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CONTROL OF HIND LIMB MUSCLE SPINDLES
FROM THE MESENCEPHALON AND DIENCEPHALON
IN THE CAT

A thesis submitted to the University of Glasgow
in candidature for the degree of
Doctor of Philosophy
in the faculty of medicine

by

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June 1990.

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ABSTRACT

Appelberg and Emonet-Dénand (1965) observed an increase in dynamic sensitivity of muscle spindles in hind limb muscles during stimulation in the mesencephalon in the approximate location of the Red Nucleus. This was seen in a flexor (Peroneus Tertius) and an extensor (Flexor Digitorum Longus). Using this and later work as a basis, Appelberg (1981) described an area covering the dorsocaudal part of the Red Nucleus and dorsal and caudal to it, which on stimulation selectively recruited hindlimb gamma motoneurons.

The aim of this project was to investigate the effect of stimulation in the region described by Appelberg (1981) on gamma motoneurons of the Tenuissimus muscle, and to assess its value as an experimental tool. Other possible areas for selective recruitment of dynamic and other types of gamma motoneuron were also to be investigated.

Two types of experiment were carried out. In one, spindles of the Tenuissimus muscle were exposed (isolated) with their nerve and blood supplies intact. Then, by direct observation of intrafusal fibres, the effect of stimulation in the mesencephalon and telencephalon was assessed. In the other type of experiment, single group Ia afferent fibres from Tenuissimus, Peroneus Tertius, and Flexor Digitorum Longus muscles were prepared in dorsal root filaments. The nature of any changes in gamma firing was inferred from changes in the afferents' response to muscle length change.

Static effects were seen in many experiments, and mixed dynamic and static in some. The static bag2 and chain fibres showed a large degree of independence in their excitation and inhibition, and were seen to be affected in opposite ways on some occasions. However, no clear area emerged as a particular site for recruitment of any type of intrafusal fibre in tenuissimus spindles.

It was also seen that the act of inserting microelectrodes into the habenulae silenced spontaneous activity in a tenuissimus spindle. This was not the case for the red nucleus or the substantia nigra.

It was concluded that tenuissimus is not affected from the area described by Appelberg (1981) and that the assertion that this area selectively recruits dynamic gammas in hind limb muscles has to be qualified until tested in individual muscles. The results from single experiments on Peroneus Tertius and Flexor Digitorum Longus support this view.

Dedication

I would like to dedicate this thesis to the memory of my former supervisor, Professor Ian A. Boyd.

Isaac Newton is reported to have said:

"If I have seen further, it is by standing on the shoulders of giants."

Having completed my brief immersion in the field of spindle physiology, I myself feel like a small midget who has perched precariously on the shoulders of giants : unable to see as far or as widely as theybut still able to enjoy the view.

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Contents

	<u>Page No.</u>
INTRODUCTION.....	1
The structure of the muscle spindle	3
Selected physiology of the spindle.....	18
Recruitment of gamma motoneurons from the mesencephalon and neighbouring parts of the brain.....	29
Aim of the project.....	41
 MATERIALS AND METHODS.....	 43
Preparatory surgery.....	46
Fine surgery and preparation for experiment.....	54
Experiment: stimulation and data gathering.....	62
Histology.....	66

RESULTS.....	69
Exposed spindle experiments.....	73
Dorsal root afferent responses.....	91
Comparison of sites recruiting known intrafusal fibres and those recruiting different categories of afferent effects.....	111
DISCUSSION.....	113
Comparison of effects seen with previous work.....	114
Recruitment of intrafusal fibre types and the types of intrafusal fibre whose contraction is responsible for characteristic changes in the Ia afferent discharge.....	119
Other effects.....	125
Validity and evaluation of effects seen.....	127
Conclusion.....	134

(cont.)

Recommendations for future work.....136

REFERENCES.....140

Figures

Opposing page

- Page 20..... Figure 1 : Known innervation of muscle spindle in 1964.
- Page 21..... Figure 2 : The dynamic index.
- Page 26..... Figure 3 : Responses of Ia afferents to frequency ramp stimulation of τ motoneurons innervating static bag2 and chain fibres.
- Page 38..... Figure 4 : Effects of mid-brain stimulation on hind limb Ia afferents seen by Appelberg and co-workers.
- Page 51..... Figure 5 : The Lumbo-sacral Plexus, showing the nerves supplying the muscles of the hip.
- Page 55..... Figure 6 : Rudimentary set-up for the exposed spindle experiments.
- Page 56..... Figure 7 : Video still of Tenuissimus spindle.
- Page 62..... Figure 8 : Experimental set-up in earlier dorsal root experiments.

- Page 63..... Figure 9 : Experimental set-up in later dorsal roots experiments.
- Page 67..... Figure 10 : Brain-stem section showing Fe³⁺ deposits and a 'tear' caused by electrode insertion.
- Page 71..... Figure 11 : Standardised set of brain-stem sections used as the basis for figs. 13-24 and figs 37-45.
- Page 72..... Figure 12 : Key to symbols used in figs. 13-24.
- Page 74..... Figure 13 : Stimulation sites in the brain-stem shown to affect dynamic bag1 intrafusal fibres.
- Page 75..... Figure 14 : Stimulation sites in the brain-stem shown to affect static bag2 intrafusal fibres.
- Page 77..... Figure 15 : Stimulation sites in the brain-stem shown to affect chain intrafusal fibres.
- Page 78..... Figure 16 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 23/2/89.

Page 79..... Figure 17 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 2/3/89.

Page 79..... Figure 18 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 2/3/89.

Page 80..... Figure 19 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 7/3/89.

Page 81..... Figure 20 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 18/5/89.

Page 82..... Figure 21 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 8/6/89.

Page 84..... Figure 22 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 15/6/89.

Page 86..... Figure 23 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 31/8/89.

Page 87..... Figure 24 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 14/9/89.

- Page 92..... Figure 25 : Ia afferent response to muscle stretch, showing measurements of the dynamic index and the length sensitivity.
- Page 93..... Figure 26 : Illustration of predominantly 'baseline' effect.
- Page 95..... Figure 27 : Illustration of predominantly 'variability' effect.
- Page 97..... Figure 28 : Illustration of increase in both 'baseline' and 'variability' effects.
- Page 98..... Figure 29 : Illustration of inhibition of both 'baseline' and 'variability' effects.
- Page 99..... Figure 30 : Illustration of inhibition of 'variability'.
- Page 100..... Figure 31 : Illustration of increased 'baseline' and 'variability' with probable dynamic admixture.
- Page 101..... Figure 32 : First response to stretch during stimulation in figure 31.
- Page 103..... Figure 33 : Example of a response to brain-stem stimulation in a Peroneus Tertius Ia afferent.

Page 105..... Figure 34 : Example of a response to brain-stem stimulation in a Flexor Digitorum Longus Ia afferent.

Page 106..... Figure 35 : Effect of different levels of anaesthesia on the length-ramp responses of a Ia afferent from a spindle with efferent innervation intact.

Page 106..... Figure 36 : Effect of stimulation in the lateral mesencephalon on the length responses of a Ia afferent.

Page 108..... Figure 37 : Stimulation sites producing effects in Ia afferents like those of fig. 26.

Page 108..... Figure 38 : Stimulation sites producing effects in Ia afferents like those of fig. 27.

Page 108..... Figure 39 : Stimulation sites producing effects in Ia afferents like those of fig. 28.

Page 108..... Figure 40 : Stimulation sites producing effects in Ia afferents like those of fig. 29.

Page 108..... Figure 41 : Stimulation sites producing effects in Ia afferents like those of fig. 30.

Page 108..... Figure 42 : Stimulation sites producing effects in Ia afferents like those of fig. 31.

Page 108..... Figure 43 : Stimulation sites producing effects in Ia afferents like those of fig. 33.

Page 108..... Figure 44 : Stimulation sites producing effects in Ia afferents like those of fig. 34.

Page 108..... Figure 45 : Stimulation sites producing effects in Ia afferents like those of fig. 36.

Page 120..... Figure 46 : Effects of stimulation of τ motoneurons to different intrafusal fibres on Ia afferent responses.

Page 121..... Figure 47 : Effects of 'ramp frequency' stimulation of τ motoneurons to different intrafusal fibres on Ia afferent responses.

INTRODUCTION

The introduction is divided into four main sections:

- 1) THE STRUCTURE OF THE MUSCLE SPINDLE.
- 2) SELECTED PHYSIOLOGY OF THE SPINDLE.
- 3) RECRUITMENT OF GAMMA MOTONEURONES FROM THE MESENCEPHALON AND NEIGHBOURING PARTS OF THE BRAIN.
- 4) THE AIM OF THE PROJECT.

Since the first three sections are closely intertwined, dividing them in this way is fairly artificial, and inevitably involves some repetition. Although this makes the introduction longer than strictly necessary, it is hoped that this will improve the clarity, and make a subject rich in vernacular more digestible for any readers who are new or tangential to it. For the sake of fluency, cross-referencing between sections is kept to a minimum.

1) THE STRUCTURE OF THE MUSCLE SPINDLE.

a) The Classical Picture.

The first description of muscle spindles in mammalian muscle was by Kühne in 1863. It was also he who gave them their name (muskelspindeln; whence comes the name in English). This name was purely descriptive, and hence has survived, since until late in the last century they were generally assumed to be centres of muscle growth.

The notion that they might be sense organs was first proposed by Kerschner (1888), after reviewing the existing morphological literature, and by Onanoff (1890) on the basis that most spindle nerve fibres degenerated following the destruction of the dorsal root ganglia. Both of these authors also noted the efferent innervation of the spindle.

