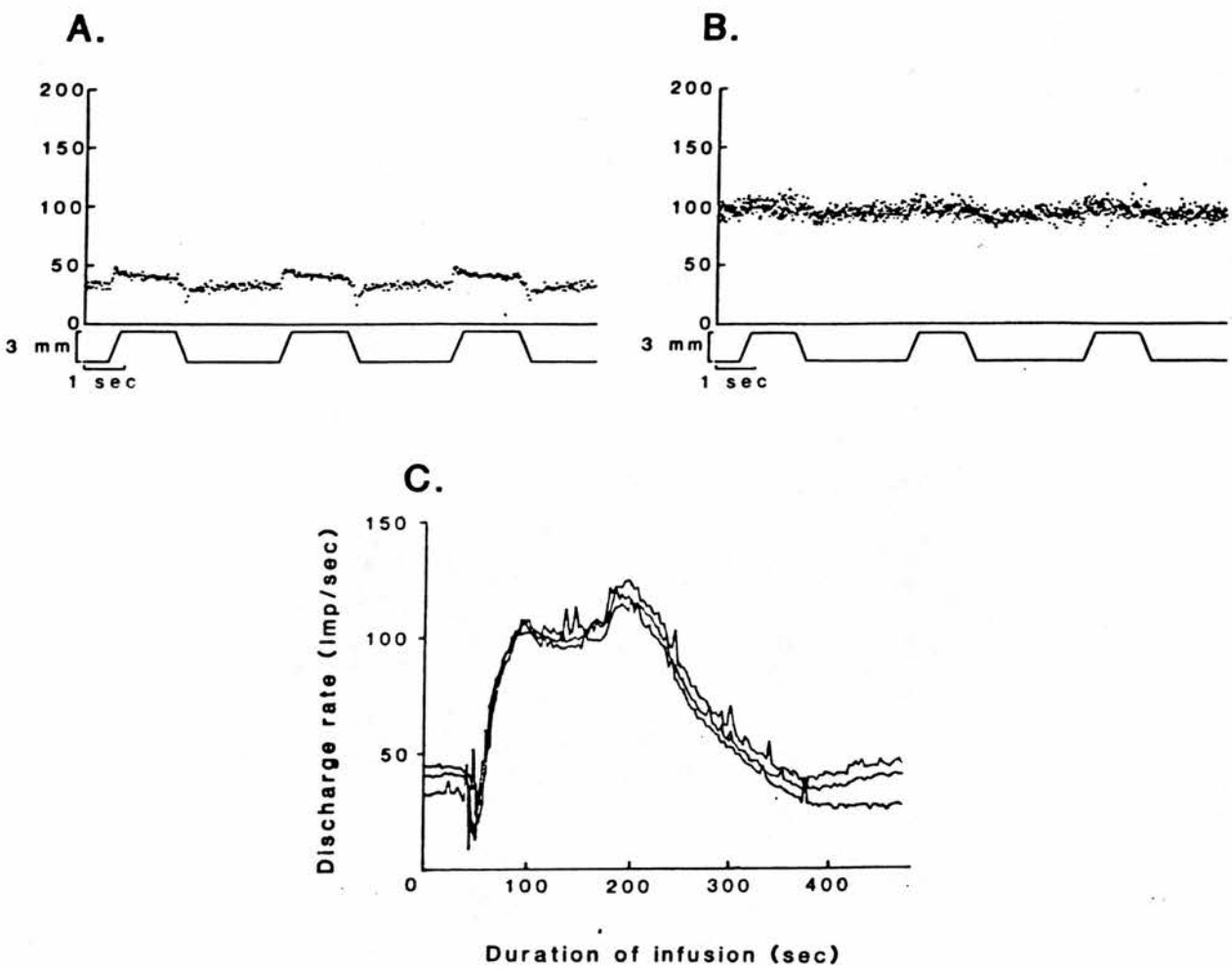


Fig. 11 A-C. The pattern of SCh activation of a third biventer type 3 afferent. Response of this afferent to ramp stretching (3 mm, 10 mm/sec) before (**A**) and after 180 seconds (**B**) of a SCh infusion at 100 μg/kg/min. **C** Time course of this activation.

this unit had an initial discharge rate of 46 Imp/sec, a dynamic response of 75 Imp/sec (neglecting the initial burst), and a position response of 58 Imp/sec, so that the dynamic index was 17 Imp/sec and the position sensitivity 4 Imp/sec/mm. About 48 seconds after the start of the SCh infusion, the initial discharge began to increase and become more variable, reaching a maximum of 100 Imp/sec after 160 seconds of infusion (Fig. 12, C); over the same period, the dynamic response and position response increased rather more steeply and nearly in parallel, so that at maximal activation (Fig. 12, B), the dynamic index of the afferent had fallen by about 30% to 11 Imp/sec, whilst the position sensitivity had nearly trebled to 11.3 Imp/sec/mm. Of the 104 afferents exposed to SCh in this series, this was the only one to show an appreciable increase in its position sensitivity. After 200 seconds of infusion, the pump was switched off, and these changes gradually reversed over the next 350 seconds.

B. AFFERENT RESPONSES TO DOSES OTHER THAN 100 μg/kg/min.

In order to ascertain whether or not the standard dose of SCh was sufficient to fully activate biventer muscle afferents, 7 experiments were performed in which, in addition to the standard dose of SCh, higher doses in the range 200-500 μg/kg/min were used; in addition, the infusions were continued for about twice as long as standard in these experiments in order to look for possible late effects. For reasons which I shall elaborate upon shortly (Discussion, p.mm), the main interest in this series centred upon the behaviour of type 2 and 3 afferents.

The main finding of these 7 experiments was essentially negative, in that the very large majority of the afferents studied showed no further changes in stretch sensitivity after a higher dose SCh infusion than they had done after the 100 μg/kg/min infusion. In Fig. 13 is illustrated the only exception to this rule, which was seen on two occasions; in the instance shown here, the afferent had been identified as type 2 on the basis of a large increase in dynamic index superimposed upon substantial biassing of the initial discharge after exposure to a 100 μg/kg/min SCh infusion. In

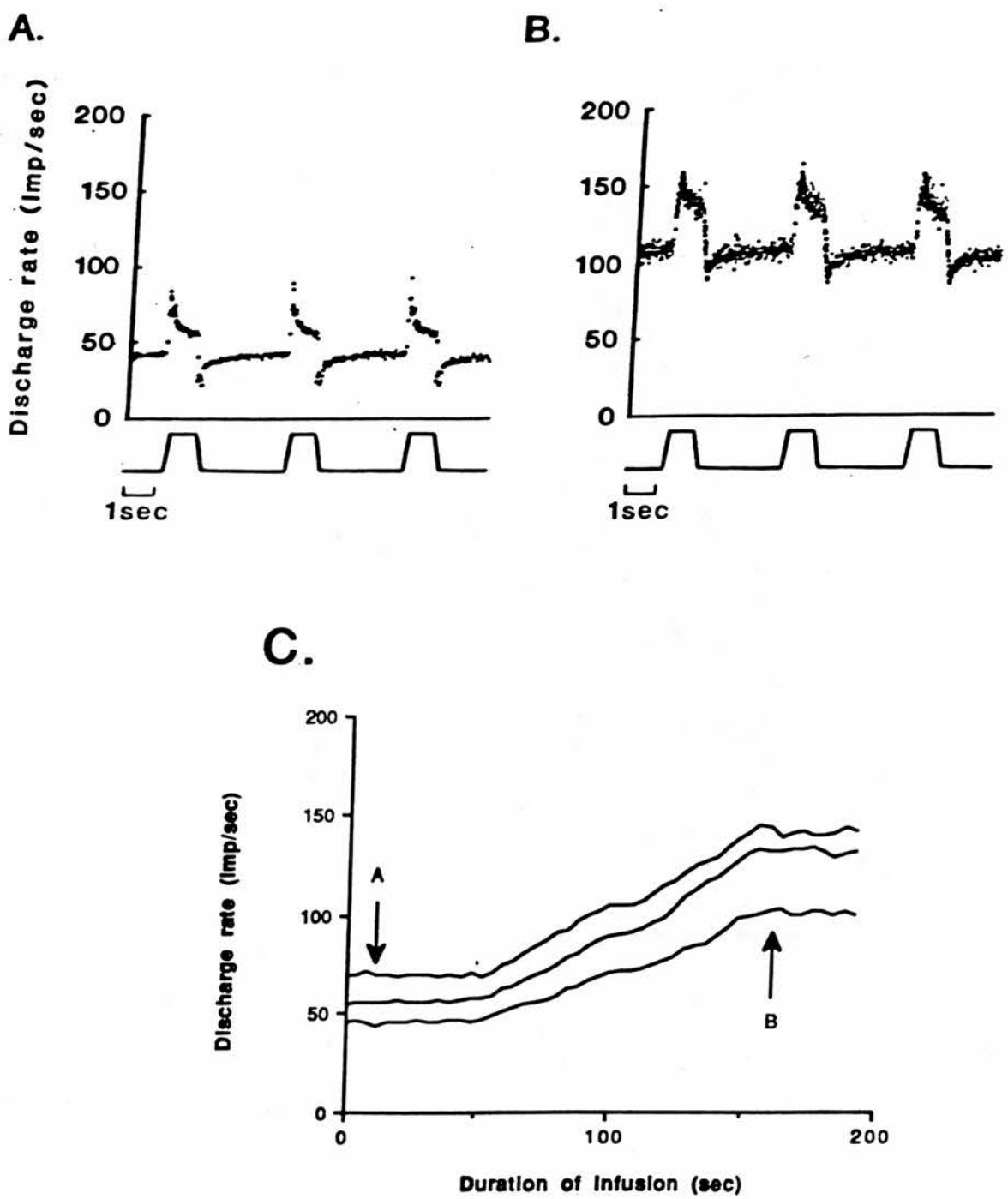


Fig. 12 A-C. The pattern of SCh activation of the only biventer type 4 afferent encountered. Response of this afferent to ramp stretching (3mm, 10 mm/sec) before (**A**) and after (**B**) SCh infusion at 100 μg/kg/min. The time course of this activation is shown in **C** in the standard format; at the point marked **A**, the responses shown in panel **A** were obtained, and at point **B**, those shown in panel **B**.

response to a prolonged 200 μg/kg/min infusion, the latter part of which is illustrated in Fig. 13, the discharge of the afferent was again substantially biassed, on this occasion to 130 Imp/sec, the dynamic index trebled to 78 Imp/sec from 26 Imp/sec, and, as was typical of this class of afferent, the position sensitivity fell by 30%. These changes were similar in magnitude, though of more rapid onset, than those provoked by the 100 μg/kg/min infusion rate; when the infusion was continued past 5 minutes, however, the discharge of this afferent became erratic, the dynamic response initially failing to reach the highest frequencies of around 240 Imp/sec which it had achieved earlier in the infusion (e.g. leftmost traces in Fig. 13); subsequently, the afferent ceased firing during the period of muscle stretching (Fig. 13, open arrows), and finally was silenced during the entire cycle of muscle stretching, holding and releasing (Fig. 13, filled arrows). Note that there was at no time an increase in the position sensitivity of this afferent; of 12 type 2 afferents which were exposed to higher doses of SCh, none showed any late changes in position sensitivity, and only one other behaved in the manner illustrated in Fig. 13.

Fig. 14 illustrates the response to a 300 μg/kg/min SCh infusion of an afferent which had been classified as type 3 after exposure to 100 μg/kg/min SCh on the basis of a marked reduction in position sensitivity superimposed upon a very variable, biassed initial discharge rate. In the control condition, this afferent had an initial discharge of 42 Imp/sec, a dynamic response of 78 Imp/sec and a position response of 68 Imp/sec, giving a dynamic index of 10 Imp/sec and a position sensitivity of 8.7 Imp/sec/mm (Fig. 14, A). About 40 seconds after the start of the higher dose infusion, the initial discharge began to rise steeply, reaching 100 Imp/sec in 44 seconds and thereafter increasing more slowly to a maximum of 120 Imp/sec after 190 seconds of infusion (Fig. 14, C); over the same period the dynamic index and position sensitivity were reduced by 50% and 64% respectively. In the maximally activated state (Fig. 14, B) the pattern of afferent response to stretch was essentially the same as that produced by a 100 μg/kg/min SCh infusion (compare e.g. Figs. 9-11, B).

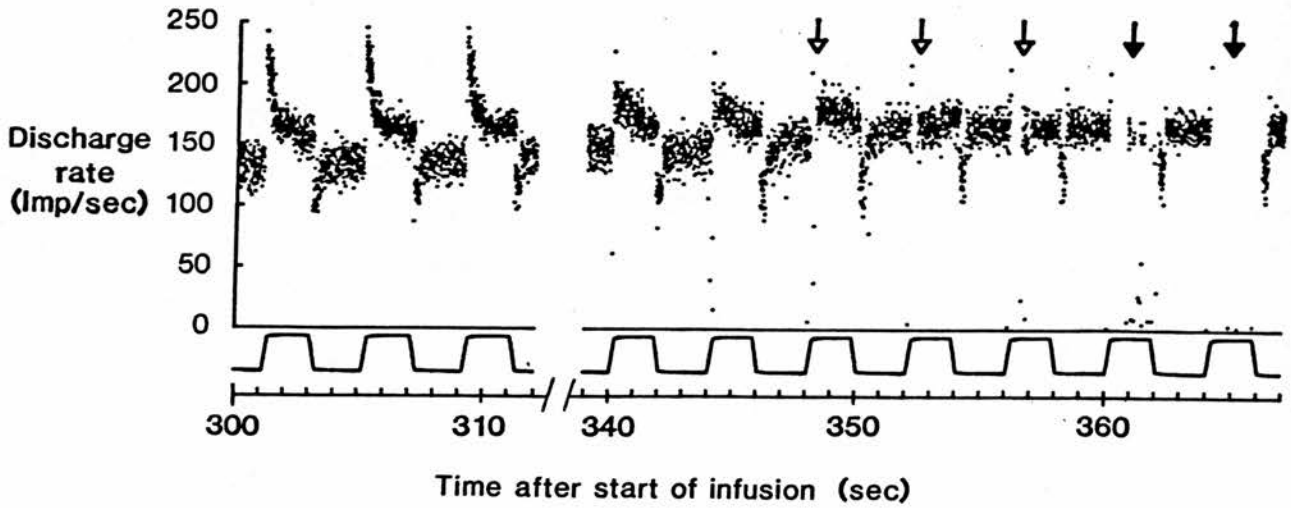


Fig. 13. Response of a biventer type 2 afferent to a prolonged infusion of SCh at 200 μg/kg/min. First three stretches show a similar response to that provoked by SCh at 100 μg/kg/min. Remaining stretches show the development of depolarisation block (open arrows) which ultimately eliminates the response to ramp stretch (filled arrows). Numbers below the length signal indicate the time since the start of the infusion.

Fig. 14 is included here for another reason, namely to illustrate the response to SCh infusion of the afferent whose ambiguous responses to contraction of the individual compartments of the biventer muscle were shown in Fig. 2 above.

A final series of 4 experiments was performed in which afferents were exposed to stepwise increases of SCh infusion rate over the range 25-200 μg/kg/min. Afferents to be exposed to this regime were actively selected on the basis of their passive properties (the means by which this was achieved will be described below p. 144) as likely to be classified as either type 2 or 3 as described above. These afferents were then exposed to the stepped dose infusion and one of four possibilities ensued. In the commonest two scenarios (9 of 14 activations), the low doses of SCh up to around 60 μg/kg/min provoked no changes in afferent response to stretching, whilst any higher dose powerfully activated the afferents to a level which was not subsequently exceeded by the 100 μg/kg/min dose; there were two scenarios here, one in which an afferent was activated in a manner typical of type 2 afferents (e.g. Fig. 8), and another in which it was activated in a manner typical of type 3 (e.g. Fig. 9).

The remaining 5 activations were more interesting, and were of two types. Fig. 15 illustrates the pattern of activation shown by 3 units in different experiments; panel A of this figure illustrates the control responses of this afferent, and panel B the first evidence of SCh-evoked activation which in this case came after about 100 seconds of an infusion of 30 μg/kg/min SCh. At this time, there was a sudden, transient period of excitation during which the initial discharge of the afferent was biassed to about 80 Imp/sec, this change being accompanied by an increase in the variability of the impulse train, and by a reduction in the dynamic index and position sensitivity of the afferent. The form of the afferent response to stretch at this point resembled a scaled down version of the maximal response of the 33 type 3 afferents identified on the basis of their response to 100 μg/kg/min SCh infusion (compare Fig.15, B and Figs. 9-11, B). This "event" lasted for about 30 seconds before suddenly and spontaneously waning, the form of the afferent response to stretch reverting to

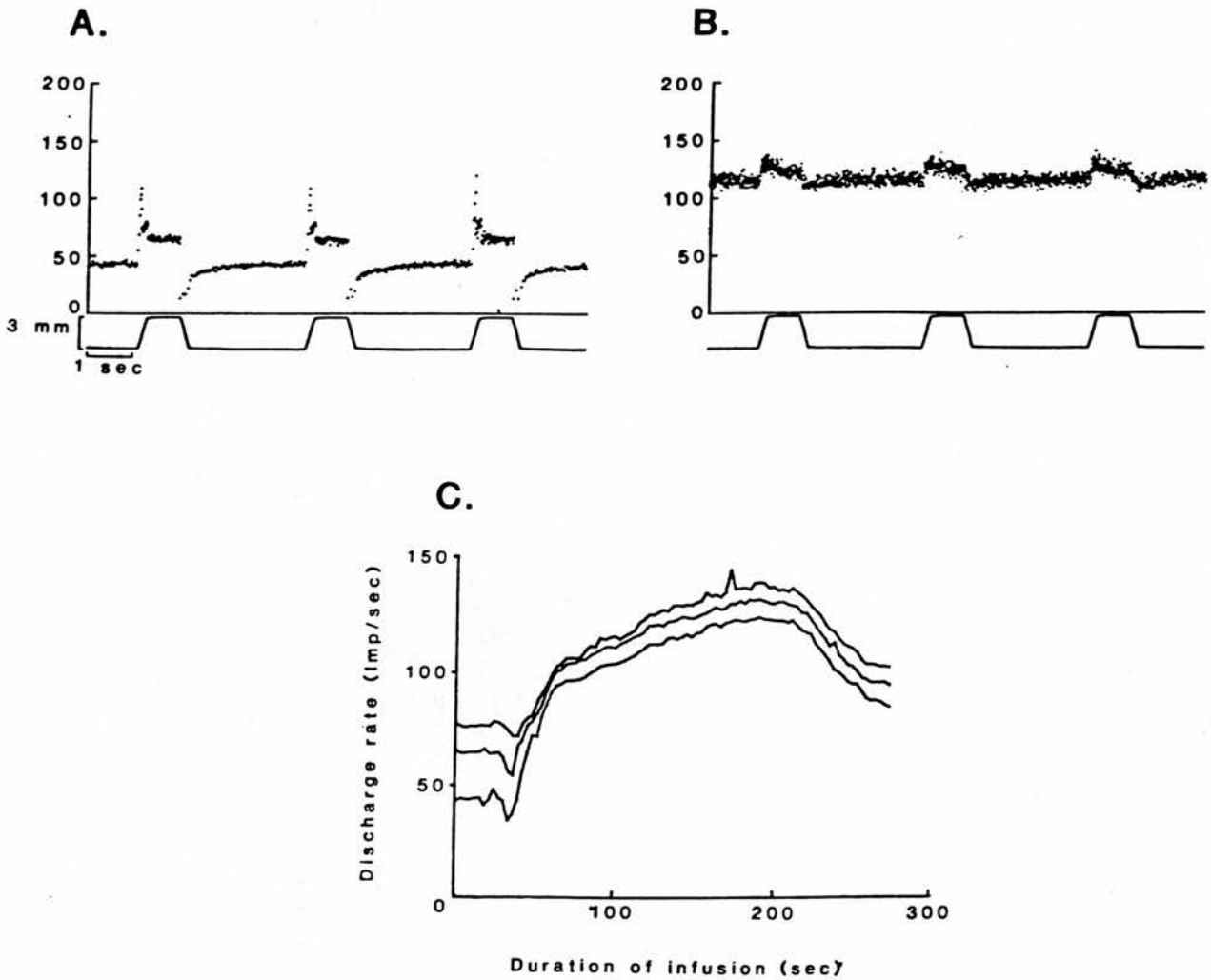


Fig. 14 A-C. Pattern of activation of a biventer group 3 afferent exposed to a SCh infusion at 300 μg/kg/min. Response to stretch (3 mm, 10 mm/sec) before (**A**) and after 196 seconds (**B**) of infusion ; time course of this activation shown in **C**.

what it had been immediately prior to the event. After a further 25 seconds of infusion, a second event of equally sudden onset occurred (Fig. 15, C, open arrow); on this occasion, the initial discharge was biassed to around 130 Imp/sec over the course of 2 stretch cycles (about 8 seconds), afferent discharge variability increased markedly again, and dynamic and position sensitivities both fell. However, these changes were followed after 10 seconds by a clear increase in the dynamic response of the afferent which reached frequencies of 250 Imp/sec, as well as by an increase in the dynamic index which reached 50 Imp/sec (Fig. 15, C, filled arrow). This second event also lasted about 30 seconds, after which the degree of biassing fell considerably, leaving the afferent with a high dynamic index but very little position sensitivity (Fig. 15, C, right hand part). Shortly after this second event the infusion rate was increased to 60 μg/kg/min, and after a delay commensurate with the washout time of the arterial cannula the afferent was rapidly activated to a level (Fig. 15, D) which was not subsequently exceeded when the dose was raised again to 120 μg/kg/min (not illustrated), and which clearly identified it as a type 2 afferent, as defined above.

The remaining two activations took the form illustrated in Fig. 16 and were also characterised by the occurrence of a series of events at low doses of SCh. As before, panel A illustrates the control response to stretch of this afferent, and panel B the occurrence of a sudden, transient biassing of the discharge rate accompanied by an increase in the discharge variability and a reduction in dynamic and position sensitivities. These changes reversed suddenly and spontaneously after about 25 seconds and rapidly restored the afferent response to stretch to what it had been immediately before the event. Three more events of similar magnitude and time course were observed over the next 120 seconds (not illustrated), after which the infusion rate was stepped up to 100 μg/kg/min, resulting in the rapid activation of the afferent to a level which was not exceeded by a further increase in the dose to 200 μg/kg/min (Fig. 16, C). In these two activations, however, there was no evidence during any of the events of an increase in the dynamic index of the afferent, nor was it increased in the

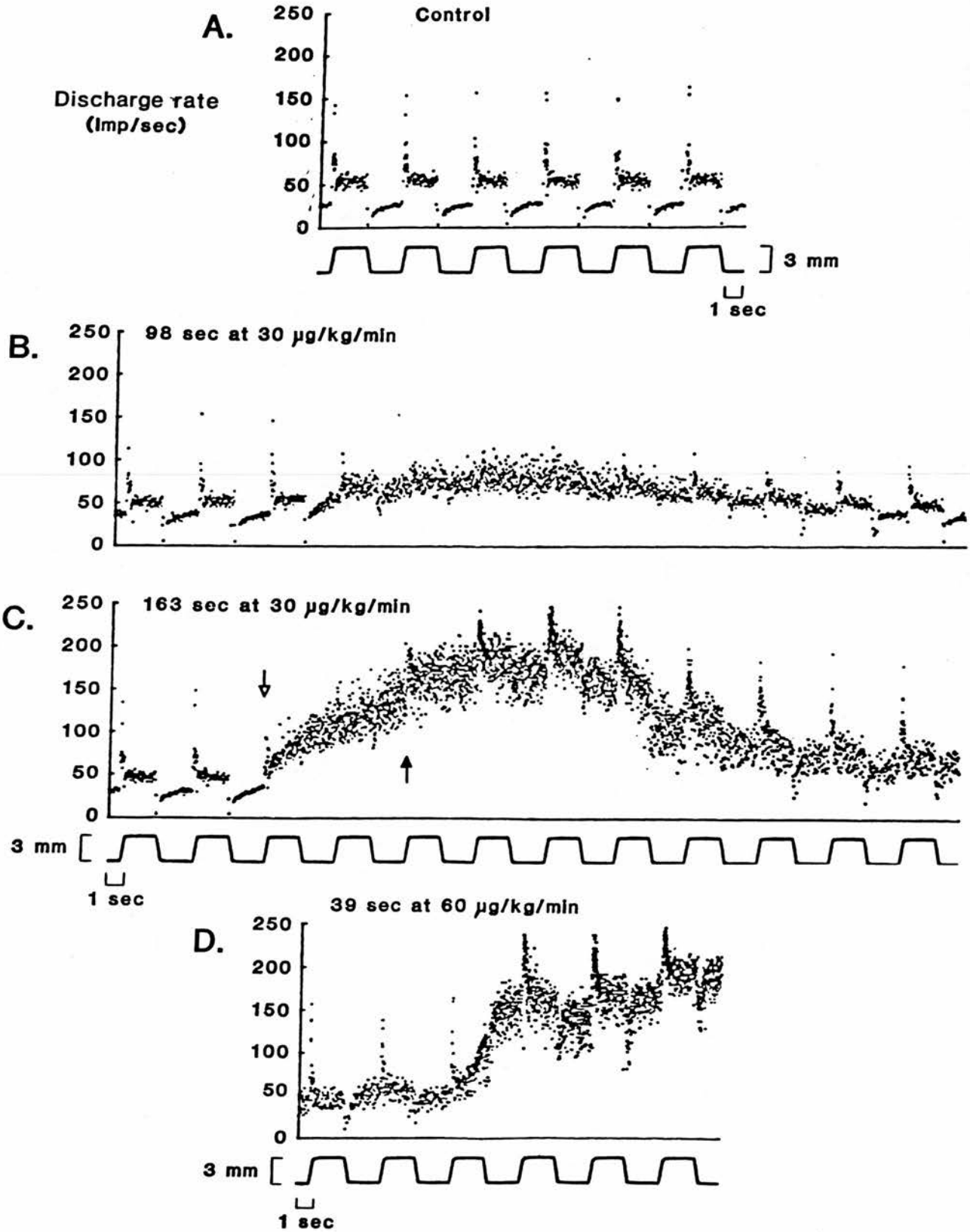


Fig. 15 A-D. Excitation by ramped-dose SCh infusion (see text) of a biventer group 2 afferent. Control responses to stretch (3 mm, 10 mm/sec) in **A. B, C** Responses to stretching at successive times during a SCh infusion at 30 µg/kg/min; the number above each panel refers to the timing of the first stretch in the sequence relative to the start of the infusion. **D** Response of this afferent when the infusion rate was increased to 60 µg/kg/min; last stretch in **D** shows the maximally activated response of this afferent, which was not exceeded by further increases in the SCh infusion rate.

maximally activated state, and these two afferents were therefore classified as type 3, as defined above.

1:3.3. PASSIVE PROPERTIES OF BIVENTER AFFERENTS.

Three passive properties of the biventer afferents were routinely studied, namely the coefficient of variation of the afferent discharge at the resting muscle length, the afferent sensitivity to sinusoidal muscle stretching and the sensitivity to longitudinal vibration of the muscle. Of the total sample of 112 afferents, 77 had all three passive properties measured successfully. For the remaining 35 afferents, only one or two of the passive properties were studied; the measurement most commonly omitted was the coefficient of variation, since it is altered unpredictably when an afferent discharge tonically at a rate below 20 Imp/sec, as was sometimes the case in these experiments (Matthews & Stein 1969b).

As was intimated in the Methods section (p. 106), there was a problem with the measurement of afferent sensitivity to sinusoidal muscle stretch which arose from a miscalibration of the length transducer. The sensitivity of an afferent to 1 Hz sinusoidal muscle stretching is reported as its discharge per millimetre of muscle stretch (i.e. with units of Imp/sec/mm), so that the modulation of an afferent's discharge in response to a stretch of exactly 1 mm amplitude is, by definition, the same as the sensitivity of that afferent. Unfortunately, straightforward division of the discharge modulation evoked by a stretch of amplitude other than 1 mm by the actual length change to yield an estimated 1 mm discharge modulation cannot be justified, since discharge modulation is known to be a distinctly non-linear function of the amplitude of stretch (Hulliger, Matthews & Noth 1977a). Instead, one of two strategies was employed to obtain a value of sinusoidal sensitivity when the afferent had not been exposed to a stretch of exactly 1 mm amplitude.

If the afferent was studied with two stretch amplitudes, one slightly less than 1mm, the other slightly greater than 1 mm, then the 1 mm value was obtained by

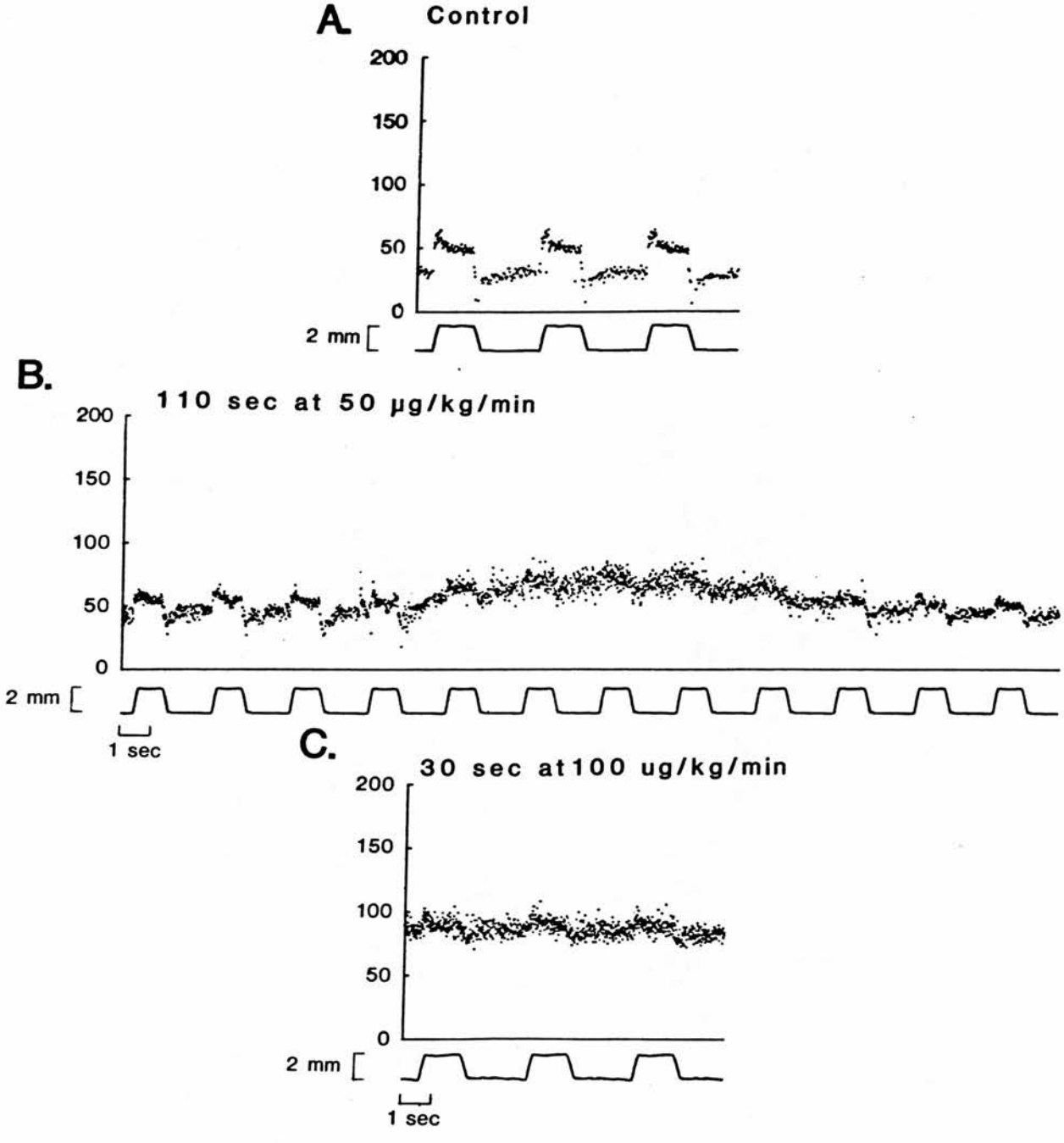


Fig. 16 A-C. Excitation of a biventer group 3 afferent by ramped-dose SCh infusion. Control responses to stretch (3 mm, 10 mm/sec) shown in **A**. **B** Response of this afferent after 110 seconds of SCh infusion at 50 µg/kg/min; several other episodes of the sort shown in **B** were seen (not illustrated) before the SCh infusion rate was increased to 100 µg/kg/min to yield the responses shown in **C**. Further increase in the SCh dose did not produce any other changes in afferent behaviour.

linear interpolation between the two points. Alternatively, where the afferent was studied only with stretch amplitudes greater than 1 mm, a 1 mm value was obtained by extrapolation using a "population slope" obtained as follows. First, 31 afferents which had all been studied with 1 mm sinusoidal stretches as well as stretches of larger amplitude were identified. Next, these afferents were grouped according to their pattern of activation by SCh, on the assumptions that the patterns of SCh activation correlated with patterns of intrafusal termination of the afferents and that afferents with similar intrafusal terminations would have comparable passive properties. Then, for each amplitude of sinusoidal stretch, a mean discharge modulation was calculated for each of the three major classes of afferent (Fig. 17), and finally for each class of afferent the slope of the line connecting the mean responses to stretch amplitudes of 1 and 1.5 mm, and that connecting the mean responses to stretches of 1.5 and 2 mm were calculated; these were the "population slopes". Given an afferent which was exposed to a stretch of 1.7 mm amplitude, and which had a SCh typing of 2, then the type 2 population slopes were used: first the 1.5-2 mm slope was used to extrapolate from the 1.7 mm response to an estimated 1.5 mm response, then the 1-1.5 mm slope was used to extrapolate to the estimated 1 mm response. It was felt that, whilst this method would still introduce errors, an attempt to take into account the inherent non-linearities in afferent response to stretch would make these errors less than those incurred by simple division of afferent response by stretch amplitude. Of the 96 afferents studied with sinusoidal stretches, 34 (35%) were not tested with 1 mm sinusoidal stretches; of these, the linear interpolation estimate was used for 16, and the extrapolation estimate for 18.

Each of the passive properties measured was distributed as a continuum with no apparent separation into sub-populations which might represent different afferent types; for example, the coefficients of variation of the 89 afferents studied formed a smooth continuum from 0.018 to 0.112. A similar situation pertains in populations of hindlimb muscle spindle afferents unless afferents are distinguished by

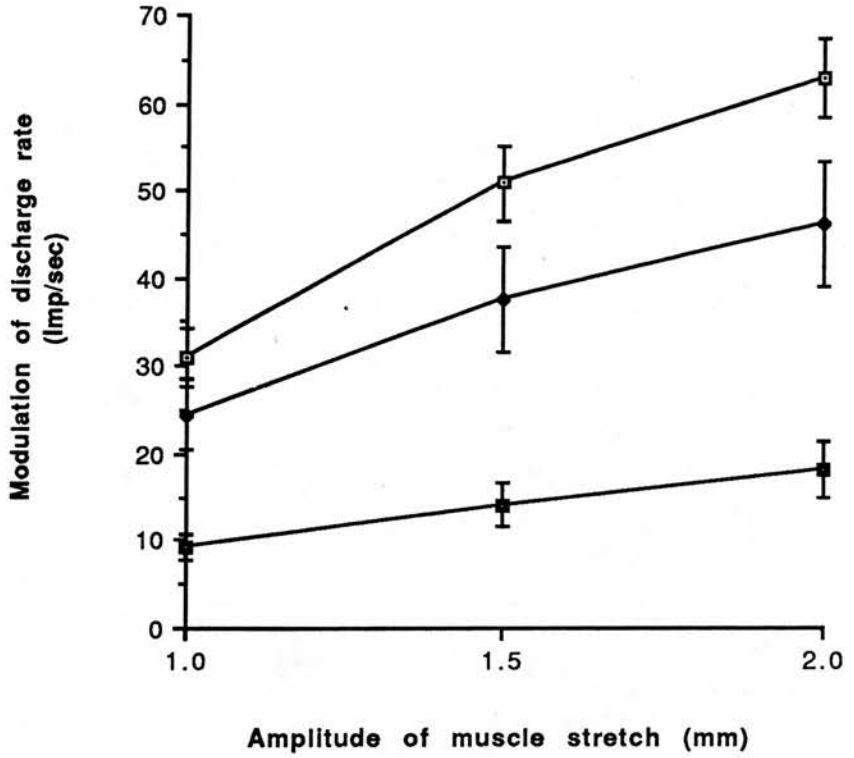


Fig. 17. Relationship between the amplitude of sinusoidal muscle stretching and the modulation of afferent discharge rate for 31 biventer afferents. The three symbols represent different patterns of SCh activation of the afferents concerned: thick-walled square: type 1 afferents (n=6); square with dot in it: type 2 afferents (n=15); filled circle: type 3 afferents (n=10). Each point is the mean response to stretch of the given amplitude of all the afferents with that pattern of SCh excitation, and the error bars show +/- 1 standard deviation from the mean.

means of their conduction velocity when it becomes apparent that secondary afferents tend to have lower coefficients of variation than primary afferents (Matthews & Stein 1969b). In the present experiments, the distinguishing characteristic used was not afferent conduction velocity but rather the pattern of SCh activation. When this was taken into account, it then became apparent that each continuum could be subdivided into two populations. Thus, the coefficient of variation continuum could be subdivided into a population of afferents with coefficients of variation less than 0.03 most of which (23 of 32) were classified as type 1, and a second population with coefficients of variation of 0.03-0.112 made up almost entirely of type 2 and type 3 afferents completely intermingled. Similar considerations apply to the measurements of sinusoidal sensitivity (type 1 afferents: all in the range 1-24 Imp/sec/mm; type 2 & 3 afferents: 40 of 44 in the range 18-84 Imp/sec/mm) and sensitivity to vibration (1:1 driving in: type 1 afferents: 20-50 Hz; type 2 & 3 afferents: 36-220 Hz).

In the case of afferent sinusoidal sensitivity, the impression that type 2 and 3 afferents had very similar passive properties was tested statistically; in order to rule out possible errors introduced by the estimation methods described above, only those afferents which had been exposed to stretches of exactly 1 mm amplitude (Fig. 17) were compared by means of a two-tailed, unpaired Student's t-test, having first determined by means of an F-test of population variances which form of the t-test was applicable. For each amplitude of sinusoidal stretching, the mean modulation amplitude of type 1, 2 and 3 afferents were compared to each other, and the results of this comparison are tabulated in Table 1. Examination of the rightmost column of Table 1 reveals that, for all amplitudes of stretching, the sinusoidal sensitivities of type 2 and 3 afferents are very significantly greater than those of type 1 afferents, whereas the responses of type 2 and 3 afferents did not differ significantly from each other at any amplitude of muscle stretching.

All three passive properties and the pattern of SCh excitation were measured for 65 afferents during these experiments, and the findings for these 65

		AFFERENT TYPE			COMPARISON OF MEAN RESPONSES		
		Type 1 (n=15)	Type 2 (n=10)	Type 3 (n=6)	Type 1:Type 2	Type 1:Type 3	Type 2:Type 3
Stretch (mm)	1	31.1 +/- 3.29 (162.8)	24.6 +/- 3.98 (158.1)	9.3 +/- 1.61 (15.5)	0.2 > P > 0.15	P << 0.001	0.005 > P > 0.001
	1.5	50.3 +/- 4.31 (278.2)	37.6 +/- 6.01 (361.7)	14.1 +/- 2.53 (38.4)	0.1 > P > 0.05	P << 0.001	P < 0.001
	2	62.85 +/- 4.5 (297.1)	46.2 +/- 7.2 (511.8)	18.2 +/- 3.2 (60.2)	0.1 > P > 0.05	P << 0.001	P < 0.001
Slope (Imp/sec /mm)	1---1.5	38.6	26.1	9.6			
	1.5---2	25.1	17.1	8.2			

Table 1. *Top left* Mean response +/- standard error of mean of the three main afferent types identified by SCh in response to sinusoidal stretching at three amplitudes as indicated. The numbers in brackets indicate the variance in the sample, on the basis of which the appropriate form of the t-test was chosen for analysis of significance. *Top right* Comparisons between afferent types of their responses to different stretch amplitudes. *Bottom* Population slopes (see text) connecting the mean responses to 1 and 1.5 mm stretching and 1.5 and 2 mm for each afferent type.

afferents are summarised in the multi-axis plot of Fig. 18. In this figure, the different patterns of SCh activation observed for the various afferents are represented by different symbols (type 1 afferents: open squares; type 2 afferents: filled circles; type 3 afferents: open triangles; type 4 afferent: filled square). Plotting all three passive properties against each other reveals that what had applied to individual properties also applies to the multi-axis plot:- as a whole, the afferents occupy a continuous volume of the three-axis plot, but if the patterns of SCh activation are taken into account two distinct populations can be discerned. In the lower left quadrant of the plot, at low coefficients of variation, low sinusoidal sensitivities and low vibration sensitivities (driving) lie the large majority of type 1 afferents. The type 2 and 3 afferents almost all have higher coefficients of variation, sinusoidal sensitivites and vibration sensitivities, but even in this multi-axis plot they are completely intermingled in the remaining quadrants, with no indication of separation into two sub-populations.

A particular use of this multi-axis plot, which was touched on above (p. 134), was that it permitted active selection of afferents for the four ramped-dose SCh experiments performed at the end of this series. In these experiments, type 2 and 3 afferents were the particular focus of attention; since the passive properties of each afferent were determined before exposure to SCh, they could be entered into the multi-axis plot to assess the likelihood that the afferent would be classified as type 2 or 3 by a subsequent SCh infusion. In this way time was saved by avoiding the needless activation of large numbers of type 1 afferents, and in fact no type 1 afferents were exposed to ramped-dose SCh infusions.

Richmond & Abrahams (1979a) have reported that three classes of presumed muscle spindle afferent from biventer cervicis can be identified on the basis of a combination of their dynamic index, discharge variability and the exact details of the response to a ramp stretch such as the presence or absence of an "acceleration response". In their hands, presumed secondary afferents had low discharge variability, low dynamic index and a smooth increase in firing frequency over the course of a ramp

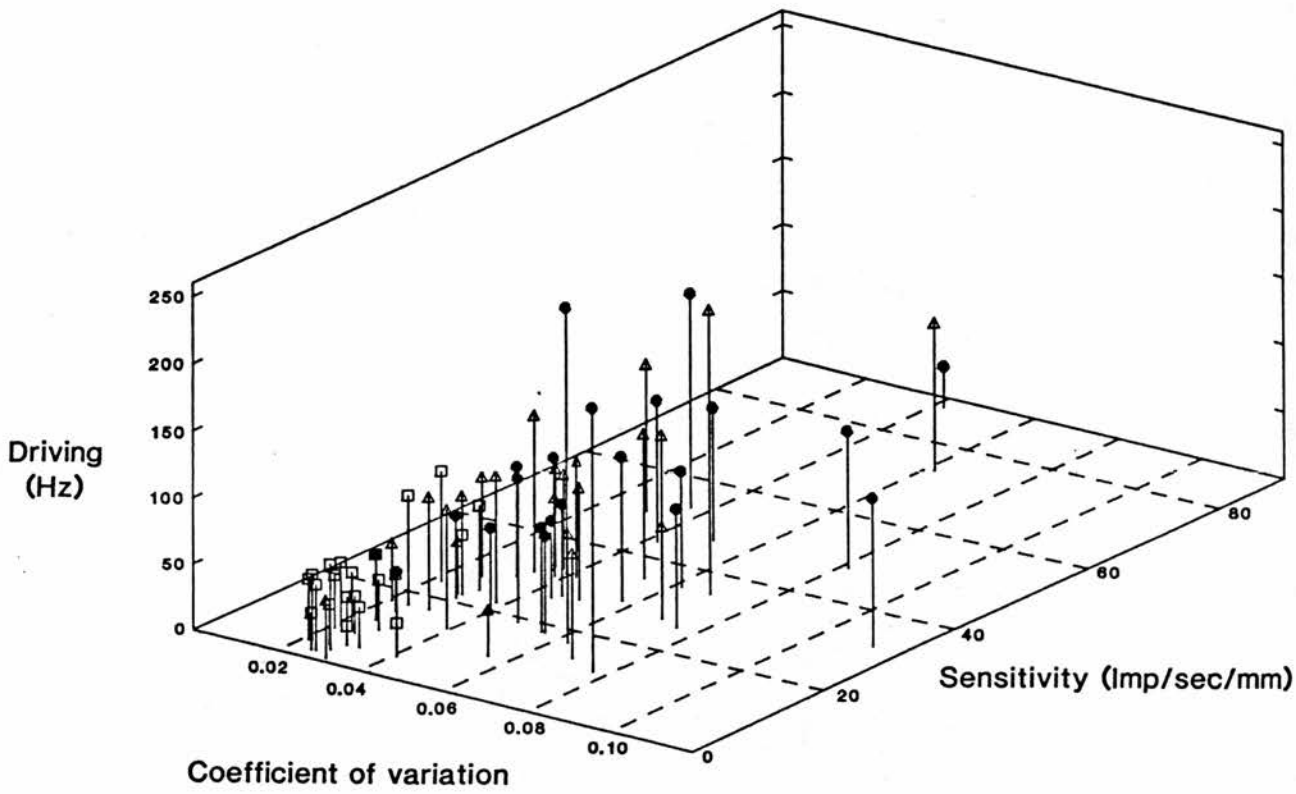


Fig. 18. Summary diagram showing the relationship between the coefficient of variation of the resting discharge, the sensitivity to 1 Hz sinusoidal stretching, and the maximum frequency to which the afferent could be "driven" 1:1 by small-amplitude vibration, for the 65 afferents in which all three measurements were obtained in addition to a characterisation by pattern of SCh activation as indicated in the symbols:- squares: type 1 afferents; filled circles: type 2 afferents; open triangles: type 3 afferents; filled suqare: the type 4 afferent.

stretch; presumed primary afferents (by which was meant b_1b_2c primary afferents, though this terminology was not in use at the time) had a high discharge variability, large dynamic indices and abrupt increases in discharge rate at the start of a ramp stretch, and an intermediate group of afferents had intermediate variability accompanied by abrupt increases in firing at the start of a stretch, but with a disproportionately small dynamic index. It has already been shown that, in the present series, discharge variability (coefficient of variation) can only be used to identify 2 rather than 3 classes of afferent. The ramp stretches used in most of the present experiments were substantially slower than those used by Richmond & Abrahams (1979a) who used velocities of 20 mm/sec routinely, but for 31 biventer afferents ramps of 3 mm amplitude and 20 mm/sec velocity were used in order to permit comparison with the work of Richmond & Abrahams; in the present experiments, however, the SCh typing of each afferent was available as an independent arbiter of the possibility that pattern of response and magnitude of dynamic index could be used to differentiate three classes of afferent.

Fig. 19 shows the relationship between the dynamic indices and coefficients of variation of these 31 afferents, the different symbols representing the SCh characterisation of each afferent as in Fig. 18. As was the case for the three passive properties described above, the dynamic indices of these biventer afferents form a continuum ranging from 4 to 58 Imp/sec, with no clustering to suggest the existence sub-types of afferent unless the pattern of SCh activation is taken into account. If this is done, it is clear that type 1 afferents tend to have the lowest dynamic indices (4-15 Imp/sec), whereas both type 2 and 3 afferents have higher dynamic indices, the majority being intermingled in the range 15-40 Imp/sec; although there is a tendency for type 3 afferents to lie in the lower part of this range (e.g. 7 of 11 have dynamic indices less than 25 Imp/sec) and for type 2 afferents to lie in the upper part of the range (9 of 13 have dynamic indices greater than 25 Imp/sec), there is nevertheless a sufficient degree of overlap between the two populations to make a diagnosis of

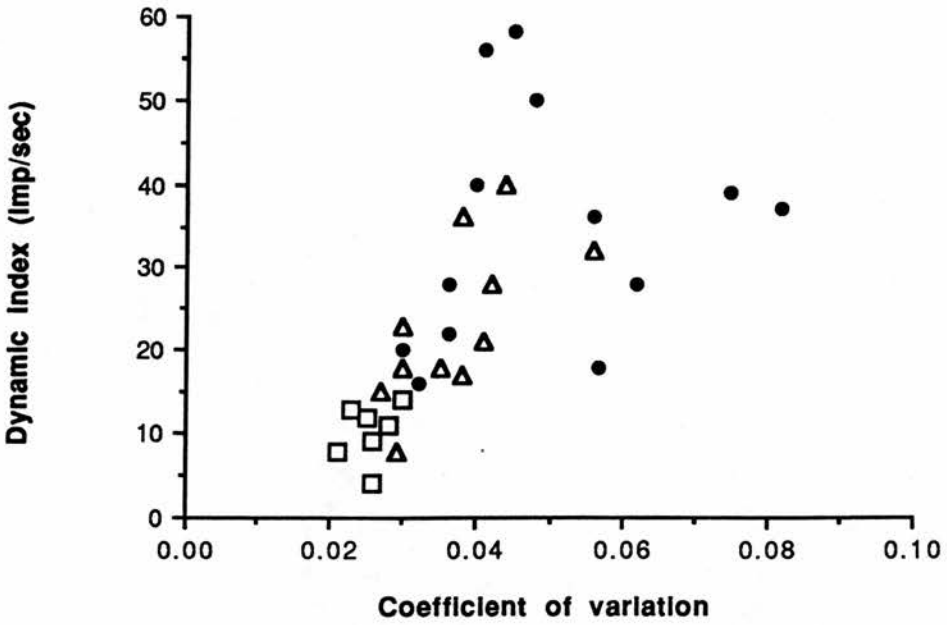


Fig. 19. Relationship between the coefficient of variation and the dynamic index in response to a stretch of 3 mm, 20 mm/sec for 31 biventer afferents. Symbols indicate pattern of SCh excitation: squares: type 1 afferents (n=7); filled circles: type 2 afferents (n=13); triangles: type 3 afferents (n=11).

afferent type based upon dynamic index and coefficient of variation alone questionable. As for the exact shape of the afferent response to a ramp stretch, this was very variable; none of the 7 type 1 afferents studied here had an acceleration response (i.e. a brief high-frequency burst at the start of the stretch), and the discharge of the afferents generally increased gradually over the course of a ramp stretch of the muscle, two features which tended to separate these afferents from the remaining 24. Only 7 of the 31 afferents showed an acceleration response, of which four were subsequently identified as type 2 and three as type 3 on the basis of their SCh activations. Both type 2 and type 3 afferents showed marked early increases in their firing rate in response to a ramp stretch of the muscle (fast rise phase, Literature Review, Part 1, p. 50), followed by a more gradual increase for the remainder of the stretching phase (slow rise phase). These features were not studied quantitatively, but no qualitative features of the afferent response to a ramp stretch could be discerned which would permit type 2 and type 3 afferents to be distinguished without reference to patterns of SCh excitation.

CHAPTER ONE

DISCUSSION

I have already put forward arguments to support the contention that the majority of the afferents studied in this series of experiments were indeed muscle spindle afferents (see p. 111). Before going on to discuss the other properties of biventer afferents, there is one feature which warrants some consideration, namely the anomalous responses to muscle contraction of biventer afferents which resulted in unusual difficulties of afferent identification.

The responses of biventer afferents to contraction of the muscle were initially rather unexpected. Richmond & Abrahams (1979a), in the only other electrophysiological experiments using biventer cervicis, had previously encountered afferents whose discharge rate was increased by contraction of the muscle, but these authors, in line with the classical description in the hindlimb, had assumed that such afferents must derive from Golgi tendon organs. It was therefore worrying in the present experiments, which were aimed at surveying muscle spindle afferents, to be finding so many presumed Golgi tendon organ afferents, that is afferents whose discharge was increased by muscle contraction. It was not until a few examples of the mixed responses of afferents as illustrated in Fig. 2 had been observed that it became clear that there was something unusual about the biventer cervicis muscle.

The most obviously unusual feature of this muscle is its architecture, for it is transected obliquely at regular intervals along its length by bands of fibrous tissue, so that from the dorsal aspect it appears to be divided into four or five compartments which are linked in series to each other. When the present experiments were carried out, the microarchitecture of the biventer muscle had been only partially elucidated, and although Richmond & Abrahams (1975a) had reported that individual extrafusal fibres could be teased out of the biventer muscle and could be seen to penetrate fibrous bands and extend through several compartments of the muscle, it was not clear what proportion of extrafusal fibres were similarly arranged. The importance of this point is that if all extrafusal fibres were to extend throughout the length of biventer, differing only in the level at which they were innervated by the segmental

nerves, then all stretch receptors lying in the belly of the muscle (including Golgi tendon organs adjacent to the fibrous inscriptions) would be mechanically in parallel with the contractile apparatus and so would be expected to show unloading responses to muscle contraction. Conversely, if extrafusal fibres were entirely restricted to one segment of the muscle, inserting into the fibrous bands rather than penetrating them, then muscle spindles lying in a given compartment of the muscle would be mechanically in parallel with the extrafusal fibres of that compartment, whereas they would be in series with the extrafusal fibres of any other compartment; in this case, mixed loading and unloading responses of the kind observed in these experiments are to be expected of muscle spindle afferents, whereas Golgi tendon organ afferents ought to be loaded by the contraction of any of the compartments.

The actual finding of mixed loading and unloading responses in some biventer afferents (e.g. Fig. 2) initially supported the strictly compartmentalised view of the microarchitecture of biventer and generated some optimism that muscle spindle and Golgi tendon organ afferents from biventer might yet be distinguishable in the standard manner (see e.g. Matthews 1972). Unfortunately, a number of practical and theoretical problems conspired to make this a forlorn hope. The most serious problem was a theoretical one, namely that knowledge of the microarchitecture of biventer was, at the time these experiments were performed, inadequate to allow assessment of the possibility that some extrafusal fibres might be constrained to lie in one compartment only, whilst others might extend through two or more compartments. A mixed arrangement of extrafusal fibres of this sort would lead to a mixed parallel/serial relationship of individual stretch receptors (both Golgi tendon organs and muscle spindles) to the contractile apparatus; the responses to muscle contraction of a particular receptor would depend upon the proportion of contracting extrafusal fibres with which it had an in-parallel arrangement compared to the proportion with which the arrangement was in-series, a situation which allows infinite variability and total unpredictability.

Technical difficulties merely added to the problems; for example, one or more of the muscle stimulation channels frequently failed at the critical moment - the surface electrodes might fall off, or the twitch responses to nerve stimulation might obviously decrease over time, suggesting that some of the motor axons were failing to conduct the impulse. Since the recording arrangements were rather precarious, the first priority was to study the pattern of SCh activation of the isolated afferent, rather than risk losing it whilst repairing the stimulation channel. Even if one assumes that all extrafusal fibres are restricted to a single muscle compartment, the finding of a loading response to contraction of all muscle compartments bar one cannot be taken as evidence that the afferent under study derived from a Golgi tendon organ, since contraction of the inactive compartment might have yielded an unloading response. A further technical problem was that under the particular experimental conditions used, at least one compartment had to be stimulated by means of surface silver ball electrodes, since its compartmental nerve was being subdivided to yield afferents. There was no means of knowing whether this route of excitation was reliably activating all the extrafusal fibres in that compartment, nor to what extent the muscle was twisting and distorting to produce confounding mechanical effects. All in all, there were too many uncertainties of interpretation of afferent responses to muscle contraction for them to be worthy of routine study.

With the passage of time, however, the position has become less uncertain. Richmond and her collaborators have recently reported the results of a detailed histochemical examination of the biventer muscle which has shown that, whilst there are regional variations in the microarchitecture of biventer, these are arranged medio-laterally rather than longitudinally (Armstrong, Rose, Vanner & Richmond 1988; Richmond & Armstrong 1988). For example, in a given compartment of the muscle, more medially placed extrafusal fibres are more often of histochemical type SO whereas more laterally placed fibres are more often type FG (Richmond & Armstrong 1988). Whilst this specialisation may have implications for

the recruitment and use of the various sub-volumes of muscle compartments (Richmond & Armstrong 1988), it would not appear, *a priori*, to have any special significance for receptor function. Very importantly, however, the finding of these authors that very few, if any, extrafusal fibres or motor axons penetrate the tendinous inscriptions of the muscle (Armstrong, Rose, Vanner & Richmond 1988) means that the relationship of stretch receptors and extrafusal fibres is known: muscle spindles are in-parallel with the extrafusal fibres of their parent compartment and in-series with all others, whereas Golgi tendon organs are in-series with all extrafusal fibres. In future experiments, provided that all compartments of the muscle are adequately stimulated, a Golgi tendon organ afferent may be identified by universal loading responses to contraction, whereas a spindle afferent is identified whenever an unloading response is encountered.

The serial arrangement of the compartments of the biventer muscle, and the consequences which this has upon the response to muscle contraction of the stretch receptors in each compartment, are not unique. A similar serial arrangement has been shown by glycogen depletion techniques to apply in the semitendinosus muscle of the cat hindlimb (English & Letbetter 1981), and mixed loading/unloading responses of muscle spindle afferents from the different compartments of that muscle have been described (Schwestka, Windhorst & Schaumberg 1981; Botterman, Hamm, Reinking & Stuart 1983) and explained by the same reasoning as was used above. Bodine, Roy, Meadows, Zernicke, Sacks, Fournier & Edgerton (1982) also give examples of a number of other muscles, such as the jaw opening digastric muscle, which have serially arranged compartments, but spindle afferent responses to contraction in these muscles have not been studied.

The special arrangement of the motor and sensory apparatus of these serially-arranged muscles has some interesting implications for their motor control. For instance, if at some time only one compartment were to be active, one would expect

much of the force which it generated to be dissipated in the visco-elastic elements of the neighbouring, relaxed compartments. This point has been considered in detail theoretically by Morgan (1985), and confirmed experimentally for the semitendinosus muscle (Bodine et al. 1982). One implication of this result is that for two serially linked compartments to function optimally both should generate similar forces and should be at similar points on their length:tension characteristic at any time; breaking these requirements might lead to gross asymmetry in sarcomere lengths in the two compartments, the more weakly active compartment being at risk of overstretching. Accurate contractile matching is thus essential, and there appear to be a number of design features of serially-linked muscles which are aimed at achieving this goal. Firstly, the maximum force which a muscle, or a compartment of a serially-linked muscle, is capable of generating is proportional to its cross-sectional area (Henneman & Olson 1965); one aspect of contractile matching of serially-linked compartments would therefore be to match their cross-sectional areas, and this appears to have been achieved for semitendinosus (Bodine et al. 1982). Another approach is to ensure that the linked compartments are always activated together even though the possibility of independent contraction exists anatomically. Again, there is experimental evidence that this occurs in practice in the form of EMG data obtained from the two compartments of semitendinosus during stepping movements; these data show a double burst of activity in semitendinosus during the step cycle, each compartment being recruited and released at the same time, at least at slow and moderate velocities of movement (English & Letbetter 1981; Murphy, Roy & Bodine 1981). A potential neural substrate for this synchronised activity in serially arranged compartments has been described by Botterman, Hamm, Reinking & Stuart (1983) in studies of the distribution of Ia-EPSPs within the semitendinosus motor nucleus. These authors demonstrated that there is a very high degree of divergence in the central connectivity of semitendinosus Ia afferents which produce large EPSPs in the majority of semitendinosus motor neurones. This situation is in contrast to that applying in most other hindlimb motor neuron pools in which Ia afferents project particularly heavily to

motor neurones in whose muscle unit the parent spindle lies, whilst projecting more sparsely or not at all to other motor units (see e.g. Binder & Stuart 1980); Botterman, Hamm, Reinking & Stuart (1983) have suggested that the unusual arrangement of Ia feedback in semitendinosus results in a degree of "hardwiring" which guarantees, through spindle afferent drive, that both compartments of semitendinosus will always be co-activated.

Some of these findings in the semitendinosus muscle have also been found to apply in the neck extensor muscles of the cat. Thus, Richmond & Armstrong (1988) have shown that the individual compartments of the biventer muscle have very similar functional cross-sectional areas, implying similar force-generating capabilities. The actual forces generated by the individual compartments of biventer have not been reported thus far; I performed three preliminary experiments in 1986 with a view to measuring these forces, but did not undertake a full-scale study. Essentially, the experimental arrangement was identical to that used in the experiments reported above, with the addition to the muscle stretching arrangement of a tension transducer; individual compartments were activated via their segmental nerves either singly or in various combinations, and either with single shocks or tetani. Two findings were of relevance to this discussion: firstly, all the compartments, when individually active, generated very similar forces both in response to single shocks and to tetani. For example, the peak tension achieved during a twitch was on the order of 0.08 N for all compartments, and during a 50 Hz tetanus was about 0.4 N; Richmond & Armstrong (1988) estimate from compartmental cross-sectional areas that the force-generating potential of a biventer compartment should be on the order of 1-2 N, the rather lower values I obtained presumably being due to the dissipation of much of the energy released by the active compartment in the visco-elastic elements of the inactive compartments. When all the compartments were simultaneously activated tetanically, the peak tension approached that predicted by Richmond & Armstrong (1988), being 0.86 N in one case and 0.74 N in the other in which this was studied. The second

finding, which in fact terminated one of the three experiments, was that tension imbalances produced by uncoordinated contraction of the separate compartments of a serially-linked muscle can have serious consequences; in this case, all compartments except that innervated by the anterior division of the C3 nerve were being tetanically stimulated, the result being that the muscle tore through the anterior C3 compartment!

Experimental support for the concept of balanced activation of serially-linked compartments in neck muscle comes from both whole animal studies and from reduced preparations. In the awake, performing cat, Loeb, Yee, Pratt, Chanaud & Richmond (1988) have demonstrated widespread synchronisation of motor unit recruitment in different compartments of various neck muscles during slow movements, and in the decerebrate animal Wilson's group (Bilotto, Schor, Uchino & Wilson 1982; Ezure, Fukushinma, Schor & Wilson 1983) have shown the cervico-collic-reflex-induced EMG activities of the several compartments of the splenius muscle always to be in phase with each other. Interestingly, however, Ezure, Fukushima, Schor & Wilson (1983) have observed that the gain of the reflex can be greater in some compartments than in others, depending upon whether the reflex is being induced by movements of the head upon the neck or by movements of the trunk relative to the neck. This suggests, at least in the reduced preparation, that the ability to separately control the serially-liked compartments of neck muscles may to some degree have been retained. Correspondingly, the possibility that central connectivity of neck muscle afferents might be hardwired to support rigid co-activation of in-series compartments, as suggested for semitendinosus (Botterman, Hamm, Reinking & Stuart 1983), does not appear to be experimentally supported. Brink, Jinnai & Wilson (1981) surveyed the pattern of projection of splenius afferents to their motor nucleus by recording EPSPs at various levels in the cervical spinal cord whilst stimulating single compartmental nerves. They demonstrated that both Group I and Group II afferents have a polarised pattern of central projection, such that afferents in a given segmental nerve project extensively to motor neurones lying at the same level in the cord (i.e. C4

afferents to C4 motor neurones), progressively less extensively to more rostrally located motor neurones, and apparently not at all to more caudally located neurones. There would thus appear to be a weighting of spindle afferent feedback in this muscle in favour of the most rostral compartments, though this might, of course, be compensated for by a reciprocal weighting of descending drive from higher centres. Another unusual feature of the central connections of neck afferents which has recently been highlighted is that there do not appear to be short-latency reflexes analogous to the monosynaptic stretch reflex of hindlimb muscles (Richmond & Loeb 1990). It may be that in muscles with more than two serially-linked compartments, and which cross many joints such as splenius and biventer, there are advantages to retaining partially or completely separate control of the individual compartments, though what these might be is a matter for speculation.

1:4.1 IDENTIFICATION OF THE FOUR CLASSES OF BIVENTER AFFERENT.

In the course of these experiments, biventer afferents have been studied with SCh under various experimental conditions, the overall conclusion from which is that four patterns of activation of the afferents can be discerned. In the Results section, these were identified by numerical labels as patterns 1 to 4, and it is my purpose here to show that corresponding to each pattern of afferent activation there is a particular pattern of intrafusal termination of that class of afferent. In the absence of other widely used diagnostic tests such as conduction velocity and response to muscle contraction, this can only be done by reference to previously reported correlations of SCh activation with these other diagnostic criteria, as provided for example by the work of Dutia (Dutia 1978, 1980; Dutia & Ferrell 1980; see also Gladden 1976; Boyd 1985a), as well as correlations of other functional characteristics of spindle afferents with these diagnostic tests.

With this in mind, an early issue is which, if any, of the patterns of SCh

activation seen for biventer afferents are at all similar to those reported for soleus afferents by Dutia (1980). Since he reported only three patterns of SCh activation for soleus spindle afferents, it is inevitable that at least one of the patterns described here will be without a hindlimb analogue, but there may be more than one pattern in this position. In fact, within limits which will be considered further below, it transpires that three of the four patterns of SCh activation seen in the present experiments can indeed be considered to be analogous to the three patterns described for soleus spindle afferents, and these will be considered in turn before moving on to consider the pattern which is specific to biventer afferents.

Type 1 afferents.

It will be remembered that, in response to 100 μg/kg/min infusion rates, the signature of this pattern of SCh activation was its unremarkability; other than producing a modest and gradual biassing of the afferent discharge to higher rates, SCh infusions had little effect upon the discharge of these afferents, and there were very small if any changes in afferent sensitivity to the static and dynamic components of a ramp stretch (e.g. Fig. 5). This pattern of SCh activation is identical to that reported for soleus secondary afferents which had been independently diagnosed as such on the basis of their conduction velocities (Dutia 1980). However, in contrast to the negative findings of Granit, Skoglund & Thesleff (1953), Dutia has also shown that some Golgi tendon organ afferents, namely those discharging at relatively low muscle tensions ("low-threshold" tendon organs), can be excited by SCh infusions (Dutia & Ferrell 1980); as is the case for secondary afferents, this excitation consists of a gradual biassing of the afferent discharge with minimal changes in sensitivity to components of the stretch. The biventer type 1 afferents are therefore open to diagnosis as spindle secondary afferents, low-threshold Golgi tendon organ afferents or a mixture of the two.

A number of features favour the conclusion that the large majority of the type 1 afferents were spindle secondary rather than low-threshold tendon organ

afferents. The first, which have already been considered above (see Results, p. 111), are the very low muscle tensions which prevailed at the muscle length used in these experiments, and the high sensitivity of the majority of afferents to muscle length changes which can have produced only very modest tension changes. The term "low-threshold" as applied to tendon organs is a relative one; unlike the majority of tendon organ afferents, "low-threshold" tendon organs discharge tonically at passive muscle tensions of 75-100 gm.wt (Dutia & Ferrell 1980), but this passive tension is substantially greater than that required to provoke tonic discharge in muscle spindle afferents (Matthews 1933). Assuming firstly that an analogous population of "low-threshold" tendon organs exists in biventer, and secondly that they have similar tension thresholds to those of their soleus equivalents, it is unlikely that they would have been tonically active at all at the resting muscle length in the present experiments, and even less so that they would have been discharging at frequencies of over 20 Imp/sec. Even the higher tensions which exist at the longer muscle length during ramp stretching were substantially lower than 75 gm.wt. and so unlikely to elicit maintained discharge from tendon organ afferents (Houk & Simon 1967). Finally, for 3 of the 28 type 1 afferents, the response to compartmental muscle twitches was of the mixed loading/unloading type which has been considered above; these afferents must therefore be identified as spindle rather than Golgi tendon organ afferents.

The second feature which goes against a diagnosis of tendon organ afferent for type 1 is to be found in the details of the magnitude and time course of the biassing action of SCh. In general, the magnitude of the biassing action of SCh on low-threshold tendon organ afferents is much less than that seen for primary and secondary spindle afferents, the maximum biassing action observed by Dutia & Ferrell (1980) being of the order of 15-20 Imp/sec. Furthermore, these authors reported that biassing of the discharge of low-threshold tendon organ afferents proceeded even more gradually than that of spindle secondary afferents, and that their discharge continued to increase for up to 10 minutes after cessation of an infusion. In contrast, the discharge

of the type 1 afferents studied in the present experiments, was biassed by between 15 and 51 Imp/sec over the course of a SCh infusion, and in none of the 28 SCh activations did afferent discharge further increase after the infusion was terminated.

The final line of evidence that type 1 afferents should be identified as spindle secondaries derives from their passive properties. Three passive properties were measured routinely for biventer afferents, namely the coefficient of variation of their resting discharge, their sensitivity to sinusoidal muscle stretch and their sensitivity to muscle vibration. The range of values found for each of these parameters was compared with the reported range for hindlimb spindle secondary afferents, and in each case was found to be similar. For example, all bar two of the type 1 afferents studied in these experiments had coefficients of variation in the range 0.018-0.032, which should be compared with the reported hindlimb range of about 0.015-0.025 (Matthews & Stein 1969b). It is clear that there is a substantial degree of overlap in these ranges as they stand, and the real extent of overlap is liable to be rather greater since the sample of Matthews & Stein (1969b) specifically excluded afferents with intermediate conduction velocities (i.e. 60-80 m/sec) from the analysis whilst such afferents will have been included in the present sample. When the ranges of sensitivity to sinusoidal stretching and muscle vibration of type 1 afferents are compared to those reported for hindlimb spindle secondary afferents, it is again apparent that there is a substantial degree of overlap between the ranges found in the two sites (sinusoidal sensitivity data from Matthews & Stein 1969a; Cussons, Hulliger & Matthews 1977; vibration sensitivity data from Brown, Engberg & Matthews 1967a; Matthews 1972).

Taking all these features into account, it was felt that type 1 afferents could be identified with reasonable confidence as spindle secondary afferents, with the possible exception of some of the more insensitive afferents which might instead have been low-threshold Golgi tendon organ afferents. Does the pattern of SCh activation of type 1 afferents fit with current knowledge of the intrafusal termination of spindle

secondary axons and with our understanding of the intrafusal actions of SCh? When Dutia performed his experiments (1975-1978), secondary axons were still widely held to form terminals almost exclusively on nuclear chain fibres, with only the occasional axon forming collateral terminals on one or both of the nuclear bag fibres (Literature Review, Part 1, p. 31-32). At the same time it had been confirmed visually that the action of SCh on nuclear bag fibres was to produce a contracture with consequent elongation of the equatorially placed sensory terminals, whilst nuclear chain fibres were simply paralysed (Gladden 1976). In this context, any changes in secondary afferent discharge provoked by exposure to SCh could not be explained by invoking a mechanism involving alterations in the physical properties of intrafusal fibres, the main alternatives being that SCh might directly influence secondary sensory terminals, or that these could be influenced indirectly by some other action of SCh. A favoured mechanism was that potassium ions, which were known to be released from paralysed extrafusal fibres, might diffuse into the spindle capsule, changing the transmembrane distribution of cations and resulting in depolarisation of sensory terminals (Paintal 1964; Kidd & Vaillant 1974); as a bonus, the same mechanism could also be invoked to explain the SCh-evoked changes in Golgi tendon organ afferent discharge.

More recently, however, it has become apparent that the large majority of hindlimb spindle secondary axons contact one if not both types of nuclear bag fibre as well as the nuclear chain fibres (Banks, Barker & Stacey 1982), and a similar situation probably also prevails in biventer spindles (Richmond, Bakker, Bakker & Stacey 1986). It is therefore no longer necessary to invoke indirect mechanisms of SCh action via potassium release, for secondary afferents are open to a direct influence from the contracting nuclear bag fibres (see Gregory & Proske 1987). The findings of Boyd and his group (e.g. Boyd 1981b, 1985a, 1986) show that this alternative mechanism can still explain the characteristic pattern of SCh activation of identified secondary afferents, since the effects of contracture of either type of bag fibre on juxta-equatorially situated secondary endings are markedly different from those on

equatorially placed primary endings. Thus, in most instances, contraction of bag_1 or bag_2 fibres biases secondary afferent discharge whilst barely affecting afferent sensitivity to dynamic and static components of a ramp stretch; when such sensitivities are altered, they are as often decreased as increased. The effect of a SCh infusion on a secondary afferent would therefore typically be to bias the discharge rate through bag fibre contraction with minimal changes in length sensitivity, and this is precisely what is seen for biventer type 1 afferents. This alternative mechanism might then be used to explain the differences in behaviour of Golgi tendon organ and spindle secondary afferents during SCh infusion as follows: firstly with regard to time course, the activation of spindle secondary afferents might be expected to proceed more rapidly than that of tendon organ afferents, since in the former effects are produced via a specific, receptor-mediated effect, whereas in the latter case it is the gradual accumulation of a by-product of this receptor-mediated effect in the vicinity of sensory terminals which produces a change in behaviour. Secondly, the immediate reversal of changes in secondary afferent behaviour upon cessation of a SCh infusion can be explained by rapid, enzyme-assisted vacation of cholinergic receptors, with consequent termination of action, whereas the continuing excitation of tendon organ afferents after the end of an infusion could be explained by the continued presence of the exciting agent, i.e. potassium ions.

Very recently, a third possible mechanism has come to light, namely SCh-induced local contraction of chain fibres. This possibility is based upon some still controversial histological studies by Arbuthnott et. al. (1982, 1985) as discussed in Literature Review, Part 1, p. 60. Boyd, Sutherland & Ward (1985) have suggested, on the basis of correlated electrophysiological and electron microscopic studies of static gamma innervation of intrafusal fibres, that chain fibres may receive two distinct forms of motor innervation, each with its own type of motor terminal and each producing a different effect on chain fibre mechanics. The one, designated the m_c ending, when active produces brief twitches of chain fibres as classically described by

Boyd and others (Literature Review, Part 1, p. 36, 54). The other, designated the m_a ending, when active does not cause twitching, and is instead thought to produce local changes in stiffness of the underlying regions of the chain fibre. It is proposed that such changes in chain fibre stiffness might modify the way in which a stretch of their polar regions is transmitted to the secondary sensory terminals investing them, and on the few occasions when the action of this class of static gamma axon on secondary afferent stretch sensitivity has been studied, it has been seen to consist of biassing and an increase in the position sensitivity (Boyd, Sutherland & Ward 1985).

This new proposal is not widely accepted, and the work upon which it is based is at a very preliminary stage, but it does suggest an alternative route by which SCh could bias the discharge of secondary afferents, namely by binding to m_a plate endings and inducing these local changes in chain fibre stiffness. However, chain fibres have always been seen to be flaccidly paralysed by ACh or SCh in visualised spindle experiments (Gladden 1976; Boyd 1985a), and to be totally unresponsive to direct electrical stimulation or to activation via static gamma axons, suggesting that they behave like extrafusal muscle fibres and develop depolarisation block in the presence of these drugs. Furthermore, in these isolated spindle experiments secondary afferents are often silent at rest; exposure to SCh at a concentration just sufficient to paralyse chain fibres without producing bag fibre activity does not provoke any afferent discharge, but when the dose of SCh is increased and the bag_2 fibre begins to contract the afferent suddenly begins to discharge tonically (Boyd 1985a). Concrete results of this sort, coupled with extensive histological work showing the frequency of secondary terminations on the bag_2 fibre support the concept of bag_2-fibre-evoked biassing of secondary afferent discharge. Nevertheless, the possibility of m_a-plate-evoked chain fibre biassing cannot be ruled out.

There are thus at least three possible mechanisms by which SCh could induce biassing of secondary afferent discharge: indirectly via potassium ion leakage, directly via bag_2 fibre contracture, and directly via local contraction in m_a poles of

chain fibres. Of course, any combination of these three possibilities might occur in reality, and it may be variation in the extent to which each mechanism is active which underlies the different magnitudes of SCh action on individual spindle secondary afferents. This matter will be considered further when discussing the SCh activation of the type 4 afferent.

Type 2 afferents.

Type 2 afferents were studied with several regimes of SCh administration which will be considered sequentially. Taking the responses to the standard 100 μg/kg/min infusion first, it will be remembered that activation proceeded in two Phases; in Phase I, there was a gradual increase in initial discharge, dynamic response and position response, the greatest change being in the initial discharge, so that the position sensitivity decreased. In Phase II, these early changes continued at about the same rate, but the dynamic response rose very much faster, so that the dynamic index increased two- or threefold. In the maximally activated state, the afferent discharge was substantially biassed, the dynamic index was increased and the position sensitivity of the afferents was substantially reduced (Figs. 7 & 8, B). These changes are not identical to any of the patterns of SCh excitation reported for soleus afferents (Dutia 1980); two of the three alternatives can, however, be discounted, namely that shown by secondary afferents (see above) and that shown by "truly intermediate" afferents (see below) since there were no similarities with the present type 2 pattern at all. Only soleus "primary" * afferents showed the large increases in dynamic index and biassing of afferent discharge shown by biventer type 2 afferents, and although there were significant differences from the later stages of the SCh activation of soleus "primary" afferents, which will be considered further below, biventer type 2 afferents were initially diagnosed as "primary" afferents as well.

* "Primary" is in inverted commas here because, since Dutia (1980) used the term, histological understanding has moved on and it is now appreciated that there are two distinct forms of spindle primary afferent, b_1b_2c primaries and b_2c primaries (Banks, Barker & Stacey 1982). With hindsight, it is clear that Dutia used the term "primary" to refer to b_1b_2c primary afferents, and I shall make this substitution henceforth for the sake of clarity.

The differences between the SCh excitations of biventer and soleus b_1b_2c primary afferents are of both degree and time course. A fuller description of the pattern of activation of soleus b_1b_2c primary afferents and its interpretation was provided in part 3 of the literature review above (see pp. 86-88) and I shall only briefly repeat the "classical" pattern here. Activation of the majority (18 of 20) of soleus b_1b_2c primary afferents proceeded in three distinct Phases: Phase I - a gradual increase in initial discharge alone resulting in a modest reduction in position sensitivity; supposed to be due to a non-specific effect of SCh; Phase II - a very large and rapid increase in dynamic response and dynamic index, attributed to SCh-evoked contracture of the dynamic bag_1 fibre; and Phase III - a later increase in the position response and position sensitivity of the afferent, supposed to be caused by contracture of the static bag_2 fibre which had been shown to be less sensitive to SCh than the bag_1 fibre (Gladden 1976). When fully activated, soleus b_1b_2c primary afferents typically had initial discharges of around 100 Imp/sec, position responses of 200-300 Imp/sec and dynamic responses of 350-500 Imp/sec.

The more minor differences between the SCh activations of biventer and soleus b_1b_2c primary afferents are in the early time course of activation, and in the degree of changes in afferent discharge, whilst the major difference is in the absence in the biventer afferents of a Phase III of activation. Dealing first with differences in time course, in biventer b_1b_2c primary afferents, the latency to the start of Phase I of excitation was 30-70 seconds when excited by intra-arterial infusions, values which are somewhat higher than those reported for soleus b_1b_2c primary afferents (10-60 seconds; Dutia 1978); similarly, the latency to Phase II of activation for biventer b_1b_2c primaries was in the range 100-140 seconds, which is to be compared with a soleus value of about 20-80 seconds. It is clear that in biventer, excitation takes longer to develop, and, once started, progresses more slowly. However, the changes in discharge occurring in biventer and soleus b_1b_2c primary afferents during Phases I & II of activation were very similar, occurred in the same sequence, and were interpreted in

the same way. The only minor difference was that, during Phase I, biventer b_1b_2c primaries routinely underwent simultaneous increases in initial discharge, position response and dynamic response from the start (e.g. Figs. 7 & 8, C), whereas the majority of soleus b_1b_2c primaries initially showed isolated increases in initial discharge which were later joined by lesser increases in dynamic and position responses.

The fairly trivial nature of these differences, which are mainly of time course, suggests that there may be a simple physical explanation for them, such as differences in circulatory delays and/or regional blood flow between the two experiments. One possible source of such differences might be a degree of circulatory compromise of the biventer muscle produced during the extensive dissection of the neck tissues required to prepare it, since its blood supply is rather precarious, arriving as it does in slender blood vessels running with the segmental nerves. In answer it must be said that this possibility was appreciated, and a great deal of time was spent during the dissection in avoiding injury to the blood supply, as a result of which complete denervation of the neighbouring complexus muscle was sometimes not achieved (see Methods, p. 97). Furthermore, in none of the experiments did the muscle appear to be hypoxic, as might have been indicated by a bluish discolouration, impaired contractility in response to electrical and chemical (SCh) stimulation or spontaneous fasciculation; nor were afferents isolated which initially behaved well and subsequently silenced, suggesting metabolic starvation. A more likely explanation of the slower progress of biventer activations has to do with the route of infusion. SCh infusions were made into a common carotid artery near the aortic arch, from where the infused drug was directed not only into the opposite carotid artery (and hence rather circuitously to the biventer muscle under study) but also into the opposite forelimb and into the descending thoracic aorta and hence to the remainder of the body. Much of an infusion was thus initially misdirected, only reaching the target after recirculation and partial catabolism. In the case of the soleus experiments, a much more direct course to

the muscle applied.

Differences between soleus and biventer b_1b_2c primary afferents in the extent of changes in afferent discharge were minimal during the early parts of a SCh activation, but increased as the infusion progressed and were marked when afferents had been maximally activated. Thus, the discharge of biventer b_1b_2c primary afferents, though typically biassed to around 100 Imp/sec which is similar to the soleus value, did not achieve nearly the same peak frequencies. Typically, the dynamic response of a biventer b_1b_2c primary afferent at maximal excitation was on the order of 160-200 Imp/sec, and even the most powerfully excited afferent only achieved a peak frequency of 230 Imp/sec, or approximately half the rate attained by some soleus b_1b_2c primary afferents; similarly, the maximum position response of biventer b_1b_2c primary afferents was typically about 100-120 Imp/sec or about half the value achieved by soleus b_1b_2c primary afferents. Most importantly, however, there was no sign in any of the 34 afferents studied during the present experiments of progress beyond Phase II to Phase-III-like behaviour with an increase in position response of the afferent. Several possible explanations for this spring immediately to mind, of which two are readily testable: (i) that the dose of SCh was inadequate in the biventer experiments to fully activate b_1b_2c primary afferents; the static bag_2 fibre, which when active is supposed to produce the changes of Phase III, may be even less sensitive to SCh than in hindlimb muscle spindles; and (ii) that the dose of SCh was adequate, but was infused for an inadequate length of time. Alternatively, one would have to assume that biventer b_1b_2c primary afferents are inherently different from their soleus counterparts. It was in an attempt to choose between these alternatives that the later experiments of this series described on pp. 130-138 were performed in which alternative regimes of SCh administration were employed.