However, it was not until after the work of Sherrington (1894) and Ruffini (1898) that the spindle's status as a sense organ under motor control began to establish itself. Sherrington investigated the afferent innervation of muscle by cutting the ventral roots and dorsal roots (central to dorsal root ganglia) and allowing the efferent fibres to degenerate towards the periphery. A substantial afferent supply to muscle was found, including axons as large as the largest motor axons. Much of this innervation was traced to the muscle spindles, demonstrating that these were - at least partly - sensory in function. Ruffini stained the spindle with gold chloride, highlighting the nerve endings. With this, he was able to give the most complete description until that date.

The clear, concurring accounts of Ruffini and Sherrington established a 'classical' picture of mammalian muscle spindle structure which, although in some respects altered, and refined or elaborated in others, remains broadly the same

today. A brief summary of their work (drawing on Matthews, 1964; Granit, 1970; Matthews, 1972; Boyd & Smith, 1984; Hulliger, 1984; Boyd, 1985) is given below here, and subsequent developments are dealt with in the three subsequent sections.

The spindle described by Ruffini consisted of a bundle of muscle fibres (one type) of various diameters (6 - 28 μm), though all generally thinner than typical (extrafusal) fibres (average dia. 50 μm approx. - Boyd (1985b)). These 'intrafusal' muscle fibres ran together in a bundle of fairly constant thickness along its length. They showed striations along most of their length, but the central 400 μm had a conglomeration of nuclei, and a relative scarcity of contractile elements. At their very ends, the intrafusal fibres attached to extrafusal muscle, or to tendon - often in series with a Golgi tendon organ. Along the central third or so of the spindle, the intrafusal fibres were contained within a connective tissue capsule, widest at the centre (or equator), and tapering towards the ends (or poles). It is this feature which gives the spindle its characteristic fusiform shape and its name. Sherrington believed that the fluid giving the capsule its turgidity was lymph, after an apparently successful attempt to inject it via the lymphatic system. It has since been shown not to be lymph, since it contains hyaluronic acid (Brzezinski, 1961; Fukami, 1986) and is presumed to be a product of the capsule itself (Fukami, 1986).

As mentioned above, Sherrington (and Ruffini) demonstrated conclusively that the spindle had an afferent nerve supply. For different reasons, both Sherrington and Ruffini concluded that the spindle received no efferent supply, but it is now well established that it does. Ruffini described three types of nerve ending. Firstly, the primary or annulo-spiral ending, which invested all the intrafusal fibres at their central region, winding regularly round their outer surface like a spiral or spring. The secondary or flower-spray endings, lying out from the centre, were less regular in appearance than the

primary. There was always only one primary ending per spindle supplied by a large myelinated axon, whereas there was a variable number of secondaries, from none to several, lying on either side of the primary and supplied by smaller myelinated axons. The third type were the plate endings, lying out towards the poles of the spindle. Ruffini concluded that these were also sensory despite their resemblance to extrafusal motor endplates. In actual fact these are motor endplates, while the primary and secondary are genuinely sensory.

The classical picture was completed by the demonstration that the plate endings were motor. This was strongly suggested by the work of Perroncito (1901) who showed that in the lizard, spindles are supplied by branches of neurones common to extrafusal fibres. Unequivocal evidence for this in mammals came following a series of selective degeneration experiments by Hinsey (1927) and Hines and Tower (1928).

In 1948, the classical picture was substantiated by Barker, studying the rabbit spindle. It is unfortunate that he chose the rabbit, as its spindles are much more like the classical picture in appearance than those of other common experimental species, and he missed the chance to observe any differences. He did, however, introduce the term 'nuclear bag', to describe the abundance of aggregated nuclei he found in the equatorial region of each intrafusal fibre.

Intrafusal Fibres.

The first refinement of the classical picture was the distinction between two different types of intrafusal fibre : the nuclear bag fibres and the nuclear chain fibres. This was

made in the cat by Boyd in a series of experiments between 1956 and 1962; and in human spindles by Cooper and Daniel during the same period.

In 1956, Boyd noted that spindles contained 2-3 large intrafusal fibres, which ran the full length of the spindle, and 2-5 smaller ones, each about half the total length. In 1958, he again described the large and small intrafusal fibres, this time also noting that the small ones had a single chain of nuclei running through their equator, while only the larger ones had an actual nuclear bag. Then, in 1959, he found that the secondary sensory ending lay almost exclusively on the small fibres, and in 1960 he named these the 'nuclear chain' fibres, as distinct from the larger 'nuclear bag' fibres. Boyd collated this and subsequent work for what became the seminal reference for feline spindle structure (Boyd, 1962).

In 1961, Barker and Gidumal reported 3 diameters of intrafusal fibre, in approximately the correct size ranges to correspond with the modern day position, though occurring in the wrong proportions. However, the idea of further intrafusal sub-divisions does not seem to have gathered any momentum at that time.

It was in 1972 that the next advance from Boyd's 1962 description was made. Ovalle and Smith (1972) separated the nuclear bag fibres into bag1 and bag2 fibres on the basis of their histochemical reactions. They also distinguished 3 types of chain fibres, though this finding has not been pursued with anything like the same vigour.

In 1974, Gladden showed that bag fibres in human spindles fell into 2 groups, with differences in the distribution of surrounding elastic tissue (Cooper & Gladden, 1974). In the same year, Barker and Laporte (Barker, 1974 - introduction) summarised material showing that, in general, mammalian bag fibres differed in length, development, ultrastructure, and histochemistry.

In 1976, Gladden established that, in the cat too, the bag fibres had different arrangements of elastic fibres; those innervated by static gamma-motoneurons having more and thicker elastic fibres in the polar region. She also showed that the bag fibre with most elastic fibres corresponded to the histochemistry of Ovalle and Smith's bag2 fibre. This allowed Boyd, Gladden, McWilliam, and Ward (1977) to equate their functionally classified 'dynamic bag fibre' with the bag1 fibre, and their 'static bag fibre' with the bag2, confirming an earlier proposal by Boyd (1976). The two bag fibres thus became known as the dynamic bag1 fibre (Dbl), and the static bag2 fibre (Sb2).

The other advance in classification of intrafusal fibres has been a degree of sub-division of the chain fibres. It was shown by Harker et al. (1977) that repetitive stimulation of high conduction velocity (c.v.) β -motoneurons (see later) led to depletion of glycogen in chain fibres, specifically the longest one. Shortly after this, Jami et al. (1979) demonstrated that the β innervation to chain fibres was static in effect, and also that the chain fibres thus innervated were once again those projecting beyond the capsule. Kucera (1980) proposed a further sub-division of chains, according to how far they projected beyond the capsule. These were 'long', 'typical', and also 'intermediate' chain fibres, with the 'typical' ending at the capsule end. The possibility of sub-types of chain fibres has not received the same attention that bags did, presumably because it does not resolve an existing dilemma, or fit into or extend upon the existing framework of physiologically obtained knowledge.

Afferent Innervation.

The structure of the afferents is, of the three broad categories, the one which has been least modified from the classical picture. By the degeneration experiments of Hinsey (1927) and Hines & Tower (1928), the annulo-spiral (or primary), and flower-spray (or secondary) endings were established as afferents, which finding has stood until the present day.

In 1948, Barker's study of the rabbit spindle stressed the similarity of the two endings; the secondary differing from the primary not in form, but only in location and size of associated nerve fibre.

Lloyd (1943) classified muscle nerve afferents into four groups, I-IV, on the basis of their size and their evoked reflex activity. Earlier, B.H.C. Matthews (1933) had made a functional sub-division of muscle afferents into: class A1 (presumed from spindles - fell silent during maximal and supramaximal twitch); class A2 (presumed from spindles - fell silent during maximal twitch, but increased during a supramaximal one); class B (presumed from tendon organs - increased during muscle twitch); and class C (unclassified, probably free nerve endings). Synthesis of these two classifications has led to the large muscle afferents supplying the primary endings being known as the Ia afferent, and the smaller afferents supplying the secondary endings the II afferent. The afferents to the Golgi tendon organs are called Ib.

The major differences between the primary and secondary endings had to wait for the discovery of two types of intrafusal fibre by Boyd (1956) and Cooper and Daniel (1956). In 1959, Boyd observed that the secondaries lay almost exclusively on the small (chain) intrafusal fibres, and substantiated this in a larger study (Boyd, 1962). Boyd also

designated sections of about 400µm, moving outwards from the primary, which the secondary ending(s) could occupy as S1, S2, S3, and S4 .

The first application of electron microscopy to the spindle nerve endings was by Merrillees in 1960, confirming the similarity (in the rat) of form of the primary and secondary. Electron microscopy was used again by Adal, in 1969. He reported a very close appositioning of the sensory nerve and muscle membranes. The nerve terminals were convex, and lay in a groove in the muscle, underneath the muscle fibre basement membrane. There was a very small gap (100-200Å) between the two membranes, but no sign of any electrical or mechanical connection.

The structure of the sensory terminals has since been pursued almost exclusively by Barker, Banks, and co-workers. Their work in the intervening years is collected in Banks, Barker and Stacey (1982). They showed that in 270 silver-stained typical tenuissimus muscle spindles, the terminal branching usually segregates the bag1 fibre, with the bag2 and chain fibres innervated somewhat collectively. In two reconstructed terminals it was also shown that the bag1 had a greater proportion of the Ia's sensory contact than the bag2 or individual chain fibres. However, the chain fibres collectively had slightly more, and this is probably of physiological significance, since there are many cross-connections between terminals on the chain fibres, particularly the shorter ones. After consideration of the arrangement of nerve terminals and intrafusal fibres, they suggested that the mechanism for sensory transduction was compression of the nerve terminals between the plasmalemma and the basement membrane. Banks again proposed this in 1986, when he also showed differences in the indentation of the sensory terminals on the different intrafusal fibres; with those on the chain fibres indented most, and those on the bag1 least.