Although the standard dose of SCh used in the present experiments (100 μg/kg/min) and the duration of the infusions were both comparable to those successfully used by Dutia during his experiments on soleus spindle afferents, the

possibility that they were inadequate still had to be entertained. Seven experiments were therefore performed in which higher doses of SCh (200-500 μg/kg/min) were infused for longer periods than standard (up to 7 minutes compared to 4-5 minutes), the afferents which were exposed to this regime having been specifically selected on the basis of their passive properties (see p. 144) as likely to be classified as b_1b_2c primaries. Even in response to these higher dose infusions, however, none of 12 b_1b_2c primary afferents showed any further changes in stretch sensitivity beyond those evoked by a 100 μg/kg/min infusion, and none showed any sign of progression to Phase-III-like behaviour as described for soleus b_1b_2c primary afferents. Instead, the main effect of the higher doses was to produce slightly faster progress through phases I and II of excitation. The only other effect on b_1b_2c primary afferents was, on two occasions when the afferents were particularly powerfully activated, to so excessively drive the afferent discharge during periods of muscle stretching as to cause initially partial and later complete silencing (Fig. 13) in a manner suggestive of the onset of depolarisation block (Kidd & Vaillant 1974); in the example shown in Fig. 13, note that although initially it is only the dynamic response which is blocked, there is still not even a transient increase in position response suggestive of Phase-III-like behaviour.

The finding of depolarisation block suggests that there may be an inherent upper limit to the discharge rate of biventer b_1b_2c primary afferents which is in the vicinity of 250 Imp/sec. This could, in fact, have been predicted *a priori* as follows. The peak discharge rate which an afferent can achieve is mainly dependent upon the duration of its absolute refractory period, and this is known to bear an inverse relationship to the diameter of the axon (Erlanger & Gasser 1937; Paintal 1965). Neck muscle afferent axons are known to be generally of smaller diameter than hindlimb muscle afferents; thus, Banks, Barker & Stacey (1982) report the range of b_1b_2c primary afferent diameters averaged for several hindlimb muscles as 3.4-12.8 μm, while the range for biventer b_1b_2c primary afferents is reported as about 3.5-7.5 μm (Richmond, Bakker, Bakker & Stacey 1986). Given that those afferents which achieve

the highest discharge rates are the largest, then for the hindlimb a 12.8 μm afferent equates to a discharge rate of around 500 Imp/sec; clearly, the maximum attainable discharge frequency must be less for biventer b_1b_2c primary afferents, and using a scaling factor derived from Paintal (1965), the predicted value is about 200 Imp/sec which is not dissimilar to the value observed in the present experiments. However, even though biventer b_1b_2c primary afferents can thus be expected to have a more restricted range of discharge frequencies with which to indicate their excitability than, for example, soleus b_1b_2c primary afferents, there is no reason not to expect their pattern of SCh activation to be simply a scaled-down version of that of soleus b_1b_2c primary afferents, including Phase-III-like behaviour.

One possibility remained to be excluded, namely that in biventer muscle spindles the static bag_2 fibre was so insensitive to the effects of SCh that before it had even been recruited by exposure to a high dose SCh infusion, depolarisation block of the afferent had already set in due to overdriving by the dynamic bag_1 fibre. An attempt was therefore made in the final four experiments to find some evidence that both bag_1 and bag_2 fibres were in fact being activated by SCh infusions by exposing afferents to gradually increasing doses of SCh from sub-threshold values up to doses of 200-300 μg/kg/min. In most cases this protocol was no more revealing than the standard regimen, but on three occasions, as illustrated in Fig. 15, some unusual responses to SCh infusion were seen. These consisted of "events" during which there were changes in position sensitivity and dynamic index which were transient, apparently independent of each other, of very sudden onset and of equally sudden termination. The earliest events, which were seen when the SCh dose was in the range 30-50 μg/kg/min, consisted of a substantial biassing of the afferent discharge, an increase in discharge variability and a reduction in position sensitivity, all of which produced a pattern of response to each ramp stretch which was quite unlike the response of these afferents at any other time before, during or after exposure to higher doses of SCh; nor did this unusual response pattern have any hindlimb analogue in the

experiments of Dutia (1980). The rapidity of onset and termination of these "events", coupled with the magnitude of the biassing produced suggested that they were generated by transient contraction of one of the intrafusal fibres, presumably as the concentration of SCh in the vicinity of the spindle fluctuated around its threshold for contraction.

Later in the infusion, as the dose of SCh at the spindle continued to increase, "events" took on a mixed form. For the first few stretches of a mixed "event", the afferent responded very much as it had done during the simpler "events" just described, though changes were even larger, but in subsequent stretches of the same event the afferent suddenly developed a large dynamic response and dynamic index, so that its overall response to ramp stretches was now very reminiscent of that seen for b_1b_2c primary afferents during 100 μg/kg/min infusions (compare Fig. 15, C with Figs. 7 & 8, B). Even later in these mixed events, the biassing action suddenly waned, whereas the increased dynamic index survived for a few stretches more. These mixed events are interpreted as ones in which the concentration of SCh was near the threshold value of two types of intrafusal fibre. Undoubtedly, one of these was the dynamic bag_1 fibre which, when active, contributed the increase in dynamic index as it has been shown in visualised spindle experiments to do (e.g. Boyd 1981b, 1985a, 1986). This leaves either chain fibres or the bag_2 fibre as the source of the biassing action in these events; although chain fibres are known to be paralysed by SCh (Gladden 1976), it is conceivable that when the SCh dose is near the value required to paralyse them, they might fasciculate, as do extrafusal muscle fibres, and thus affect afferent sensitivity to stretch.

Against this suggestion, and favouring the bag_2 fibre as the source of the biassing action, are the following points: (i) the degree of biassing during an event increases steadily for periods of up to 30 seconds, whilst over the same period some fasciculating chain fibres would be expected to become paralysed so that their

presumed biassing action would be steadily removed rather than added to; (ii) the size of the biassing events increased with each subsequent event; again the paralysis of more and more chain fibres as the infusion proceeds would not be expected to yield this result; (iii) the form taken by the mixed events suggests that the bag_1 fibre makes little if any contribution to the degree of biassing of the afferent discharge, instead contributing the increase in the dynamic index; if this is also the case when the afferent is exposed to a 100 μg/kg/min SCh infusion, then the fibre which does contribute the biassing action is not paralysed during 5 minutes or more of infusion, since the biassing of the discharge rate does not wane until the infusion is switched off; (iv) the pattern of response seen during the simple events, i.e. a biassed, variable discharge and a dramatically reduced position sensitivity, is very similar to the response of tenuissimus b_1b_2c primary afferents to ramp stretching when it had been visually confirmed that only the bag_2 fibre was contracting (e.g. Boyd 1981b, 1985a, 1986).

Taking all these points into account, it seems most likely that the simple events are to be interpreted as the result of transient contractions of the static bag_2 fibre, whilst the mixed events are the result of first bag_2 fibre and later superadded dynamic bag_1 fibre action. The conclusion is, therefore, not only that the standard 100 μg/kg/min SCh infusion rate, and any greater dose, is ample to cause both the bag_1 and the bag_2 fibres to go into contracture since these events occurred at SCh doses of 50-60 μg/kg/min, but also that in biventer spindles, in contrast to hindlimb spindles, the bag_2 fibre may actually be more rather than less sensitive to SCh than the bag_1 fibre. The differences between the SCh activations of biventer and soleus b_1b_2c primary afferents cannot therefore be ascribed to non-recruitment of certain intrafusal fibres. Instead it would appear that there is a fundamental difference between b_1b_2c primary afferents from the two muscles, either in the mechanical consequences of bag_2 fibre contraction, or in the way contributions to afferent excitability from active bag_1 and bag_2 fibres combine to produce the final impulse train.

In the late 1970s, when Dutia carried out his experiments with SCh, the

effects of bag fibre action on spindle afferent discharge had not been fully elucidated; under the prevailing models, it was entirely reasonable to postulate, as he did, that contraction of the bag_2 fibre generated the increase in position sensitivity which was seen to occur in Phase III of activation of most soleus b_1b_2c primary afferents. With the passage of time, however, Boyd's group and others (Literature Review, Part 1, p. 49 et seq.) have provided very detailed descriptions of the action on afferent discharge of activity in the several intrafusal fibres, from which it has become clear that contraction of the bag_2 fibre results in either no change, or a reduction in the position sensitivity of b_1b_2c primary afferents, rather than the large increase seen during Phase III in soleus. The findings from the present experiments appear to fit very well with this later observation as has just been discussed, leading one to favour the view that the presence of a Phase III of SCh activation in soleus, but not in biventer, b_1b_2c primary afferents is due to some inherent difference in the way the excitant inputs from jointly active bag_1 and bag_2 fibres are combined to produce the final impulse train. One of the aims of the second series of experiments (see Chapter 2) was to attempt to confirm Dutia's findings with regard to b_1b_2c primary afferents in order to cast more light on this issue. Discussion of this point will therefore be deffered until these later experiments have been reported (Chapter 2, Discussion, pp. 228 et seq.).

Moving on to consider the passive properties of biventer b_1b_2c primary afferents, the main point of interest is whether these properties of the afferents are comparable to the reported properties of their extensively studied hindlimb analogues. As was mentioned in the Literature Review part 2 (pp. 74-75), the anatomical studies of Richmond's group (e.g. Richmond, Bakker, Bakker & Stacey 1986) provide no structural evidence to suspect that there would be any functional differences, so that the finding of passive properties comparable to those of hindlimb b_1b_2c primary afferent would lend support to the SCh-based identification, as was the case for SCh-identified biventer secondary afferents. One proviso must be mentioned, namely that in the hindlimb experiments there was no possibility of separating b_1b_2c and b_2c primary

afferents which were identified as "primary" purely on the basis of their conduction velocity (see e.g. Matthews 1972), so that the passive properties of hindlimb "primary" afferents are those of both subtypes; fortunately, b_2c primary afferents are generally rather rare in the hindlimb muscles which were used to study spindle afferent characteristics (usually soleus or tenuissimus), so that the extent of "contamination" of b_1b_2c primary properties by those of b_2c primaries is unlikely to be unduly large. A comparison of the properties of biventer b_1b_2c primary and hindlimb "primary" afferents would therefore seem justifiable.

Starting with coefficients of variation, the values for biventer b_1b_2c primary afferents occupied the range 0.028-0.12 which is almost identical to that reported for hindlimb primary afferents under comparable conditions of resting afferent discharge (Matthews & Stein 1969b). As was also the case in the hindlimb experiments, the coefficients of variation of b_1b_2c primary afferents were generally larger than those of secondary afferents,but in the present experiments the degree of overlap between the ranges for secondary and b_1b_2c primary afferents was rather greater than that seen in the hindlimb experiments. The likely explanation for this observation is that Matthews & Stein (1969b) actively excluded afferents with intermediate conduction velocities from their analysis; if there were a positive relationship between axonal conduction velocity and coefficient of variation, as appears to hold for dynamic indices in response to ramp stretches (Matthews 1963), then the removal of those with intermediate conduction velocity would be very likely to restrict the degree of overlap between the coefficients of variation of primary and secondary afferents.

Considering next the sinusoidal sensitivities of b_1b_2c primary afferents and beginning with general features, the sinusoidal sensitivities of biventer b_1b_2c primary afferents are comparable to those of soleus primary afferents in that they are significantly greater than those of secondary afferents (Figs. 17 & 18; Table I), and in

that the afferent discharge was often silenced during cycles of muscle shortening, a feature which secondary afferents never displayed in the present experiments and which was also unusual in experiments on soleus secondary afferents (Matthews & Stein 1969a; Cussons, Hulliger & Matthews 1977; Hulliger, Matthews & Noth 1977a). With regard to a comparison of the ranges of sinusoidal sensitivity of biventer and soleus secondary afferents, there is a difficulty in that Hulliger, Matthews & Noth (1977a) displayed their findings graphically as a mean response +/- a standard deviation; however, given that 95% of a population will lie within 2 standard deviations of the population mean (Swinscow 1982), analysis of their Fig. 5 (Hulliger, Matthews & Noth 1977a) yields a range of about 29-58 Imp/sec/mm. The overall range of values in the present experiments was rather wider than this (5-84 Imp/sec/mm), and a number of possible contributory factors must be considered.

Most important is the fact that for 35% of the afferents in the present experiments, the sensitivity value had to be estimated from the response to a stretch amplitude of other than 1 mm, and it was recognised that this could be introducing errors. Examination of Fig. 18 reveals, however, that the wideness of the observed range is largely due to the presence of two outlying units with sensitivities respectively of 5 and 84 Imp/sec/mm; the vast majority of afferents had sensitivities in the narrower range of 18-53 Imp/sec/mm which is much more in agreement with the estimated hindlimb range. Furthermore, examination of the properties of a small subset of the population of afferents in the present experiments all of which were exposed to stretch amplitudes of exactly 1 mm amongst others, reveals (Fig. 17) a mean and standard deviation of the 1 mm response of 31 +/- 4 Imp/sec/mm (95% of population in range 23-39 Imp/sec/mm); the similarity of this range to the overall range for the whole population of b_1b_2c primary afferents suggests that the errors introduced by the estimation methods are unlikely to be very substantial, leading to the conclusion that the ranges of sinusoidal sensitivity of biventer b_1b_2c primary and soleus primary afferents are very similar. The properties of the outlying units may then be due to

errors in estimation, but may equally be real values; it should be pointed out that the soleus data (Hulliger, Matthews & Noth 1977a) was based on only 12 afferents, whereas the present experiments report the properties of nearly three times this number of afferents.

The final passive property routinely studied was the vibration sensitivity of the afferents which was assessed by determining the maximum frequency to which the afferent discharge could be driven 1:1 by longitudinal vibration of the muscle. The criterion by which failure of driving was assessed was the appearance in a display of instantaneous afferent discharge frequency of intervals at any sub-multiple of the stretching frequency. This criterion was rigidly applied, but it should be noted that this does not mean that the afferent was not driven by higher frequencies of vibration than those reported here. On the contrary, a vibration frequency of 300 Hz might drive an afferent 1:2, producing an afferent discharge rate of 150 Imp/sec, whilst the recorded vibration sensitivity of this same afferent might be only 110 Imp/sec since at this frequency exact 1:1 driving failed.

The range of vibration sensitivities obtained in this way for biventer b_1b_2c primary afferents (42-220 Hz, with most in the range 80-120 Hz) seems initially to be skewed towards the low end given that hindlimb primary afferents are said to be driven by vibration frequencies of up to 500 Hz (e.g. Matthews 1972); however, re-examination of the data on which this statement is based (Brown, Engberg & Matthews 1967a) reveals that these authors used a rather looser definition of driving than the one applied in the present experiments, since from their published figures (e.g. Brown, Engberg & Matthews 1967a, Figs. 12, 13) it would appear that they included 1:2 driving. It is, however, possible to derive an analogous measure to that used here from their figures; thus, in response to a stretch of 25 μm amplitude, the average response of primary afferents crossed the 1:1 driving line (i.e. failed to drive exactly 1:1) at a vibration frequency of just over 100 Hz, and in response to a 10 μm stretch at about 90 Hz (Brown, Engberg & Matthews 1967a, Fig. 12). A presumably separate set of

afferents, in response to 10 μm stretch amplitude, failed to drive 1:1 at a vibration frequency of 50 Hz (Brown, Engberg & Matthews 1967a, Fig. 13). Since there was no information as to the standard deviation of these mean responses, the true range of frequencies at which 1:1 driving failed in soleus primary afferents is unknown, but the mean values themselves cover the range 50-110 Hz, and the true range will by definition be rather greater. This range is very similar to the range of vibration sensitivities observed in the present experiments for biventer b_1b_2c primary afferents in response to comparable stretch amplitudes, and is at higher values than are seen for secondary afferents from soleus (Brown, Engberg & Matthews 1967a) and biventer (Fig. 18) muscles. As was the case for the other passive properties, this observation therefore supports the identification by SCh of type 2 afferents as b_1b_2c primaries.

The type 4 afferent.

Only one afferent was classified as type 4, but this is considered before the more numerous type 3 afferents because its pattern of SCh activation had a hindlimb analogue in the "truly intermediate" afferents of Dutia (1980). This afferent differed from all the others encountered in the present experiments in that it underwent a marked increase in position sensitivity which developed gradually over the course of the SCh infusion and was accompanied by a substantial biassing of the afferent discharge. The degree of biassing (64 Imp/sec), and its relatively steep rate of rise are suggestive of a substantial direct input to the biassing via intrafusal fibre contraction, rather than a non-specific effect such as potassium ion release; the fact that there was no increase in dynamic index militates against a contribution from the bag_1 fibre, which leaves the bag_2 fibre and chain fibres as the source of the biassing action. Classically, (Gladden 1976), chain fibres are held to be paralysed by exposure to SCh which would seem to rule them out as contributing the biassing action to the type 4 afferent. However, in discussing the SCh activation of secondary afferents, it has already been argued that the recent correlated ultrastructural and electrophysiological

studies of Boyd's group (Arbuthnott, Ballard, Boyd, Gladden & Sutherland 1982; Boyd, Sutherland & Ward 1985) raise the possibility that m_a plate endings on chain fibres might provide an alternative route for SCh to directly affect afferent sensitivity. Not only may such a mechanism produce biassing of secondary afferent discharge, but it may also produce a marked increase in their position sensitivity (Boyd, Sutherland & Ward 1985). Chain fibre action of this sort could therefore be responsible for the increased position sensitivity of the type 4 afferent in these experiments, and there would be no need to assume bag_2 fibre contact.

Alternatively, this afferent may have had substantial terminal contact upon the bag_2 fibre to which the increase in position sensitivity is to be attributed. Two morphologies of this type can be envisaged, namely b_2c primary afferents and secondary afferents with significant bag_2 fibre contact, both in terms of contact area and effect of this contact on afferent mechanosensitivity. However, as was described above in detail (see pp. 52-53), the action of the bag_2 fibre on primary afferent position sensitivity is generally to reduce it, so the large increase in this parameter displayed by this type 4 afferent suggests that it cannot have had a primary termination within its spindle of origin. Instead, this pattern of SCh activation is interpreted as that of a secondary afferent. It is not possible to decide whether the effects were produced by a substantial bag_2 fibre action or by the postulated m_a-plate-induced chain fibre action, though the latter possibility is very much more speculative than the former, being based on the preliminary results of only one group of experimenters. Numerically, also, there is no further clue as to the correct alternative, since 88.6% of hindlimb secondary afferents have bag_2 fibre contact (Banks, Barker & Stacey 1982), and the proportion of spindles in which at least one pole of the chain fibres bears m_a plate endings would appear to be of a similar order (Sutherland, Arbuthnott, Boyd & Gladden 1985).

That bag fibre action can certainly influence secondary afferents in vivo is shown by the fact that Dutia (1978) also described a "truly intermediate" afferent which showed evidence of bag_1 fibre action on its sensitivity. This afferent behaved

basically like the type 4 afferent here, except that it underwent a doubling of its small dynamic index as well an increase in position sensitivity; that it was not a primary afferent was attested to by a combination of the smallness of the changes, the absence of a marked increase in discharge variability and the overall shape of the response to a ramp stretch which was quite unlike that of primary afferents (Dutia 1978). In the present experiments no such afferents were identified, though this may simply be the result of differences in placement of the threshold above which a significant increase in dynamic index was considered to have occurred. Consider, for example, Fig. 6 above. I have classified this afferent, and 2 others which behaved similarly, as a secondary, even though there was an increase in its dynamic index of about 30%, since I was not confident that such a small change in dynamic index, which amounted to only 3 Imp/sec, could not have been purely artefactual. Should this afferent perhaps have been identified as "truly intermediate"?

When Dutia (1980) coined the term "truly intermediate" afferent, he was using it to describe the properties of what was then felt to be a rather rare form of spindle afferent. Since it was supposed to be a rare form of ending, perhaps it had special properties which might be used by the central nervous system to special effect, and so it was of interest to distinguish its afferent from "normal" primary and secondary afferents. I would argue that this concept has become redundant, now that it has been appreciated that the majority of secondary afferents in fact have bag fibre contact of some sort (Banks, Barker & Stacey 1982). It has already been argued (Literature Review, Part 2, p. 78 et seq.) that this finding suggests that the functional characteristics of secondary afferents will form a continuum, afferents with no bag fibre input occupying one extreme and those with substantial input from both types of bag fibre the other, rather than two distinct populations - secondary and "truly intermediate" - as identified by Dutia (1980). Instead, the finding of "truly intermediate" behaviour should be viewed the other way around, as: "Why is the "truly intermediate" pattern of SCh activation so uncommon, when the anatomical substrates

for it, whether bag fibre contact or the bearing by chain fibres of m_a plate endings, are apparently so common?".

From the point of view of bag fibre action on secondary afferent mechanosensitivity, the answer to this question would seem to lie in a high degree of variability of bag fibre action (Literature Review part 1, p. 53). Thus, in many instances, bag_2 fibre contraction provokes a degree of biassing of secondary afferent discharge, and this has already been discussed in the context of the genesis of SCh-evoked biassing of afferent discharge (see p. 161). However, the changes in the position sensitivity of secondary afferents provoked by bag_2 fibre action range from reductions, through no change to increases. Similarly, bag_1 fibre action on secondary afferent discharge ranges from nothing to a substantial increase in dynamic index in about 10% of cases (Boyd, Sutherland & Ward 1985). If the dynamic and/or position sensitivity (or both) of an afferent were either unchanged or slightly reduced by the action of SCh, then this afferent would be diagnosed as deriving from a secondary ending, by which Dutia (1980) meant a juxta-equatorial ending contacting only chain fibres; it will be appreciated, however, that many secondary afferents which have bag fibre contact would behave in the same way. This variability in bag fibre action on secondary afferents might be the result of any number of variables such as the relative contact area on bag and chain fibres of sensory terminals, or the exact point on the bag fibre at which the chain fibre collaterals reach it (S1 or S5), but its effect is to make it impossible to say that an afferent which did not show an increase in static or dynamic sensitivity did **not** have bag fibre contact. The position is made even more confused if one allows that chain fibre activity induced by m_a plate endings may significantly affect afferent mechanosensitivity too.

These various considerations would seem to so undermine the term "truly intermediate" as to make it meaningless. I have therefore abandoned it, and propose to call the biventer type 4 afferent a secondary afferent, possibly with bag_2

fibre contact. Of course, if in the future it were to transpire that SCh does not activate m_a plate endings on chain fibres, and that increases in static and dynamic sensitivity of secondary afferents are produced solely by bag fibre action, then it might be useful to be able to differentiate secondary afferents which have bag fibre contact. This could still be done with SCh, as shown by Dutia (1980) and in the present experiments, but I am not sure that it is worth confusing the terminology by introducing another name for afferents with this pattern of SCh excitation in the meantime.

Turning briefly to the passive properties of the type 4 afferent, suffice it to say that comparison of these with the properties of undifferentiated secondary afferents (Fig. 18, open and filled squares) showed them to be compatible with its identification as a secondary afferent also. Its properties did tend to lie at the top end of the secondary range in each case, which suggests that it was one of the larger secondary afferents (Matthews 1963), and this would be compatible with the idea that it had substantial bag_2 fibre contact (Banks, Barker & Stacey 1982).

Type 3 afferents.

Coming at last to type 3 afferents, these were the only biventer afferents whose pattern of SCh activation did not have a hindlimb analogue. Their SCh activation consisted of a large and rapid biassing of the afferent discharge, accompanied by increased variability and a marked reduction in position sensitivity, so that when fully activated the afferents were rather insensitive to muscle stretching. The size and rate of biassing favour a direct genesis via intrafusal fibre contraction as has been discussed above for b_1b_2c primary afferents (e.g. p. 165 et seq.), as do the "events" occurring during the response of type 2 afferents to stepped-dose SCh infusions (see p. 169 et seq.). Characteristically, type 3 afferents showed a reduction in dynamic index after exposure to SCh, suggesting that the active intrafusal fibre could not be the bag_1 fibre, which anyway tends to have only moderate biassing action (Boyd 1981b); this leaves the bag_2 fibre or the chain fibres as potential sources of this biassing action.

The possibility that m_a-plate-evoked chain fibre action might affect afferent sensitivity has been considered in depth above. The effects on secondary afferents described by Boyd's group are, of course, totally incompatible with the pattern of activation of type 3 afferents, since in the former position sensitivity increases substantially, whilst in the latter it is dramatically reduced. Boyd's group does not, however, seem to have reported the action of m_a-plate-evoked chain fibre activity on the behaviour of primary afferents yet, so the possibility that this mechanism might effect the reduction in position sensitivity seen for type 3 afferents cannot be ruled out. There is, however, no need to resort to such esoteric possibilities, for it is well known from the work of Boyd's group that bag_2 fibre action on primary afferents produces exactly those changes in sensitivity to muscle stretch which are seen for type 3 afferents exposed to SCh (see e.g. Boyd 1981b, 1985a, 1986; Boyd & Smith 1984). Furthermore, during an isolated spindle experiment using the tenuissimus muscle, Gladden and others recently recorded by chance from a primary afferent which was later shown histologically to be a b_2c primary afferent. They were able to record from this afferent whilst stimulating a static gamma axon which had been seen to produce bag_2 fibre activity in a different spindle capsule, and the pattern of response to ramp stretching of this afferent which they saw during stimulation of this gamma axon was indistinguishable from the response to stretch of biventer type 3 afferents when maximally activated by SCh (Gladden's observations on the b_2c primary afferent were reported at a meeting of the Physiological Society in Glasgow in 1987 but have not been published; other findings from those experiments were reported in Boyd & Sutherland 1987).

I have already argued, from the behaviour of b_1b_2c primary afferents in the present experiments, that the standard dose of SCh (100 μg/kg/min) used was more than adequate to activate both the bag_1 and the bag_2 fibres (pp. 168et seq.). The absence in the high-dose SCh experiments of any further changes in response to stretch of type 3 afferents (Fig. 14) indicates that the apparent pureness of the bag_2 fibre input

to type 3 afferent discharge at standard doses of SCh was not an artefact caused by failure of the bag_1 fibre to contract in some spindles, so that b_1b_2c primary afferents were misidentified. Thus, the most reasonable interpretation of the behaviour of type 3 afferents is that it is that provoked in b_2c primary afferents by exposure to SCh. Finally, this interpretation would also fit with the results of the experiments using ramped-dose SCh infusions which produced activations, the early parts of which were punctuated by "events" which have already been discussed (p. 167 et seq.). In the case of the 2 type 3 afferents which were exposed to this regime of SCh administration, the events which occurred were of only the simple type, resembling scaled-down versions of the ultimate response to stretch after the full dose of SCh was applied (Fig. 16, B), and presumably reflecting transient contractions of the bag_2 fibre when the SCh dose near the spindle was around its threshold. In these afferents, there was never any sign of a bag_1 fibre input which would have been revealed in mixed events as shown by b_1b_2c primary afferents (Fig. 15, C).

Other evidence which can be adduced to support the identification of type 3 afferents as b_2c primary afferents includes the following. Firstly, sheer numbers; 33 afferents, or over a third of the total sample, were placed into type 3, so whatever morphology underlies them must be common. Tandem spindles, and therefore b_2c primary afferents, are common in neck muscles, and in fact b_2c primary afferents have been estimated to make up about a third of all primary afferents from them (Richmond & Abrahams 1975b). The actual frequency of presumed b_2c primary afferents in the present experiments is even higher than this figure, approaching a half, which may reflect a sampling bias in favour of medium-sized afferents brought about by the technical difficulty of splitting peripheral nerves rich in connective tissue. Allowing for this, the observed frequency of presumed b_2c primary afferents is not incommensurate with that expected on histological grounds.

Further evidence that b_2c primary afferents have been correctly

identified by SCh comes from examination of their passive properties. Examination of Fig. 18 shows that each of the passive properties of type 3 afferents covers a very similar range of values to that of b_1b_2c primaries, so that the two populations are indistinguishable other than on the basis of their patterns of SCh activation. This suggests that type 3 afferents are indeed primaries, in fact b_2c primaries, and the finding that their passive properties are indistinguishable from those of b_1b_2c primary afferents is entirely in agreement with that of Banks, Ellaway & Scott (1980) that passive properties of peroneus brevis and tertius muscle spindle afferents cannot be used to differentiate two classes of primary afferent corresponding to b_1b_2c and b_2c primary afferents respectively.

In conclusion, it would appear that SCh can be readily used, as was hoped at the start of these experiments, to identify b_2c primary afferents. The remaining parts of this thesis are mainly concerned with providing corroborating evidence for this conclusion firstly on the basis of further studies of passive properties of spindle afferents, especially conduction velocity (Chapter 2), and then on the basis of a correlated electrophysiological and histological study (Chapter 3).

CHAPTER TWO

Experiments performed on the medial gastrocnemius muscle of the hindlimb.

2:1 EXPERIMENTAL RATIONALE.

It seemed desirable to repeat the biventer study in the hindlimb for a number of reasons. Firstly, as was described above (p. 165 et seq.), the details of the SCh activation of b_1b_2c primary afferents from biventer were significantly different from those reported for b_1b_2c primary afferents from soleus (Dutia 1980). Secondly, the fact that biventer and soleus b_1b_2c primary afferents were differently affected by SCh infusion raised the possibility that the b_2c primary afferents from hindlimb spindles might also be differently activated than their counterparts from neck muscle spindles. One aim of this second series of experiments was, therefore, to examine the SCh activations of hindlimb primary afferents of both b_1b_2c and b_2c type. A second aim was to measure the conduction velocities of identified b_2c primary afferents which, for technical reasons elaborated upon above (p. 104), was not possible during the experiments performed on biventer spindles. The anticipated value of conduction velocity measurements was not that they would provide a definitive means of identification of b_2c primary afferents, since the diameters of b_2c primary afferent axons measured at the spindle are known to overlap extensively with those of the smallest b_1b_2c primary afferents and those of the largest (S1) secondary afferents in both neck and hindlimb muscles (Banks, Barker & Stacey 1982; Richmond, Bakker, Bakker & Stacey 1986). Rather, it was felt that the **range** of conduction velocities measured for afferents identified as b_2c primaries by their pattern of SCh activation might provide support for this identification.

Finally, these experiments on hindlimb spindle afferents were undertaken since they would allow one to compare the classification of spindle afferents on the basis of their pattern of SCh activation with that obtained for the same population of afferents on the basis of their conduction velocities. The impact of the uncertainties in the conduction-velocity-based system which have long been appreciated (Literature Review, Part 2, p. 75 et seq.) could then be assessed. The medial gastrocnemius muscle was selected for this study since, at the time it was

performed, this muscle was thought to contain the highest proportion of tandem muscle spindles in the hindlimb (Richmond, Stacey, Bakker, Bakker 1985); more recently, it has been realised that the peroneus brevis muscle would have been a better choice (Scott & Young 1987).

2:2.1 SURGICAL PROCEDURES.

Many of the methods described above for the biventer experiments were also used in this second series of experiments using the medial gastrocnemius muscle of the cat hindlimb. In this section, I therefore propose only to describe methods specific to the gastrocnemius experiments.

A total of 10 animals weighing 1.8 to 3.4 kg was used in this series of experiments. Animals 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 also placed in the trachea and in the left femoral artery. The femoral arterial cannula was advanced centrally until its tip was judged to lie in the vicinity of the aortic bifurcation since it was to be used to infuse SCh into the right hand external iliac artery and thence the right hand femoral artery in order to activate spindles in the right hand medial gastrocnemius muscle.

LUMBOSACRAL LAMINECTOMY.

An incision was made in the midline of the back from the level of the third lumbar to the second sacral spine and opened out laterally. The lumbodorsal fascia thus exposed was slit open over the length of the incision in the plane between the medial portions of the longissimus dorsi muscle and the multifudus muscle on each side and the longissimi dorsi were pulled laterally. The multifudi were then rapidly cut away from the laminae of the lumbar vertebrae; in the absence of cautery equipment, the considerable bleeding which this procedure caused was

staunched by placing dental packing swabs which had been soaked in hot saline onto the cut muscles. After a few minutes, the remaining shreds of tissue could be scraped off the vertebral laminae to expose them fully. Starting with the L4 vertebra and working caudally, the vertebral laminae were removed by repeated application of the following technique. The spinous process caudal to the lamina to be removed was grasped with a towel clip and lifted to accentuate the point of articulation between the two vertebrae; using a large pair of bone rongeurs, the mamillary, articular and spinous processes of the upper vertebra were removed. The tips of a finer pair of rongeurs were introduced under the caudal articular facets and small chips of bone were repeatedly removed working forwards and sideways until the whole lamina had been removed. Sharp protrusions of bone were removed from the lateral walls of the vertebrae, and any small bleeding points were clamped or otherwise compressed until the bleeding ceased. Generally, the dura mater investing the cord and cauda equina was exposed from about L4-S1 in this way. Finally, a wire frame was stitched to the margins of the skin incision to fashion a pool; initially, this was packed with tissues soaked in warm saline whilst the remainder of the surgery was performed. Subsequently the pool was filled with warm paraffin oil maintained at 32-35 degrees centigrade by radiant heat from a lamp.

PREPARATION OF THE RIGHT HINDLIMB.

Preparation of the right hindlimb involved the division of all its nerves bar that to the medial gastrocnemius muscle in order to reduce as far as possible the neural traffic in the dorsal roots. This started with the exposure, on the anterior aspect of the thigh, of the femoral neurovascular bundle. The femoral nerve was sectioned at its point of exit from the abdominal cavity, and, in the medial part of the same incision, the obturator nerve innervating the adductors of the thigh was found and divided. This incision was sutured shut, the animal was turned to lie on its belly, and a small incision was made over the upper part of the thigh extending

a few centimetres towards the knee from the sciatic notch of the pelvis. By partially reflecting the biceps femoris muscle, the sciatic nerve was exposed and followed towards the knee. The large bundle of branches supplied to the hamstring muscles was divided, as was the branch to the tenuissimus muscle and any other small branches encountered. The nerve was then traced proximally as far as possible and as many as possible of the branches to the tail and pelvis were cut or, where the presence of blood vessels precluded this, crushed. This incision was also sutured shut.

A final incision was then made over the calf, running approximately in the midline from somewhat above the knee to below the ankle. This was opened out by blunt dissection to reveal the two heads of the gastrocnemius muscle and the vascular popliteal fat pad. This fat pad was carefully removed by progressively lifting it, tying off any blood vessels thus revealed with pairs of sutures and cutting the blood vessels between the sutures. Its removal exposed the lower part of the course of the sciatic nerve, its branches and its division into the tibial and common peroneal nerves. The sural nerve, common peroneal nerve and any other small branches were all divided and the medial edge of the proximal pole of the lateral gastrocnemius muscle was drawn laterally to reveal the sheath of branches of the tibial nerve which run to innervate a number of the muscles of the calf and foot. The branches running to the foot were divided as were those to the lateral gastrocnemius, plantaris and soleus muscles. By grasping the severed central ends of these nerve branches and drawing them craniad, it was possible to dissect them back towards the main sciatic nerve and remove the tissue entirely, leaving just the branches to medial gastrocnemius attached to the sciatic nerve; it was thus ensured that a bipolar stimulating electrode subsequently applied to these branches would only activate axons innervating medial gastrocnemius.

The last step in preparing the hindlimb was to separate the medial gastrocnemius tendon from those of the lateral gastrocnemius and soleus muscles and

to sew a length of stout thread (EPA 2, Douglas & Geck) to it for subsequent attachment to the muscle puller. The medial gastrocnemius muscle was separated from its neighbours sufficiently to ensure the optimum transmission of muscle stretches to its muscle spindles. Dessication was prevented by stitching a wire frame to the margins of the skin incision and filling the pool thus contrived with paraffin oil maintained at 32-35 degrees centigrade by radiant heat from a lamp.

2:2.2 PREPARATIONS FOR RECORDING.

The animal was 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 fixing the knee joint the other the ankle joint. A strong stainless steel pin was passed between two of the upper lumbar spinous processes, deep to the lumbodorsal fascia and supraspinous ligaments and was fastened to the brass frame. The hindquarters of the animal were thus fixed firmly.

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 exit level was identified by its position (approximately level with the cranial extremities 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 each then lifted with a fine glass hook, 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 in order to de-efferent muscle spindles in the medial gastrocnemius muscle.

The several natural divisions of the L7 and S1 dorsal roots were teased apart before being placed individually over a bipolar silver hook electrode. The details of the recording apparatus used are the same as those used in the biventer experiments (p. 99 and Fig.1) with the sole modification of the addition in some experiments of a second, identical recording channel so that a number of afferents could be studied simultaneously. Using this apparatus, each dorsal root division was screened for the presence in it of medial gastrocnemius afferents by applying supramaximal shocks (0.01 msec duration; 3-5 X threshold voltage required to produce muscle contraction) to the medial gastrocnemius muscle nerve in the periphery and looking for compound action potentials of fixed latency in the dorsal root division. Divisions which showed compound action potentials were put to one side; subsequently they were repeatedly subdivided until functionally single unit filaments were obtained for study.

Muscle spindle afferents were identified by the reduction in their firing rate during contraction of the medial gastrocnemius in the standard manner (Matthews 1933), 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. Since the main focus of attention was on the properties of b_1b_2c primary and b_2c primary afferents, more rapidly conducting units were selectively sampled, although a number of slower axons were also studied. Units were subjected to the same set of tests, performed in the same order, as was used in the biventer experiments (pp. 103-104), with the addition that, in this second series of experiments, the dynamic response of afferents in response to ramp stretches of 3 mm amplitude and 10 mm/sec velocity was also routinely determined. Finally, each afferent was exposed to an intra-arterial infusion of SCh, generally at a dose of 100 μg/kg/min with three exceptions in which it was 180, 200 and 220 μg/kg/min due to a miscalibrated weighing scale.

At the end of each experiment, the sciatic nerve was exposed over its

entire length and the distance between the stimulating cathode at the muscle nerve and the more distal recording electrode at the dorsal roots was measured. The conduction velocity of the afferents could then be calculated as the conduction distance divided by the conduction delay.

CHAPTER TWO

RESULTS

A total of 88 medial gastrocnemius afferents were studied in the L7 and S1 dorsal roots of 10 cats in this second series of experiments. In contrast to the biventer muscle afferents, most of the medial gastrocnemius afferents were readily shown to be muscle spindle afferents on the basis of their response to contraction of the medial gastrocnemius muscle (Matthews 1933). However, 12 afferents did not discharge tonically at the resting muscle length, so that it was not possible to demonstrate an unloading response to muscle contraction. These afferents were nevertheless identified as spindle afferents because they did **not** show a loading response to muscle contraction, because they were all very sensitive to small amplitude vibration of the muscle, and because they were subsequently activated by SCh in a manner quite atypical of even low-threshold Golgi tendon organ afferents. Attempts were made to induce tonic discharge in these afferents, but were unhelpful because the problem appeared to be one of twisting of the muscle rather than its absolute length. If the muscle was twisted in such a way as to make previously silent afferents discharge tonically, then other afferents which had discharged tonically before would fall silent. This may result from the pennate architecture of the medial gastrocnemius muscle in which the extrafusal muscle fibres, and the muscle spindles, run obliquely between large aponeuroses of origin and insertion (Swett & Eldred 1960a).

2:3.1 AFFERENT RESPONSES TO SCh.

All 88 spindle afferents could be classified into one of four types on the basis of their pattern of SCh excitation, as was the case in the biventer experiments. There were more similarities than differences between the four patterns of SCh activation of biventer and medial gastrocnemius spindle afferents, and in order to avoid confusion the temporary labels used in the biventer experiments (e.g. "type 1 afferents") were used in this second series to describe similar patterns of SCh excitation.

Type 1 afferents.

This group contained 11 afferents, which, like their biventer analogues, were distinguished by a gradual, moderate degree of biassing of the afferent discharge unaccompanied by marked changes in afferent mechanosensitivity. The patterns of response of two afferents in this type are illustrated in Figs. 20 and 21.

Turning first to Fig. 20, A, it can be seen that this afferent had, in the control state, an initial discharge of 26 Imp/sec, a dynamic index of 18 Imp/sec and a position sensitivity of 9.5 Imp/sec/mm. The time course of the SCh activation of this afferent is not illustrated, but was very similar to that of biventer type 1 afferents (e.g. Fig. 5, C) and proceeded as a single, unpunctuated process; after 220 seconds of infusion at 100 μg/kg/min, the response to stretch, which had been unchanged for some 20 seconds, was as shown in Fig. 20, B. The initial discharge had been modestly biassed by 27 Imp/sec to 53 Imp/sec, this being accompanied by a very slight increase in the discharge variability. Over the same period, the dynamic index of the afferent was reduced by 66% to 8 Imp/sec, and the position sensitivity by 16% to 8 Imp/sec/mm. The infusion was switched off shortly after the last stretch in Fig. 20, B, and the changes in afferent discharge reversed as gradually as they had developed. Most of the afferents placed into type 1 behaved like the one shown here.

The activation of a different afferent of this type is illustrated in Fig. 21. This figure is included mainly for comparison with the biventer afferent whose activation is shown in Fig. 5 above (see Discussion, p. 227). This afferent was rather insensitive to muscle stretch, both in the control (Fig. 21, A) and fully activated states (Fig. 21, B), and this behaviour was not altered by increasing the resting length of the muscle. In the control state, the initial discharge was 28 Imp/sec, the dynamic index about 4 Imp/sec and the position sensitivity about 2 Imp/sec/mm. Over the course of 250 seconds of SCh infusion at 100 μg/kg/min, this behaviour was very gradually transformed into that shown in Fig. 21, B; the afferent discharge was biassed by 18

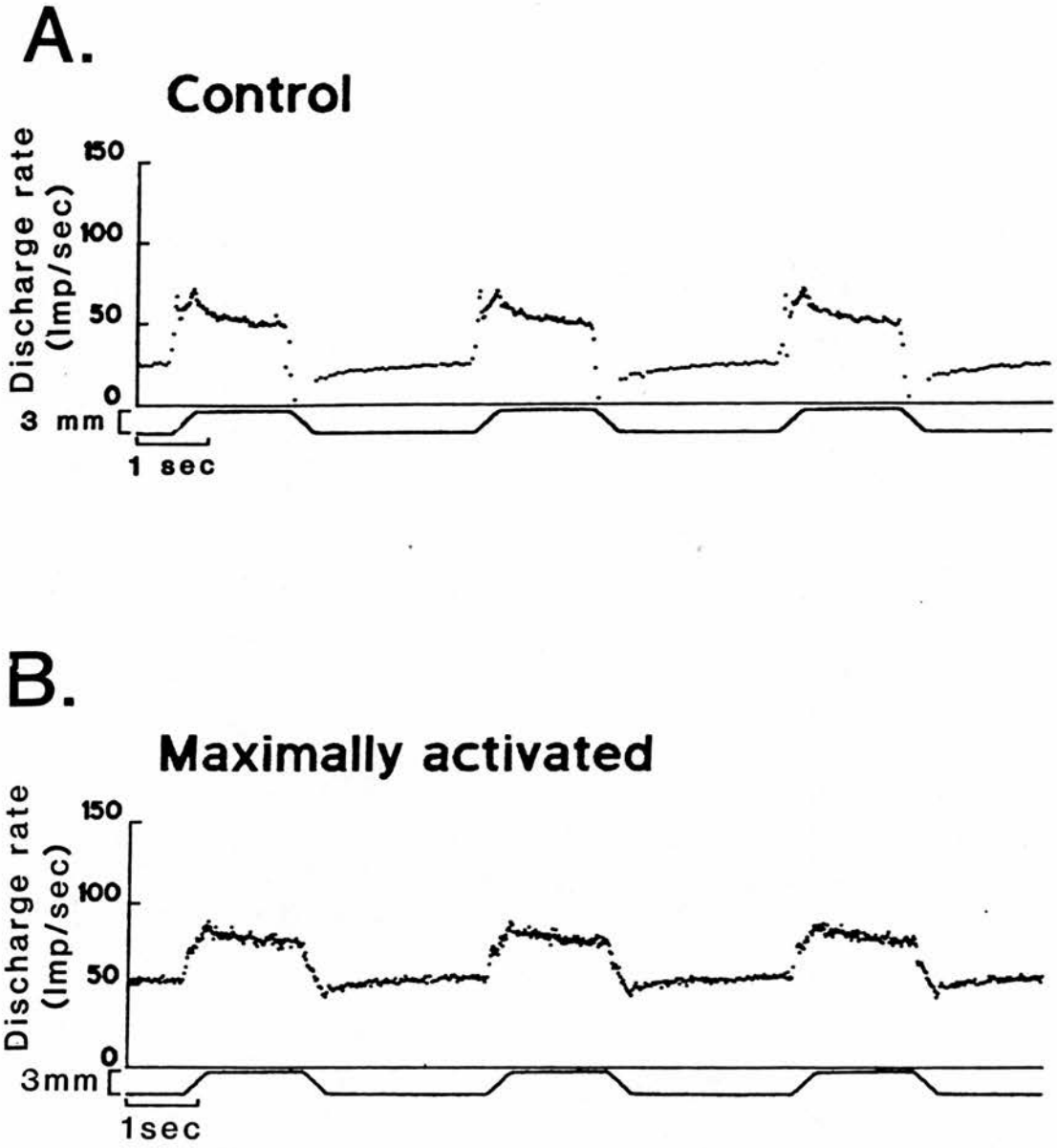


Fig. 20 A, B. Pattern of SCh activation of a medial gastrocnemius type 1 afferent. Response to three stretches (3 mm, 10 mm/sec) of this afferent before (**A**) and after 220 seconds of SCh infusion at 100 μg/kg/min (**B**). Conduction velocity of this afferent was 50 m/sec.

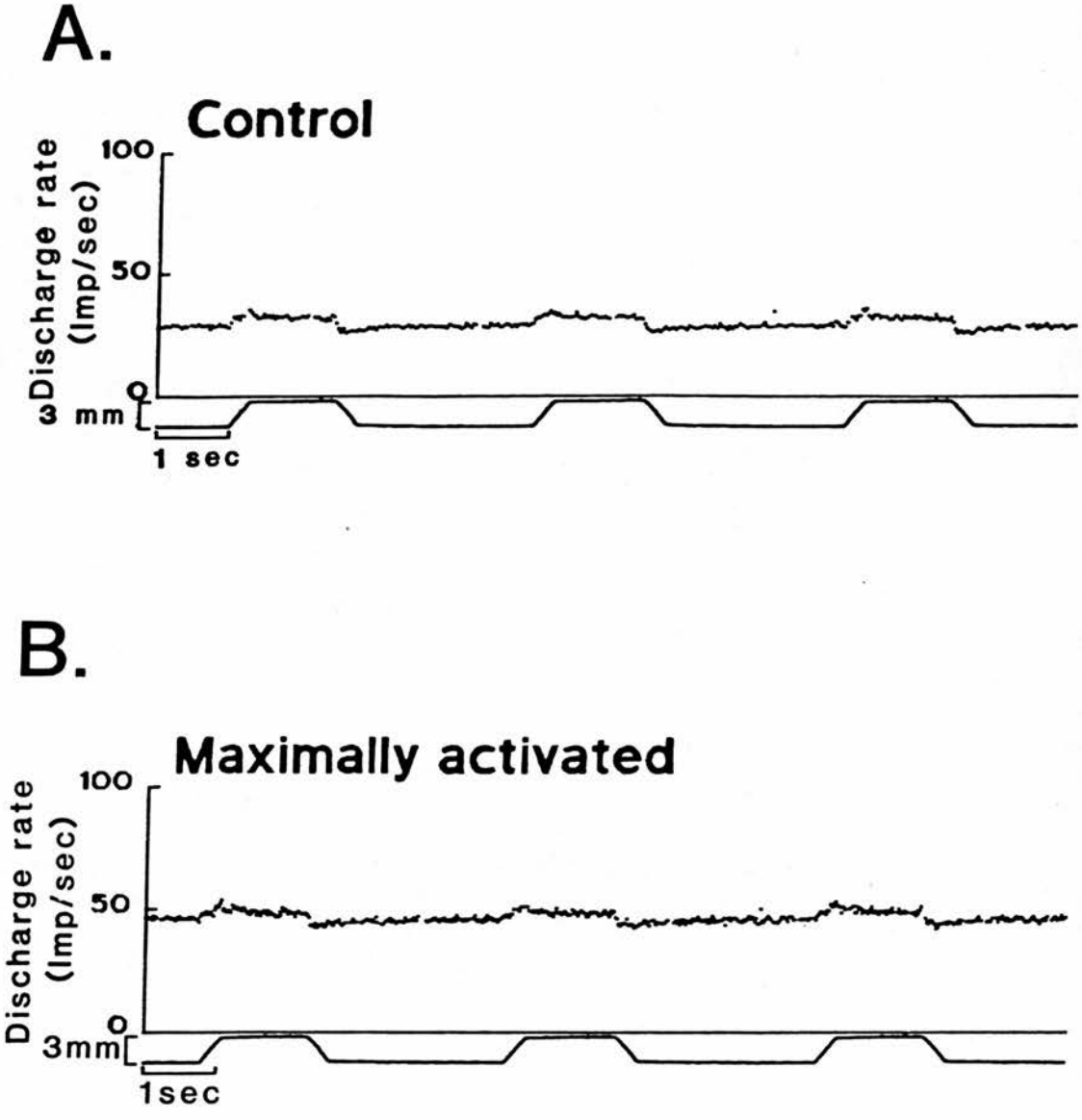


Fig. 21 A, B. Pattern of SCh activation of a different medial gastrocnemius type 1 afferent. Response to ramp stretching before (**A**) and after 250 seconds (**B**) of SCh infusion at 100 μg/kg/min. Conduction velocity of this afferent was 44 m/sec.

Imp/sec to 46 Imp/sec, the dynamic index fell marginally to perhaps 3 Imp/sec (hard to measure) and the position sensitivity to 1.5 Imp/sec/mm. The infusion was then switched off, and these unremarkable changes reversed over the course of 5 minutes.

Type 2 afferents.

Fifty-three afferents were classified as type 2 on the basis of their excitation by SCh. As for biventer type 2 afferents, all afferents of this type underwent a large increase in dynamic response and dynamic index, but these changes were accompanied by variable changes in other parameters of afferent response to stretch. Illustrations of a number of the activations of this class of afferent are therefore included.

The afferent whose activation is shown in Fig. 22 was fairly typical of this class in that its behaviour was intermediate between two extremes which will be illustrated later. In the control state, this afferent had an initial discharge of 8 Imp/sec, a dynamic index of 19 Imp/sec, and a position sensitivity of 7.6 Imp/sec/mm (Fig. 22, A). The afferent was then exposed to a SCh infusion at 100 μg/kg/min, and the time course of events thereafter, which was measured by hand from a hard copy of the activation (c.f. the biventer experiments), is illustrated in Fig. 22, C. The activation appeared at first glance to proceed as a single, unpunctuated event, but on closer inspection could be split into two phases: Phase I of activation began about 56 seconds after the start of the infusion, at which time the initial discharge, dynamic response and position response all began to drift up together. Over the next 50 seconds, the discharge of this afferent was biassed by 42 Imp/sec to a maximum of 50 Imp/sec; changes in dynamic response and position response over the same period were roughly parallel, such that 100 seconds after the start of the infusion, the dynamic index of the afferent had slightly more than doubled to 46 Imp/sec, and the position sensitivity to 16.7 Imp/sec/mm.

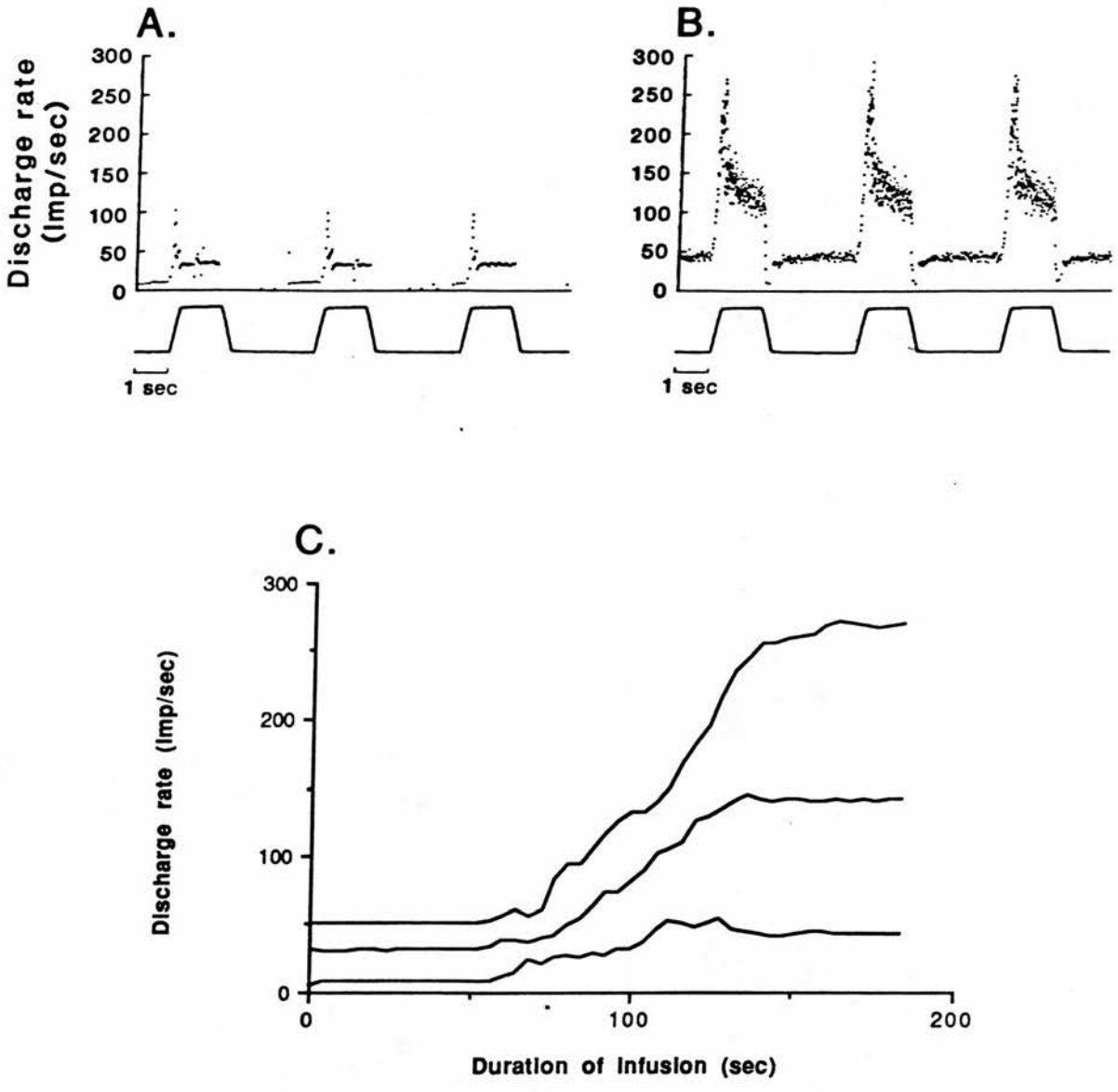


Fig. 22 A-C. Pattern of SCh activation of a medial gastrocnemius type 2 afferent. Response to ramp stretching before (**A**) and after 164 seconds (**B**) of SCh infusion at 100 μg/kg/min. Time course of the activation is shown in **C**; these data were measured by hand from a pen recorder copy. Conduction velocity of this afferent was 82 m/sec.

Phase II of this activation started after 100 seconds of infusion, and was characterised by a much steeper rise in dynamic response, which reached peak frequencies of 280 Imp/sec or so; this was accompanied by a continued rise in the position response, whilst the initial discharge reached a plateau and then fell slightly. When fully activated, the initial discharge of this afferent was 48 Imp/sec, the dynamic index had increased 670% to 127 Imp/sec, and the position sensitivity had risen 430% to 32.6 Imp/sec (Fig. 22, B). The infusion was actually continued longer than Fig. 22, C suggests, but there were no further changes in afferent response to stretching. In particular, there were no late increases in position response, nor any other reason to suspect that a third phase of activation had started. The infusion was eventually stopped after 240 seconds, and the afferent discharge returned gradually to its control form over a few minutes.

The SCh activation of the afferent illustrated in Fig. 23 lay at one extreme of the range seen for medial gastrocnemius type 2 afferents; only one other afferent behaved similarly. In this instance, the afferent had an initial discharge of 20 Imp/sec, a dynamic index of 21 Imp/sec and a position sensitivity of 3.6 Imp/sec/mm (Fig. 23, A). The time course of events after the start of a 100 μg/kg/min SCh infusion is shown in Fig. 23, C, the particular feature of this activation being that it proceeded in three phases: Phase I, which began 24 seconds after the start of the infusion, consisted of a gradual biassing of the initial discharge by about 10 Imp/sec, with minimal change in the dynamic and position responses. This phase lasted for about 20 seconds, after which Phase II was heralded by rapid increases in both dynamic and position responses, accompanied by continued, but lesser, increases in initial discharge; over the next 64 seconds, these changes produced an increase in dynamic index of 470% to 99 Imp/sec, and in position sensitivity of 750% to 27 Imp/sec/mm. In the majority of afferents, as was just illustrated in Fig. 22, C, this would represent the end-stage of the SCh activation, and the position response of this afferent did appear to be levelling out from about 90 seconds onwards (Fig. 23, C). There was, however, a sudden further

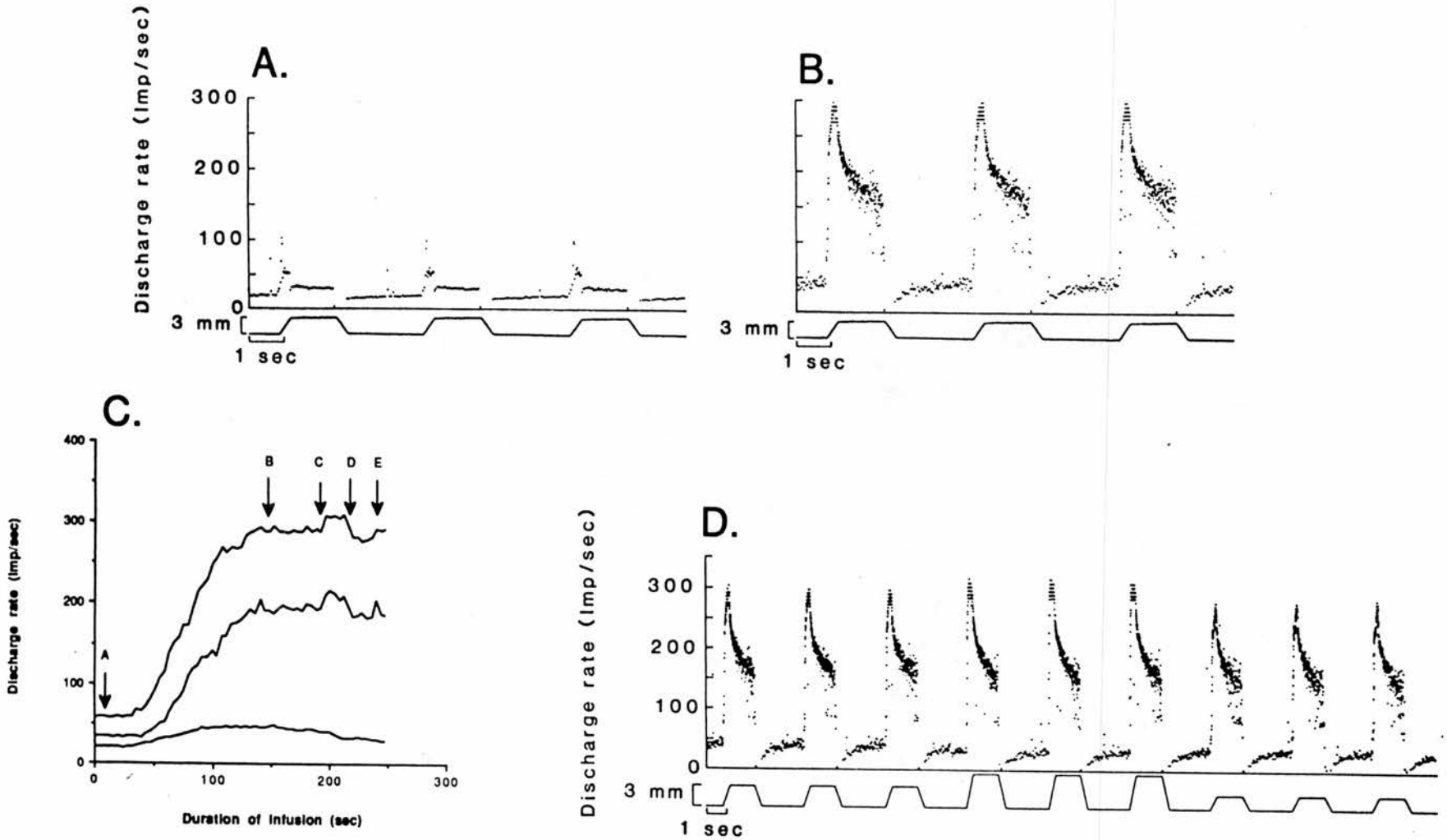


Fig. 23 A-D. Pattern of SCh activation of a different medial gastrocnemius type 2 afferent. Response to stretching before (**A**) and after 140 seconds (**B**) of SCh infusion at 100 μg/kg/min. Timecourse of this activation is shown in **C** (data derived by hand from a pen recorder output). In **C**, A and B indicate the times at which the responses to stretch shown in panels **A** and **B** respectively were obtained; C-E refer to responses shown in **D** in which the amplitude of the ramp stretch was varied between 2 mm and 5 mm as indicated. Conduction velocity of this afferent was 85 m/sec.

increase in position response starting 112 seconds into the infusion; this was accompanied by a slightly smaller increase in dynamic response, whilst the initial discharge of the afferent remained steady at 41 Imp/sec, with the result that 140 seconds into the infusion the dynamic index was slightly reduced from its maximum attained at the end of Phase II to 83 Imp/sec, and the position sensitivity was further increased over its Phase II value to 42.2 Imp/sec/mm, nearly 12 times the control value (Fig. 23, B, C). The changes which took place between 112 seconds and 140 seconds after the start of the infusion were identified as Phase III of activation in this afferent.

The infusion was continued for a further 92 seconds after the full development of Phase III, to a total duration of 248 seconds without eliciting any other changes in afferent sensitivity, other than those marked at C, D & E in Fig. 23, B; these were produced by changes in the stretch amplitude, as illustrated in Fig. 23, D, which were introduced in order to determine whether the afferent was capable of discharging at even higher frequencies than those achieved during Phase III of activation. At the point marked C in Fig. 23, C, the stretch amplitude was increased from 3 to 5 mm (velocity unchanged) which resulted in further increases in both dynamic and position responses above the frequencies attained in response to the 3 mm stretch amplitude (compare the second set of three stretches in Fig. 23, D with the first set of three stretches). At point E (Fig. 23, C), the stretch amplitude was reduced to 2 mm and both dynamic and position responses were correspondingly lower than the 3 mm values (last three stretches of Fig. 23, D). Shortly after this the infusion was stopped, and these changes in afferent stretch sensitivity reversed steadily over a number of minutes to restore the control response to stretch.

The other extreme form of type 2 activation seen in these experiments is shown in Fig. 24. In the control state, this afferent had a dynamic index of 22 Imp/sec; its exact position sensitivity was impossible to ascertain, since it was not discharging tonically at the resting muscle length, but was at least 10 Imp/sec/mm (Fig. 24, A). The time course of the activation of this afferent by a 100 μg/kg/min SCh infusion is shown

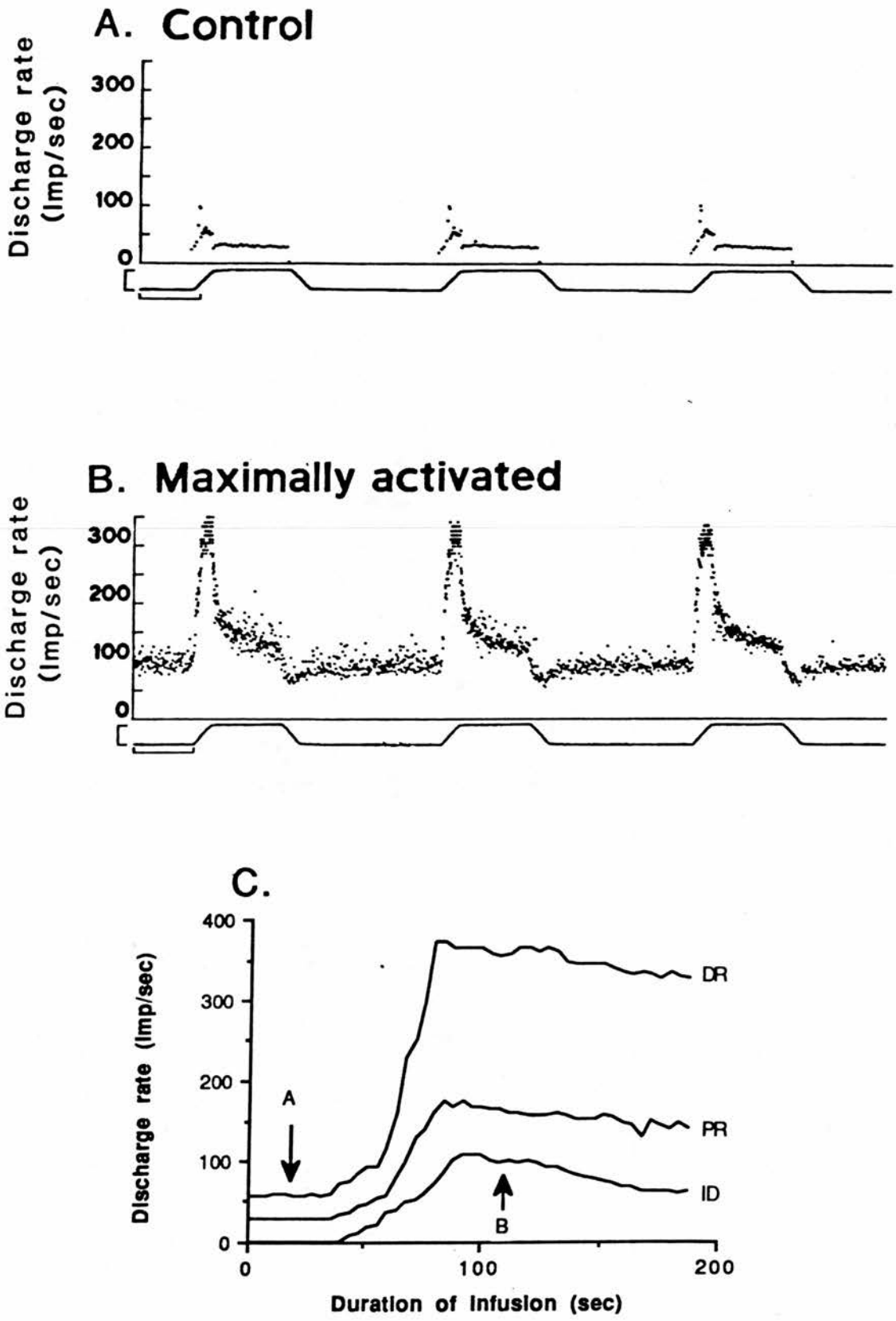


Fig. 24 A-C. Pattern of SCh activation of another medial gastrocnemius type 2 afferent. Response to stretching (3 mm, 10 mm/sec) before (**A**) and after 108 seconds (**B**) of SCh infusion at 100 μg/kg/min. The time course of activation of this afferent (measured by hand) is shown in **C**; here, **A** shows the time at which the responses in panel **A** were obtained, **B** the timing of the responses shown in panel **B**. Conduction velocity of this afferent was 107 m/sec.

in Fig. 24, C, from which it is apparent that activation proceeded in only two phases. Phase I started after 40 seconds of infusion and consisted of a biassing of the initial discharge to 35 Imp/sec, accompanied by roughly parallel increases in the dynamic and position responses such that the dynamic index was only slightly increased and the position sensitivity slightly reduced compared to control. Phase II began after 56 seconds of SCh infusion, and consisted of a very rapid increase in the dynamic response of the afferent to a peak frequency of 350 Imp/sec; the concomitant increases in position response and initial discharge were much less substantial, so that whilst, in the maximally activated state (Fig. 24, B), the dynamic index had increased nearly ten-fold to 210 Imp/sec, the position sensitivity, at 14.6 Imp/sec/mm, was only slightly increased, if at all given that its value in the control state was not known exactly. Despite continuing the infusion for 80 seconds after the responses shown in Fig. 24, B were obtained, no further changes in afferent sensitivity revealed themselves, and the infusion was terminated after 184 seconds, whereupon the changes in sensitivity provoked by SCh reversed over the next few minutes.

This pattern of excitation, with marked increases in dynamic index but only small changes in position sensitivity, was shown by 8 other afferents. Fortuitously, one of these other afferents was excited with a non-standard dose of SCh, namely 220 μg/kg/min, and this activation yielded further useful data. In its earlier stages it was very similar to that just shown (Fig. 24), but differed in its later stages which are shown in Fig. 25. The earliest data shown in this figure were obtained after 160 seconds of infusion, the stretches on the left hand side showing a pattern of response to stretch very similar to that of the previous afferent at maximal activation (Fig. 24, B). From the third stretch onwards, however, the dynamic response of the afferent no longer reached the peak frequencies of nearly 300 Imp/sec which it had done earlier, and a gap in the discharge of the afferent during the period of ramp stretching gradually appeared (Fig. 25, arrows). During this gap, the afferent was not totally silenced, but the impulses which it did discharge clustered around a frequency

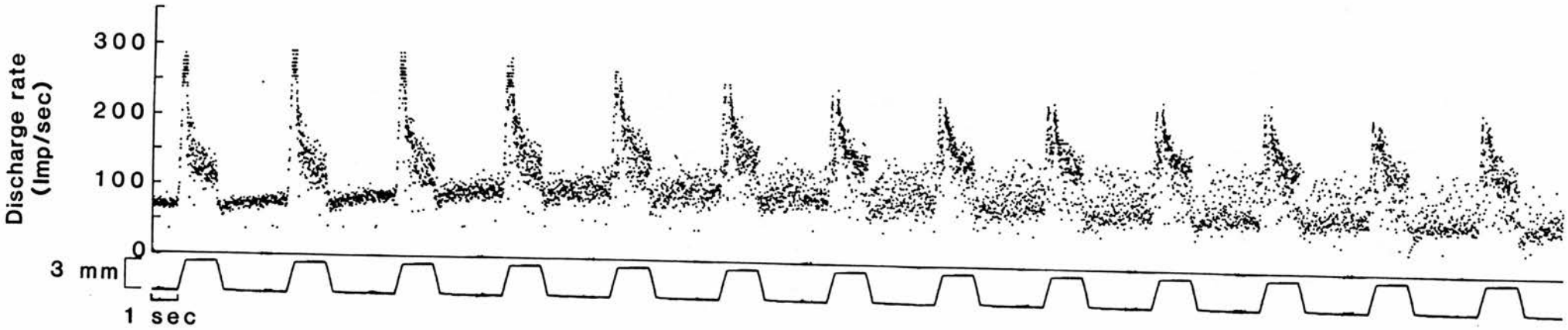


Fig. 25. Pattern of excitation of a medial gastrocnemius type 2 afferent excited by a SCh infusion at 220 μg/kg/min, showing the development of depolarisation block. The leftmost stretch of this sequence was applied 160 seconds after the start of the SCh infusion. Conduction velocity of this afferent was 106 m/sec.

of about 150 Imp/sec, or about half the peak rate achieved earlier (see e.g. the sixth and seventh stretches of Fig. 25). While this change in dynamic response was occurring, the initial discharge of the afferent underwent a further biassing of about 20 Imp/sec, accompanied by an increase in discharge variability, but there was no evidence of an increase in position sensitivity analogous to the Phase III of activation shown by some type 2 afferents (Fig. 24); from the ninth stretch of this sequence, the initial discharge became extremely variable and its mean level began to wane, leaving behind a steady level of position response and thus giving the appearance of an increase in position sensitivity (Fig. 25), but this negative event should be contrasted with the positive development of such a change shown in, for example, Fig. 24.

In any given experiment, the whole range of SCh activations of type 2 afferents described above could be seen, and no features of afferent response to stretch, nor of other afferent properties, could be identified on the basis of which the eventual pattern of SCh activation could be predicted. For example, the final responses to stretch of four type 2 afferents after exposure to a 100 μg/kg/min infusion of SCh are shown in Fig. 26, together with their axonal conduction velocities. The top two afferents both showed marked increases in their position sensitivities, the bottom two did not; the degree of biassing of the afferent discharge was similar in all four cases, and there was no evidence of a relationship between afferent conduction velocity and the nature of changes in position sensitivity evoked by SCh. Finally, one afferent which showed a Phase III of activation in response to SCh was allowed to recover fully before the muscle was shortened by 5 mm; the SCh activation was then repeated, but there was no change in the afferent response compared to that obtained at the original muscle length, and in particular the Phase III of activation was not lost. Likewise, lengthening the muscle by 3 mm from the control length did not change the pattern of SCh activation when this was reassessed.

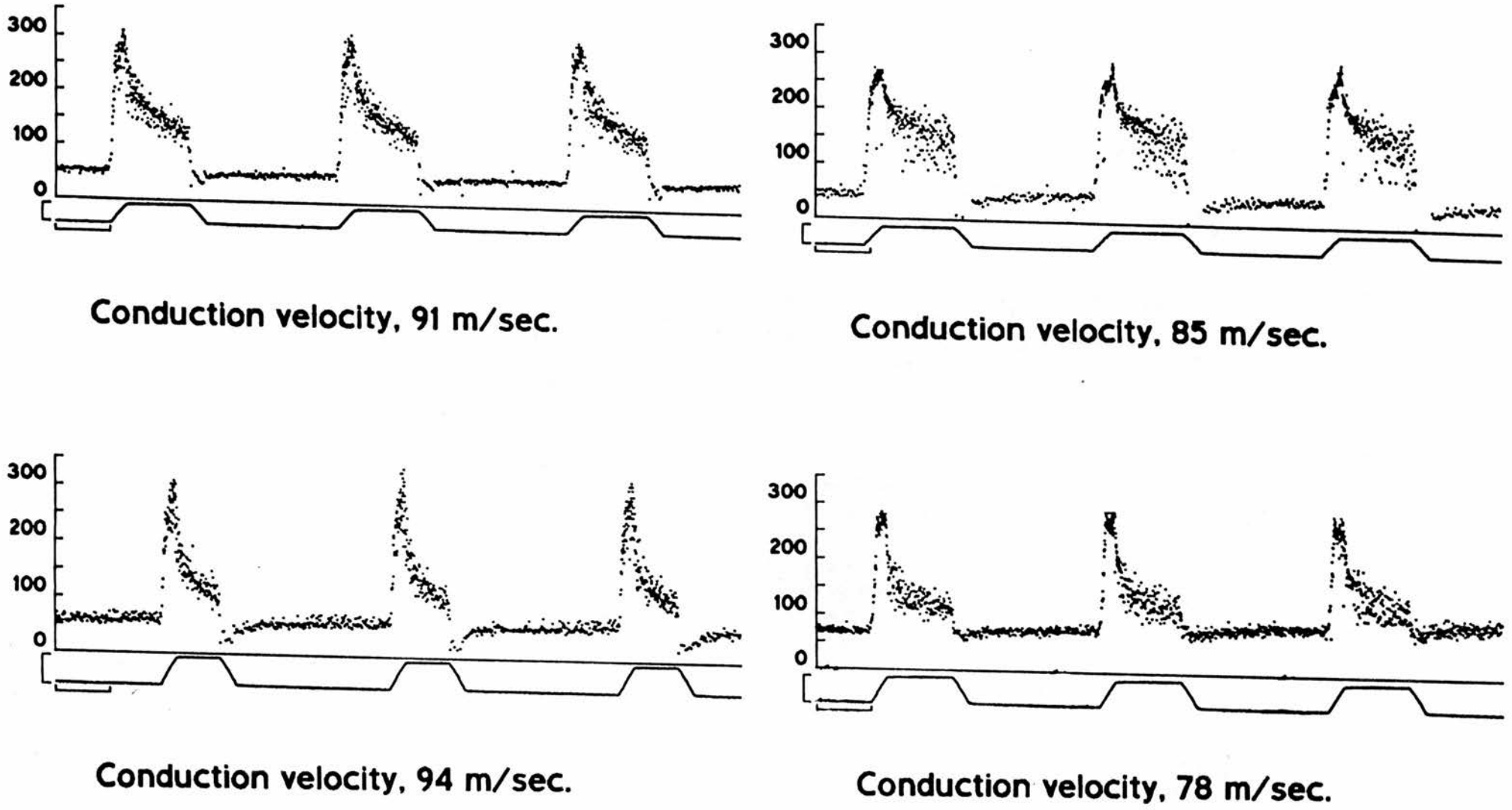


Fig. 26. The response to ramp stretching (3 mm, 10 mm/sec) of four medial gastrocnemius type 2 afferents when maximally activated by infusion of SCh at 100 μg/kg/min. Note the variability in position sensitivity of these type 2 afferents. The conduction velocity of each afferent is indicated beneath the panels.

Type 3 afferents.

Twelve afferents were classified as type 3, and the behaviour of three of these is illustrated in Figs. 27-29. The behaviour of the afferent illustrated in Fig. 27, A,B was typical of the majority of the medial gastrocnemius type 3 afferents, whilst the other two afferents illustrated showed unusual features but were nevertheless confidently placed into type 3.

The afferent whose behaviour is shown in Fig. 27 had a control initial discharge of 47 Imp/sec, a dynamic index of 11 Imp/sec and a position sensitivity of 2.5 Imp/sec/mm (Fig. 27, A). The time course of activation of this afferent is not illustrated, but was very similar to that of biventer type 3 afferents (e.g. Fig. 9, C) both in terms of latency to the first changes in discharge rate and in terms of the rate of rise of the initial discharge once activated. Thus, after about 50 seconds of SCh infusion at 100 μg/kg/min, and over the course of 6 stretches, or 24 seconds, the initial discharge of this afferent increased by 71 Imp/sec to 118 Imp/sec (Fig. 27, B); in the same period, the dynamic index was almost totally abolished, and the position sensitivity fell by about 38% to about 1.5 Imp/sec/mm (Fig. 27, B). In fact, when it was maximally activated by SCh, this afferent had been rendered so insensitive to stretching that it was rather difficult to discern its response to each ramp. This pattern of response to SCh persisted until the infusion was switched off after 220 seconds, upon which the biassing gradually waned over the course of a few minutes to restore the control response to stretch.

The second type 3 afferent illustrated (Fig. 28) was rather more sensitive to stretch of medial gastrocnemius than the one just shown. Thus, it had an initial discharge of 14 Imp/sec, a dynamic index of 22 Imp/sec (ignoring an initial burst), and a position sensitivity of 13 Imp/sec/mm (Fig. 28, A). After 160 seconds of exposure to 100 μg/kg/min SCh, the response to stretch of this afferent had evolved into that shown in Fig. 28, B, and did not change any further with continued infusion.

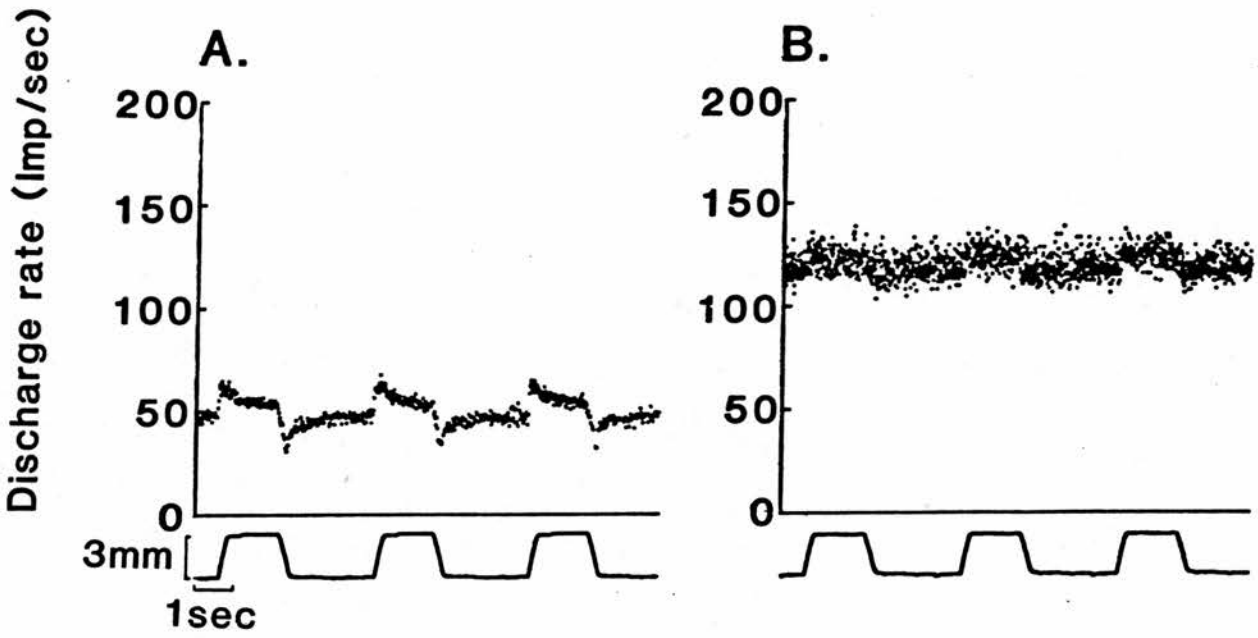


Fig. 27 A, B. Pattern of SCh activation of a medial gastrocnemius type 3 afferent. Response to ramp stretching (3 mm, 10 mm/sec) before (**A**) and after 212 seconds (**B**) of SCh infusion at 100 μg/kg/min. Conduction velocity of this afferent was 94 m/sec.