In 1989, Banks presented work on the re-innervation of spindles by native (i.e. spindle) afferents. Although not stated in the abstract (Adal and Banks, 1990), he showed that (formerly) secondary afferents occupying the equatorial part of the spindle after re-innervation now displayed the qualitative features of a typical Ia response, while retaining their II conduction velocity. A similar exchange happened to former primary afferents. This physiological experiment lent support to the earlier anatomical observations that the difference between the two endings was in their location, and not in their structure.

Efferent innervation (τ motoneurons).

Following the confirmation by Hinsey (1927) and Hines & Tower (1928) of the efferent status of the plate endings, came the demonstration by Leksell (1945) that the smaller efferent axons to muscle influenced spindles.

Leksell applied pressure to the nerve, preferentially blocking the larger axons. He found a small wave in the L7 ventral root when stimulating the soleus nerve, which had a higher threshold and a lower conduction velocity (c.v.) than the large axons. Erlanger & Gasser (1937) had earlier named the large axons "alphas" (α), and after a comparison of the conduction velocities, Leksell christened his small efferents "gammas" (τ). He also tested the effects of these τ after blocking the α conduction. He found that they caused no measurable increase in the tension in the muscle, but did increase the afferent discharge, which he assumed to come from spindles.

A few years later, in a paper containing many firsts, Kuffler, Hunt, & Quilliam (1951) stimulated single efferent axons in the first split ventral root preparation. They found effects

on spindles from the efferents of c.v. between 15 and 50 m/sec.; and extrafusal contraction only from the efferents of c.v. greater than 55 m/sec. They thus showed that the small, slow τ efferents innervated spindles, and did not innervate extrafusal fibres. The converse - that fast efferents do not innervate spindles is not true. There are axons conducting in the α range which also send collaterals to spindles. These are known as beta axons (β -axons) (see later). The suggestion by Hunt & Paintal (1958), that nerve fibres to spindles should be known as fusimotor fibres, has since been used to embrace both τ and β axons.

After discovering in 1956 that there were two sizes of intrafusal muscle fibre, Boyd went on in 1958 to investigate differences in their innervation. He described discrete end-plates on the large (or bag) intrafusal fibres, and a network-like distribution of terminals on the small (or chain) intrafusal fibres. He suggested that the network on the chains was analogous to the endings on tonic fibres in lower vertebrates (review - Morgan & Proske, 1984), and that these would provide the means for slow, graded contraction, while the plates provided the capacity to twitch.

In 1961, Boyd elaborated this description, adding differences in diameter and location between the τ_1 (plates) and τ_2 (network) endings, and re-iterated his position in his 1962 paper. This was that the large nuclear bag intrafusal fibres were innervated by discrete endplates belonging to the thicker τ_1 axons, in a group at the polar part of the spindle. The small nuclear chain fibres were innervated by a network of endings belonging to the thinner τ_2 axons, closer to the spindle equator. As a result of the nature of the terminals and the relative quantities of myofibrils and mitochondria, he concluded that on the balance of evidence the bag fibres twitched, and the chain fibres contracted in a graded manner. However, he was puzzled by the fact that chain fibres atrophied more quickly after ventral root section - more like extrafusal fibres.

In fact, Boyd had been misled about the nature of the contraction in the two different intrafusal fibre types. Smith (1966) and Boyd (1966) both showed that stimulation caused rapid twitches in the small intrafusal fibres, and slower local contractions in the larger ones.

Shortly before this, Matthews (1962) discovered two functional types of τ -motoneurone - 'dynamic' and 'static' - and suggested that these were equivalent to τ_1 and τ_2 respectively.

Boyd's classification of τ s was challenged by Barker in 1962, at the beginning of a dispute which would not be resolved for over a decade.

At a symposium in Hong Kong on muscle receptors (Barker, 1962), Barker objected to Boyd's proposal that the two categories of intrafusal fibre had mutually exclusive innervation, as he (Barker) had seen single τ terminating on large and small intrafusal fibres.

In 1965, Barker & Ip coined the term 'trail ending', rather than τ_2 -network, which became accepted. They also demonstrated that trail and plate endings could both innervate each of the intrafusal fibre types. Further to this, Barker (1966) found a second type of plate ending at the ends of spindle poles, making 3 types of ending in all. He called the type at the ends of the poles p1 plates. These were very similar to extrafusal plates, and could sometimes be seen to be innervated by axons common to extrafusal fibres. The former 'plate endings' were mostly in the mid polar region, and these became known as p2 plates. The trail endings lay in a diffuse structure, typically juxta-equatorially. Barker also remarked on the similarity between the trail endings and the 'en grappe' endings in mammalian extraocular muscles, which cause local non-propagated potentials. He proposed that the functionally defined static τ and dynamic τ exerted their effects not by exclusive - or even preferential - innervation of the different types of intrafusal muscle fibres, but by the

different kinds of ending acting in different ways on intrafusal fibres generally, as happens in arthropod muscle. Barker, Stacey, & Adal (1970) confirmed this pattern of innervation in a variety of muscles, showing also that the number of p1 plates increased in fast muscle, while the no. of p2 plates was reduced.

In 1971, however, Boyd (1971) presented data conceding that while some τ -innervation was distributed to both fibres, this was only 10%. He interpreted this as demonstrating an anatomical basis for functional separation. The implication was that the 10% of mixing was 'overspill', of little physiological significance.

In 1973, however, there were findings which starkly contradicted Boyd's demarcation. In a histophysiological study, Barker, Emonet-Dénand, Laporte, Proske, & Stacey (1973) reduced the motor innervation of tenuissimus to a single τ , and allowed 7 or 12 days for degeneration of all others. Then the τ was categorised as static or dynamic, and its distribution examined. Coincidentally, all 10 successful experiments had a static τ (τ -s) remaining, and these ended in trail endings. In 6 spindles examined closely, it was twice as common for τ -s to innervate both bags and chains as it was to innervate either alone.

The situation was resolved by the discovery that bag fibres were of two types. Ovalle & Smith (1972) showed that bag fibres fell into 2 different histochemical classes, with at least one of each per spindle. In 1974, at a spindle symposium (Barker; 1974) this became accepted as fact.

Following this, Boyd, Gladden, McWilliam, & Ward (1975) showed that in spindles isolated in vivo, functionally identified dynamic τ (τ -d) excited one bag fibre, while τ -s excited chain

fibres and/or the other bag fibre. In a later study (Boyd et al., 1977) they established these as the Dbl fibre, and the Sb2.

Hereafter, in vivo isolated spindles are referred to as exposed spindles to distinguish this preparation from the more common in vitro preparation.

There was, however, one area remaining to be resolved. Barker, Emonet-Dénand, Harker, Jami and Laporte (1976) had found that, using glycogen depletion as an indicator, the bag1 fibre was innervated by τ -s about as often as the bag2. Now, it is accepted that glycogen depletion might have given misleading results so far as the dynamic bag1 fibre is concerned. In 1981, Gladden published work showing that after impalement of identified dynamic bag1 (Dbl) fibres in exposed spindles, stimulation of τ -s did not cause any intracellular potential changes, and Banks, Barker, & Stacey (1981) showed that there is very little trail innervation of the bag1 fibre. The reason for the glycogen depletion in the bag1 fibres remains a mystery. Barker & Stacey (1981) suggested that the high rate of τ -s activity may release sufficient K^+ to activate the Dbl.

As one chapter began to close, another one opened. It was also in 1981 that Boyd showed the effects of contraction of the different types of intrafusal fibre on the two afferents. In his 1981 paper, Boyd clearly demonstrated differences in effect from the two intrafusal fibres of the 'static system', though he did not comment on the implications of this for the efferent control of the spindle.

In 1982, Arbuthnott et al. examined with electron microscopy the ultrastructure of motor terminal endings on intrafusal fibres. They identified four basic structural types of ending (m_a , m_b , m_c , m_d), and another with intermediate properties ($m_{a,b}$). The distribution of these was not random and showed varying degrees of selectivity. The m_b endings lay only on the Dbl, and the Dbl supported only m_b endings; the m_c endings lay

only on chains, though the chains also had m_a and m_d endings; and m_d endings lay only on the long chain fibre. m_a endings, however, were distributed to both Sb2 and chains. In addition to all this, they noted that chain fibres with either an m_a or an m_c plate at one pole never also had the other plate at that pole.

In 1982, Boyd introduced a new test to 'diagnose' the intrafusal fibres involved from the response of the Ia and II afferents (Boyd & Ward, 1982). This was a 'frequency ramp' of stimulation, which is described completely in the section on Physiology, and examples of which are shown in fig. 3.

Using this technique, Boyd (1986) carried out an intensive functional study, with this diagnosis backed up by exposure and observation of as many intrafusal fibre contractions as possible, and also by subsequent electron microscopy. He found that 35 of 37 τ -s showed a preference in their innervation; always innervating Sb2 or always chains, accompanied by varying admixtures of supply to the other fibre. Only 2 appeared to innervate only chains in some of their spindles, and only Sb2 in the others they supplied. Boyd drew a parallel with these results, and the ultrastructural findings of Arbuthnott et al. (1982) and proposed two types of τ -s: a 'static bag τ -motoneurone' terminating on m_a plates, mainly on the Sb2 intrafusal fibre; and 'static chain τ -motoneurones' ending in m_c plates on the chain fibres.

More recently, following a complete analysis of the histology of Boyd's (1986) and subsequent work Arbuthnott, Boyd, Gladden, Sutherland and Ward (unpublished work), found that the distribution of their different classes of end-plate suggested that any given τ could follow one of three patterns of distribution. This prompted a re-assessment of the corresponding physiological responses, which by then also included rather more. When these were found to be in agreement with the histology, they felt confident enough to propose yet another sub-division of static τ . The three groups proposed were:

Group 1 - ending in m_c plates only, and lying only on chain fibres. This is a strict relationship: m_c plates are only found on chain fibres, so any axon terminal ending at one must belong to group 1.

Group 3 - group 1's opposite - ending in m_a plates always on the Sb2 fibre and showing a definite preference for that fibre. However, it also has some chain fibre involvement (also m_a plates) at the level of overspill, or 'ontological accident'. This consists of an occasional one or perhaps two in some of the spindles supplied.

Group 2 - lying between groups 1 and 3 in the scheme - ending in m_a plates with a fairly indiscriminate distribution: a substantial chain fibre involvement (>2) in all spindles supplied, and frequent Sb2 innervation in addition.

It should be pointed out that sub-division of static τ is not universally accepted. Banks, Barker & Stacey (1985) dismissed the distribution pattern of Boyd's 2 types as due to random variation. Also, while Banks (1988) evidently accepts Boyd's means to test the intrafusal fibre innervated by τ -s, and used it to assess their distribution to bag2 and/or chains, he arrived at a different conclusion. He found that the τ 's conduction velocities correlated with the pattern of intrafusal distribution, with the faster ones supplying bag2, and the slower ones, chains. He maintains that this represents a single type of τ -s, distributed along a continuum.

If the two ends of this continuum were to be recruited separately by the CNS - and there is ample evidence that they are (e.g. Gladden, 1981) - then I find it hard to see any great distinction between Banks' proposal, and two or three separate types. I feel that reconciliation between the two positions is not far off.

Efferent innervation (β motoneurones).

Most lower species do not have the discrete fusimotor innervation of mammals (evidently birds are an exception to this generalisation: a fusimotor effect on duck spindles was shown by Dorward, 1970). Intrafusal contraction is brought about by collaterals of axons supplying extrafusal fibres. This 'skeletofusimotor' innervation is presumed to provide intrafusal contraction, maintaining the afferent discharge in the face of extrafusal shortening. In mammals however, intra- and extrafusal contraction has been largely uncoupled with the advent of the τ system(s). The natural conclusion is that this provides a more versatile control system. It is not true though, that mammals have no skeletofusimotor innervation. It is now well established that there are both dynamic β axons and static β axons (see Laporte et al. (1981) for summary). The conventional position on these has been that they are evolutionary relics. However, in some muscles they are not uncommon, and may even preponderate over the same type of τ -motoneurone (Emonet-Dénand, personal communication). Since the muscles where this happens are involved in relatively precise movements (Peroneus Brevis, Adductor Digiti Quinti Medius) this makes the conventional position less tenable.

SELECTED PHYSIOLOGY OF THE SPINDLE.

In 1933, B.H.C. Matthews divided the afferents from muscle into response type subgroups : A, B, and C. Of these, the A-type afferents fell silent during muscle twitch. Muscle spindles were known to give rise to sensory fibres and to lie in parallel with extrafusal fibres, hence liable to be unloaded by their contraction. So, he suggested that his A receptors arose from spindles.

12 years later, Leksell (1945) found that selective activation of the small efferent (τ) nerve fibres to muscle increased their afferent discharge. He was not able to show conclusively that they did not cause any contraction, but suggested that this was so.

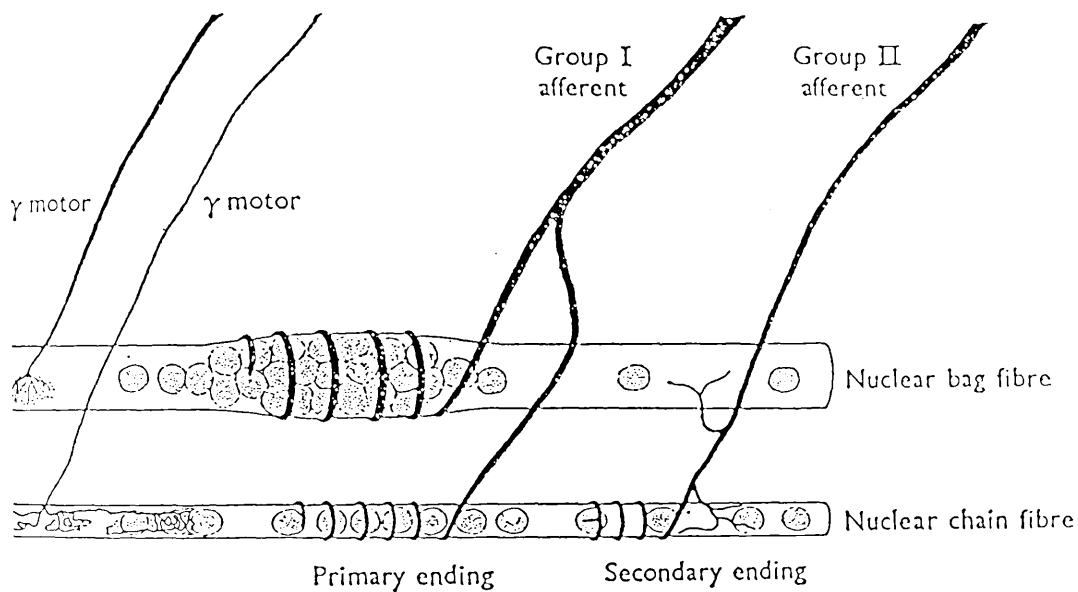
Kuffler, Hunt, and Quilliam (1951) integrated the findings of B.H.C. Matthews and of Leksell by using a preparation of dorsal and ventral roots, split down to strands containing single functional units. They were thus able to look at the uncontaminated effects of single efferent fibres on individual spindles. They found that the distinct group of small diameter, or τ , efferents (ca. 1/3 of total) increased the firing of A-type afferents, while the larger diameter group gave rise to muscle twitches. In some cases, there was a 1:1 relationship between stimulation pulses and the afferent spikes. This phenomenon has since become known as 'driving'. One year later, using the same preparation, Hunt and Kuffler (1951a, 1951b) laid many of the ground rules of spindle physiology. The small diameter efferents were shown to increase afferent firing even during contraction of extrafusal motor units. All A-type (spindle) receptors were found to be affected, and each spindle was innervated by several gammas, whose effects could sum or even potentiate each other. They also found that the converse was true; and that any one τ supplied several spindles. The discharge of spindles could also be increased by stimulation of the larger efferents, but only if initial tension in the muscle was high.

Skeletofusimotor (β) innervation in mammals was not known at this time, and they ascribed this only to (local) intramuscular tension changes, concluding that spindles were not directly affected by large diameter efferents.

Later, combining his earlier observations (Merton, 1951) on the silent period in muscle with the fact that the small gamma efferents increased the afferent discharge, Merton (1953) proposed that movement and posture were effected via a servo loop. He had concluded that the large efferents fell silent following an imposed twitch because their major input - from the spindle afferents - was momentarily removed as the extraneous twitch unloaded them. Given this, and the fact that gammas could increase the afferent discharge, he suggested that the central command to movement would initiate the required impulse frequency in the appropriate τ . This would then be followed by the alphas via the afferents. The advantage of this method, which became known as the follow-up servo hypothesis, was that unexpected differences from, or disturbances in the chosen muscle length were automatically compensated for by the negative feedback of the stretch reflex. This meant that only the desired muscle length had to be coded for in the central command, with no need to estimate the load.

In 1958, Hunt and Paintal studied the afferent connections to the small, or gamma, fibre cell bodies within the spinal cord. Their evidence strongly suggested that they were activated by afferents only via diffuse, polysynaptic pathways. They also suggested the term 'fusimotor' for neurones motor to the spindle, as this was strictly functional, avoiding the assumption that all small nerve fibres supplied spindles. As said earlier, 'fusimotor' is a useful term, as it includes both gamma (τ) fusimotor neurones, and also the larger diameter beta (β) fusimotor neurones. It is used in this inclusive sense throughout the thesis.

Figure 1 : Known innervation of muscle spindle in 1964.



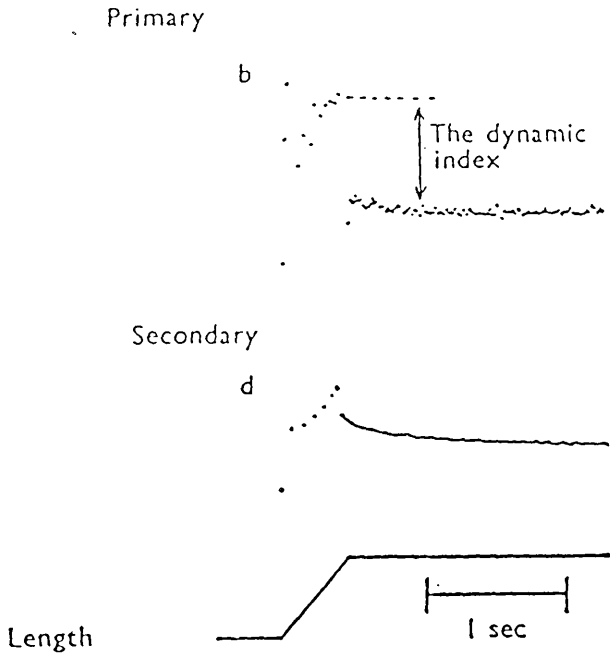
(From Matthews, 1964)

In 1961, Cooper looked at the secondary endings, and compared their responses to the primaries'. She found that only the primaries showed a large dynamic component in their firing during a 4mm stretch.