At maximal activation, the initial discharge was more variable than in the control state and had been biassed by 73 Imp/sec to 87 Imp/sec, and the position sensitivity of the afferent had again fallen to virtually zero. This afferent showed a somewhat anomalous change in its dynamic index which appeared to take on a negative value, that is that the discharge rate of the afferent **increased** by 14 Imp/sec immediately following the end of the stretch, rather than falling as in the classical dynamic index. Nevertheless, there was little hesitation in placing this afferent into type 3 on the basis of the marked biassing and reduction in position sensitivity produced by the action of SCh. As before, these changes in afferent discharge all reversed over the course of a few minutes when the SCh infusion was switched off.

The third type 3 afferent, whose behaviour is shown in Fig. 29, had an initial discharge of 21 Imp/sec, a dynamic index of 28 Imp/sec and a position sensitivity of 10.8 Imp/sec/mm (Fig. 29, A). After 180 seconds of exposure to SCh at 100 μg/k/min the response to stretching changed to that shown in Fig. 29, B; the initial discharge was markedly more variable than in the control condition, and had been biassed by 109 Imp/sec to about 130 Imp/sec. The position sensitivity had fallen by 52% to 5.2 Imp/sec/mm, but the dynamic index had actually increased, nearly doubling to 47 Imp/sec (Fig. 29, B). The magnitude of the dynamic index at full activation was, however, much less than that shown by type 2 afferents encountered during the same experiments (see e.g. Fig. 26), and the most prominent changes are the biassing and reduction in position sensitivity on which basis this afferent was still classified as type 3. These changes in afferent sensitivity reversed over the course of a few minutes when the SCh infusion was terminated.

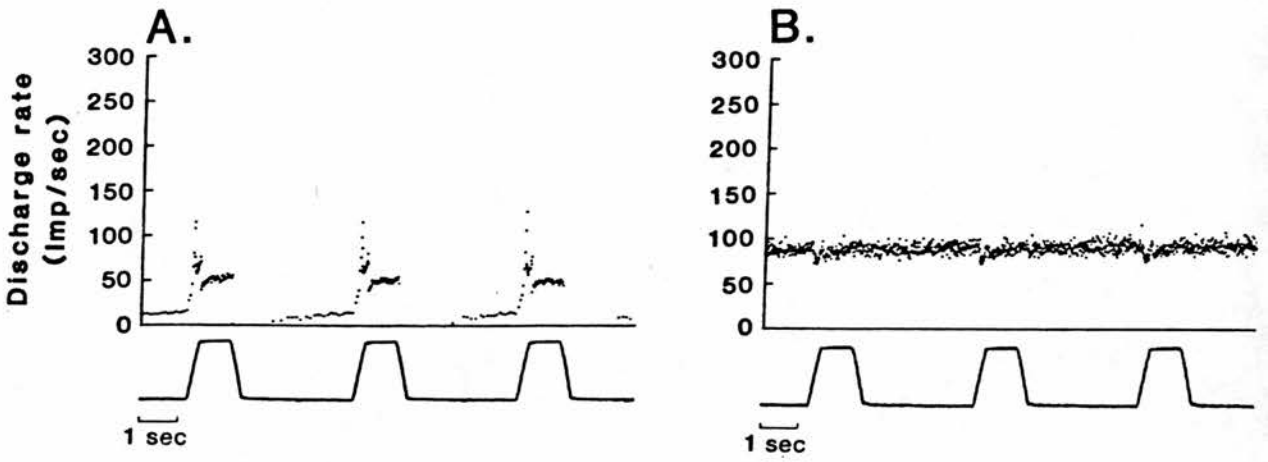


Fig. 28 A, B. Pattern of SCh activation of a different medial gastrocnemius type 3 afferent. Response to stretching (3 mm, 10 mm/sec) before (**A**) and after 160 seconds (**B**) of SCh infusion at 100 μg/kg/min. Conduction velocity of this afferent was 29 m/sec.

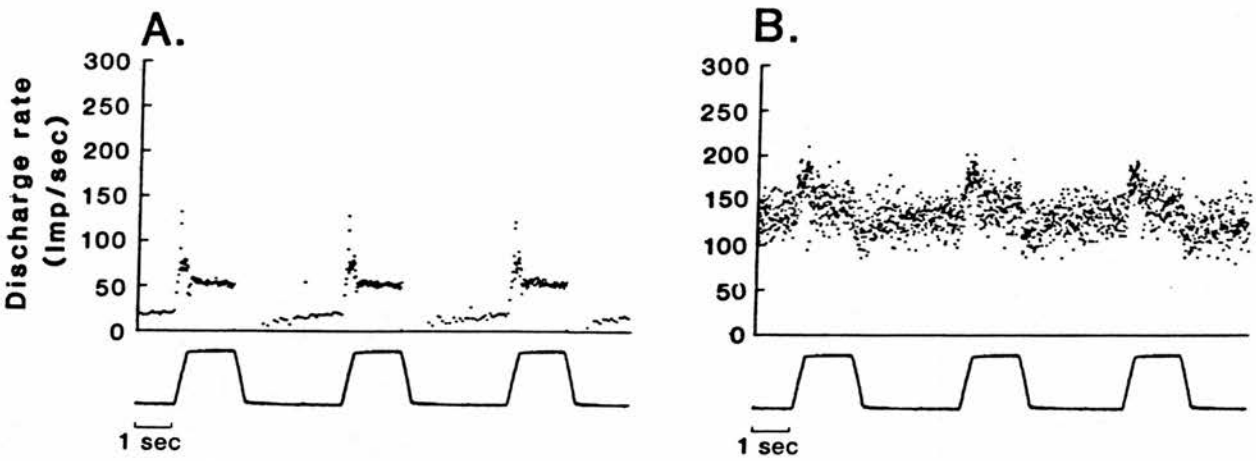


Fig. 29 A, B. Pattern of SCh activation of another medial gastrocnemius type 3 afferent. Response to stretching (3 mm, 10 mm/sec) before (**A**) and after 180 seconds (**B**) of SCh infusion at 100 μg/kg/min. Conduction velocity of this afferent was 89 m/sec.

Type 4 afferents.

This final group of afferents contained 12 members, none of which could convincingly be placed into one of the other classes. The SCh-evoked changes in afferent discharge consisted of a moderate to substantial biassing action, with only a slight increase in discharge variability (Figs. 30-32), these changes being accompanied by variable increases in dynamic index and position sensitivity on the basis of which two subtypes of behaviour were identified.

The commonest change (11 out of 12 afferents) is shown for two afferents, whose behaviour encompasses the two extremes seen in this subtype, in Figs. 30 & 31. The afferent whose SCh activation is illustrated in Fig. 30 was relatively sensitive to muscle stretch, having in the control state an initial discharge of 40 Imp/sec, a dynamic index of 25 Imp/sec and a position sensitivity of 11.5 Imp/sec/mm (Fig. 30, A). Over the course of the next 160 seconds, the action of 100 μg/kg/min SCh transformed the behaviour of this afferent to that illustrated in Fig. 30, B, this modification taking place as a smooth progression over time similar to that illustrated for the biventer type 4 afferent in Fig. 12. When maximally activated by SCh, the initial discharge had been biassed by 51 Imp/sec to 91 Imp/sec, the dynamic index had more than trebled to 80 Imp/sec, and the position sensitivity had nearly doubled to 20 Imp/sec/mm. What distinguishes this afferent from type 2 afferents (see e.g. Figs. 23-26) and places it into type 4 in spite of these substantial increases in stretch sensitivity is a combination of the rather low discharge variability, given the level to which the discharge rate has been biassed, and the fact that the axon had a conduction velocity of only 58 m/sec.

The activation of a second afferent placed into this subset of type 4 is shown in Fig. 31, this afferent being more representative of the majority than the one just described. In this case, the control initial discharge was 26 Imp/sec, the dynamic index 17 Imp/sec and the position sensitivity 9.4 Imp/sec/mm (Fig. 31, A). Over the

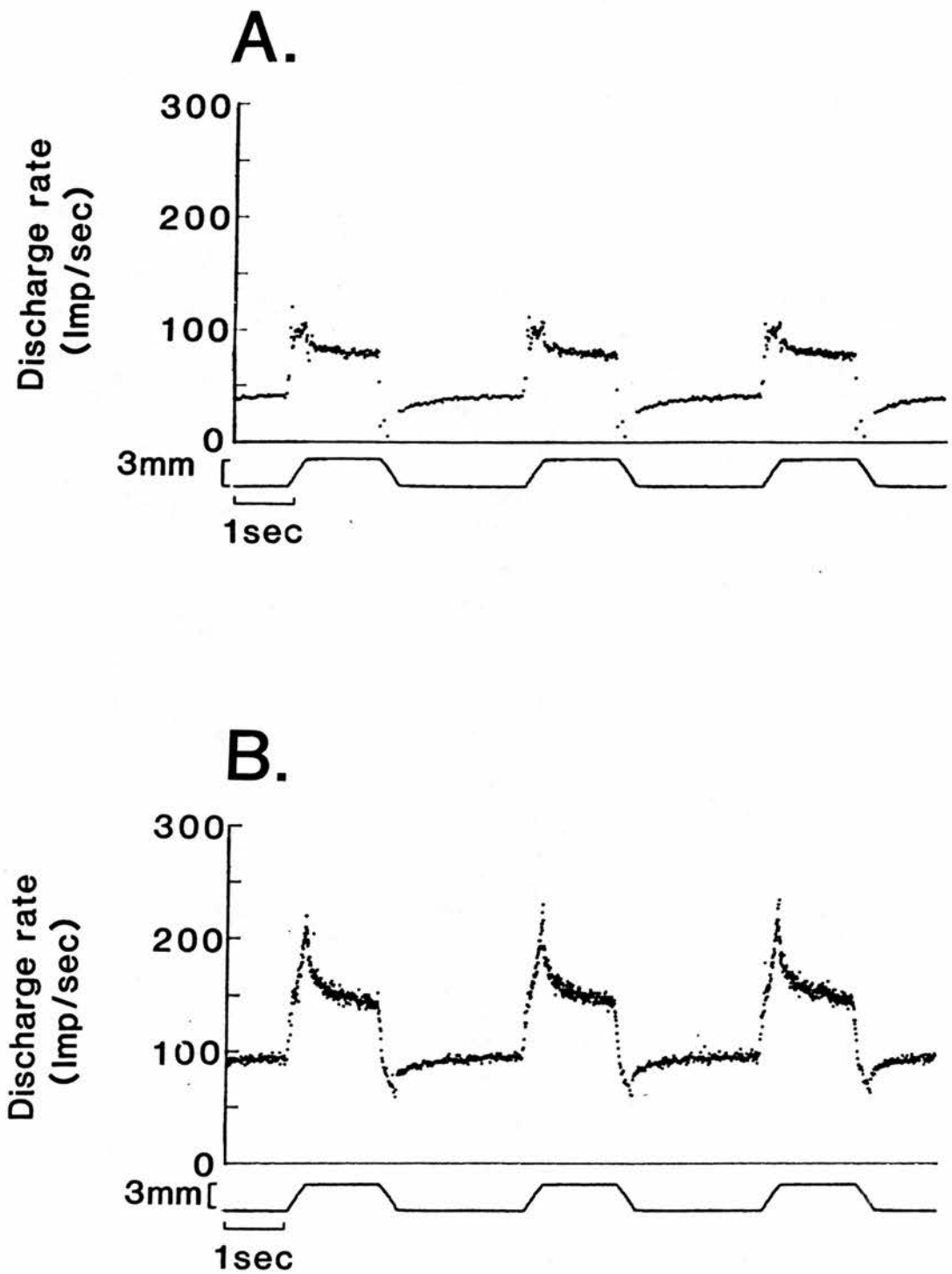


Fig. 30 A, B. Pattern of SCh activation of a medial gastrocnemius type 4 afferent. Response of this afferent before (**A**) and after 152 seconds (**B**) of SCh infusion at 100 μg/kg/min. **C** Timecourse of this activation (hand measured). Conduction velocity of this afferent was 58 m/sec.

course of 140 seconds of a 100 μg/kg/min SCh infusion the initial discharge was biassed by 44 Imp/sec to 70 Imp/sec, the dynamic index increased by 164% to 28 Imp/sec, and the position sensitivity rose by 154% to 14.5 Imp/sec/mm (Fig. 31, B); these changes again took place as a single, smooth process, and reversed in similar fashion over the course of a few minutes when the infusion was switched off after the last stretch shown in Fig. 31, B. The particular feature of this subtype of type 4 afferent was thus that although both dynamic index and position sensitivity rose over the course of a SCh infusion, the increases in dynamic index outweighed those in position sensitivity.

Only one afferent was encountered which showed the reciprocal of these changes in its response parameters, and its activation is illustrated in Fig. 32. In the control state, this afferent had an initial discharge of 18 Imp/sec, a dynamic index of 10 Imp/sec and a position sensitivity of 4.3 Imp/sec/mm (Fig. 32, A). Exposure to SCh provoked a biassing of the initial discharge by 25 Imp/sec to 43 Imp/sec, accompanied by 150% increase in the dynamic index to 15 Imp/sec and a 230% increase in the position sensitivity to 9.9 Imp/sec/mm (Fig. 32, B). As was the case for all the other type 4 afferents, these changes occurred smoothly and gradually over the course of the infusion, and reversed equally gradually when the infusion was stopped.

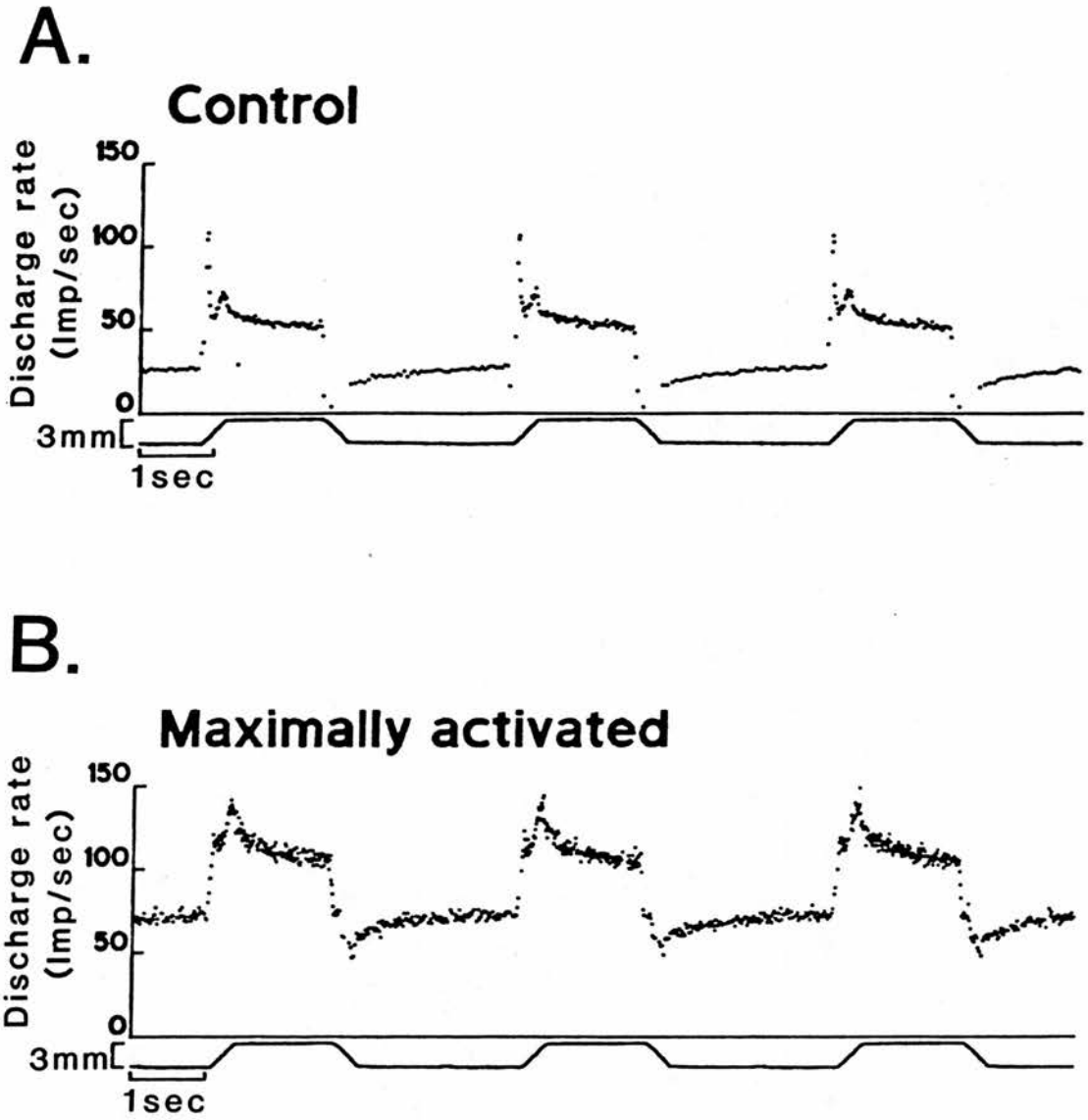


Fig. 31 A, B. Pattern of SCh activation of a different medial gastrocnemius type 4 afferent. Response to stretch (3 mm, 10 mm/sec) before (**A**) and after 140 seconds (**B**) of SCh infusion at 100 μg/kg/min. Conduction velocity of this afferent was 72 m/sec.

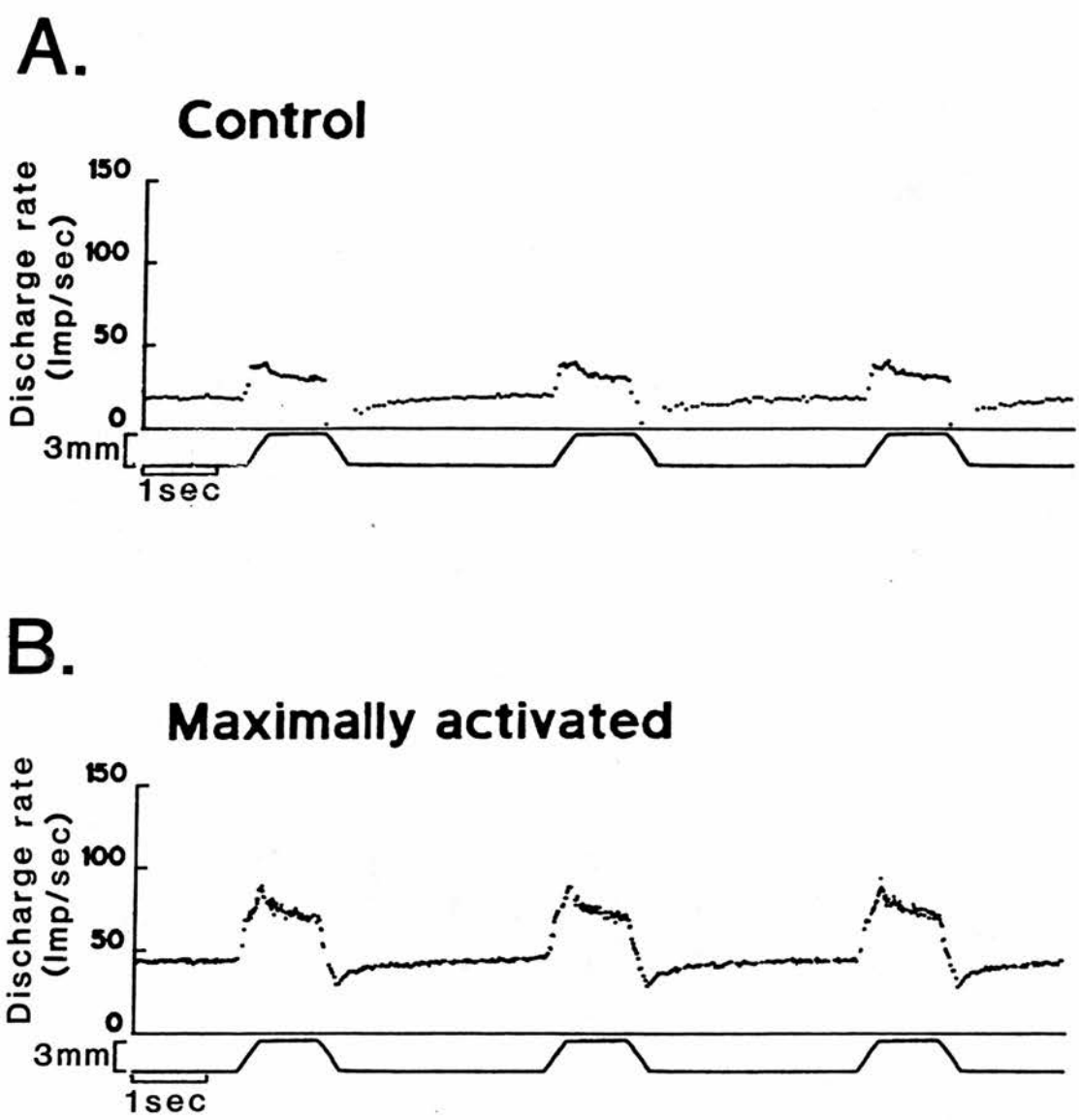


Fig. 32 A, B. Pattern of SCh activation of another medial gastrocnemius type 4 afferent. Response to stretch (3 mm, 10 mm/sec) before (**A**) and after 140 seconds (**B**) of SCh infusion at 100 μg/kg/min. Conduction velocity of this afferent was 66 m/sec.

2:3.2 PASSIVE PROPERTIES OF GASTROCNEMIUS SPINDLE AFFERENTS.

The same passive properties which were measured for biventer afferents were also measured for the medial gastrocnemius spindle afferents. In this second series of experiments, sinusoidal stretch amplitudes were always exactly 1 mm, so that there were no uncertainties in the measurement of sinusoidal sensitivity. The dynamic index was also routinely measured for all afferents, rather than for a subset. The passive property whose measurement presented technical difficulties in this series was the coefficient of variation of the resting discharge; this was mainly a problem for type 2 afferents, since some of these were not tonically active at the resting muscle length, and others discharged at less than 20 Imp/sec, under which circumstances the coefficient of variation is altered unpredictably (Matthews & Stein 1969b). Afferents affected by such problems were not included in the analysis. The most important addition to the passive property assessments in the medial gastrocnemius experiments was the measurement of axonal conduction velocity which was not feasible in the biventer experiments.

The first four figures of this section show plots of each of the four passive properties against axonal conduction velocity, the different symbols representing the different patterns of SCh excitation of the afferents. In Fig. 33, the coefficient of variation is plotted against axonal conduction velocity for those 69 afferents in which both measurements could be made in addition to a SCh typing. The observed conduction velocities ranged smoothly from 29 to 128 m/sec, and the coefficients of variation from 0.018 to 0.112. Within these continua, there was a tendency for afferents with higher conduction velocities to have higher coefficients of variation, with one obvious outlying unit, the type 3 afferent conducting at 29 m/sec, which had a far higher coefficient of variation than other slowly conducting afferents; there was, however, no obvious clustering of afferents which might allow subtypes to be identified on the basis of their passive properties alone. If the afferent classification on the basis of pattern of SCh activation was also taken into account, on the other

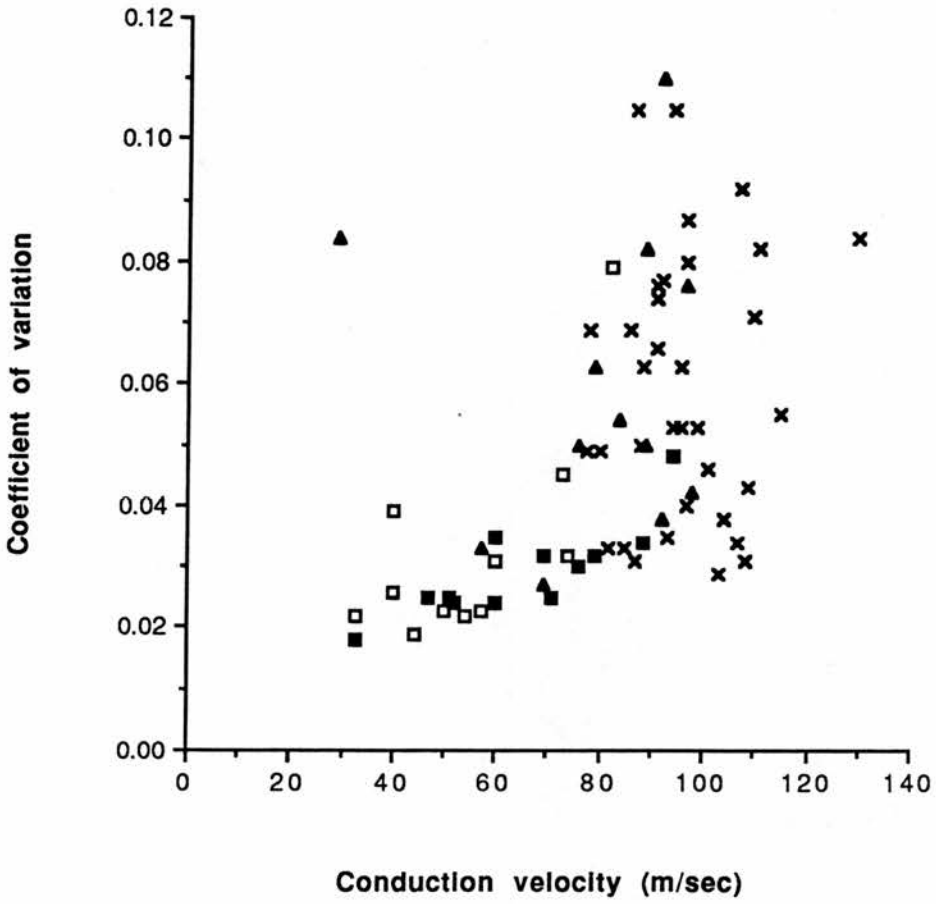


Fig. 33. Relationship between afferent conduction velocity and the coefficient of variation of the resting discharge for 69 medial gastrocnemius muscle spindle afferents in which both parameters were measured. Symbols indicate the pattern of SCh excitation of each afferent:- open squares: type 1; crosses: type 2; filled triangles: type 3; filled squares: type 4.

hand, some clustering did become apparent. Thus, eight of eleven type 1 afferents (Fig. 33, open squares) had conduction velocities of less than 60 m/sec and coefficients of variation under 0.03; two of the three remaining afferents had conduction velocities in the 60-80 m/sec range, and the final afferent had a conduction velocity of 82 m/sec. In contrast, thirty-two of thirty-four type 2 afferents (Fig. 33, crosses) had conduction velocities greater than 80 m/sec and coefficients of variation greater than 0.03, the final two type 2 afferents both having conduction velocities of 78 m/sec.

The other two classes of afferent identified by their pattern of SCh activation had passive properties which overlapped to a greater or lesser extent with those of type 1 and type 2 afferents. Thus, type 3 afferents (Fig. 33, filled triangles) had conduction velocities in the range 29-97 m/sec and coefficients of variation of 0.027-0.112. These ranges overlapped rather more with those of type 2 than with those of type 1 afferents; thus over half of the type 3 afferents (seven of twelve) had conduction velocities greater than 80 m/sec, and another quarter (three afferents) had conduction velocities in the range 60-80 m/sec. The range of coefficients of variation of type 3 afferents was almost identical to that of type 2 afferents, whilst only three of eleven type 1 afferents had similar coefficients of variation.

Reciprocal findings to those just described for type 3 afferents apply to type 4 afferent properties, for these overlapped more with those of type 1 than with those of type 2 afferents. Half (six of twelve) of the type 4 afferents had conduction velocities of 60 m/sec or less, and another four had conduction velocities in the 60-80 m/sec range; the final two type 4 afferents conducted at 88 and 94 m/sec. The coefficients of variation of type 4 afferents discharge ranged from 0.018 to 0.049, with nine of twelve under 0.032 and thus very similar to those of type 1 afferents.

Figs. 34-36 show similar plots for the other passive properties measured, namely the sinusoidal sensitivity of the afferents, their sensitivity to vibration of the muscle, and their dynamic indices in response to 3 mm, 10 mm/sec

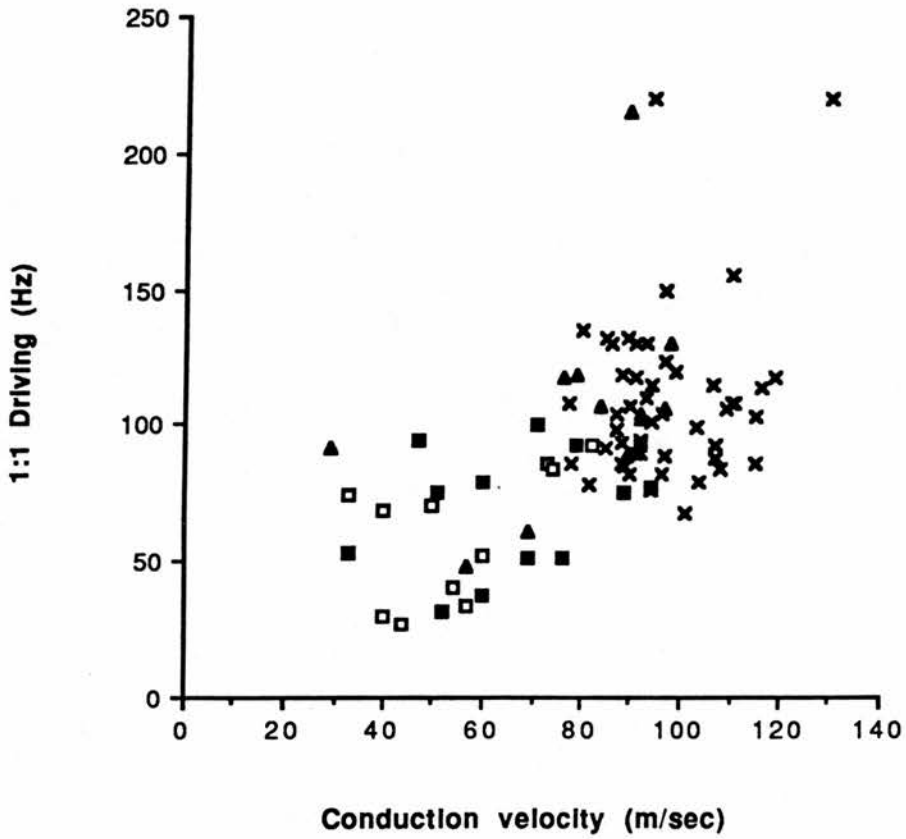


Fig. 34. Relationship between afferent conduction velocity and the highest frequency to which afferent discharge could be "driven" 1:1 by small-amplitude vibration for 82 medial gastrocnemius muscle spindle afferents. Symbols indicate pattern of SCh activation:- open squares: type 1; crosses: type 2; filled triangles: type 3; filled squares: type 4.

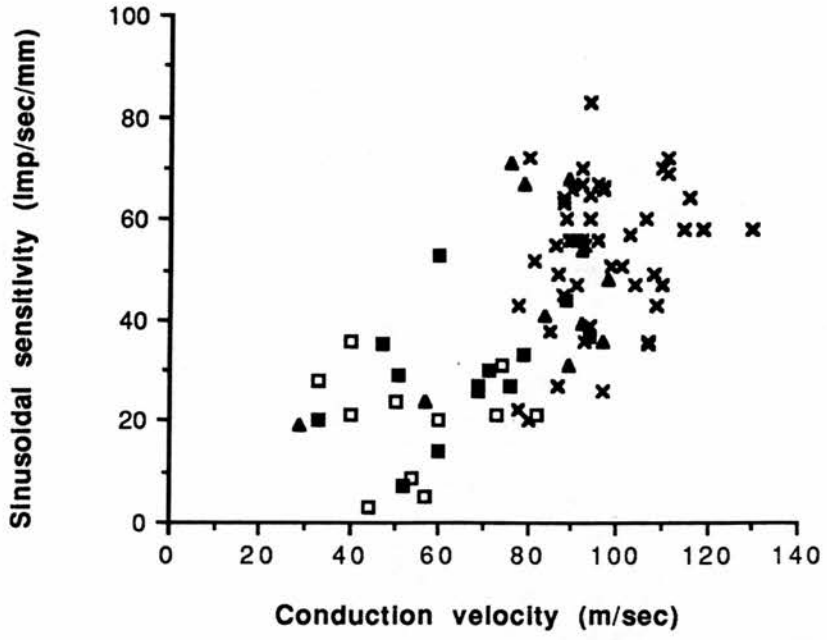


Fig. 35. Relationship between afferent conduction velocity and sensitivity to sinusoidal muscle stretching (1 Hz, 1mm) for 83 medial gastrocnemius muscle spindle afferents in which both measurements were made. Symbols indicate pattern of SCh activation:- open squares: type 1; crosses: type 2; filled triangles: type 3; filled squares: type 4.

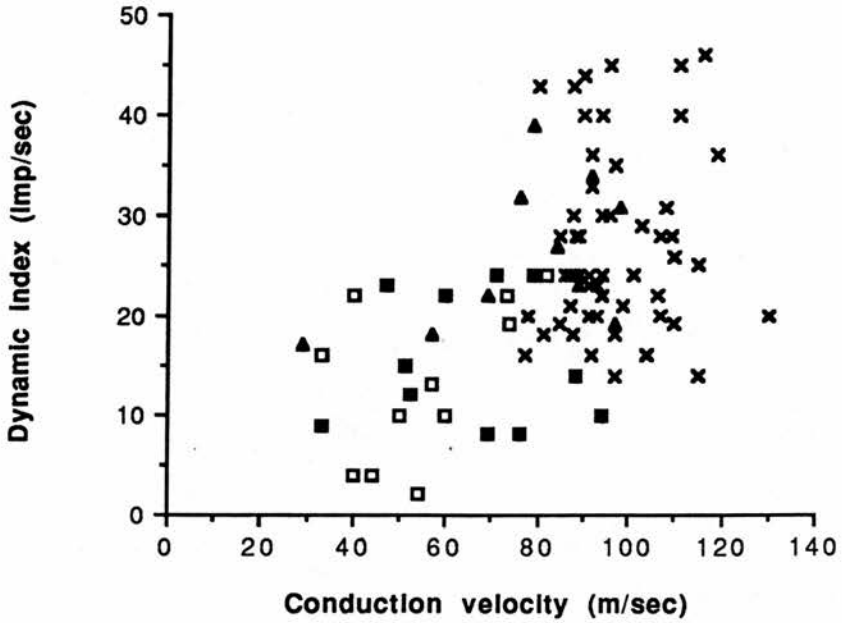


Fig. 36. Relationship between afferent conduction velocity and dynamic index in response to 3 mm, 10 mm/sec ramp stretching of 83 medial gastrocnemius muscle spindle afferents. Symbols indicate pattern of SCh activation:- open squares: type 1; crosses: type 2; filled triangles: type 3; filled squares: type 4.

ramp stretching. Each of these plots shows that, as was the case for coefficients of variation, afferents with higher conduction velocities tend to have higher values of the passive property illustrated in that figure, and that overall the passive properties of the whole population of afferents form continua, with no clustering which could be reliably utilised in the differentiation of subtypes of afferent. Again, taking into account the SCh activation of the afferents does reveal clustering of passive properties, with variation in the degree of overlap between the ranges of properties of each afferent type depending upon which passive property is considered. For example, the ranges of 1:1 driving of the different afferent types (Fig. 34) show relatively little overlap between type 1 and type 2 afferents: eight of eleven type 1 afferents are driven by vibration to less than 80 Hz, whilst forty-four of forty-seven type 2 afferent are driven to over 80 Hz. Of twelve type 3 afferents, ten are driven to over 80 Hz and thus behave almost identically to type 2 afferents, whereas nine of twelve type 4 afferents are driven to less than 80 Hz and closely resemble type 1 afferents in this respect. The plots of sinusoidal sensitivity (Fig. 35) and dynamic index (Fig. 36) against conduction velocity show rather more extensive overlap between the properties of the four classes of afferent, but it is again apparent that type 3 afferents behave more like type 2 than type 1 afferents, and vice versa for type 4 afferents.

Other authors have reported improved differentiation of afferent subtypes on the basis of passive properties alone when two of these are considered at once, for example by plotting a measure of the dynamic sensitivity of the afferents against a measure of their static sensitivity (e.g. Jami & Petit 1979). In the present experiments, four passive properties were measured, and plotting these against each other in pairs yielded 6 two-axis plots. I do not propose to illustrate all of these here; suffice it to say that in no instance was there an improvement in the ability to identify afferent subtypes on the basis of their passive properties alone. Only by taking the pattern of SCh activation into account could this be done. An example of this type of plot is shown in Fig. 37 where afferent sinusoidal sensitivity is plotted against dynamic

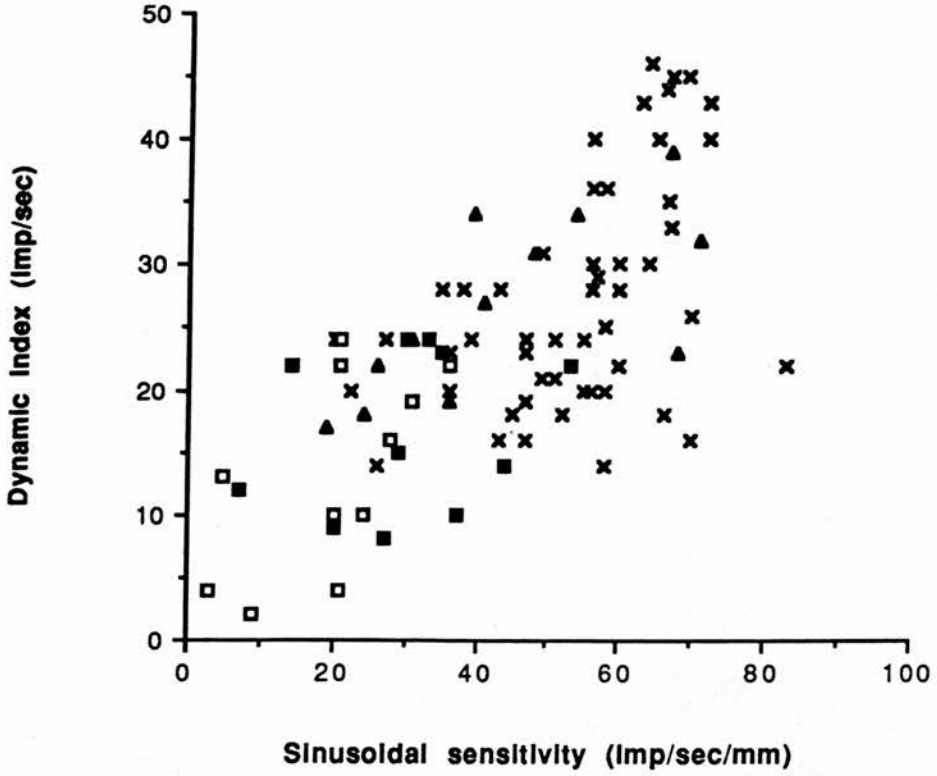


Fig. 37. Relationship between afferent sensitivity to sinusoidal muscle stretching and dynamic index in response to 3 mm, 10 mm/sec ramp stretching for 83 medial gastrocnemius muscle spindle afferents in which both measurements were obtained. Symbols indicate pattern of SCh excitation:- open squares: type 1; crosses: type 2; filled triangles: type 3; filled squares: type 4.

index. It is clear that, as in the plots of conduction velocity against passive properties, there is a continuum ranging from afferents with low values of each parameter to those with high values of each; there is no obvious clustering, and it is only when patterns of SCh activation are taken into account that it becomes clear that type 2 and 3 afferents tend to lie towards the upper right hand region of this plot, whereas type 1 and 4 afferents lie towards the lower left.

CHAPTER TWO

DISCUSSION

2:4.1. SCh activations of medial gastrocnemius afferents.

In the course of this second series of experiments, 88 medial gastrocnemius muscle afferents were studied in detail. In contrast to the position in the biventer experiments (Chapter 1), all of the medial gastrocnemius afferents could be positively identified as muscle spindle afferents on the basis of their responses to muscle contraction in the standard manner (Matthews 1972). It was therefore reassuring to find that medial gastrocnemius afferents showed four basic patterns of SCh activation, and that these were closely analogous to those seen for biventer afferents during the first series of experiments (compare Figs. 5-12 with Figs. 21-29); this suggests that the biventer afferents, which were identified only indirectly as spindle afferents, were probably correctly so classified. This observation extends to those biventer secondary afferents which were rather insensitive to stretch and the effects of SCh (e.g. Fig. 5), which, it was feared, might have been misidentified Golgi tendon organ afferents (see p. 158), since equally insensitive afferents encountered during the present medial gastrocnemius experiments were definitely identified as muscle spindle afferents (see Fig. 21).