In the same year, Harvey & Matthews also found the primary endings to be more sensitive than the secondaries to changes in length, and that τ -motoneurons could have one of two effects on the relationship between muscle length and afferent discharge frequency. Either the slope of the relationship was increased, or this remained the same, with a general increase in the level of afferent firing.

Also in 1961, Jansen and Matthews compared the responses of afferents before and after de-efferentation (either by curarisation or by cutting the ventral roots) in the decerebrate cat. They found that while in the case of the secondaries the efferents exerted a continuous increase in their static response, the primaries could have either their static responses increased, or their dynamic responses, or a mixture of both. It was known by this time that the secondary endings lay almost entirely on chain fibres, whereas the primaries lay on both bags and chains (Boyd, 1962). Jansen and Matthews (1961) pointed out the appealing link between the anatomy and the physiology, and proposed that chain fibres were responsible for the increased static response, and bags for the dynamic ones (see fig. 1). In 1962, they repeated this finding (Jansen & Matthews, 1962) and also showed that various manoeuvres such as stimulation of labyrinth, cerebellum, or contralateral popliteal nerve could influence the dynamic and static sensitivities of primary endings independently of each other. In this paper, they also introduced a quantitative (if admittedly arbitrary) measure of dynamic enhancement. This was

Figure 2 : The dynamic index.



(From Brown, Crowe & Matthews, 1965)

the 'dynamic index' : the drop in the afferent discharge from the firing rate immediately before the end of stretching, to the rate 0.5 seconds after that point (see fig. 2).

Matthews (1962) went on to examine the effects of single τ fibres on the dynamic and static properties of primary endings, to see if the variation in these was controlled by individual τ -fibres. He found that although all τ increased the primary response by an approximately equivalent amount at a fixed length, during stretch their effects fell into two categories. The 'dynamic fusimotor fibres' also increased the dynamic sensitivity of the ending, and did so at all stimulation frequencies. The 'static fusimotor fibres' were defined rather negatively in that they did not increase, or could even reduce, the dynamic response of the primary.

In 1963, Matthews tested systematically that the dynamic sensitivity of the primary endings to stretch increased with increasing velocity, and that at all velocities they were consistently more sensitive in this respect than the secondary endings. Following this, Crowe and Matthews (1964a) showed that as well as increasing the dynamic index per se, dynamic τ (τ -d) increased the slope of the relationship between dynamic index and velocity. Static τ on the other hand reduced, or did not affect the slope of this relationship. They also noted that there were two components to the dynamic index : a rapid fall followed by a slower decay. This slow decay was not affected by the velocity of the preceding stretch. In a succeeding paper (Crowe & Matthews 1964b), they showed that : any particular τ -motoneurone exerted the same effect (static or dynamic) on all the spindles innervated by it ; that static and dynamic τ -motoneurones had such a large overlap in conduction velocity that they could not be reliably distinguished by this means ; and that during a sinusoidal length stretch, tonic stimulation of τ -d caused an increase in the modulation of afferent firing, with silence during the

shortening period; and static τ caused a reduction in modulation, accompanying an overall increase in the average firing rate.

In 1966, Matthews' schema that dynamic qualities are subserved by bag fibres, and static ones by chains, received support from the finding of Appelberg, Bessou and Laporte (1966) that during simultaneous recording of primaries and secondaries from any given spindle, on all occasions τ -s excited the secondaries, whereas τ -d (only one case out of eight) rarely did so.

Also in 1966, Boyd used his isolated spindle preparation (Boyd 1958) to look at the contraction of intrafusal fibres during graded stimulation of the muscle nerve. He found that although single stimulus pulses caused no visible effect in the intrafusal fibres, contraction was evident at a rate of 10/sec and maximal at about 100/sec. When they contracted, the chain fibres worked as a single unit, contracted rapidly, and caused the 'driving' of the primary afferent first remarked on by Kuffler et al. (1951). Contraction of the bag fibres was slower, and took place further towards the spindle poles than that of the chains. He also noted that sometimes one of them contracted in addition to the chains. In the same year, Smith found that when stimulated directly, large (presumably bag) intrafusal fibres contracted more slowly than small (presumably chain) intrafusal fibres. He also noted that treatment with suxamethonium appeared only to affect the large (bag) fibres.

In 1968, Bessou, Laporte & Pagès (1968a) developed the technique of accumulating a 'frequencygram', consisting of the overlaid instantaneous frequency discharges of many successive responses to single stimulation pulses. With certain qualifications, they asserted that the form this took was a reflection of the mechanical distortion of the sensory spirals by intrafusal contraction. Using this technique in an

accompanying paper (Bessou, Laporte and Pagès, 1968b), they showed that while τ -d could elicit one of two possible responses : no (detectable) response or a small one ; τ -s could effect 3 possible responses : no response, a small response, or a large amplitude response. They did not comment on whether or not any one τ was consistent in its effect in all spindles, but did point out that only the static axons which produced the large amplitude responses 'drove' the primary discharge, presumably by each response being sufficiently large to provoke a discharge. They also speculated that the heterogeneity of static effects could be due to activation of intrafusal fibres of different contraction speeds, but did not go any further on this matter. 4 years later, in 1972, Bessou and Pagès found that on penetrating intrafusal fibres of unknown type, dynamic τ only ever produced a non-propagated potential to a single stimulus pulse, while static τ gave a non-propagated response in some fibres and propagated action potentials in others.

Although the latter two physiological studies were generally in agreement with Matthews' scheme of things, their peculiarities acquired new significance following the unequivocal findings of Barker, Emonet-Dénand, Laporte, Proske, and Stacey (1973). In the same year, Bessou and Pagès, and (significantly) Boyd, Gladden, McWilliam and Ward carried out experiments where they identified τ s as static, and then exposed the spindles they lay in. It was found that some stimulated chains only, and some stimulated chains and one of the bag fibres. Bessou and Pagès also found that some τ stimulated one bag fibre only.

Taken together, all this work caused a new appreciation of the histochemical findings of Ovalle & Smith (1972) (see structure - intrafusal fibres)

These new findings - that static τ commonly innervated bags and chain fibres, and that there were apparently two sub-types of bag fibre - provoked new research which established the 3 sub-divisions of intrafusal fibres as genuine in 1974 (Barker, 1974).

Following this, Bessou and Pagès (1975) re-examined the effects of the different types of τ on intrafusal fibres, looking at the different types. They found that one third of static τ innervated chain fibres exclusively, one third a bag fibre exclusively, and one third both bags and chains. They also confirmed that chain fibres contracted as a single unit, and noticed that the bag fibres innervated by static τ had a visibly faster contraction time than those supplied by the dynamic τ .

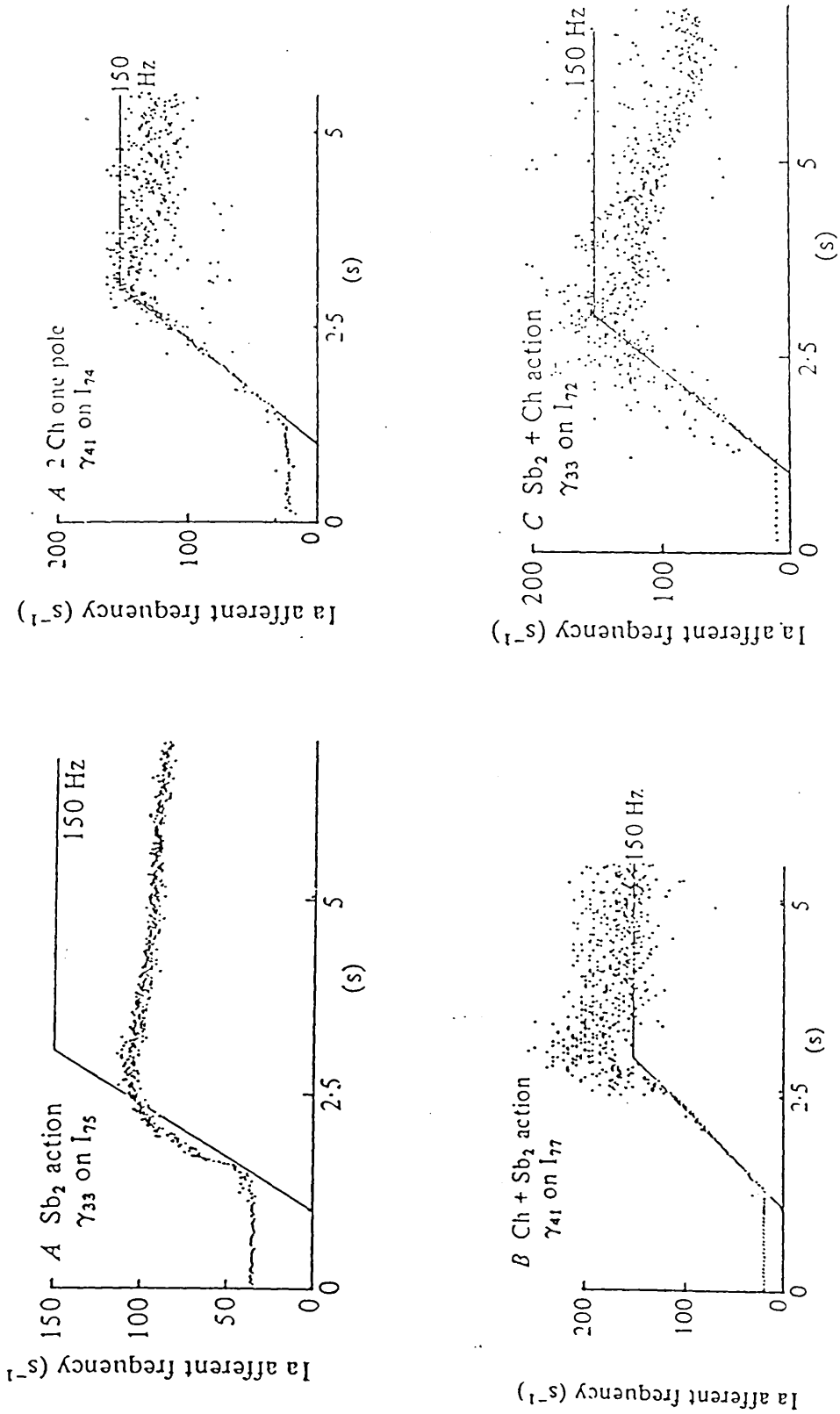
Earlier in 1975, Boyd, Gladden, McWilliam and Ward established that in any spindle one bag fibre was activated only by τ -d, and the other only by τ -s. They dubbed them the 'dynamic bag fibre' and the 'static bag fibre' accordingly. Also in 1975, Boyd and Ward recruited individual unidentified τ in the muscle nerve by use of graded stimulation, providing results in complete agreement with those of Bessou & Pagès.

In 1976, Boyd quantified the results of these and other experiments. Like Bessou and Pagès (1975), he found that the two bag fibres were of different contraction speeds. He named them the 'slow nuclear bag fibre' and the 'fast nuclear bag fibre'. The fast nuclear bag fibre had about the same contraction speed as the twitching chain fibres. The previously quoted minimum effective stimulus (Boyd, 1966) of 10 impulses/sec was observed to produce an oscillation in the chain fibres, and a smooth contraction in both bags. Single stimuli usually gave a twitch in the chain fibres, sometimes produced a slight contraction in the fast nuclear bag fibre, and never gave anything in the slow nuclear bag fibre. Contraction in the different fibres reached a maximum level at different rates of stimulation. At body temperature, both sets of bag fibres did not increase their response after 75 - 100

Hz stimulation (slow bag tending to be slightly lower), whereas the chains generally continued to be increasingly excited up to 150 Hz stimulation. Boyd also noticed the phenomenon of 'creep' in the slow nuclear bag fibre. In this, the outer parts of the fibre would yield slightly following a passive stretch, allowing the sensory spirals to shorten to about 75% of the maximum opening at the end of the stretch. He proposed that the fast nuclear bag fibre could be equated with the static bag fibre, and the slow nuclear bag fibre with the dynamic bag fibre. This relationship was confirmed in 1977 by Boyd, Gladden, McWilliam and Ward by direct observation of the mechanical properties of intrafusal fibres activated by identified static or dynamic fusimotor neurones. Also, by dye injection of the dynamic bag fibre, and subsequent histology, this fibre was established as being the bag1 of Ovalle and Smith (1972); and the static bag as their bag2 (Gladden, 1976). Thus arose the names dynamic bag1 fibre (Dbl), and static bag2 fibre (Sb2).

The establishment of separate static and dynamic bag fibres, whilst retaining the (static) chain, made necessary a reassessment of static and dynamic effects. This was done by Emonet-Dénand, Laporte, Matthews and Petit in 1977. They found that the sub-division of τ into static and dynamic was still valid, though there was some qualitative variation in static effects. The criteria they used to diagnose dynamic or static action had all been remarked on before, but they found that the consistent effect of dynamic action which persisted throughout static admixture was the slow decay of Crowe and Matthews (1964b), and of static action, the ability to maintain afferent firing even during fairly rapid shortening. In 1981, Gladden impaled identified intrafusal fibres in spindles which were in continuity (afferent and efferent) with the central nervous system (CNS). She found that Sb2 fibres had a greater degree of spontaneous activity in the lightly anaesthetised state than did chains or Dbl. She also found that Sb2 fibres were the most easily recruited during central

Figure 3 : Responses of Ia afferents to frequency ramp stimulation of γ motoneurons innervating static bag2 and chain fibres.



(From Boyd, 1986)

stimulation, and Db1 more easily than chain fibres. Furthermore, after spinalisation above the T12 vertebra, the spontaneous activity of the Db1 was unaffected, while that of the Sb2 was reduced considerably, and that of the chains profoundly (Gladden & McWilliam, 1977). Thus, quite clear differences in central influences on the static and dynamic systems were shown. But what was novel, and what was stressed, was that the Sb2 and chains were affected differently in these different circumstances.

The different effects of central control on Sb2 and chains strongly suggested that the two intrafusal fibres were innervated by separate populations of τ .

Also in 1981, Boyd showed the effects on spindle afferents of stimulation of various types of τ whose intrafusal distribution was determined subsequently by exposing the spindles. He found that when it was the Sb2 which contracted, the discharge of the Ia afferent was strongly biased. Contraction of the Sb2 during a ramp stretch decreased the dynamic sensitivity of the Ia, and either decreased, or had no effect on, its length sensitivity. The τ recruiting exclusively Sb2 had only minor, probably insignificant effects on the group II afferent. Gammas to the chain fibres could drive the Ia at a ratio of 1:1 up to about 60 Hz. It is not really appropriate to consider length sensitivity or dynamic index during driving, but - as measured - these were reduced. As would be expected from the anatomy, only the chain fibres had a large effect on the II, exciting them powerfully. During stretch the II's length sensitivity was increased, often by a lot, and their dynamic sensitivity decreased. The τ recruiting the Db1 fibre increased the dynamic and length sensitivities of the Ia, and had very little effect on any II endings.

Having accepted the possibility that the different types of intrafusal fibre could be under different central control by different populations of τ , Boyd - working mainly with Gladden and Ward - went on to put to the test any possible distinction in the effects of different static τ s.

Boyd and Ward (1982) devised the frequency ramp test (see fig. 3), and Boyd, Gladden and Ward (1983a, 1983b) used it to determine the range of effects of different τ -s on several different spindles. Individual τ -s were stimulated at a frequency progressing linearly from 0 Hz to 150 Hz. Activation of the 2 different intrafusal fibres was recognised by the chains' ability to drive up to about 75 Hz, and by the fact that their maximal effect on the afferent (without driving) is reached at 150 Hz. The Sb2 does not drive the Ia, and has its maximal effect at about 100 Hz. Activation of both together always drives up to about 100 Hz. After this point, the pattern of the Ia discharge resembles an Sb2 effect, but is more irregular.

The test was subsequently refined by Boyd, Gladden and Ward (1983a) who showed that chains alone usually drove 1:1 up to about 75 Hz, and thereafter at a submultiple (1:2) of the stimulation frequency. Static τ innervating both Sb2 and chains always drove 1:1 up to 75 Hz, and often up to 100 Hz. Boyd, Murphy and Mann (1985) showed that driving was most likely to occur at intermediate muscle lengths, and that changing from a short to an intermediate length could convert 1:2 into 1:1 driving.

In 1986, taking into account all the accumulated data from the above, and also the ultrastructural end-plate studies of Arbuthnott et al. (1982), Boyd proposed that there were 2 separate populations of static τ , although there was a fairly large degree of overspill on to the non-preferred intrafusal fibre. One of these would have a clear preference for the Sb2, supplying it in all innervated spindles. Any connections by these axons to chain fibres would only have a weak, insignificant effect on the primary, but a possibly powerful one on the secondaries. The other kind would always innervate chain fibres, driving the Ia discharge and strongly biasing the secondaries' firing. Extra connections onto Sb2 fibres apparently enhanced the axons driving ability, making possible 1:1 driving up to 100 Hz.

Recently, in the light of increasing resources of anatomical data, the position has been re-assessed by Gladden (unpublished work). It is now considered by the Glasgow group that there are 3 types of τ -s (see structure - efferent innervation). The additional physiological effects of this further sub-division seem to be : m_c end plates on chain fibres (Group 1 axons) have a high safety factor and are able to drive 1:1 up to high (75 Hz) stimulation frequencies ; m_a end plates on chain fibres (Group 2 axons which have no additional Sb2 involvement) have a low safety factor and tend to drive at sub-harmonics (1:2 or 1:3) of the stimulation frequency. Sb2 involvement in Group3 and Group2 axons seem to enhance driving ability, allowing 1:1 driving up to 100 Hz.

RECRUITMENT OF GAMMA MOTONEURONES FROM THE MESENCEPHALON AND NEIGHBOURING PARTS OF THE BRAIN.

The first to investigate the effects of central stimulation on τ -motoneurones were Granit and Kaada (1952). In cats anaesthetised with chloralose and dial, they set out to test the effects from areas of the brain known to exert an influence on the motor system. They assessed any changes in τ firing during stimulation both by direct recording of ventral root fibres (small spikes assumed to be τ) and by inference from any changes in the discharges of A-type afferents. They found excitatory areas in - notably for this project - the mesencephalic tegmentum and the contralateral habenulae ; but also in the pontine tegmentum, dorsal hypothalamus, midline nuclei of the thalamus, cortex, pyramidal tract, and some areas of the anterior lobe of the cerebellum. Repetitive stimulation in any of these produced an acceleration of afferent firing which persisted after cessation of stimulation and decayed only slowly over several tens of seconds.

In some of their ventral root recordings they found 1:1 driving of the τ discharge by the mid-brain stimulation (32 imp/sec). This would imply a fairly direct connection from the mid-brain to the τ cells. Strangely however, in these cases also, the discharge persisted after stimulation stopped.

The next year, in 1953, Eldred, Granit and Merton compared the effects of various central manipulations on α and τ motoneurones, with a view to predicting the probable spindle afferent discharge. They found that α firing evoked by cortical stimulation was accompanied by sufficient τ 'bias' to maintain afferent firing over the physiological range of muscle lengths, and also that τ discharge preceded α discharge. At the time this was seen as strong support for the servo hypothesis of spindle function. They also noted that while de-afferentation profoundly depressed spontaneous α

firing, it had little effect on τ , echoing Hunt and Paintal's later (1958) finding that afferents only have polysynaptic connections with τ -motoneurones.