The four patterns of SCh activation seen for medial gastrocnemius muscle spindle afferents were interpreted in exactly the same way as their analogues in the biventer experiments. I shall therefore not repeat the reasoning here, and instead shall simply state that the correlation between SCh typing and anatomical typing was deduced to be: type 1 afferents: spindle secondaries; type 2 afferents: b_1b_2c primaries; type 3 afferents: b_2c primaries; type 4 afferents: secondaries, possibly with bag fibre collaterals; for a full justification, see Chapter 1, pp. 158-183. The sample in the present series was intentionally biased towards more rapidly conducting afferents in order to ensure an adequate sample of b_1b_2c and b_2c primary afferents for study. In this discussion, I shall concentrate on those details in which medial gastrocnemius afferents differed from their biventer analogues, and in a later section I shall adduce more evidence to support the SCh-based classification of the medial gastrocnemius

afferents.

Essentially, the pattern of SCh excitation of secondary afferents was identical in the biventer and medial gastrocnemius experiments; the only minor difference was that medial gastrocnemius secondary afferents were probably activated slightly more quickly by SCh than biventer secondary afferents, although I have not illustrated time courses for the medial gastrocnemius afferents since these differences were small and felt merely to reflect a more direct path from the site of SCh infusion to the spindles in the medial gastrocnemius experiments (see p. 165-166). Similarly, the only difference in the SCh activations of b_2c primary afferents from biventer and medial gastrocnemius muscles was in the faster time course in medial gastrocnemius spindle afferents. More significantly, b_2c primary afferents were much commoner in the biventer series than in the medial gastrocnemius series; thus, nearly 50% of the presumed biventer primary afferents were of b_2c type, compared to about 18% for medial gastrocnemius. In the biventer experiments, b_2c primary afferents were probably somewhat over-represented, given the histological finding that about a third of biventer primary afferents derive from b_2c spindle units (Bakker & Richmond 1981), whereas in the medial gastrocnemius experiments the observed incidence is only slightly lower than the expected 23% of all primary afferents (Swett & Eldred 1960b). This difference probably reflects the greater sampling errors in the biventer experiments introduced by the technical difficulties involved in splitting peripheral nerves rich in connective tissue, rather than dorsal roots as in the medial gastrocnemius experiments. Allowing for this, the relative frequencies of presumed b_2c primary afferents from biventer and medial gastrocnemius are broadly as expected anatomically, which lends support to the SCh-based classification scheme.

There were more substantial differences between the patterns of SCh activation seen for biventer and medial gastrocnemius b_1b_2c primary afferents. Thus, although medial gastrocnemius b_1b_2c primary afferents were identified as such on the

grounds that they shared a substantial degree of biasing of their discharge as well as a marked increase in dynamic index with their biventer analogues when exposed to SCh, there was a most striking difference between b_1b_2c primary afferents from the two muscles in that, in all cases except perhaps that illustrated in Fig. 24, the medial gastrocnemius b_1b_2c primary afferents underwent a definite increase in position sensitivity upon exposure to SCh; this ranged widely in magnitude from a doubling of the control value to a twelve-fold increase. In contrast, biventer b_1b_2c primary afferents invariably showed a reduction in position sensitivity after exposure to SCh. It will be remembered that one point of difficulty in interpreting the biventer experiments was that the SCh activation of b_1b_2c primary afferents differed from that classically described by Dutia (1980) for soleus primary afferents in that there was no late increase in afferent position sensitivity (Phase III of activation). Although the medial gastrocnemius b_1b_2c primary afferent stuided in the present series therefore behaved more like the soleus primary afferents of Dutia (1980) than did the biventer b_1b_2c primary afferents, significant differences between the activations of soleus and medial gastrocnemius b_1b_2c primary afferents still exist, most importantly in the time course of the changes provoked by SCh infusion. Thus, in the present medial gastrocnemius experiments, the changes in stretch sensitivity of all but two b_1b_2c primary afferents afferents developed in only two Phases, whereas 18 of 20 soleus primary afferents developed substantial additional increases in position sensitivity during a distinct third Phase of activation in addition to those which developed during earlier phases of the activation. Only two medial gastrocnemius b_1b_2c primary afferents showed a third phase of activation analogous to that seen for soleus primaries (see e.g. Fig. 23), but this was not nearly as clearcut as that described by Dutia (1980), nor was the magnitude of the additional increase in position sensitivity which occurred during this third phase of activation as great as that seen for soleus primary afferents.

In his soleus experiments, Dutia (1978) found that most primary afferents exposed to intravenous infusions of SCh did not develop a Phase III response,

but that this situation reversed if the drug was infused intraarterially. Since activation of the bag_2 fibre was, at the time, held to produce an increase in position sensitivity of primary afferents (Literature Review, Part 3, p. 88), this finding was attributed to partial catabolism of an IV dose of SCh in the lungs and plasma whilst en route from the infusion site to the soleus muscle, such that the final dose at the spindle was insufficient to excite the bag_2 fibre. In the present experiments, the magnitude of the changes in stretch sensitivity of the medial gastrocnemius b_1b_2c primary afferents and the peak discharge rates achieved when they were maximally excited by SCh were both substantially less than those found for soleus primary afferents by Dutia (1978), and one possible interpretation of these findings is that the SCh levels produced in the vicinity of the medial gastrocnemius spindles was insufficient to recruit the bag_2 fibre in order to produce Phase III of activation; those increases in position sensitivity which did occur might simply have arisen from the incomplete adaptation of the afferent discharge from the very high rates achieved under bag_1 fibre influence at the peak of a ramp stretch.

Against this suggestion are the following observations: (i) the dose of SCh used routinely (100 μg/kg/min) was the same as that which had proved successful in Dutia's soleus experiments, as was the route of infusion via the contralateral common iliac artery (Dutia 1978); the blood supply to the soleus and medial gastrocnemius muscles is from the same trunk arteries and should be equally accessible via this route; (ii) there were marked variations within, as well as between, experiments in the degree of change in position sensitivity provoked in b_1b_2c primary afferents by this dose of SCh (see Fig. 26); in the extreme case, one b_1b_2c primary afferent in a given experiment might show a distinct Phase III of activation, whilst another might undergo only a very modest increase in position sensitivity over two phases of activation. In the present experiments, as in Dutia's soleus experiments (1978), there was very little surgical intervention in the vicinity of the muscle under study, and there was no reason to believe that either it, or its blood supply, were in less than optimal

condition; (iii) in three of the present experiments, a miscalibrated weighing scale resulted in the use of larger SCh doses than had been anticipated, and in these experiments increases in position sensitivity of b_1b_2c primary afferents were equally varied; in one instance, a b_1b_2c primary afferent was so powerfully excited by SCh that it developed depolarisation block (Kidd & Vaillant 1974), but there was no evidence at any time of late Phase-III-like behaviour, even though the SCh dose was 2.2 times the normal (Fig. 25); and (iv) the behaviour of presumed b_2c primaries from both biventer and medial gastrocnemius muscle spindles strongly suggested that the bag_2 fibre was in fact excited by the standard 100 μg/kg/min dose of SCh. It would thus seem unlikely that the differences in behaviour of medial gastrocnemius and soleus b_1b_2c primary afferents could be attributed to incomplete activation of the intrafusal fibres in some muscle spindles.

More recent studies of the effects of bag_2 fibre action on primary afferent sensitivity have shown this to consist of a biassing of the afferent discharge rate, with either no change, or a reduction, in position sensitivity (Boyd 1981b, 1986), rather than the large increase seen by Dutia (1980) in Phase III of activation. Nevertheless, a Phase III of activation undoubtedly occurs regularly in soleus primary afferents, and may also be seen in occasional medial gastrocnemius b_1b_2c primary afferents, so that some mechanism other than superadded bag_2 fibre action must be invoked to explain it. Alternatives which might be considered include: (a) SCh might affect individual bag_2 fibres differently, in some causing substantial shortening whilst in others causing only regional changes in stiffness and hence producing different inputs to b_1b_2c primary afferent discharge; these differences might reflect structural differences between bag_2 fibres such as the number of motor endings available for SCh to act upon; (b) there might be differences between b_1b_2c primary afferents in the distribution of sensory terminals to the several intrafusal fibres of a spindle, such that in some spindles the bag_1 fibre has a relatively greater influence upon the final Ia discharge than in others; (c) there might be differences between individual b_1b_2c

primary afferents in the way in which signals generated in the sensory spirals on each intrafusal fibre interact to determine the final afferent discharge; (d) the models developed from studies of other preparations to explain how the signals generated in different Ia sensory spirals interact to determine the final afferent discharge might not be applicable when SCh has been used to excite spindle afferents; SCh may produce a far more powerful activation of bag fibres than would ever occur *in vivo*, both in terms of a prolonged duration of activation compared to that generated via synaptic activation, and possibly also by acting on extra-synaptic acetylcholine receptors; the activity in primary sensory spirals must certainly be somewhat atypical when SCh has been used to activate a spindle, for only the bag fibre terminals will be excited, whereas when combined static and dynamic fusimotor action is used to excite a spindle, the sensory spirals on all three intrafusal muscle fibre types will be active; (e) some intrafusal fibre other than the bag_2 fibre may be responsible for generating Phase-III-like behaviour such as those chain fibres with m_a plate endings; the frequency with which chain fibres bear m_a plate endings in soleus and medial gastrocnemius spindles might then be supposed to vary; or (f) there may be some other difference between the experimental arrangements used in the various studies; for example, the SCh activations of individual b_1b_2c primary afferents might be dependent upon the resting length of the spindles involved, or upon the fractional length change produced by a muscle stretch as opposed to the absolute length change, and these might have been different in the soleus (Dutia 1980), biventer and medial gastrocnemius experiments (present work).

For most of these possibilities, there is insufficient information with which to decide their contribution to Phase-III-like behaviour in b_1b_2c primary afferents. For example, there has not yet been a systematic survey comparing the carriage rate of m_a plates on chain fibres in different muscles, and, similarly, whilst there is certainly variation between spindles in the number of motor terminals applied to individual intrafusal fibres (Banks, Barker & Stacey 1985), studies have not been

designed to examine systematic variations in this parameter between the spindles of several muscles. The possibility that SCh might affect different bag_2 fibres in different ways can, however, probably be discounted by the following reasoning: in visualised spindle experiments, bag_2 fibres all show substantial shortening when exposed to SCh rather than local stiffness changes (Smith 1966; Gladden 1976; Boyd 1985a); although rather few spindles have been examined in this way, and none of these have been biventer or medial gastrocnemius spindles, Boyd made an observation which suggests that his group's more extensive work using fusimotor-evoked bag fibre activity can be adduced to support the contention that bag_2 fibres all behave rather similarly when active. Thus, Boyd (1985a) showed for three visualised tenuissimus spindles that exposure to increasing SCh concentrations provoked progressive bag_2 fibre shortening and that the maximal shortening of the bag_2 fibre provoked by exposure to SCh was exactly the same as the shortening produced by high-frequency stimulation of all the static gamma axons innervating the bag_2 fibres in these spindles; sub-maximal doses of SCh induced incomplete contraction of the bag_2 fibre which could be made to contract maximally by superadded static gamma axon stimulation, and the combined effect of various levels of SCh and static gamma action equalled the maximal effect produced by either alone. These findings imply that SCh acts on the bag_2 fibres at the level of their motor terminals rather than extra-synaptically, and that it will not generate unphysiologically powerful contraction which might artefactually produce Phase-III-like behaviour. Furthermore, it suggests that the bag_2 fibre activity produced by stimulating static gamma axons is comparable to that provoked by the action of SCh, and here there is a much larger body of evidence which shows that bag_2 fibres all behave very similarly when activated (Boyd, Gladden, McWilliam & Ward 1977; Boyd 1981b, 1986; Boyd, Murphy & Moss 1985; Boyd, Murphy & Mann 1985; Boyd, Sutherland & Ward 1985). Finally, in the experiments reported here, the uniformity of the response to SCh of presumed b_2c primary afferents from both medial gastrocnemius and biventer also favours the conclusion that bag_2 fibres all behave similarly when active, undergoing substantial shortening to which the large biassing of

primary afferent discharge is to be attributed.

The possibility that the differences between muscles in the SCh-evoked changes in position sensitivity of b_1b_2c primary afferents might be dependent upon the resting length of the muscle or upon the fractional change in length of their spindles produced by a 3 mm ramp stretch, as was generally used in the present experiments and in Dutia's soleus experiments (1978), cannot be completely ruled out since the influence of these factors was not systematically assessed. However, the following points can be made: (i) the lengths of the muscular portions of soleus and medial gastrocnemius are similar at about 5 cm (Reighard & Jennings 1935), as are the mean lengths of their simple (i.e. non-tandem) spindles (Swett & Eldred 1960b), so that the fractional length changes with which soleus (Dutia 1980) and medial gastrocnemius (present experiments) b_1b_2c primary afferents were studied are likely to be comparable; the velocities of stretching in the two experiments were also comparable at 10-12 mm/sec; (ii) when b_1b_2c primary afferents were studied with several different amplitudes of ramp stretching (e.g. Fig. 23, D), the differences in afferent response were quantitative rather than qualitative; thus, the b_1b_2c primary afferent whose responses to 2, 3 and 5 mm ramp stretches when maximally activated by SCh are shown in Fig. 23, D would always have been classified as showing a Phase III response pattern, even though there was variation in the absolute discharge frequencies attained. Likewise repeating the SCh activation of this afferent when the resting muscle length had been reduced by 5 mm or increased by 3 mm produced only quantitative changes in behaviour, but did not convert the time course of the SCh activation from a three-phase pattern to a two-phase pattern; and (iii) even though the fractional length change of biventer muscle spindles produced by a 3 mm muscle stretch is quite likely to have been less than that applied to medial gastrocnemius spindles, since the biventer cervicis muscle is about 50% longer than medial gastrocnemius, b_2c primary afferents nevertheless behaved very similarly in the two muscles; by extension, the behaviour of the bag_2 fibre in b_1b_2c spindle units is unlikely to have been markedly different in the

two muscles, and yet their respective b_1b_2c primary afferents showed very different changes in position sensitivity when activated by SCh.

One factor which might well significantly influence the behaviour of different b_1b_2c primary afferents, and which is known to vary substantially between hindlimb muscles, is the intrafusal branching pattern of the Ia axon. Banks, Barker & Stacey (1982) have shown that there are two main patterns of branching, termed segregated and mixed. Most hindlimb Ia afferents (84%) divide to form 2 first-order branches; in the segregated pattern of distribution, one of these innervates the bag_1 fibre only, the other co-innervating the bag_2 and chain fibres, whereas in the mixed pattern the first-order branch supplying terminals on the bag_1 fibre also innervates bag_2 and/or chain fibres. Among tenuissimus Ia axons, 73% had a segregated distribution, whereas in the superficial lumbrical muscle 83% had a mixed distribution; soleus was intermediate with 53% segregated and 46% mixed. Given the current understanding that each sensory spiral is capable of generating action potentials, that the impulses arising in first-order branches of Ia axons interact by collision and antidromic invasion at branch points to determine the final afferent discharge, and that dynamic fusimotor action is readily occluded by static action (Literature Review, Part 1, p. 56), it would seem reasonable to suppose, as intimated by Banks, Barker & Stacey (1982), that b_1b_2c primary afferents with a segregated distribution of terminals might be more powerfully influenced by bag_1 fibre activity than those with a mixed distribution, since the bag_1 fibre input would interact at a relatively late stage (at the first branching node) with the impulses arising in the other sensory spirals and might be less readily occluded; such afferents might therefore have a relatively high dynamic sensitivity. In the mixed pattern of terminal distribution, the bag_1 fibre input might be expected to be partially occluded by early interaction with inputs from other intrafusal fibres to yield a mixed signal in the first-order branch supplying the bag_1 fibre, which would then undergo further interaction at the first branching node with impulses travelling in the other first-order branch; such Ia axons might be expected to receive a

relatively greater static than dynamic input. Hulliger & Noth (1979) have shown that static fusimotor action on primary afferent discharge readily occludes dynamic fusimotor action during the troughs of large-amplitude sinusoidal stretching cycles as long as the static-action-induced trough response is 25% or more greater than the dynamic-induced trough response, whereas at the peaks of stretching the static and dynamic actions partially summate; assuming this also to be the case for ramp stretching, it is conceivable that those Ia axons with a segregated terminal distribution would not show Phase-III-like behaviour when excited by SCh because the static input does not sufficiently exceed the dynamic input to totally occlude it at the short muscle length and at the long muscle length simply partially summates with it. Those Ia axons with a mixed terminal distribution might, as a SCh infusion progresses, develop a significantly greater static than dynamic input to the Ia discharge, so that the dynamic action is occluded at the short muscle length and summated with at the longer length, effectively increasing the change in discharge rate produced by each length change and hence increasing the position sensitivity of the afferent; this effect might only develop later in an infusion when the static drive sufficiently outweighs the dynamic drive, and thus might appear as a separate phase of the SCh activation, such as Dutia's Phase III of activation.

Physiological support for this idea is not very extensive, but nor is evidence to the contrary; that there are several spike initiation sites in Ia axons has been shown by a variety of workers studying pacemaker switching and dominance in Ia axons as was described in part 1 of the Literature Review (pp. 55-58) but only three reports suggest that individual Ia axons might vary with respect to the dominance and interaction of their pacemakers. Firstly, Hulliger & Noth (1979) have studied the effects of fusimotor action at fixed muscle length on the interspike intervals of soleus Ia axons, and have observed that, whilst in every case dynamic and static fusimotor action both altered the distribution of interspike intervals, in some Ia axons combined stimulation of both types of efferent caused a greater change than either alone had

produced (i.e. there was some summation of action), whereas for others combined stimulation produced no changes over and above those produced by static fusimotor action alone (i.e. there was occlusion of the dynamic by the static action). Hulliger and Noth (1979) attributed this variability in Ia response to "probabilistic mixing" (Eagles & Purple 1974) of the impulse trains arising in several first-order branches, but it is intriguing to speculate that it might instead be due to differences in the terminal distribution pattern of the Ia axons. Occluding responses to co-stimulation of static and dynamic fusimotor axons were seen for 62% of the eight Ia afferents studied, and summation for 38%; the reported frequency of segregated versus mixed terminal branching patterns for seventeen soleus Ia axons is 47% and 53% respectively (Banks, Barker & Stacey 1982); the hypothesis outlined above would predict that the proportion of occluding responses and mixed terminal distribution would be similar, as would be the proportions of summating responses to combined fusimotor stimulation and segregated terminal distribution. Although the observed values do not exactly match this expectation, they are only about 50% in error. It would be very interesting to make a comparison of this sort on the basis of a much larger sample of afferent responses and of reconstructed Ia branching patterns. Cheney & Preston (1976b), in a survey of fusimotor action on baboon soleus primary afferents, found summation of dynamic and static action for 68% of primaries and occlusion for 32%; these proportions are intriguingly close to the proportion of segregated and mixed Ia terminal distribution patterns of cat soleus, but the branching patterns of baboon soleus Ia axons are not available for comparison. The final piece of work along these lines is that of Schafer & Awiszus (1989) who studied the time course of the slow fall phase of adaptation after a ramp muscle stretch in the presence and absence of fusimotor activity, and found that for some Ia axons, the major determinant of the afferent discharge appears to be the input from the bag_1 fibre, whereas for others it appears to be an input from bag_2 and/or chain fibres. A correlation between the main determinant of Ia afferent discharge and the intrafusal branching pattern of the axon as proposed

above remains to be established; likewise, it is not yet known whether medial gastrocnemius Ia axons are particularly likely to have a segregated terminal distribution pattern, and biventer afferents to have a mixed distribution pattern, as would be expected under the proposed model, given the patterns of SCh excitation of their b_1b_2c primary afferents. Nevertheless, I personally favour some variant of this type of model over the alternatives mentioned above (p. 231-232) to explain the presence or absence of Phase III of SCh activation in b_1b_2c primary afferents.

Moving on to consider the type 4 pattern of SCh activation, the principle difference between the biventer and medial gastrocnemius patterns lay in the nature of the changes in stretch sensitivity which accompanied changes in afferent discharge otherwise reminiscent of presumed secondary afferents (type 1). In the biventer experiments, only one type 4 afferent was encountered, and this showed a clear increase in its position sensitivity; this afferent was identified as a secondary afferent, possibly with bag_2 fibre collaterals although this could not be stated with certainty, since the possibility that SCh might activate chain fibres bearing m_a plate endings to produce an increase in position sensitivity could not be ruled out (see p. 176 et seq.). In contrast, all eleven medial gastrocnemius type 4 afferents studied showed a clear increase in their dynamic index after exposure to SCh; for ten of the eleven afferents, this was accompanied by a smaller increase in the position sensitivity, though the eleventh afferent underwent a greater increase in position sensitivity than in dynamic index. These afferents were again identified as secondaries, but in this case, as in similar soleus afferents (Dutia 1980), it could be confidently stated that these secondary afferents had collateral terminals on the bag_1 fibre, since only contraction of this fibre has ever been shown to increase the dynamic index of secondary afferents (Boyd 1985a; Boyd, Murphy & Moss 1985); in particular, it has never been suggested (or shown) that contraction in chain fibres produced by m_a plate endings might increase dynamic index. The interpretation of the concomitant increase in position sensitivity shown by these ten medial gastrocnemius secondary afferents is, however,

subject to the same uncertainties with regard to bag_2 fibre versus m_a-chain fibre effects as hampered the interpretation of the biventer experiments, although it is clear from histological studies that the very large majority of secondary afferents which have bag_1 fibre collaterals also have bag_2 fibre collaterals (e.g. Banks, Barker & Stacey 1982), so that a bag_2 fibre contribution to the increased position sensitivity of these afferents is very likely to be present.

The relative frequency of presumed secondary afferents with bag_1 fibre collaterals was far higher in the present medial gastrocnemius experiments than it was in the soleus experiments reported by Dutia (1980) in which secondaries with presumed bag_2 fibre collaterals predominated. Only one analogous afferent was studied in the biventer experiments, and it did not have evidence of a bag_1 fibre input to its discharge. Histological studies directly comparing the intrafusal branching patterns of soleus and medial gastrocnemius secondary afferents do not appear to have been performed, but the survey of 14 hindlimb muscles, including soleus, carried out by Banks, Barker & Stacey (1982) revealed no systematic variation in the extent to which secondary afferents in different muscles formed collateral terminals upon the bag_1 and bag_2 fibres. If the proportion of all secondary endings which lie in the S1 position is taken as a rough index of the percentage of secondary afferents with bag_1 fibre contact, then about 79% of soleus secondary afferents have such contact as compared to 76% of peroneus brevis secondaries, 78% of extensor digitorum brevis secondaries and 83% of posterior interosseus secondaries (derived from data of Banks, Barker & Stacey 1982); a similar proportion of secondary endings would appear to terminate in the S1 position in biventer muscle spindles, and, as in hindlimb muscle spindles, these generally have collateral terminals on both the bag_1 and bag_2 fibres in addition to their chain fibre terminals (Richmond, Bakker, Bakker & Stacey 1986).

Variation between muscles in the incidence, during physiological studies, of secondary afferents with presumed bag_1 fibre collaterals presumably reflects, therefore, variation in the size of the input to the final secondary afferent

discharge deriving from equally common bag_1 fibre terminals, rather than variation in the frequency of bag_1 fibre contact (cf. Jami & Petit 1979). As was suggested above for Ia afferents, the origin of this variation in bag_1 fibre influence on the final afferent discharge may lie in the terminal branching patterns of secondary afferents, and in this case the known variation in the branching patterns of secondary axons provides a satisfying explanation for the relative scarcity of secondary afferents with presumed bag_1 fibre input in physiological experiments even though they are common anatomically. Thus, although as many as 73% of hindlimb secondary endings have bag_1 fibre terminals (Banks, Barker & Stacey 1982), a pattern of termination which might be envisaged as providing a high bag_1 fibre input to the final secondary afferent discharge, namely one in which a separate first-order branch supplied the bag_1 fibre whilst another branch co-innervated bag_2 and chain fibres, is only found for about 23% of secondary afferents. It is much more common to find one first-order branch supplying bag_1 and chain, or bag_1, bag_2 and chain fibres, whilst the other supplies bag_2 and chain fibres, and this mixed terminal distribution might, by interaction of pacemakers as was described above for Ia axons, be expected to diminish the influence of the bag_1 fibre on the final afferent discharge. It is not yet known whether medial gastrocnemius secondary afferents differ in terms of their terminal branching patterns from soleus secondaries in a way which would favour this hypothesis. An alternative source of the variation in the level of bag_1 fibre influence on secondary afferent discharge might be fluctuation in the relative areas of terminal contact on the bag_1, bag_2 and chain fibres; Banks, Barker & Stacey (1982) have examined the lengths of secondary endings distributed to bag fibres, as well as the number of terminals on each bag fibre, in a number of hindlimb muscles, but they have not reported their findings in a way which enables one to determine whether there are systematic differences in these parameters between muscles, so that a verdict on this possibility cannot yet be reached.

The numbers of secondary afferents with presumed bag fibre collaterals studied in both the present experiments and those of Dutia (1980) using the soleus

muscle were small, so that there is a risk of over-interpreting the results, but it is interesting to note that the medial gastrocnemius is a rapidly contracting, phasic muscle, whereas both soleus and biventer are tonic, anti-gravity muscles. A relatively large input to the sensitivity of secondary afferents from dynamic elements of the spindle apparatus may be a useful adaptation to the control of a phasic muscle, whereas a relatively large input from static elements might be appropriate to the control of tonic muscles, and these considerations might account for the higher proportion of secondary afferents with functional inputs from the bag_1 fibre in medial gastrocnemius than in soleus and biventer. If Phase-III-like behaviour of b_1b_2c primary afferents (as discussed above) is viewed as providing increased position sensitivity, then the absence of such behaviour in medial gastrocnemius b_1b_2c primary afferents, and its frequency in soleus primary afferents, might similarly be seen as adaptations to the control respectively of phasic and tonic muscles. These suggestions are the functional analogues of the anatomically-based hypothesis of Banks, Barker & Stacey (1982; in the cat), and Walro & Kucera (1985; in the rat) that the relative richness of lumbrical muscle spindles in bag_1 fibres compared to spindles in other muscles, might provide them with a high sensitivity to small length changes as an adaptation to the control of muscles involved in fine movements.

2:4.2. Evidence supporting the SCh-based diagnosis of afferent type.

As was the case in the biventer experiments, other evidence can be adduced to support the SCh-based identification of medial gastrocnemius spindle afferent types. In this case, it takes two forms: firstly, as in biventer, the passive properties of the various afferents can be considered, and secondly their conduction velocities can be taken into account. In fact, both pieces of information have been considered together by plotting each passive property against the conduction velocity of the afferent (Figs. 33-36). It is probably easiest to take one of these figures, e.g. Fig. 33, and discuss it specifically; most of the points made also apply to the plots of the

other passive properties versus conduction velocity.

The first feature to note from Fig. 33, in which the coefficient of variation of the resting discharge is plotted against conduction velocity, is that both the coefficient of variation and the conduction velocity vary smoothly, and the range of values for each parameter is very similar to that previously reported by other authors for hindlimb spindle afferents. Thus the coefficient of variation of the resting discharge varies from 0.018 to 0.112, and, as might be expected, this is very similar to the range reported for other hindlimb spindle afferents (0.016-0.16; Matthews & Stein 1969b); the range is also identical to that seen in the present series for biventer afferents (0.018-0.112; p. 140). The conduction velocities of the medial gastrocnemius spindle afferents varied from 29-128 m/sec, and this range is also similar to that previously reported for hindlimb spindle afferents (Matthews 1972). Within the continuum formed by the smooth variation of these two parameters, there is no evidence of grouping which might be used to identify different afferent types. However, if the pattern of SCh activation of the afferents is taken into account, clustering does become apparent; thus 73% of presumed secondary afferents (open squares) had, as would be expected, conduction velocities of less than 60 m/sec, and all but one of the rest conducted at 60-80 m/sec. Similarly, secondary afferents had, as expected (Matthews & Stein 1969b), low coefficients of variation of their resting discharge; if the coefficients of variation of those secondary afferents identified by SCh which also had conduction velocities in the classical secondary range (<60 m/sec), are considered separately, then the range of coefficients of variation observed was 0.018-0.039, with most in the range 0.018-0.026 which agrees reasonably well with the range seen by Matthews & Stein (1969b) for soleus secondary afferents. Taken together, the combination of afferent conduction velocity and coefficients of variation provide substantial support for the SCh-based identification of these afferents as secondaries. A similar situation applies when considering the coefficients of variation and conduction velocities of presumed b_1b_2c primary afferents, and also when the other passive properties are compared with earlier

reports (dynamic index values: Matthews 1963; vibration sensitivity: Brown, Engberg & Matthews 1967; Trott 1976; sinusoidal sensitivity: Cussons, Hulliger & Matthews 1977; Hulliger, Matthews & Noth 1977a).

That medial gastrocnemius b_2c primary afferents (filled triangles in Figs 33-36) have in fact been correctly identified by SCh in the present experiments is favoured by the facts that: (i) more than half of the presumed b_2c primary afferents had conduction velocities in the primary range (>80 m/sec), whilst another quarter of the afferents had conduction velocities in the intermediate 60-80 m/sec range; this range of conduction velocities is in good agreement with that expected on the basis of anatomical work (e.g. Banks, Barker & Stacey 1982) which indicates that the intrafusal diameters of hindlimb b_2c primary afferents overlap extensively with those of the smaller b_1b_2c primary afferents; and as well as with the larger secondary afferents; and (ii) the values of the four passive properties observed for presumed b_2c primary afferents also overlap more extensively with those of b_1b_2c primary afferents than they do with those of secondary afferents (compare the crosses, filled triangles and squares in Figs. 33-36). Analogous arguments, but applied the other way around, favour the view that the afferents classified by SCh as secondaries with bag fibre collaterals were accurately identified; thus, most of these afferents either had conduction velocities in the secondary range (<60 m/sec), or in the intermediate range, as would be expected from the anatomical studies (Banks, Barker & Stacey 1982; Boyd 1985a), and their passive properties tended to overlap more extensively with those of other secondary afferents than with those of primary afferents.

The data in Figs. 33-36 also reveal substantial new information which earlier studies could not. Thus, it has been known since the work of Hunt (1954) that there is no absolute cut-off point in the conduction velocity distribution below which a spindle afferent can be definitely identified as a secondary and above which as a primary; this has led to the adoption of an arbitrary range of 60-80 m/sec for hindlimb muscles, such that any spindle afferent with a conduction velocity in this range is said

to be "intermediate" and can only be reclassified as primary or secondary by some other test (Literature Review, Part 2, p. 75 et seq.). Often this has been on the basis of a passive property of the afferent; for example, an afferent conducting at 70 m/sec would initially be called "intermediate", but might be reclassified as a secondary afferent on the basis of a low coefficient of variation in the same range as that seen for "typical" secondary afferents conducting at less than 60 m/sec. There are two assumptions inherent in this method: (i) that all afferents conducting at less than 60 m/sec are secondaries and at more than 80 m/sec are primaries; and (ii) that the range of values of a particular passive property seen for "typical" primary or secondary afferents applies also to those with intermediate conduction velocity. The results shown in Figs. 33-36 allow a judgment to be passed on the accuracy of these assumptions, since there is an independent arbiter of afferent type, namely the pattern of SCh excitation.

Examination of Fig. 33 reveals firstly that the vast majority of afferents conducting at less than 60 m/sec would also be classified as secondary afferents, with or without evidence of bag fibre collaterals, on the basis of their patterns of SCh excitation. Likewise, the vast majority of those conducting at over 80 m/sec would be classified by SCh as primary afferents of both b_1b_2c and b_2c type. The exceptions to this for secondary afferents are the two b_2c primary afferents (filled triangles) which conduct at 29 and 58 m/sec respectively. The SCh activation of the afferent conducting at 29 m/sec is shown in Fig. 28, and there is little doubt that this afferent showed the b_2c primary pattern; it did, however, also have a remarkably high coefficient of variation, which went totally against the trend evident in Fig. 33 for more slowly conducting axons to have lower coefficients of variation, and it is possible that this afferent's conduction velocity was artefactually low, perhaps as a result of partial conduction block produced by damage to the axon during its isolation. In the other direction, there were 3 secondary afferents which conducted at over 80 m/sec, two of which showed evidence of bag fibre collaterals (filled squares) and one of which did not (open squares); again, there was little doubt from the patterns of SCh activation

that these afferents were not primaries. All in all, when afferents are classified as primary or secondary on the basis of conduction velocities less than 60 m/sec or greater than 80 m/sec, the level of contamination in the sample is likely to be very small. The passive properties of afferents conducting at under 60 m/sec therefore truly represent those of secondary afferents, and those of afferents conducting at over 80 m/sec represent those of primary afferents.

The assumption that afferents conducting at intermediate velocities have passive properties in the same range as their more typical analogues, and that these can be used to reclassify them as primaries or secondaries, is less well supported. Thus, it is apparent from Fig. 33, and even more so from Figs. 34-36, that there is a distinct trend in the data such that more rapidly conducting axons have higher coefficients of variation, dynamic indices *et cetera.* Trends of this sort have been seen before for hindlimb muscle spindle afferents in the relationships between dynamic index and conduction velocity (Matthews 1963; cat), coefficient of variation and conduction velocity (Andrew, Leslie & Thompson 1973; rat) and coefficient of variation and conduction velocity (Cheney & Preston 1976a; baboon); the present findings extend some of these observations to cat hindlimb muscle spindles. *A priori,* this suggests that it is unlikely to be true, for example, that secondary afferents with intermediate conduction velocities will have coefficients of variation in the same range as those secondaries which conducted at less than 60 m/sec; rather, they may be expected to have somewhat greater coefficients of variation, and likewise those primary afferents with intermediate conduction velocities might be expected to have somewhat lower coefficients of variation than primaries conducting at over 80 m/sec. Using the pattern of SCh activation to classify afferents conducting in the 60-80 m/sec range, it is, in fact, apparent, that the four recognisable afferent types are equally represented, and that for each passive property there is very substantial overlap between values seen for primary (crosses and filled triangles in Figs. 33-36) and secondary (open and filled squares in Figs. 33-36) afferents, such that it would not be

feasible to use the passive properties of these afferents alone to reclassify them; this explains the failure of early attempts to identify b_2c primary afferents on the basis of passive properties alone (Richmond & Abrahams 1979; Banks, Ellaway & Scott 1980). Only the afferents' patterns of SCh excitation can be used to differentiate them reliably, and the caution shown by earlier workers when studying afferents with intermediate conduction velocities (e.g. Matthews 1963; Trott 1976) is seen to be amply justified.

The ability of SCh to classify afferents with intermediate conduction velocities which would otherwise be unclassifiable permits construction of more accurate ranges of the passive properties of primary and secondary afferents, since all such afferents will be included in the construction of the range, not just those with afferent conduction velocities in a restricted band. If this is done, the ranges are generally somewhat wider than those found when afferents were classified by conduction velocity. For example, the range of sinusoidal sensitivity of secondary afferents changes from 4-36 Imp/sec/mm when conduction velocity is used to identify secondaries to 4-53 Imp/sec/mm when pattern of SCh excitation is used. This results in increased overlap between the passive properties of primary and secondary afferents, and makes it even more difficult to justify the classification of spindle afferents on the basis of passive properties alone. Faced with problems of overlapping ranges of passive properties of this sort, other workers have plotted several passive properties against each other in an attempt to reveal clustering of properties which was not otherwise apparent and which might be used to identify afferent types (Literature Review, Part 2, p. 77). In the biventer experiments reported above, this process failed to help in the differentiation of afferent types (see Fig. 18), and this was also the case in the present medial gastrocnemius series. All four passive properties were plotted against each other in pairs, but only one such plot is shown in Fig. 37 (sinusoidal sensitivity versus dynamic index). Neither in this case, nor in any of the others, was there any way of differentiating the various afferent types other than on the basis of

their patterns of SCh excitation.

A final point of interest in Figs. 33-36 is that, whilst it is almost always true that an afferent conducting at more than 80 m/sec is a primary afferent, this does **not** mean, as has often been tacitly assumed by other workers, that the afferent in question is a b_1b_2c primary. From the data presented here, it would appear that only medial gastrocnemius afferents conducting at over 100 m/sec can reliably be assumed to be b_1b_2c primaries without further investigation, and this cut-off point would presumably vary in different muscles. Medial gastrocnemius muscle spindle afferents conducting impulses at 80-100 m/sec must be considered to have been incompletely characterised until the SCh test has been performed to differentiate b_1b_2c from b_2c primary afferents. Although b_2c primary afferents from hindlimb muscles are generally quite rare, and only about half of them conduct at over 80 m/sec, so that the potential for misidentification is low, there are exceptions which must be borne in mind, particularly the peroneus tertius and medial gastrocnemius muscles in which perhaps 1 in 8 "primary" afferents might be b_2c type (Swett & Eldred 1960b; Scott & Young 1987).

CHAPTER THREE

Experiments performed on the tenuissimus muscle of the hindlimb.

3:1 EXPERIMENTAL RATIONALE.

This final series of experiments was performed in an attempt to histologically identify the spindles of origin of identified b_2c primary afferents in order to confirm that they did indeed originate in single-bag spindles. Thanks to its architecture, the tenuissimus muscle suggested itself as the most suited to such an attempt, since its complement of muscle spindles is arranged in a line down the middle of the muscle with relatively little overlap between the subvolumes of the muscle occupied by individual spindles (Boyd 1956). Thus, particularly towards the ends of the muscle, a transverse section of tenuissimus generally only contains one spindle, and if more than one are present their respective equators are usually separated longitudinally by 4 to 5 mm (Fehr 1965; Kucera 1982b).

Given a method of accurately defining the point of origin in the muscle of a spindle afferent, the desired correlation of electrophysiology and histology should therefore be feasible. Such a method has in fact been described by Bessou & Laporte (1962) who used it to identify primary and secondary afferents originating in the same tenuissimus spindle capsule. The reported spatial resolution of the technique, whose application is described below, is on the order of 0.5 mm, and it has been used before to great effect by Boyd's group to assist in the localisation of spindles during their isolated spindle experiments. The proposed experiment was therefore to isolate tenuissimus spindle afferents in the lumbosacral dorsal roots, to classify them by their pattern of SCh activation, to localise any interesting afferents thus encountered with the technique of Bessou & Laporte (1962) and to mark their position in the muscle for subsequent histological examination.

Unfortunately, there is a drawback to the use of the tenuissimus muscle in this experiment, namely that tandem spindles are rather rare in it:- in a study of the spindle content of the mid-portion of tenuissimus, Kucera (1982) found only about 6% of primary axons to come from b_2c spindle units. The problem is

compounded by the small number of spindles contained in the average tenuissimus muscle, which Boyd (1956) states to be about 15; this leads to the expectation that a given tenuissimus muscle is likely to contain only one b_2c spindle unit (6% of 15). This figure may be a slight underestimate since it is based on the spindle content of the middle part of the muscle, whereas there is evidence that b_2c spindle units are more common near tendons (Bakker & Richmond 1981), but even allowing for this there are unlikely to be more than 2 b_2c spindle units per tenuissimus muscle.

3:2.1. SURGICAL PROCEDURES.

Seven cats weighing 1.9-2.6 kg were used in this final series of experiments. Details of the anaesthetic and general surgical procedures are the same as for the two previous series. Cannulae were placed in the trachea and an external jugular vein, but no arterial cannula was inserted. The right hindlimb was extensively denervated, leaving only the thin nerve to tenuissimus intact. A lumbosacral laminectomy was performed, the L6-S1 ventral roots were divided and the L6-S1 dorsal roots were prepared for splitting and recording as in the medial gastrocnemius experiments. The stretching and recording apparatus was identical to that used in the medial gastrocnemius experiments.

The tenuissimus, a thin, straplike muscle 9-12 cm long and a few millimetres wide (Boyd 1956), has its origin on the transverse process of the second caudal vertebra from where it runs obliquely beneath the biceps femoris muscle towards the knee. In the lower part of its course, it fuses with biceps femoris and joins the same fascia to insert into the upper lateral part of the tibia (Reighard & Jennings 1935). In the present experiments, the muscle was exposed from the area of the sciatic notch to as close to the knee as possible where it was cut and attached by a stout thread to the muscle puller. The animal was placed in a supporting brass frame and the leg was held horizontally by hip pins and by clamps fixing knee and ankle joints. The tenuissimus nerve was placed over a bipolar hook electrode for

stimulation and the tenuissimus was stretched to about its in situ length.