In 1955, Granit and Holmgren pursued further the earlier findings on driving. They proposed that this was mediated by one of two pathways from the brain-stem to τ motoneurones, the other being that subserving the diffuse acceleration of τ . They found that τ filaments could be excited on a 1:1 basis with a latency of 7-12 ms from almost anywhere (in the mesencephalic tegmentum) more than 3mm from the mid-line on the contralateral side. By systematic lesioning of the spinal cord, they found this pathway was interrupted by damage to the area containing the reticulo-spinal and rubro-spinal tracts. Since they found in this study that not only the τ , but also the afferents were driven by the stimulation, it seems certain that the intrafusal fibres excited were largely or entirely chain fibres. Regarding their other pathway - the one recruiting afferents diffusely - they did not specify whether or not it did not drive τ as well as not driving afferents, so it is not possible to say whether the relationship broke down in the spindle or centrally. Their earlier experiments suggest that the τ were not driven either.

In 1961, Koizumi, Ushiyama and Brooks found that a single stimulation pulse in the reticular formation (exact location unspecified) could inhibit spontaneous firing of τ in ventral rootlets. They did not try to reconcile their findings with those of earlier workers, but if it was the medullary reticular formation they were using, then an inhibitory site was reported there by Granit and Kaada (1952).

In 1962, Shimazu, Hongo and Kubota (1962a) looked at the effect of stimulation of various brain areas - including the mesencephalic tegmentum - on τ motoneurones, and how this was altered by depth of anaesthesia (pentobarbital) and intensity of stimulation. Under light anaesthesia, all intensities of stimulation recruited a diffuse excitation bilaterally in flexors and extensors from all areas tested. Under deep

anaesthesia, a reciprocal pattern of excitation of flexors and inhibition of extensors was seen from all brain areas, at all stimulation strengths. If anaesthesia was moderate, then low intensity stimulation gave the diffuse pattern, but a high intensity evoked the reciprocal one. They remarked that in the mesencephalic tegmentum, the diffuse effect was more persistent, but still succumbed. Since the two effects could be separated by lesioning, they concluded that these findings were a reflection of two different controlling pathways to τ , which were of different synaptic susceptibility to pentobarbital. The same year (Shimazu, Hongo and Kubota, 1962b) they found that stimulation of the medullary reticular formation in the decerebrate cat had quantitatively different excitatory and inhibitory effects on primaries and secondaries of spindles in the plantaris muscle. The fact that the influences on the two afferents were also differently affected by injection of pentobarbital led them to add to their list a third pathway from the brain-stem to spindles.

The advent of the separation of static and dynamic τ -motoneurons by Jansen and Matthews (1962) prompted the search for different effects on them from the CNS. This was an appealing prospect since it was the independent variation in static and dynamic sensitivity caused by the gross procedures of decerebration and de-efferentation that had led to their discovery.

Slightly earlier, Appelberg (1962a, 1962b) had looked at the effect of stimulation in the region of the red nucleus on hind limb spindle afferents. He had found that spontaneous firing of τ s in the ventral roots was inhibited. So too were the afferents, with the II slightly less so. At the same time, the dynamic sensitivity of the Ia was increased, and he ascribed this to inhibition of the chain fibres : unloading both sensory endings, but increasing the proportion of tension during an extension taken up by the bag fibres, which lay in

parallel to the chains. At the time it was thought that both the bag fibres subserved the dynamic features of spindle response.

In 1963, Appelberg and Kosary extended these findings on the red nucleus and claimed two areas exerting effects on spindles: a dorsal area (seen above) which inhibited fusimotor fibres generally ; and a ventral area which had reciprocal effects - exciting τ to flexors, and inhibiting those to extensors.

Looking more closely at the dynamic sensitivity of spindles, Appelberg and Emonet-Dénand (1965) again stimulated in the mesencephalon. In the approximate region of the red nucleus - the exact location unconfirmed by histology - they found an area which increased the dynamic sensitivity of spindles in both a flexor (peroneus tertius) and an extensor (flexor digitorum longus : a physiological extensor, despite its name), both ipsi- and contralaterally. Neighbouring regions gave excitation or inhibition of static properties with no accompanying effect on dynamic sensitivity. They concluded that the reason for the discrepancy between this and the earlier studies, which had found inhibition of τ , was the level of anaesthesia. Here, they were most successful using a mixture of pentobarbital and urethane, which afforded them greater control and hence the ability to work with light anaesthesia. Comparison of their figures (see fig. 4) with the findings of Emonet-Dénand et al. (1977) reveals that while their contemporary measure of dynamic activity, the dynamic index, is clearly increased, there is definitely also some static admixture : none of their responses is a 'pure' (Type 1 of Emonet-Dénand et al.) dynamic response.

Appelberg and Molander (1967) went on to histologically locate the effective site. They actually found two : one, as before, in the caudal red nucleus; and another, further down, in the inferior olive. In these experiments they used inhalation anaesthesia (halothane) which is adjustable along a continuum, and allows adjustment to the critical level. In a companion

study, Appelberg (1967) demonstrated that these two sites were different stations in the same system. Stimulation applied in either area also caused a potential in the posterior lobe of the cerebellum after a short latency. The arithmetic of the different latencies, and the interplay of refractory periods between mesencephalon and inferior olive (IO) provided persuasive evidence that the three regions were connected. If a double shock was given in the mesencephalon, the response in the cerebellum to the second shock was reduced if given soon after the first, conditioning shock. The same result was seen if the conditioning shock was given in the IO. Lesions in the IO greatly reduced both the cerebellar and the peripheral responses to mesencephalic stimulation. Despite the fact that in these experiments only the effects on one muscle - flexor digitorum longus - were tested, Appelberg proposed an area for general dynamic control, naming it the MesADC, an acronym for 'mesencephalic area for dynamic control'.

Appelberg and Jeneskog (1969) set out to identify the course of this system in its descent through the nervous system. By lesioning the spinal cord, they showed that the dynamic effects were abolished by destruction of the dorsolateral funiculus (DLF) of the cord, on the side contralateral to the stimulation, and ipsilateral to the spindles tested. They did not reconcile this convincingly with the bilateral effects seen by Appelberg and Emonet-Dénand (1965). In response to single shocks in the MesADC, a double-wave response was seen in the DLF of the cord. By lesions of the cortico-spinal tract (CST), and the rubro-spinal tract (RST) - in the medulla, where they run separately - Appelberg and Jeneskog succeeded in abolishing separately the two waves of the response in the cord. However, the dynamic response in the periphery remained. They concluded that : a) the MesADC system descended in the DLF; b) it was not subserved by the CST or the RST; c) it took a different course through the medulla from the CST or the

RST; d) since there was no accompanying descending volley, it was probably a diffuse, polysynaptic pathway. This may also be partly why repetitive stimulation was necessary.

Also working in the brain-stem around that time, Vedel and Mouillac-Baudevin (1969a) looked at the effects on soleus spindles of stimulation in the reticular formation of the mesencephalon. They found that the effects obtained depended very much on the level of anaesthesia. When light, exclusively static effects were obtained ; when moderate, predominantly dynamic ones ; and when deep, both were depressed. Extending these findings to the pontine and medullary reticular formations (Vedel and Mouillac-Baudevin, 1969b), they found mainly static effects from the pons, except in a small anterior region, which increased dynamic responses. From the ipsilateral medulla only, stimulation of the lateral part evoked dynamic effects, and of the medial part, static. Lesions of the cord located these effects to the ventral and ventrolateral funiculi (not the DLF).

In 1972, Appelberg and Jeneskog found 2 areas around the red nucleus (NR) which recruited τ -motoneurons. A dorsal area, whose effects descended in the DLF of the cord, and which produced dynamic effects ; and a ventral area which gave mixed static and dynamic effect, and ran laterally and ventrolaterally in the cord. They did not try to reconcile this with the dorsal and ventral areas found in the NR by Appelberg and Kozary (1963), which were reported to inhibit τ generally, and to have reciprocal effects on τ in flexors and extensors, respectively.

In 1974, Jeneskog demonstrated that virtually identical areas which both overlapped the dorsal NR caused peripheral dynamic activity, and a potential in the contralateral paramedian lobule of the cerebellar cortex respectively. The stimulation site ventral to the NR evoked a response in a more restricted area of the cerebellum.

Appelberg, Jeneskog and Johansson (1975) looked at the effect of NR stimulation on the different types of τ -motoneurone (static or dynamic) to different types of hindlimb muscle (flexor or extensor). Recording τ -motoneurons extracellularly, they identified the associated muscle by antidromic volley from the cut peripheral nerves, and as dynamic or static, according to whether or not it was affected by stimulation of the site dorsal to the NR : the MesADC. They achieved selective stimulation of the NR and the MesADC by monitoring the descending volley in the cord, over the RST. Selective stimulation of RST was achieved by stimulation of interposito-rubral fibres which feed monosynaptically onto the NR cells supplying the RST. The pre-synaptic terminals lie ventral to the NR and require a stimulus only 1/10 that of the RST cells themselves (Baldissera, Lundberg and Udo, 1971). Their activation is signalled by a shift in latency of the cord volley after advancing down through the NR. Thereafter, the stimulus strength is reduced. From the MesADC, dorsal to the NR, a response in the paramedian lobule of the cerebellum and a volley in the RST can be recruited at high stimulus strengths. However, at the (lower) stimulus strengths used, the rubrospinal volley disappears, leaving only the cerebellar potential. This is taken to indicate that the MesADC is being stimulated, but not the NR. Using this method they found that τ -s followed the pattern found for α -motoneurons (Hongo, Jankowska and Lundberg, 1968), with generally excitation of those going to flexors, and a mixture of excitation and inhibition on extensors. Dynamic τ followed a strictly reciprocal pattern, with excitation of flexors, and inhibition of extensors. My objection to this finding is that it is based on the premise that only τ -d are affected by the MesADC ; something which I do not think had been established, even then.