Dutia (unpublished findings) has found that even intra-arterial infusions of SCh do not reliably activate tenuissimus muscle spindles, so it was decided to apply SCh topically by dripping it onto the surface of the muscle over the region containing the spindle whose afferent was being studied; it was hoped that, since the tenuissimus is nowhere more than 2 or 3 mm thick (personal observations), the SCh would diffuse in to the spindles in sufficient quantities to excite the intrafusal fibres. As a result of this stratagem, the customary paraffin oil pool could not be used to prevent dessication since it would have restricted access of the SCh to the muscle surface; instead, the majority of the exposed muscles were covered with pieces of tissue paper soaked in warm physiological saline, and warm saline was subsequently periodically sprayed onto the tissues with an atomiser. Tenuissimus was kept moist during the isolation of single units by slackening the muscle until it lay in a pool of saline which formed at the bottom of the leg incision; when an afferent was being studied, the muscle was stretched out again, saline was sprayed onto it periodically, and a tent of moist tissues was arranged to cover the muscle without touching it so that stretches could still be applied. During the administration of SCh, the saline in which the drug was dissolved also served to keep the muscle moist.

3:2.2. PREPARATIONS FOR RECORDING.

The nerve to tenuissimus enters the muscle about half way along its length where it divides into ascending and descending branches which supply axons to the spindles as they run towards the ends of the muscle (Boyd 1956). When a tenuissimus spindle afferent was isolated in the dorsal roots, its spindle of origin was localised with a roving surface stimulating electrode (Bessou & Laporte 1962) as illustrated schematically in Fig. 38. The two poles of a bipolar silver ball electrode were placed on the muscle surface next to the point of entry into the muscle

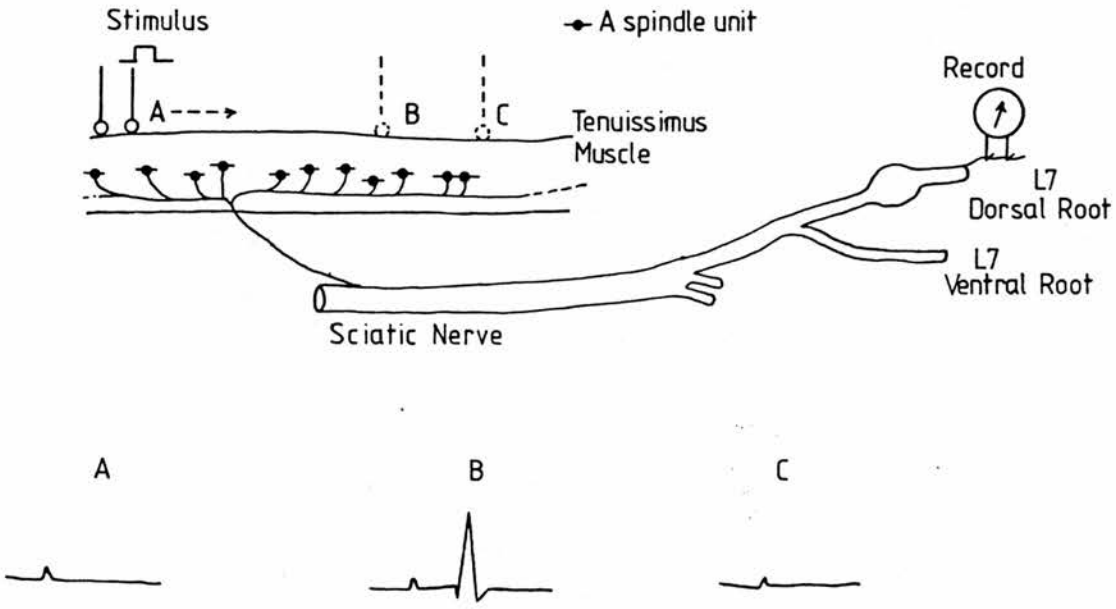


Fig. 38. Schematic representation of the method of localisation of the spindle of origin of tenuissimus muscle spindle afferents. *Top* Representation of the tenuissimus muscle, its innervation and the arrangement of spindle units within it. *Below* Schematic representation of traces recorded from dorsal root filaments in response to stimuli applied to the muscle surface at points A-C shown at top. Stimuli applied at point A produce the trace in panel A, those applied at point B the trace in panel B and so on. See text for more details.

of one of the divisions of the tenuissimus muscle nerve (Fig. 38, point A), and brief shocks (0.01 msec; 3-5 X threshold for producing visible muscle contraction) were applied about once per second. These shocks electrically activated axons in the muscle underlying the cathode, but in this initial position did not activate the afferent which was being studied in the dorsal root filament, as shown by the flat trace after the stimulus artefact in panel A of Fig. 38. When the stimulating electrode was moved to point B (Fig. 38) so that shocks now activated axons in the other main division of the tenuissimus muscle nerve, an evoked potential then appeared in the dorsal root filament with constant latency after the muscle stimulus, indicating that the parent spindle of the axon under study was in the half of the muscle served by that division of the nerve (Fig. 38, panel B).

Having determined which half of the muscle the spindle lay in, the ball electrode was then moved in steps from the nerve entry point towards the appropriate end of the muscle whilst continuing to stimulate. Action potentials continued to be evoked in the dorsal root filament, though at gradually increasing latency due to the extra conduction distance from the point of activation; beyond point C (Fig. 38), however, the shocks no longer evoked an action potential in the dorsal root filament (Fig. 38, panel C), implying that the electrode had been moved past the afferent's point of origin in the muscle, i.e. past the capsule of the parent spindle. Under microscopic guidance, the electrode was then moved in very small steps around this area to identify the exact point at which afferent action potentials could no longer be evoked by muscle stimulation; the relationship of some surface feature of the muscle to this point was noted so that SCh could subsequently be applied directly over the afferent's parent spindle. For selected afferents, the localisation was repeated after they had been fully studied, and the positions of the spindles were marked by placing 9/0 silk sutures through the muscle proximal and distal to them; the position of each spindle was noted relative to these two markers, for example as 1/3 of the way from the proximal marker to the distal marker, in order to

allow for shrinkage during subsequent histological processing. The placement of marking sutures often transected the intramuscular nerve, disconnecting spindles beyond the sutures from the dorsal roots, so that the yield of marked spindles was rarely more than two per experiment, one from each half of the muscle.

In this series of experiments, afferents with a relatively short conduction latency (< 2 msec) between muscle nerve and dorsal root filament were selected for further study in an attempt to preferentially study primary afferents. Having located an afferent's parent spindle as has just been described, the muscle was subjected to repeated ramp-and-hold stretches of 3 mm amplitude and 10 mm/sec velocity while SCh was allowed to drip from a red Luer cannula onto the muscle surface immediately above the spindle; this was continued until there was no further change in the afferent response to stretch after which the muscle was irrigated with warm saline to wash off the SCh, and the afferent response to stretch was monitored until it had returned to its control state before another afferent was tested. Initially, the concentration of SCh used was 250 μg/ml, but this was subsequently reduced to 100 μg/ml since the higher dose appeared to cause depolarisation block in some afferents. The passive properties measured in the first two series of experiments were not measured during this final series.

3:2.3. HISTOLOGICAL PROCESSING.

The marked blocks of muscle were cut out at the end of the experiment and allowed to relax in a moist atmosphere for about 15 minutes before being pinned out at their relaxed length on pieces of wax. The processing of blocks is summarised in Fig. 39. Serial transverse sections were cut right through the block on a rotary microtome (Reichert-Jung), generally at a thickness of 6 μm, and every twentieth section was floated out on a warm water bath and mounted on gelatin-coated glass slides. Sections which were not mounted initially were kept so that any sections damaged during processing could be replaced with a neighbouring section from the

block. Batches of slides were then processed for routine Haematoxylin and Eosin staining (Fig. 40), and the stained sections were examined under an inverting microscope (Nikon Labophot, with camera attachment); the epimysial sutures marking a spindle were located in the sections, and since their positions relative to the spindle capsule they marked were known, the sections likely to contain the spindle could be identified. Any spindles seen in this region of the block, were traced through as many serial sections as possible, making a note of the number and size of intrafusal fibres present, and photomicrographs were made of representative sections. A graticule marked out at 10 μm intervals was used to calibrate measurements, but no corrections were made for shrinkage during processing.

PROCESSING PROTOCOL FOR BLOCKS OF TENUISSIMUS

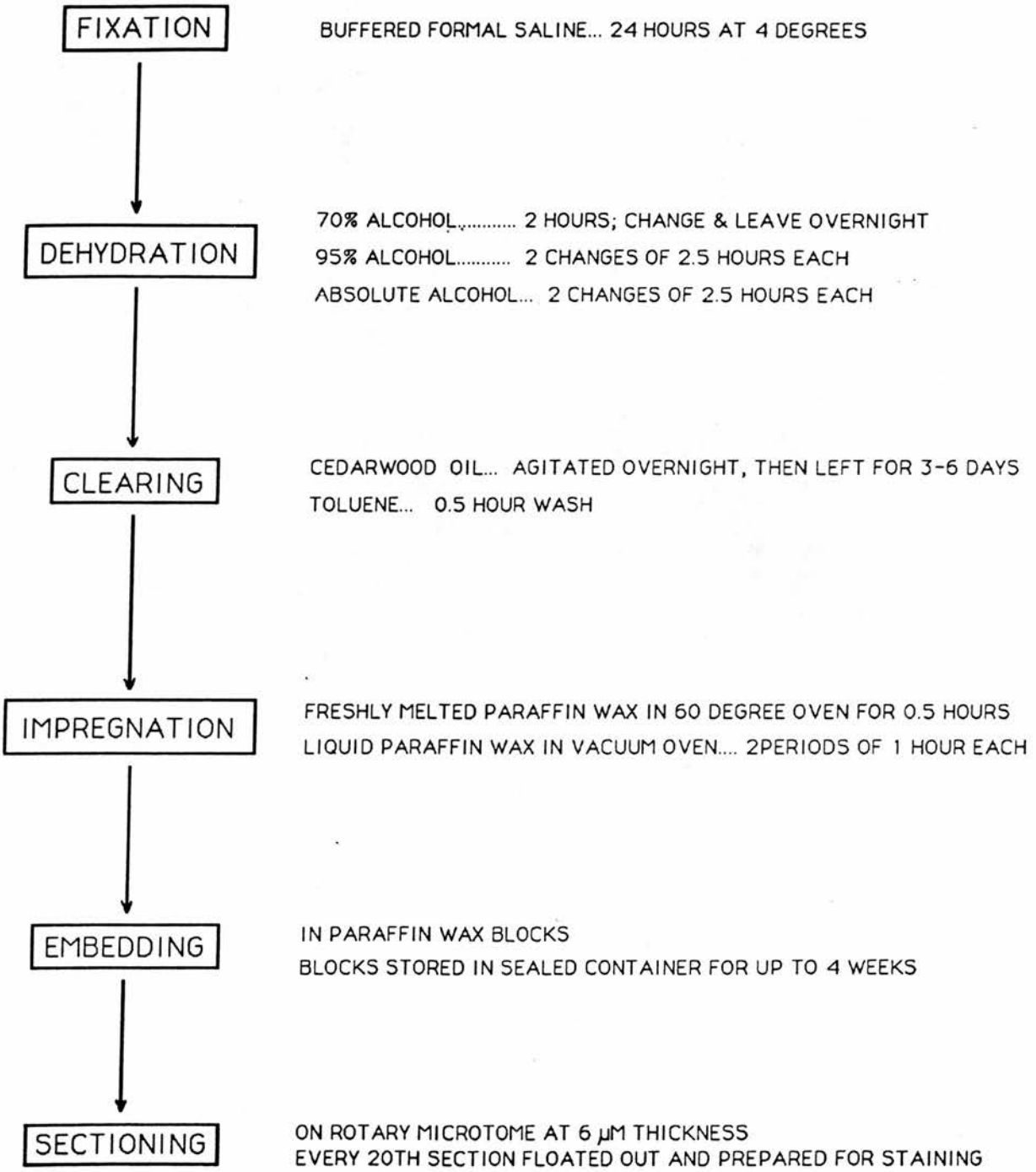


Fig. 39

STAINING PROTOCOL FOR TENUISSIMUS SECTIONS

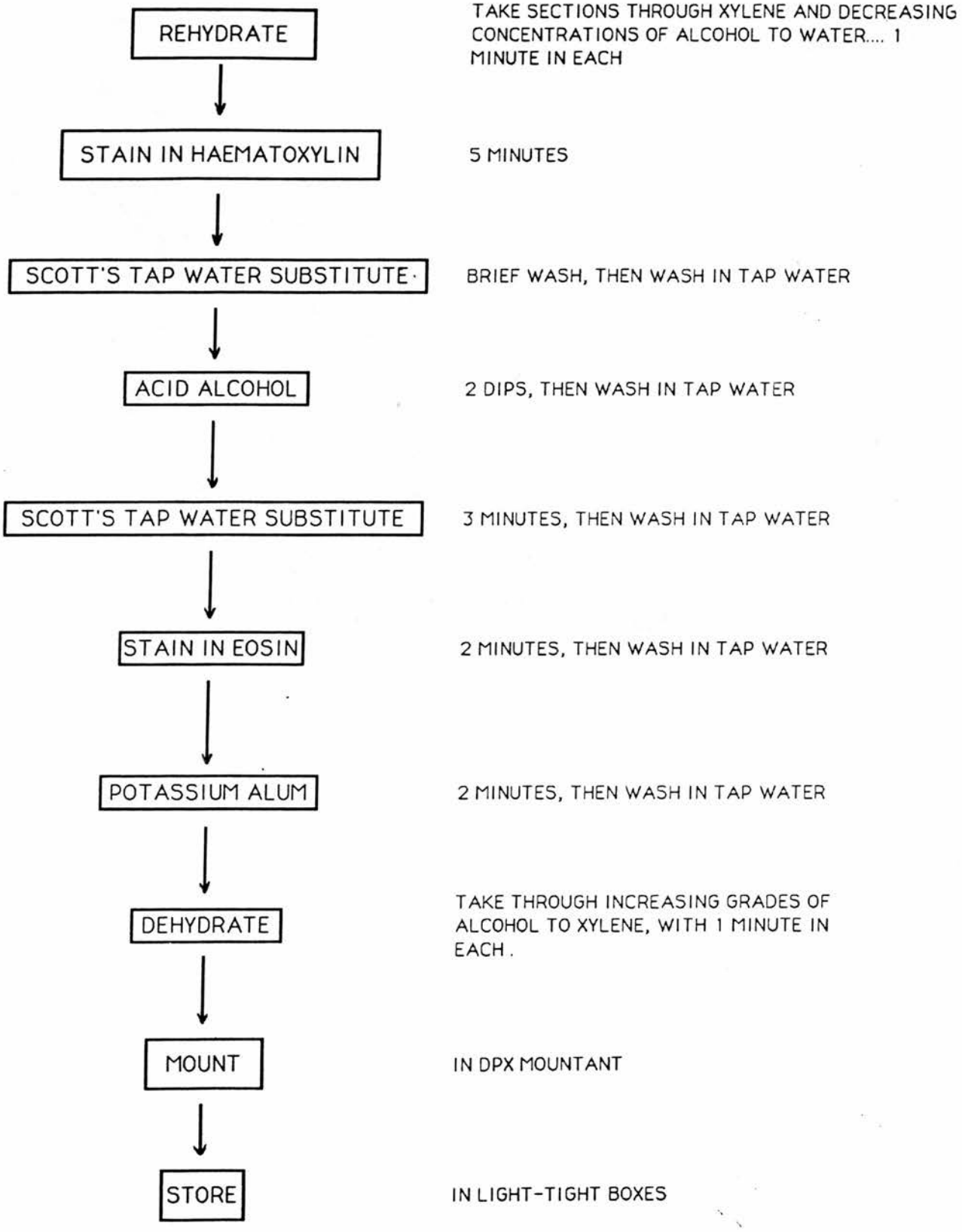


Fig. 40

CHAPTER THREE

RESULTS

In the course of these 7 experiments, a total of 34 muscle spindle and 3 Golgi tendon organ afferent axons were isolated in the L6 and L7 dorsal roots. As was described above, the fastest conducting of the muscle spindle afferents were selectively sampled, on the assumption that, as in the medial gastrocnemius muscle, b_2c primary afferents would have conduction velocities which overlapped heavily with those of b_1b_2c primary afferents. The cut-off point used was arbitrarily set at a conduction latency of 2 milliseconds to the dorsal roots, which, under the experimental conditions described above, corresponded to an afferent conduction velocity of 60-70 m/sec. Of the 34 muscle spindle afferents encountered, 14 were selected for further study on the basis of their conduction latency, and of these the region of the muscle from which the afferent originated was determined for 8 afferents and subsequently examined histologically.

In this final series of experiments, only the afferents' pattern of excitation by SCh was determined, and this was done by topical application of the drug rather than by the intra-arterial infusions that were used in the earlier series. Although the SCh activations produced by this route of administration were rather more rapid than those produced by intra-arterial infusions, reaching maximal activation in under 1 minute from the time of application of the drug, nevertheless the details of the changes in stretch sensitivity of tenuissimus spindle afferents provoked by exposure to SCh were very similar to those previously observed for medial gastrocnemius spindle afferents.

Afferent responses to SCh fell into three classes corresponding to types 1, 2 and 3 as defined above. The largest group, 12 of the 14 afferents, were excited by SCh in the manner illustrated in Figs. 41 and 43 and were identified as type 2 afferents. The afferent whose response is shown in Fig. 41 had an initial discharge rate of 18 Imp/sec, a dynamic response of about 60 Imp/sec and a position response of 28 Imp/sec in the control condition, from which it can be calculated that the dynamic index was around 32 Imp/sec and the position sensitivity about 3.3 Imp/sec/mm (Fig.

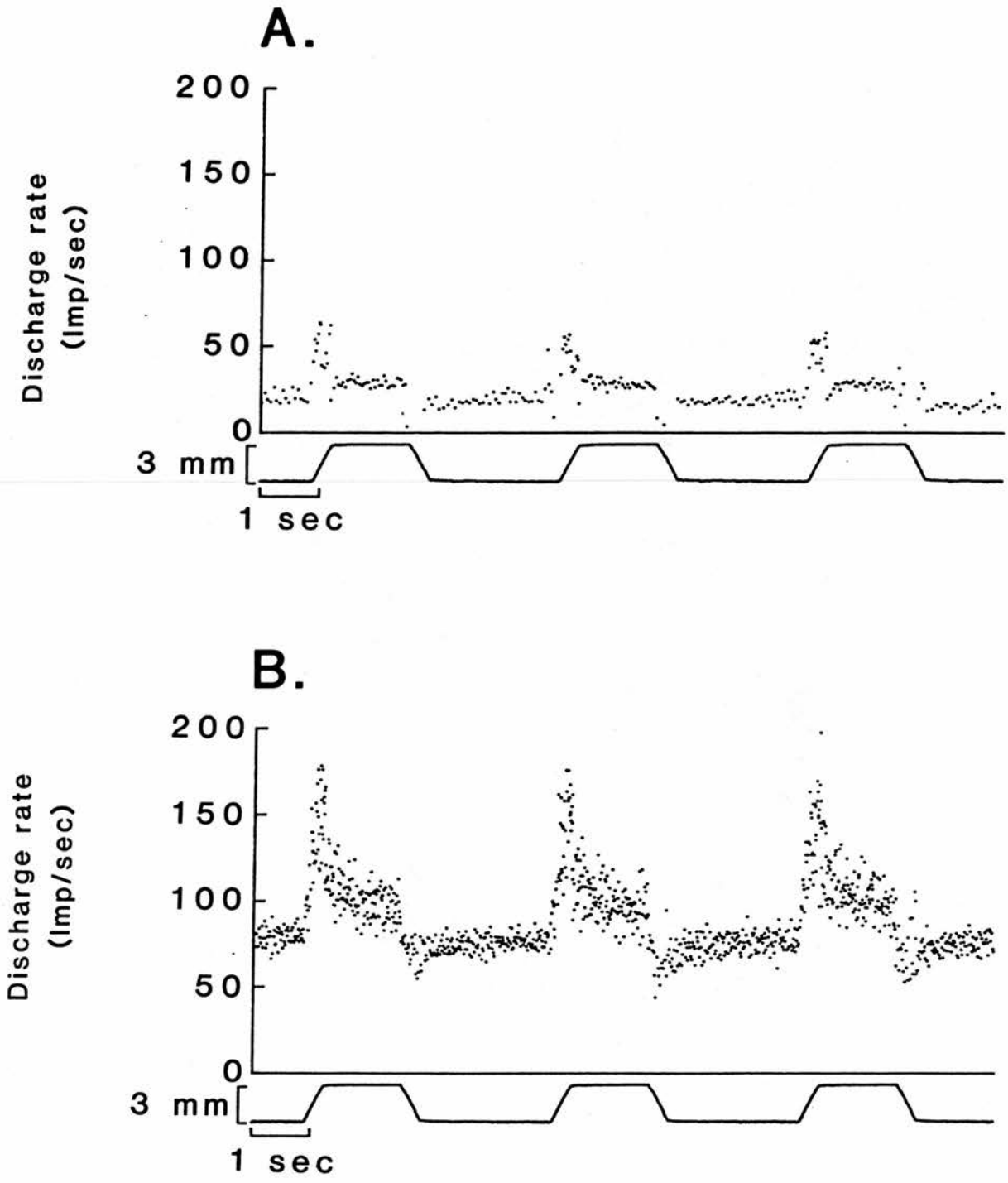
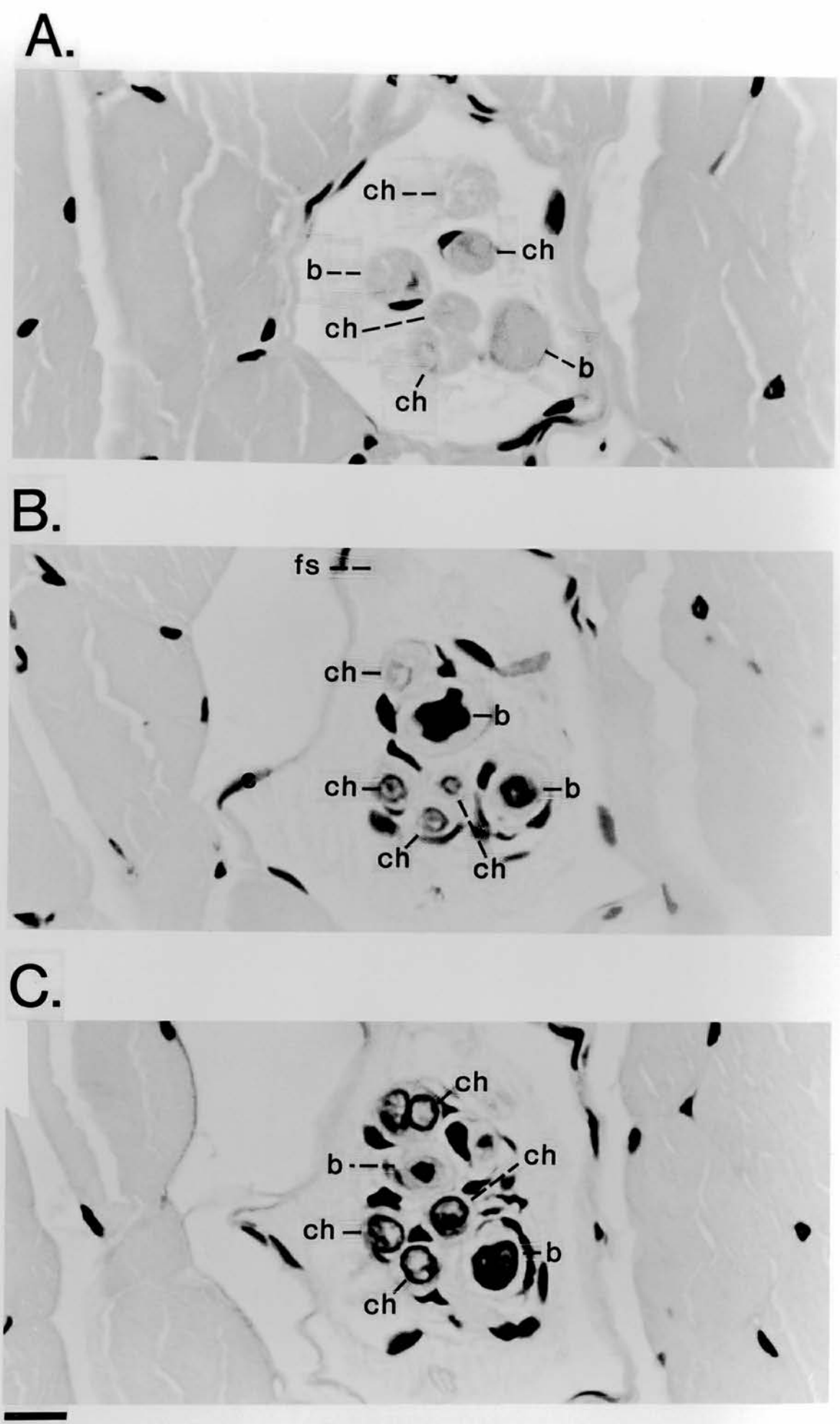


Fig. 41 A, B. Pattern of SCh excitation of a tenuissimus type 2 afferent. Response of this afferent to stretching (3 mm, 10 mm/sec) before **(A)** and 60 seconds after **(B)** topical application of SCh (100 μg/ml) to the surface of the muscle overlying the spindle of origin of this afferent. Conduction velocity of this afferent was 69 m/sec.

41, A). After exposure to topically applied SCh in a concentration of 100 μg/ml, the afferent response rapidly evolved into that shown in Fig. 41, B, the initial response being biassed by 62 Imp/sec to 80 Imp/sec and the dynamic response increasing substantially to peak values of about 180 Imp/sec; the position response increased less markedly during the exposure to SCh, reaching a maximum of 103 Imp/sec, so that the dynamic index of this afferent increased by 240% to 77 Imp/sec, whilst the position sensitivity increased by an equal proportion to 7.9 Imp/sec/mm. shortly after the responses shown in Fig. 41, B were obtained, warm normal saline was used to elute the SCh and the afferent responses to ramp stretching returned over the course of about 5 minutes to the control state. This particular type 2 afferent was one of those whose spindle of origin was localised by the method of Bessou & Laporte (1962), and in Fig. 42, A-C are shown photomicrographs of representative H&E stained transverse sections of the labelled region of the tenuissimus muscle. Only one muscle spindle was found in the excised block of muscle, and it can be seen from the section of its juxta-equatorial region (Fig. 42, A) that this spindle encapsulation contained six intrafusal fibres in all, four smaller fibres about 8 μm in diameter and two larger ones about 10 μm in diameter. Two sections of the equatorial region of these intrafusal fibres are shown (Fig. 42, B, C); the first (Fig. 42, B) was obtained 360 μm further into the block than the section in Fig. 42, A, and it is clear that each of the smaller fibres contains only a single nuclear profile in section, whereas one of the larger diameter fibres contain several overlapping nuclear profiles in section. The position with regard to the other large intrafusal fibre is not clear in this section, but an adjacent section, taken from 30 μm further into the block, shows that the second large diameter intrafusal fibre also clearly contained several overlapping nuclear profiles in a single transverse section. Only one of the smaller intrafusal fibres contained more than a single nuclear profile (Fig. 42, C, topmost fibre), but the two nuclei which it contained were adjacent to each other rather than clustered and overlapping.

Fig. 42

P.T.O.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ch
b
ch
ch
C.
ch
b
ch
ch
b
ch

The SCh activation of a second type 2 afferent, encountered during a different experiment, is illustrated in Fig. 43. In this case, the afferent had a control initial discharge of 24 Imp/sec, a dynamic response of 136 Imp/sec and a position response of 59 Imp/sec, yielding a control dynamic index of 77 Imp/sec and a position sensitivity of 11.6 Imp/sec/mm (Fig. 43, A); after exposure to SCh (100 μg/ml), there was no change at all in the initial discharge rate, but the dynamic response had risen to 250 Imp/sec and the position response to 95 Imp/sec, so that the dynamic index had increased by 213% to 155 Imp/sec, and the position sensitivity had doubled to 23.3 Imp/sec/mm (Fig. 43, B). This afferent was also localised and studied histologically, and representative cross-sections of its spindle of origin are shown in the photomicrographs of Fig. 44, A, B. Again, only one spindle encapsulation was found in the excised block of muscle, which in this case also contained a total of six intrafusal muscle fibres, four of about 6-8 μm diameter, and two larger ones of 10-12 μm diameter (Fig. 44, A). Examination of the equatorial region of these fibres reveals that each of the four smaller fibres contained only a single nuclear profile in cross-section, whereas both of the larger fibres contained several nuclear profiles in section, four in one fibre, and perhaps six in the other (Fig. 44, B). None of the smaller intrafusal fibres showed multiple nuclear profiles in any of the adjacent sections of the nucleated regions of the fibres.

Ten other type 2 afferents were excited by SCh in a manner intermediate between the two extremes illustrated in Figs. 41 and 43, but in every case there was a substantial increase in the dynamic index and position sensitivity of the afferent. Five of these afferents were localised and studied histologically as just described, and in every case the spindle of origin contained at least 2 large diameter intrafusal fibres which showed clusters of nuclear profiles in their equatorial regions, as well as a variable number (4 to 7) of smaller intrafusal fibres containing only single nuclear profiles in their equatorial zones.

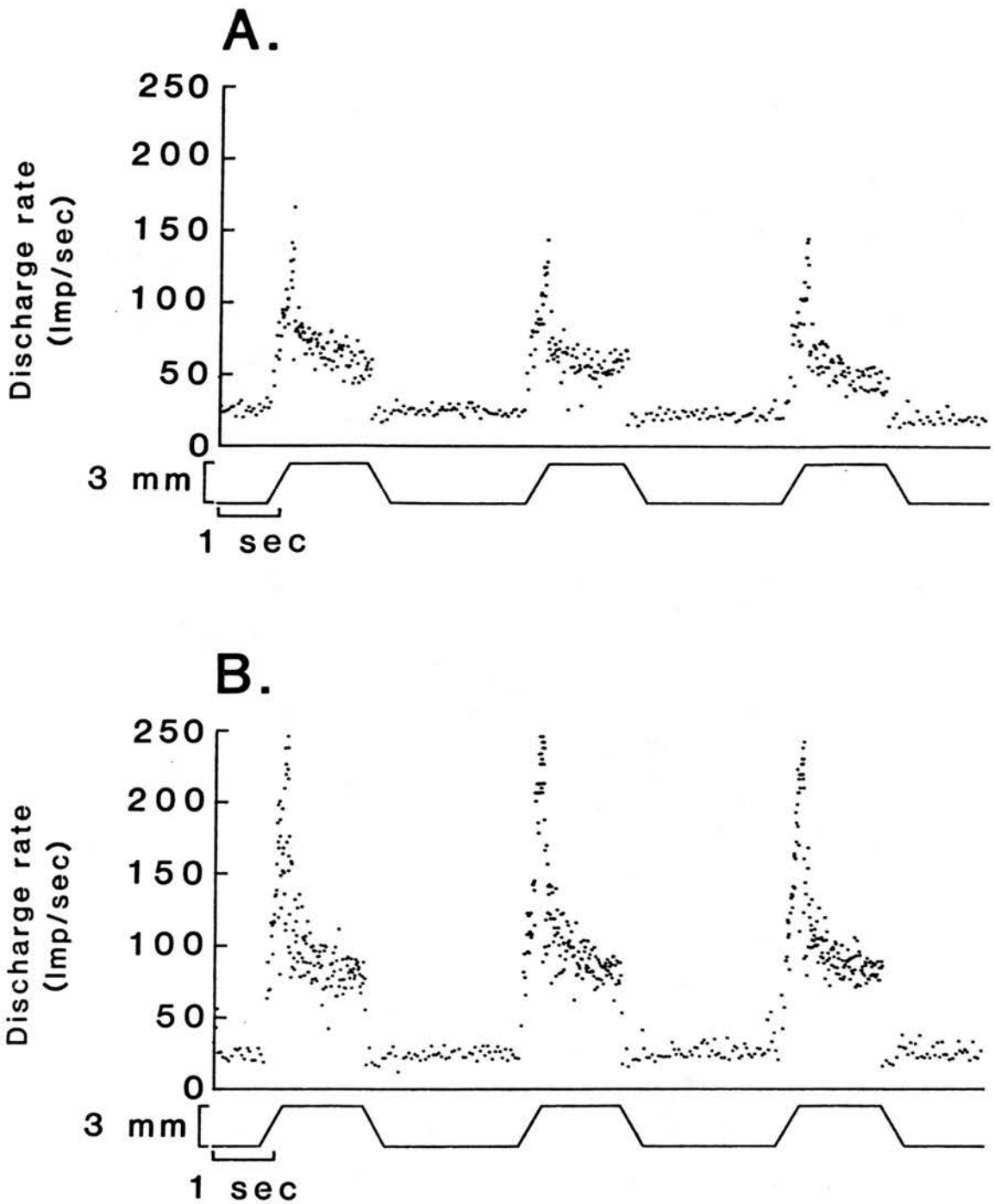
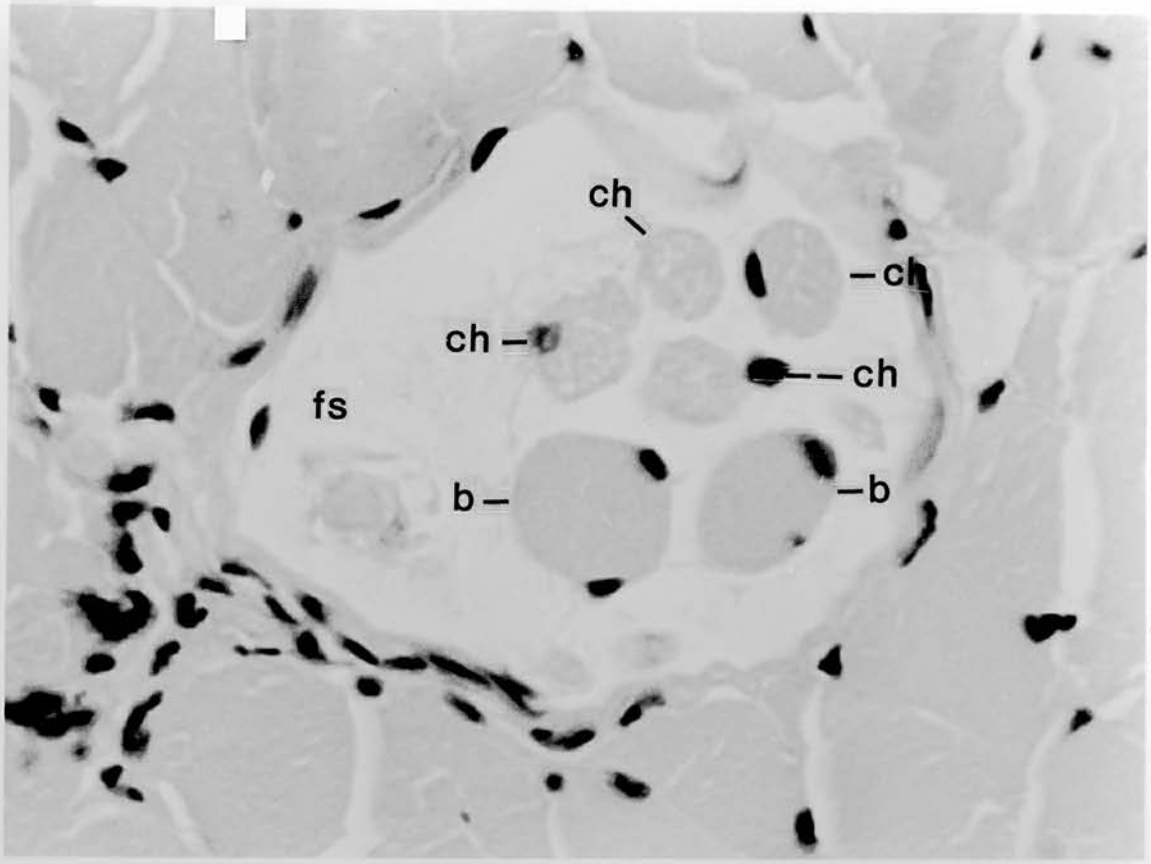


Fig. 43 A, B. Another example of the SCh excitation of a tenuissimus type 2 muscle spindle afferent. Response to stretch (3 mm, 10 mm/sec) before (**A**) and after (**B**) topical application of 100 μg/ml SCh to the muscle overlying the afferent's parent spindle. Conduction velocity of this afferent was 62 m/sec.

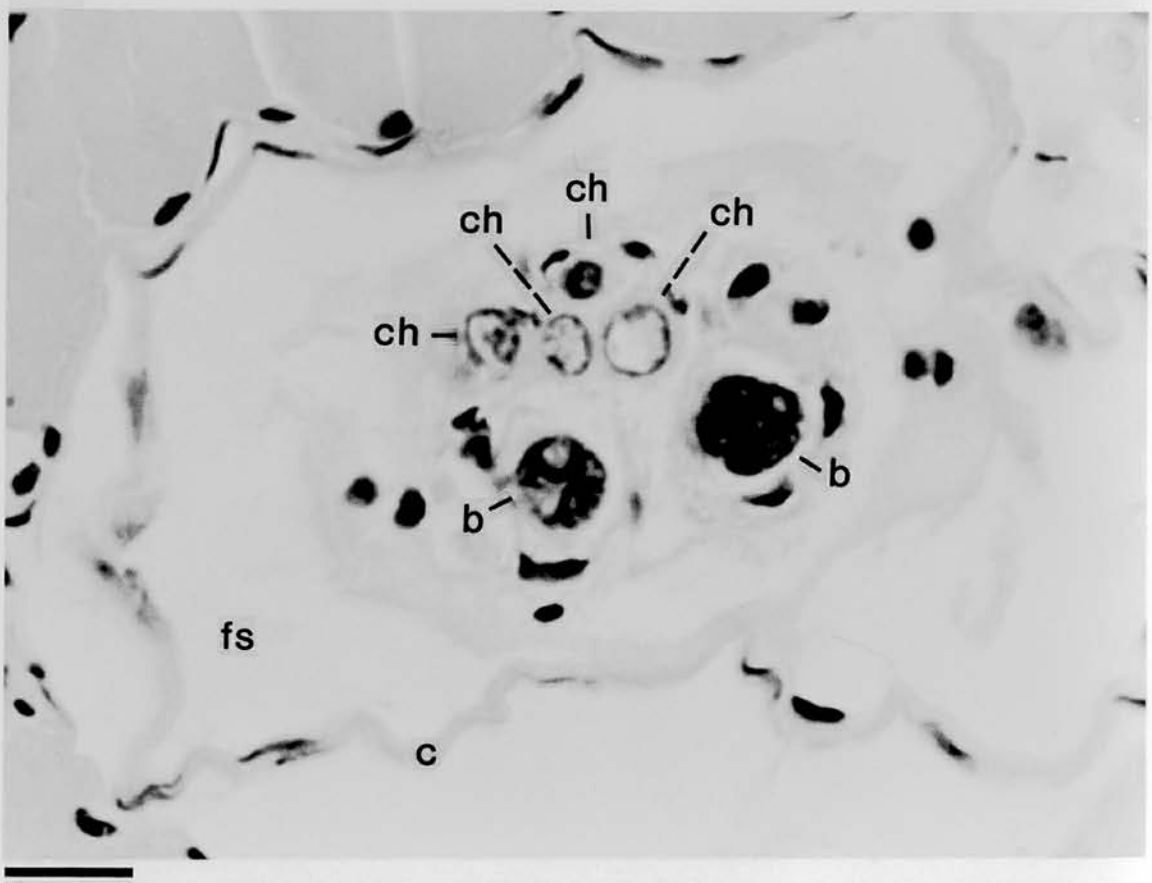
Fig. 44

P.T.O.

A.



B.



Of the remaining two afferents tested with SCh, one behaved in a very similar manner to biventer and medial gastrocnemius type 1 afferents (see e.g. Fig. 5), was not localised and will not be considered further here. The SCh activation of the final afferent is shown in Fig. 45. This afferent had a control initial discharge of 14 Imp/sec, a dynamic response of 81 Imp/sec and a position response of 46 Imp/sec, yielding a dynamic index of 35 Imp/sec and a position sensitivity of 7 Imp/sec/mm (Fig. 45, A); exposure to SCh (100 μg/ml) provoked a biassing of the discharge rate to about 73 Imp/sec which was accompanied by an increase in the discharge variability. The dynamic response rose to around 100 Imp/sec, but an increase in the position response to around 95 Imp/sec meant that the dynamic index of the afferent was substantially reduced to 5 Imp/sec; the position sensitivity of the afferent was effectively unchanged at 7.3 Imp/sec/mm, although the increase in discharge variability gave the appearance of a reduction in position sensitivity (Fig. 45, B). This afferent was identified as a type 3 afferent.

The spindle of origin of this type 3 afferent was identified as before, and in Fig. 46, A, B are shown photomicrographs of representative H&E stained cross-sections of the only spindle capsule which lay in the region of the tenuissimus muscle labelled during the experiment. Fig. 46, A shows a cross-section of one polar region of this spindle, from which it can be seen to contain five intrafusal muscle fibres, four of approximately equal diameter at about 6 μm and a single larger fibre about 10 μm in diameter. Fig. 46, B shows a section obtained about 250 μm further into the block at the equatorial zone of this spindle, in which the nuclei of the intrafusal muscle fibres can be seen; it is apparent that the four smaller intrafusal muscle fibres contain either a single nuclear profile or two adjacent nuclei, as was the case in the sections sections of the nucleated regions of these fibres adjacent to the one shown in Fig. 46, B; in contrast, the larger intrafusal muscle fibre shows four overlapping nuclear profiles in a single cross-section. This large intrafusal fibre was traced in serial sections out of this capsule, through the belly of the muscle for about 1.2 mm, and into a second

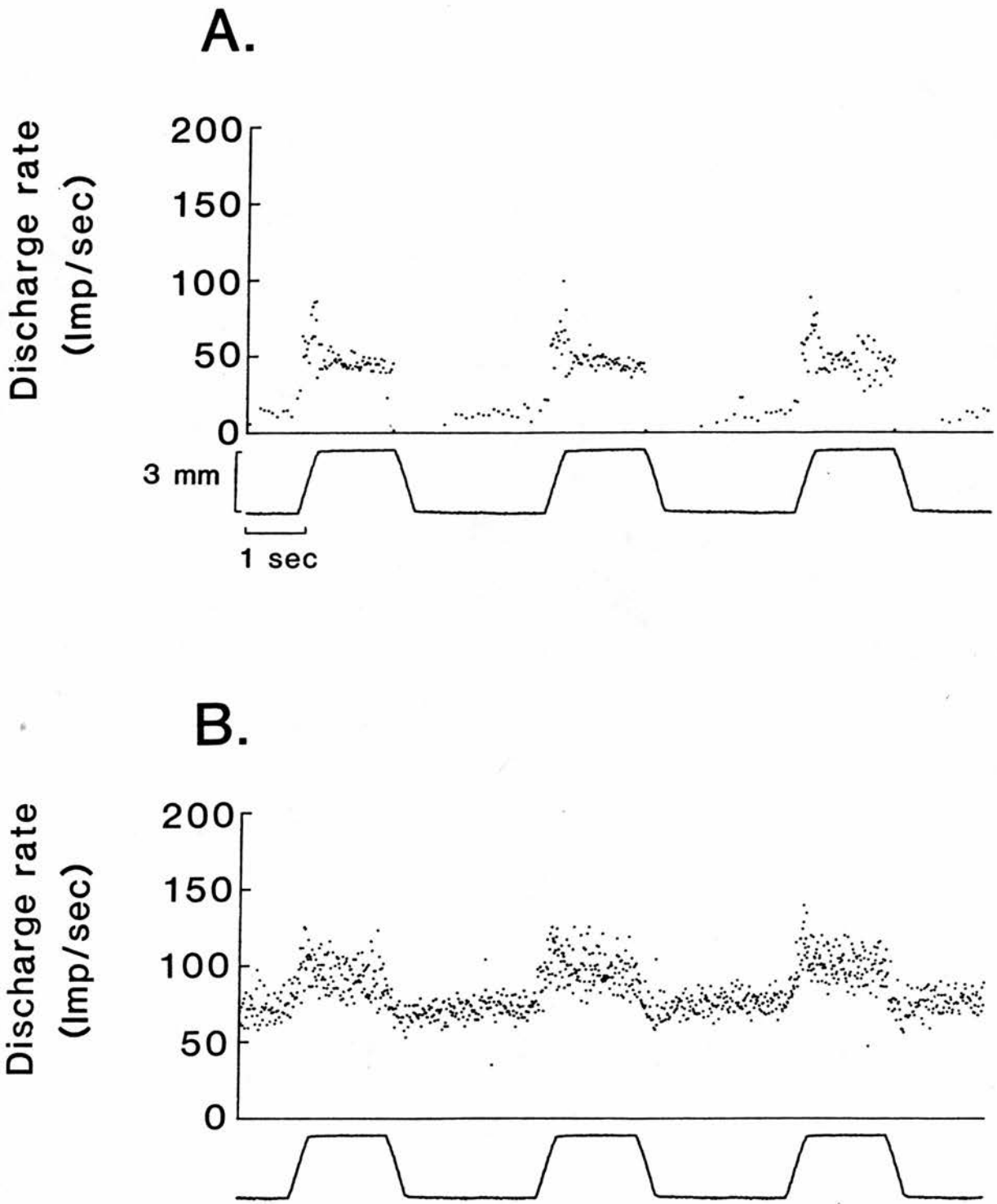
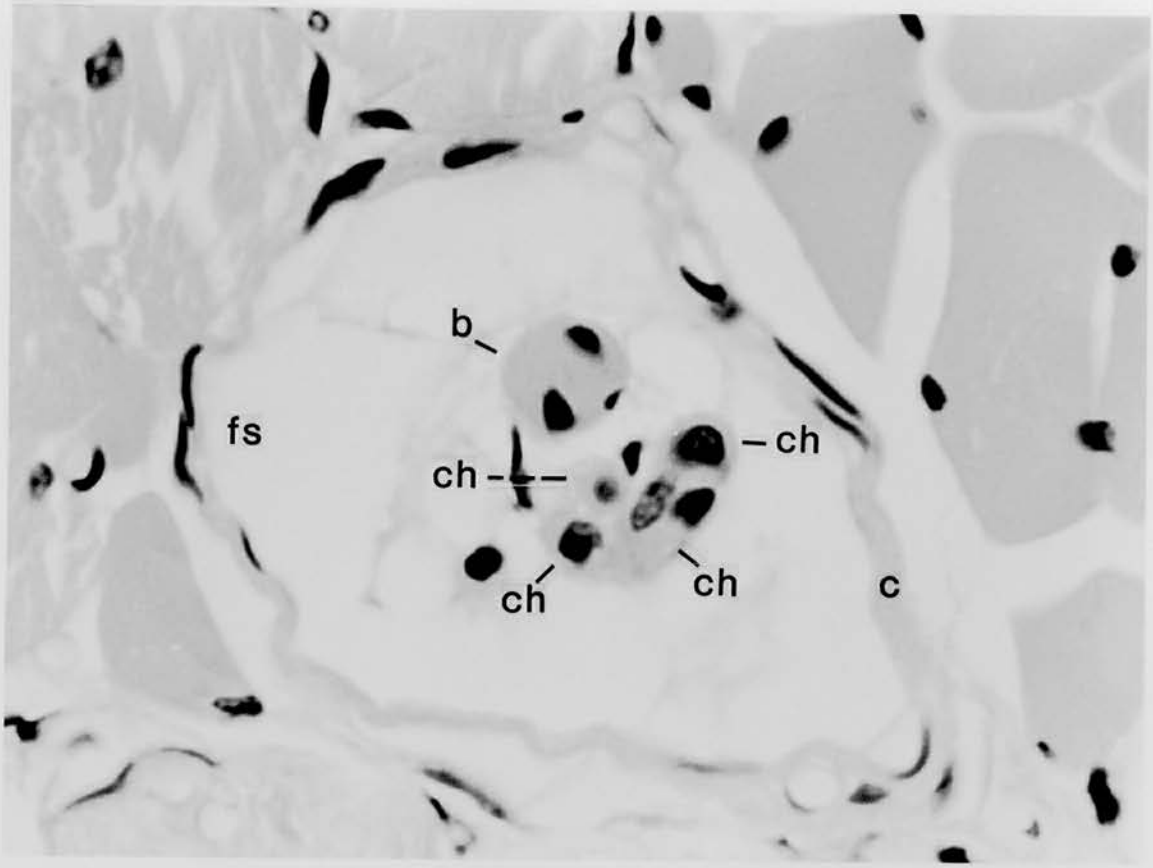


Fig. 45 A, B. Pattern of SCh activation of the single tenuissimus type 3 afferent encountered during these experiments. Response of this afferent to stretch (3 mm, 10 mm/sec) before (**A**) and after (**B**) topical application of 100 μg/ml SCh. Conduction velocity of this afferent was 64 m/sec.

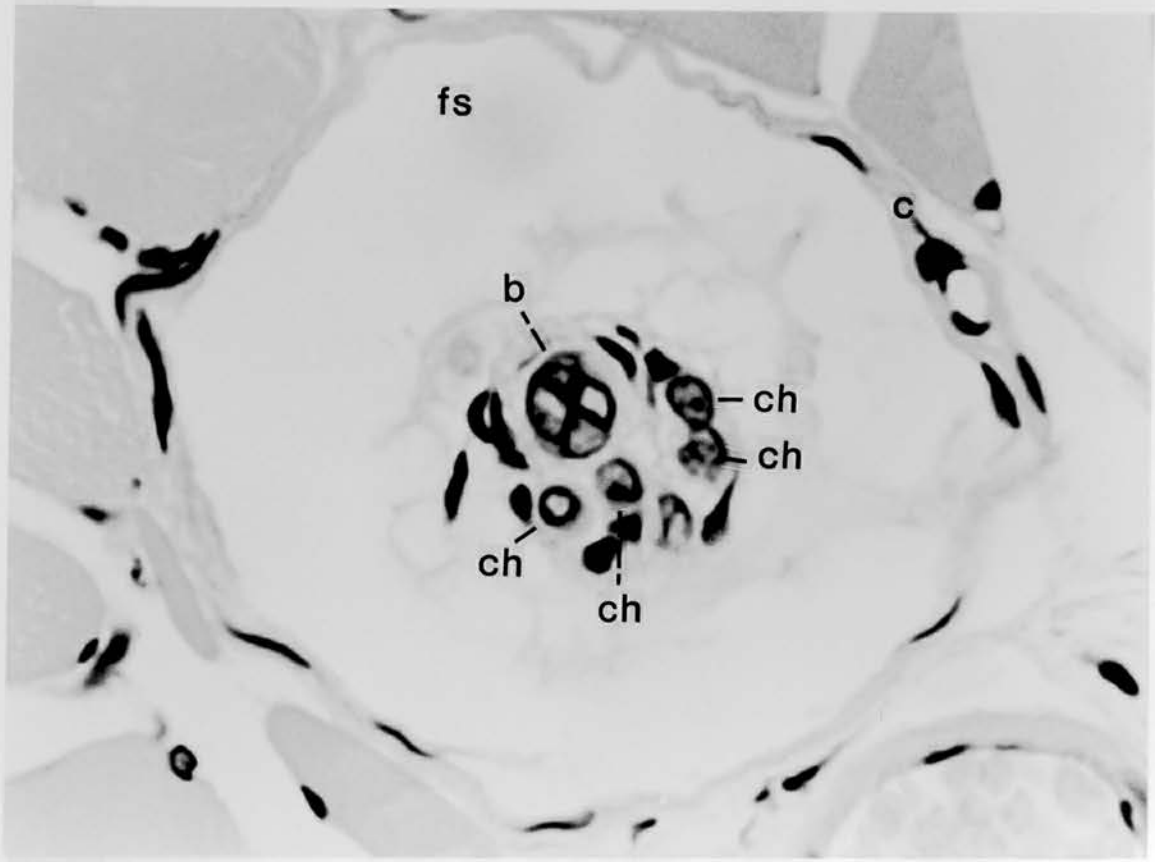
Fig. 46

P.T.O.

A.



B.



encapsulation which lay at the far end of the excised block of muscle. This second encapsulation contained two large diameter intrafusal fibres and five smaller ones; unfortunately, the excised block of muscle did not include the equatorial zone of this second encapsulation, so that the equatorial arrangement of nuclei in these intrafusal fibres is not available to help in their identification.

CHAPTER THREE

DISCUSSION

In this final series of experiments, the spindles of origin of eight tenuissimus afferents which had been studied electrophysiologically were located using the technique of Bessou & Laporte (1962) and subsequently studied histologically. The afferents selected for this study were the most rapidly conducting of each experiment in an attempt to sample primary afferents selectively. In the event conduction velocities ranged from 60 to 70 m/sec, which is rather lower than the values typically reported for tenuissimus primary afferents of over 80 m/sec (Boyd, Gladden, McWilliam & Ward 1977). The probable explanation for this is that the muscle and its nerve were at less than body temperature since they were not protected by warmed paraffin oil; examination of the results of Boyd (1986) indicates that in his isolated spindle experiments on tenuissimus, in which the muscle temperature was 33-35 degrees centigrade, many primary afferents conducted at 60-70 m/sec as in the present experiments (this can be deduced from Boyd's illustrations, since he routinely uses terminology of the form I_{63} in which the I stands for Group I (primary afferent) and the subscript is the conduction velocity of the afferent in m/sec). This does not, however, significantly affect the interpretation of afferent responses to SCh in the present experiments, since Boyd's reports of bag and chain fibre action on spindle afferent sensitivity in his 1986 experiments (when the muscle was cool) do not differ appreciably from his earlier reports.

In all but one case, complete serial reconstruction of the excised block of muscle identified as the source of a studied afferent revealed the presence of only a single spindle encapsulation, so that there was no potential for confusion as to which encapsulation the afferent studied had originated in. Considering these cases first, it transpires that in every instance the afferent which had been studied had a pattern of SCh activation of the type illustrated in Figs. 41 & 43. Although a topical route of administration of SCh was used in the present experiments, it would seem that the spindle afferents were nevertheless powerfully excited, this being borne out by the rate of change of the response to stretch after exposure to the drug, in some cases by the

size of the biassing and increased discharge variability provoked by SCh (Fig. 41, B), and in all by the form of the afferent response to ramp stretch when maximally activated by the drug (Figs. 41 & 43, B). The afferent whose response to SCh is illustrated in Fig. 41, for example, showed a very marked increase in its dynamic index and position sensitivity after exposure to SCh, the overall response to stretch being very reminiscent of that exhibited by many medial gastrocnemius b_1b_2c primary afferents (e.g. Fig. 23) in which there was good evidence that both bag fibre types were fully activated. Finally, in the first experiments of this series a topical SCh dose of 250 μg/ml caused what appeared to be depolarisation block in the afferent (not illustrated, but see e.g. Fig. 25); in later experiments, the SCh dose was reduced to 100 μg/ml, which produced the same changes in afferent sensitivity as those generated by the higher dose, but without causing depolarisation block.

The interpretation of the behaviour of these spindle afferents followed the same lines as that of their biventer and medial gastrocnemius analogues. Thus, these afferents had been selected as likely to be primaries on the basis of their conduction velocity, and subsequent changes in their stretch sensitivity provoked by SCh were those expected of primary afferents acted on by the combined contracture of bag_1 and bag_2 fibres (see p. 164 et seq. for justification). They were therefore identified, on the basis of their pattern of SCh excitation, as b_1b_2c primary afferents.

The histological findings on reconstructing the parent spindles of these afferents provided further support for this identification. Two of the seven reconstructed spindles from which b_1b_2c primary afferents originated are shown in Figs. 42 and 44; the other five were essentially the same. As shown in these photomicrographs, the parent spindles of b_1b_2c primary afferents usually contained six or seven intrafusal fibres, of which two types were readily identified. The majority of the fibres were fairly small, generally about 6-8 μm in diameter, and showed mainly mainly single nuclear profiles in their equatorial regions; these features are characteristic of chain fibres (Barker 1948), which is what these small intrafusal fibres

were therefore identified as. The presence in a few of these presumed chain fibres of two nuclear profiles adjacent to each other in a single transverse section is not incompatible with their identification, since in the region of the primary ending chain fibres can have small clusters of nuclei (see e.g. Fig. 7, Banks, Barker & Stacey 1982). In addition to several presumed chain fibres, all these spindles contained either two or three substantially larger fibres, generally about 10-12 μm in diameter, which regularly showed four or more overlapping nuclear profiles in their equatorial regions, both these being features of bag fibres (Barker 1948). As would be expected if the SCh based diagnosis is correct, b_1b_2c primary afferents thus all originated in spindles containing at least two bag fibres. Although enzyme histochemistry was not done to prove it, these bag fibres were very likely to have been a bag_1 fibre and a bag_2 fibre, since this is by far the commonest form of two-bag spindle unit (Banks, Barker & Stacey 1982; Kucera 1982b).

Of the remaining two afferents tested with topical SCh, one had an activation typical of secondary afferents and was not studied further. This leaves the single afferent whose pattern of SCh activation is shown in Fig. 45. This afferent was rapidly and powerfully excited by exposure to SCh, and, except that it did not undergo a reduction in position sensitivity, when maximally activated by SCh it responded to ramp stretching in a very similar way to biventer and medial gastrocnemius b_2c primary afferents. Although a fall in position sensitivity is one of the main diagnostic criteria for b_2c primary afferents when exposed to SCh, I had little hesitation in identifying this tenuissimus afferent as a b_2c primary afferent, being persuaded by the absence of an increase in its dynamic index, as well as by the similarity between Fig. 45, B and, for example, Figs. 28 & 29, B. The absence of a reduction in position sensitivity of this afferent is still compatible with a substantial bag_2 fibre input to the discharge of a primary afferent, for Boyd has shown in some of his isolated spindle experiments that bag_2 fibre action on primary afferents is simply to produce biassing of the afferent rate and an increase in discharge variability (Boyd, Murphy & Moss

1985) {in other cases he has shown a reduction in position sensitivity in addition, e.g. Boyd 1981b}. An alternative explanation for the absence of a reduction in position sensitivity of this afferent is that the ramp stretches used were relatively larger, in terms of percentage change in length, than those used in either the biventer or the medial gastrocnemius experiments, and so may have represented a more substantial drive to afferent firing which was harder for the bag_2 action to overcome. This interesting afferent was located and later studied histologically, and it was this reconstruction which revealed the presence in one excised block of muscle of two spindle encapsulations.

These capsules were separated longitudinally by about 1.2 mm (though *in vivo* this figure would have been somewhat larger since no corrections have been made for shrinkage during processing), but only one of the capsules was complete, the other lying at the edge of the excised block of tissue. I estimate that the equatorial regions of these two capsules, which would bear the primary endings, were about 1.8 mm apart, even ignoring shrinkage, making it reasonable to assume, given a reported spatial resolution of 0.5 mm for the location technique of Bessou & Laporte (1962), that the parent spindle of the presumed b_2c primary afferent was correctly identified as the complete one which lay in the middle of the marked block of tissue. It can be seen from the photomicrographs of Fig. 46 that this spindle unit contained five intrafusal fibres which are presumably to be identified as a single bag fibre and four chain fibres on the basis of their diameter and equatorial nucleation pattern (Barker 1948). Again, enzyme histochemistry was not performed to prove it, but this single bag fibre is most likely to have been a bag_2 fibre, as is attested to by the following points. Kucera (1982b) in a study of 341 tenuissimus spindle encapsulations found only one b_1c spindle unit but twenty b_2c spindle units, making it at least 20 times more likely that the single bag fibre in the spindle studied here was a bag_2 fibre than that it was a bag_1 fibre. In fact the odds are probably even more in favour of a diagnosis of bag_2 fibre, since other workers have not described b_1c spindle units at all (Banks, Barker &

Stacey 1982 in a sample of 310 spindle units from various hindlimb muscles, including tenuissimus, found 40 b_2c spindle units but no b_1c spindle units; Richmond, Bakker, Bakker & Stacey (1986) in a sample of 107 tandem spindle units in biventer found 49 b_2c spindle units, but no b_1c spindle units). Finally, even Kucera (1982b) has not described a tandem linkage which includes a b_1c spindle unit, but the single bag spindle unit in the present experiments appears to have taken part in a tandem linkage, for the bag fibre could be traced out of this encapsulation, through the belly of the muscle and into a second encapsulation where it was joined by another large diameter intrafusal fibre. The equatorial region of this second encapsulation was not contained in the excised block of tissue so that the nucleation patterns of its intrafusal fibres are not known, but it seems reasonable to suppose that the second large intrafusal fibre was a bag_1 fibre and that the linked encapsulation was the b_1b_2c spindle unit of a double tandem spindle (Barker & Ip 1962).

Taking all these points into account, it would seem reasonable to conclude that the tenuissimus afferent which was diagnosed by its response to SCh as a b_2c primary afferent did in fact arise in a b_2c spindle unit, and conversely that those identified as b_1b_2c primary afferents originated in b_1b_2c spindle units. A point which has recently been raised in a critique of part of this work (Matthews 1989) is that single-bag spindles may differ from typical two-bag spindles in some other way to which the unusual behaviour of their Ia afferents is to be attributed, and in particular, it was noted that "long chain" fibres might also be absent from the intrafusal fibre complement of single-bag spindles. In answer to this, I would say that, whilst it certainly appears to be the case that b_2c spindle units rarely contain a long chain fibre (see p. 70), this could only conceivably produce the response to SCh described for presumed b_2c primary afferents in the present experiments if it were also the case that the long chain fibre differed from typical chain fibres in **not** being paralysed by SCh (it would seem extremely unlikely that the absence from some spindles of a fibre which was paralysed when exposed to SCh could be responsible for the substantial changes in

the discharge of their Ia afferents seen in the present experiments). If this were the case, then the long chain fibre might contribute some input to the discharge of primary afferents which, when absent from the input to a b_2c primary afferent, causes it to show the type of response seen in the present experiments. Whilst a potential mechanism for such a difference in behaviour in long chain fibres may conceivably exist in the guise of their different motor terminals (plate endings of static beta axons) which might have different SCh sensitivities compared to motor endings on typical chain fibres, it has yet to be demonstrated that SCh produces anything other than paralysis in nuclear chain fibres. In contrast, there is no need to invoke unusual SCh sensitivities in intrafusal fibres if the effects are interpreted, as has been done here, as those produced by the SCh-evoked contracture of the bag_2 fibre in isolation; such contracture has been visually confirmed to occur in the presence of SCh, and its effects on primary afferent discharge are well documented. Furthermore, the incidence of long chain fibres in tenuissimus spindle capsules is only about 12% (Kucera 1980); if one assumes that all b_2c spindle units exclude long chain fibres, and that the incidence of b_2c spindle units in tenuissimus is about 6% (Kucera 1982b), then about 13% (12 of 94) of b_1b_2c primary afferents will have terminals on a long chain fibre but 87% will not. The large majority of b_1b_2c primary afferents will thus also lack the proposed input from the long chain fibre, the absence of which is supposed to cause the unusual behaviour of b_2c primary afferents. Finally, even if the absence of long chain fibres does partially contribute to the behaviour of b_2c primary afferents, this does not detract from the unique ability of SCh to identify these afferents, since whether or not a b_1b_2c primary afferents has an input from a long chain fibre, it will presumably always manifest a powerful bag_1 fibre input to its discharge and so could not be confused with Ia afferents arising in single-bag spindles.

RÉSUMÉ

In attempting to identify and characterise b_2c primary afferents, three series of experiments have been performed. In the first two, using a neck and a hindlimb muscle (Chapters 1 & 2), a number of passive properties of spindle afferents were studied, as well as their patterns of SCh excitation. Examination of the patterns of SCh activation of the spindle afferents encountered led to the conclusion that two classes of primary afferent exist in both neck and hindlimb muscles, in addition to secondary afferents. The first, which has been studied before with SCh, shows evidence of an input from both the bag_1 and the bag_2 fibre to its discharge and was therefore identified as the b_1b_2c primary afferent which most workers have tacitly assumed they were studying when looking at "primary" spindle afferents. The passive properties of these b_1b_2c primary afferents from neck and hindlimb spindles were very similar to those reported by earlier workers for "primary" afferents, thus lending support to the diagnosis of afferent type based upon the pattern of SCh activation.

The second class of primary afferent, which was common in the neck muscle studied, but markedly less so in the hindlimb muscles, showed evidence only of a strong input from the bag_2 fibre to its discharge, with no sign of a bag_1 fibre contribution. That this was not an artefact caused by failure to recruit the bag_1 fibre of a b_1b_2c spindle unit, so that a b_1b_2c primary afferent was misidentified, was attested to by the facts that, (i) other primary afferents in the same experiment using the same dose of SCh were often diagnosed as b_1b_2c primary afferents, (ii) using doses of SCh up to five times the standard value failed to produce any signs of a bag_1 fibre contribution to the discharge of these afferents, yet (iii) there was evidence from the neck muscle experiments, in the form of afferent responses to ramped-dose SCh infusions, that both classes of bag fibre were activated by doses in the region of 60 μg/kg/min (the standard infusion rate was 100 μg/kg/min, and the highest dose used was 500 μg/kg/min). These afferents were therefore identified as b_2c primary afferents; that they were very common in the biventer muscle, and rather rarer in the medial gastrocnemius muscle fits well with histological understanding of the frequency

of b_2c spindle units in these two muscles.

The passive properties observed for the presumed b_2c primary afferents supported their identification by SCh. In the case of the neck muscle afferents, the three passive properties measured routinely were indistinguishable from those of b_1b_2c primary afferents but significantly different from those of secondary afferents. This observation was compatible with the finding of Banks, Ellaway & Scott (1980) that the passive properties of peroneal spindle primary afferents cannot be used to identify two subtypes of primary afferent corresponding to the b_2c and b_1b_2c morphologies. In the present hindlimb experiments, the extent of overlap between the passive properties of b_2c and b_1b_2c primary afferents was less marked than in the neck experiments, but it was nevertheless impossible to identify the two subtypes of primary afferent other than on the basis of their pattern of SCh activation. In the hindlimb experiments, it was also possible to use axonal conduction velocity to classify afferents, and when this was done for b_2c primary afferents it was found that over half would have been classified as primary afferents on this basis; the range of conduction velocities seen for b_2c primary afferents was in good agreement with the range expected of them on the basis of the known range of their axonal diameters. Both these facts lend further support to the SCh-based classification of these afferents.

In the final experiments (Chapter 3), spindle primary afferents from the tenuissimus muscle were classified as b_2c or b_1b_2c type on the basis of their activation by SCh, and the spindles of origin of some of these were located and studied histologically. The majority (12 of 13, 92%) of tenuissimus primary afferents were identified as b_1b_2c primaries, and reconstruction of the parent spindles of seven of these afferents revealed the presence in all of them of at least two bag fibres, as well as a variable number of chain fibres. Although not proven by enzyme histochemistry, these pairs of bag fibres are very likely to have been a bag_1 and a bag_2 fibre, so that, as expected, b_1b_2c primary afferents were found to originate in b_1b_2c spindle units. The proportion of the primary afferents encountered which were identified by SCh as

b_1b_2c type (92%) was in good agreement with that expected from histological surveys (about 94% Kucera 1982b).

Although its pattern of SCh activation was not identical to that seen for its biventer and medial gastrocnemius analogues, one tenuissimus afferent was nevertheless identified as a b_2c primary. Reconstruction of the parent spindle of this afferent revealed firstly that it contained only one bag fibre, and secondly that it probably took part in a tandem linkage. Enzyme histochemistry was not performed to prove it, but this single nuclear bag fibre is most likely to have been a bag_2, so that on the one occasion in which it was possible to correlate the electrophysiology and anatomy of a b_2c primary afferent, it was found that the b_2c primary afferent indeed originated in a b_2c spindle unit as expected.

The overall conclusion is, therefore, that b_2c primary afferents can be reliably identified by their response to SCh administration. This opens the way for studies of their role in motor control, and also allows a verdict to be passed upon some of the speculations on this topic which are currently in the literature.

It has, for example, been proposed that, since it lacks an input from the bag_1 fibre, the b_2c primary afferent ought to be less sensitive to dynamic components of a muscle stretch than b_1b_2c primary afferents (see Boyd & Gladden 1985; Richmond, Stacey, Bakker & Bakker 1985), and candidate b_2c primary afferents have on occasion been identified on this basis (e.g. Richmond & Abrahams 1979a; Inoue, Morimoto & Kawamura 1981). From the results of the present work and those of Banks, Ellaway & Scott (1980), however, it would appear that this suggestion can be ruled out, at least for the de-efferented muscle spindle, since in both neck and hindlimb muscles there is very extensive overlap between measures of the dynamic sensitivity (e.g. dynamic index; sensitivity to muscle vibration) of b_1b_2c and b_2c primary afferents.

Alternatively, Banks, Barker & Stacey (1982) have suggested that the b_2c primary afferent might provide a signal with enhanced static sensitivity, and that this might be particularly beneficial in the control of tonically active anti-gravity muscles. This is analogous to the suggestion of Banks, Barker & Stacey (1982) in the cat, and of Walro & Kucera (1985) in the rat that the relative richness in elements of the dynamic intrafusal system of lumbrical muscle spindles is a specialisation aiding their fine control. Several findings, however, do not sit well with this idea. For example, as pointed out by Richmond, Bakker, Bakker & Stacey (1986), tandem spindles, and hence b_2c spindle units, are as common in the splenius muscle, a head rotator, as they are in the major anti-gravity muscle of the cat neck, the biventer cervicis (Richmond & Abrahams 1975b). Similarly in the cat hindlimb, soleus, a major anti-gravity muscle, is sparingly supplied with tandem spindles in comparison to medial gastrocnemius. Furthermore, the experimental findings of the present series fail to provide support for the notion of enhanced static sensitivity in b_2c as compared to b_1b_2c primary afferents, and a final difficulty is that it is not apparent why the signal from spindle secondary afferents could not be used to provide static length sensitivity.

Another suggestion which has been made is that the b_2c primary afferent does not actually have any specific function, but represents instead aberrant development of some muscle spindles (Kucera 1982b). For example, if some primary axons were to arrive in a muscle later than others, they might contact intrafusal myotubes partway through spindle morphogenesis and be incapable of inducing the formation of a bag_1 fibre. Alternatively, even if all primary axons reached a muscle at the same time, there might be limitations on the number of intrafusal myoblasts available for innervation, leading to the formation of single-bag spindles. Interestingly, tandem spindles are reputedly commoner near tendinous junctions (Bakker & Richmond 1981; Kucera & Walro 1987), and the capsule of a tandem spindle which lies closest to the tendon is liable to be the b_2c spindle unit (Richmond, Bakker, Bakker & Stacey 1986), both of which could be due to a scarcity of intrafusal

myoblasts near tendons. Reexamining the distribution of tandem spindles in various muscles, it would appear that they are commonest in muscles with large myotendinous contact areas. For example, in the neck extensor muscles, which are very heavily endowed with tandem spindles, there are several tendinous inscriptions in the belly of the muscle which greatly increase the myotendinous contact area; similarly, in hindlimb muscles such as medial gastrocnemius and peroneus tertius, which have relatively high myotendinous contact areas as a result of a pennate architecture, tandem spindles are unusually common. Whatever the origin of the b_2c spindle unit might be, up to a third of neck muscle spindle primary afferents must originate in such encapsulations (Bakker & Richmond 1981), and it seems somewhat unreasonable to dismiss these afferents as functionally insignificant aberrations.

The similarity of the properties of identified b_2c and b_1b_2c primary afferents in the present experiments indicates that, in the de-efferented spindle, the bag_1 fibre is not the major determinant of afferent dynamic sensitivity. Thus the phenomena of stretch activation (Poppele & Quick 1981) and creep (Boyd, Gladden, McWilliam & Ward 1977), which are undoubtedly restricted to bag_1 fibres, do not appear to contribute greatly to the functional characteristics of primary afferents in the passive spindle as they were classically supposed to (Dutia & Price 1990). The probable explanation for this finding lies in a recent reexamination of the measurement of afferent dynamic sensitivity; this has revealed that an afferent response to ramp stretch can be split into several parts, namely the fast rise and fall phases and the slow rise and fall phases (Boyd, Murphy & Moss 1985). These are differently affected by bag_1 and bag_2 fibre action, such that the slow rise and fall phases are sensitive to bag_1 fibre action and the fast rise and fall phases to bag_2 fibre action. The slow fall phase, when present, is probably contributed by creep in the bag_1 fibre, whereas the fast fall phase is probably caused by an ionic mechanism of the type studied by Hunt & Ottoson (1975). Examination of the control responses to ramp stretching of b_1b_2c primary afferents studied in the present experiments (e.g. Figs. 23-25, A) reveals that

the main contributor to their dynamic indices is the fast fall phase, there being very little, if any, slow fall phase. Correspondingly, some bag_1 fibres are known to show very little creep in the passive spindle, this effect only becoming noticeable when the bag_1 fibre has been activated by dynamic fusimotor action (Boyd & Smith 1984). Similarly, b_2c primary afferents identified in the present experiments showed mainly a fast fall phase after a ramp stretch; since the fast fall phase is probably caused by an ionic mechanism and is unaffected by bag_1 fibre action, there would not seem to be any reason to expect b_2c and b_1b_2c primary afferents to have different dynamic indices in the passive state.

Only when the bag_1 fibre begins to contribute a slow fall phase to the afferent response would a difference in dynamic sensitivity between b_1b_2c and b_2c primary afferents be expected to appear, as happens when the bag_1 fibre is activated by a SCh infusion. In vivo, b_1b_2c and b_2c primary afferents will therefore only behave differently when there is background dynamic fusimotor drive, which will only affect b_1b_2c primary afferents due to the severely restricted motor supply of b_2c spindle units (p. 72 et seq.). The restriction of the motor supply to b_2c spindle units has led to the suggestion by Richmond, Bakker, Bakker & Stacey (1986) that the CNS might use the b_2c primary afferent signal as a "benchmark" against which to compare a simultaneous signal from b_1b_2c primary afferents; this might be of use in separating out dynamic and static components of the afferent signal for further processing.

In Fig. 47 is shown the result of a hypothetical subtraction of the b_2c primary afferent response to a ramp stretch from the response of a b_1b_2c primary afferent; the responses of these two afferents, which are real data digitised from SCh-activated afferents, are similar to those that would be induced in vivo by combined activity in static bag_2 and dynamic fusimotor axons. It will be appreciated that a comparison of this type could permit the CNS to extract a signal with high dynamic sensitivity even under conditions of strong biassing and high discharge variability provoked by the static fusimotor activity. A similar "benchmark" arrangement might

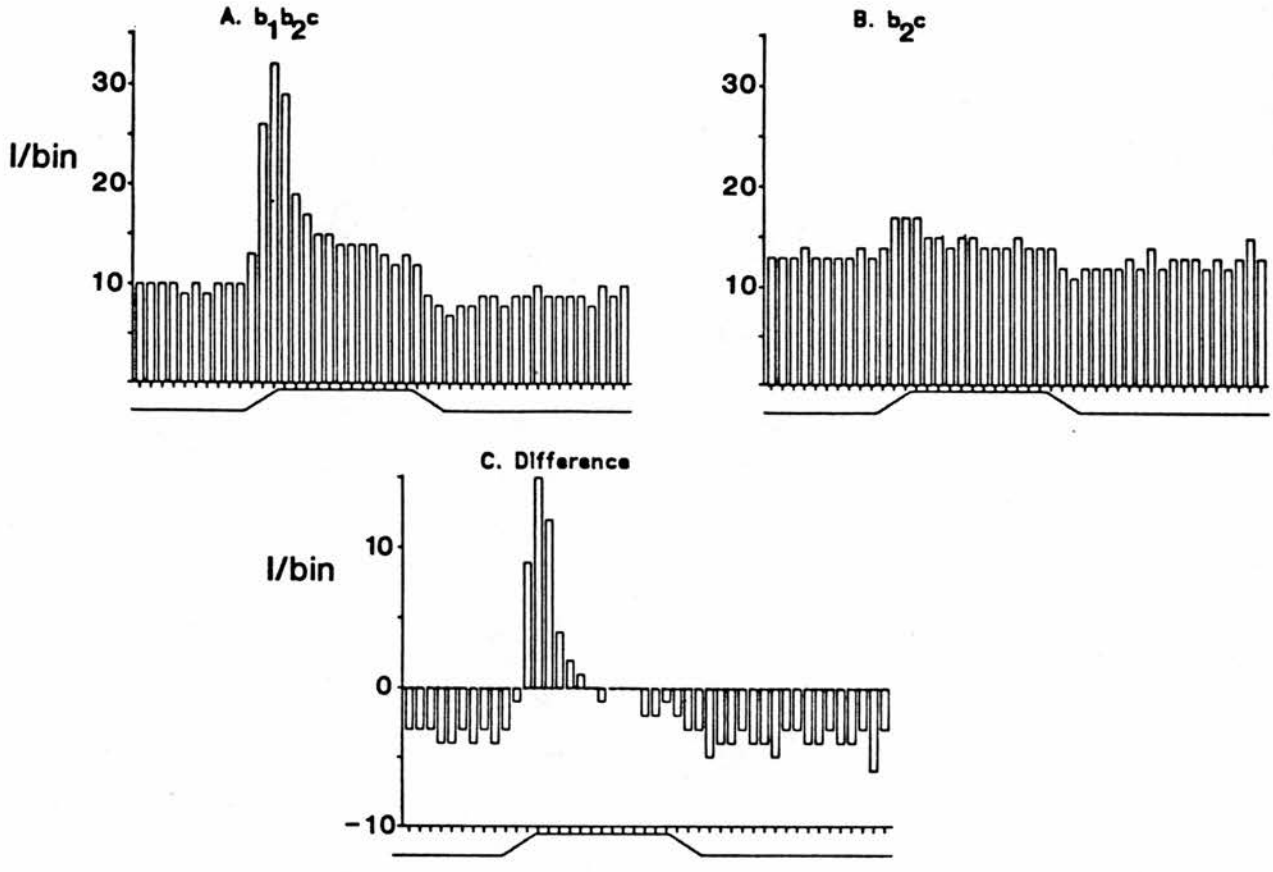


Fig. 47 A-C. Comparison of the response to stretch of a medial gastrocnemius b_1b_2c primary afferent (**A**) with that of a b_2c primary afferent (**B**). The histograms show the number of afferent spikes counted in 100 msec bins during one ramp stretch, applied when the afferents were maximally activated by a SCh infusion. (**C**) shows the result of subtracting the two histograms in **A** and **B**, to yield a signal with high dynamic sensitivity despite the level of biassing in the two inputs.

also exist to differentiate subtypes of static gamma action. If two classes of static gamma axon do exist (i.e. the static chain and static bag_2 gamma axons of Boyd 1986), and if only one type were distributed to b_2c spindle units, for example static bag_2 gamma axons, then a comparison of the behaviour of b_2c and b_1b_2c primary afferents might yield useful data in an analogous manner to that shown in Fig. 47. If such processing of afferent signals does indeed occur in the CNS, it would seem to be necessary to propose that the signal from b_2c primary afferents is differently routed than that from b_1b_2c primary afferents, perhaps by contacting an inhibitory interneuron before converging onto higher-order cells. Given that b_2c primary afferents can now be readily identified, it should now be feasible to investigate this proposal, as well as alternatives such as that suggested by Matthews (1989) that much of the discrete information provided by the various classes of muscle spindle afferent may in fact be discarded by the CNS, which instead generates an overall ensemble average signal which no one afferent could have provided.

SCh has again proved to be a powerful and versatile tool in the continuing efforts of electrophysiologists to identify, functionally, afferent subtypes which have been described by the anatomists. The work of Dutia (1980), and the results of the present experiments, suggest that the SCh test comes as close to providing an anatomically-based diagnosis of the intrafusal termination of an afferent as can be achieved without isolating the spindle and visually defining it. This is manifest both in the ability of SCh to differentiate b_1b_2c and b_2c primary afferents when all alternative means fail, and in its ability to categorise afferents with intermediate conduction velocities (60-80 m/sec) which would have been unclassifiable under alternative schemes. SCh may be particularly helpful in this latter guise for those workers who are beginning to investigate the functional characteristics of muscle spindles in non-hindlimb muscles of the cat, in which it is to be expected from histological and early electrophysiological studies that the classical means of identifying spindle afferent types will not be applicable (Literature Review, Part 2 p.

76 et seq.). Recent reports suggest that the SCh test is beginning to be used in this way (e.g. Taylor & Durbaba 1990).

The SCh test will surely also continue to be used to classify muscle spindle afferents studied by chronic implantation techniques (Prochazka & Wand 1981; Prochazka, Trend, Hulliger & Vincent 1989), although the way in which it is used at present might be extended to include the differentiation of b_2c and b_1b_2c primary afferents, as well as the identification of secondary afferents with bag_1 fibre collaterals. Although not many experimental details are provided, these authors appear to be using the work of Rack & Westbury (1966) and classifiying muscle spindle afferents which undergo an increase in dynamic index upon exposure to SCh as primaries (presumably b_1b_2c type), and those which do not show such an increase as secondaries. It will be appreciated, however, that techniques have moved on since 1966, and the results of the present work and of Dutia(1980) show that some secondary afferents with bag_1 fibre terminals can also undergo rather large increases in their dynamic indices (see e.g. Fig. 30). It would be very simple for these workers, and others in their field, to use the SCh test in the manner described by Dutia (1980) and in the present experiments in order to avoid the possibility of afferent misidentification.

One problem with using the SCh test routinely in the form described here is that one is required to infuse the drug over a number of minutes, invariably causing paralysis of respiration and necessitating ventilation of the animal. Furthermore, the whole cycle of drug infusion and recovery regularly takes twenty minutes to half an hour, during which time other experimentation is halted. Since the experiments reported here were performed, we (Price & Dutia) have modified the SCh test to make it easier to apply. Currently, we achieve equally good identification of afferent types by using bolus intraarterial injections of 1-3 mls of 100 μg/ml SCh solutions; this regime excites afferents transiently, but sufficiently powerfully to reproduce the patterns of response to stretch illustrated in this thesis, it is rarely necessary to ventilate the animal, and the effects of the SCh wear off within a matter of

minutes so that the experiment can proceed. Altogether, the test is now barely more difficult to apply than it is to measure the conduction velocity or other functional parameters of a spindle afferent.

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