In 1981, Appelberg collated all his earlier work in a definitive paper on the MesADC explaining how to achieve its selective stimulation (Appelberg, 1981). For this, two

electrodes should be placed around the NR : one in the MesADC (by stereotaxis), and one ventral to the NR in the terminal part of the interposito-rubral tract. The latter is to stimulate the NR alone, to distinguish it from MesADC stimulation. Stimulation of NR evokes a descending potential in the DLF of the cord, and a potential in the paramedian lobule of the cerebellum with a latency of 12.5 ms. Stimulation of MesADC produces no cord potentials, and an earlier potential in the cerebellar region (8 ms).

Appelberg, Hulliger, Johansson and Sojka (1982), for unspecified reasons decided to re-assess the effect of RST stimulation on τ -motoneurons whose type was identified by MesADC stimulation. Earlier, Appelberg, Jeneskog, and Johansson (1975) had found strictly reciprocal excitation of flexor τ -d and inhibition of extensor τ -d. This time, it was found that τ -d followed the same pattern as α and τ -s; namely, predominantly excitation in flexors, and equal amounts of excitation and inhibition in extensors. The only reason suggested for the differing results on the two occasions was that on the first, the τ -motoneurons were recorded extracellularly, and so those tested tended to be spontaneously firing. On the second occasion intra- and juxta-cellular recording was used, so sampling a representative cross-section of the whole population. Implicit in this is that in extensor motoneurone pools the NR has opposite effects on spontaneously firing τ -d (inhibition), and silent τ -d (excitation). They do not, however, remark upon or seek to discuss this.

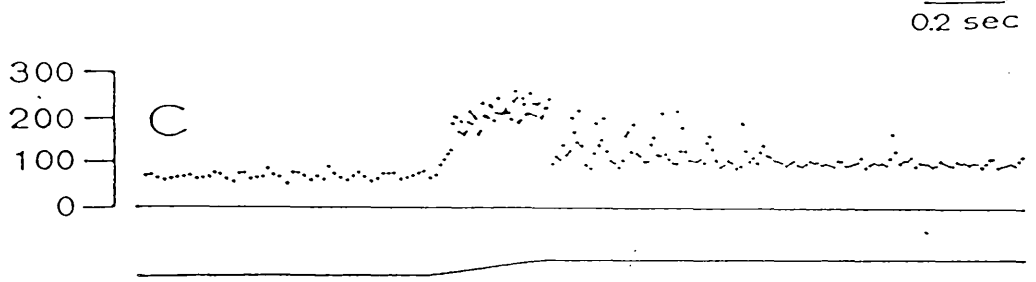
In 1983, Appelberg, Hulliger, Johansson and Sojka carried out a series of experiments (Appelberg et al., 1983a, 1983b), looking at the effects on different τ -motoneurone populations (τ -d/ τ -s; flexor/extensor) of graded electrical stimulation of peripheral nerves. Whether or not they set out to so confirm the validity of MesADC is not said, but they presented the two sets of results as mutually supportive (see below). They found that, as a whole, group II afferents generally excited

τ -motoneurones. In flexors, there was a clear preference for τ -d (ca. 65 % of τ excited), and in extensors a less marked preference (ca. 45% of τ excited). Group III afferents had a preference for τ -s, with no marked difference between flexors and extensors.

They sought to confirm their findings for group II (Appelberg et al., 1982, 1984) by the natural stimulation of muscle afferents by muscle stretch. Pull on posterior biceps/semitendinosus (PBSt) generally increased the dynamic sensitivity of Ia afferents in gastrocnemius/soleus (GS). 30% of Ia afferents showed an increased dynamic sensitivity, and 12% showed an increased static response. In the electrical stimulation experiments, group II stimulation in PBSt nerve excited GS τ -d and τ -s in the percentages 9% and 2%. Thus the proportions of τ identified as τ -d by MesADC stimulation, and excited by electrical stimulation at group II strength corresponded fairly well with the proportion of τ identified as τ -d by their action, and excited by natural group II stimulation. Any difference between the two sets of figures can be adequately explained by single shock stimuli being less effective than a sustained volley of afferent discharge; and by branching of τ -motoneurones to more than one spindle. These parallel findings by two independent means of τ -motoneurone identification support the idea that the MesADC does have a predominant effect on τ -d motoneurones since this is certainly the most straightforward connection between the two sets of results. However, there is not necessarily a link between the two; and, even if there is it does not establish the MesADC as exciting selectively τ -d.

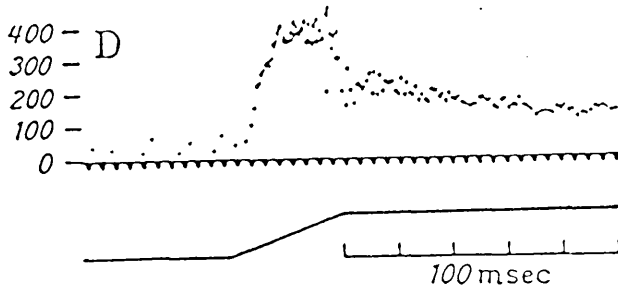
In 1984, Appelberg et al. demonstrated that muscle afferents from the contralateral GS could also provoke more dynamic effects than static in GS (64:25). Since then, using excitation from MesADC for τ -motoneurone identification, Johansson and Sojka (1985) showed that effects from cutaneous nerves are qualitatively the same for τ -d and τ -s, but that τ -d require lower stimulus strengths ; and Johansson,

Figure 4 : Effects of mid-brain stimulation on hind limb la afferents seen by Appelberg and co-workers.

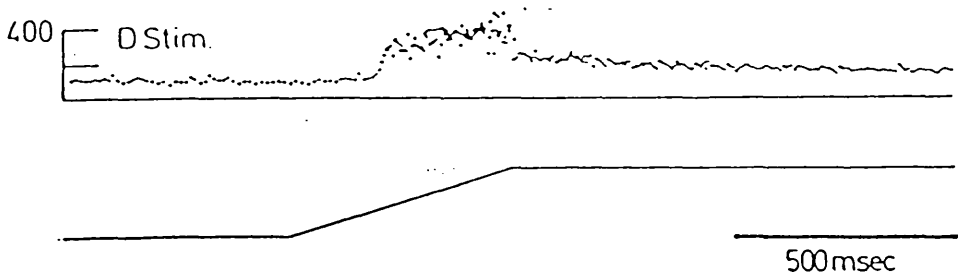


(From Appelberg & Emonet-Dénand, 1965)

IMPULSES/SEC



(From Appelberg & Molander, 1967)



(From Appelberg & Jeneskog, 1971)

Sjölander and Sojka (1986) that τ -d and τ -s are affected in the same way by stimulation of a joint nerve (posterior articular nerve). In 1988, Johansson collated all the work on effects on τ cells from the periphery and from the NR. He found that there was a weak tendency for similar effects on τ cells from the NR and from peripheral inputs; but, in a sample of 120 τ , he was not able to draw any firm conclusions.

Apart from their habit of ignoring previous contradictory results from their own and others' work, my lingering doubt about the work of Appelberg's group is a small one. It is their seeming insistence on the MesADC as an area recruiting τ -d selectively or exclusively. While the generally dynamic nature of their effects is clear, and supported by some other work (Taylor & Donga, 1989; see below), direct evidence for its exclusive nature is scanty. It has not been re-assessed since the use of solely dynamic index to gauge τ activity was tempered by Emonet-Dénand et al. (1977); nor since the advent of the bag1/bag2 distinction aroused the possibility of further sub-divisions of types of τ . According to the criteria of Emonet-Dénand et al., there is evidence of static admixture (albeit weak) in the records of (see fig.4) Appelberg and Emonet-Dénand (1965), in those of Appelberg & Molander (1967), and in some of those of Appelberg & Jeneskog (1972) - their last published ramp stretch response. Static admixture is indicated by the irregular profile of the response, and by the high ongoing discharge. In one case at least, this can be in no way due to initial tension, as the ramp stretch apparently has to take up some slack (fig. 4, bottom trace).

During the 1980s, 2 other groups have looked at the effects of mid-brain stimulation on τ -motoneurons. Donga, Gottlieb, Jüch and Taylor (1985) stimulated in and around the NR and found that this increased the dynamic index in all of 19 spindle afferents tested (presumably all primaries), but expressed doubts as to the specificity of the effect. Later, in 1988, they used MesADC stimulation to try to categorise 2 groups of

unidentified τ which were distinct in their reflex response patterns. One group showed modulated behaviour - accompanying α -motoneurons - during reflex movements ; and the other group fired tonically. They wanted to see if one type was static, and the other dynamic. They found that of 12 τ , 6 were modulated and were not affected by MesADC stimulation ; and 6 fired tonically and were affected. They assigned the tonically firing τ as dynamic, and the modulated τ as static.

Taylor and Donga (1989) found dynamic effects on afferents from triceps surae and jaw muscle from stimulation sites around the NR, including the area corresponding to MesADC. They also found dynamic effects from a more anterior site : the retroflex bundle, which runs between the habenular nuclei and other mid-brain structures, and is an output from the limbic system. They proposed that this could be the path for the increased dynamic activity accompanying certain states of arousal. They noted that in many cases, there was static 'contamination' with variable or mixed effects. This static admixture must have been confined to Sb2 fibres, since they saw no significant effects on secondary afferents. They felt able to propose the following areas as recruiting particular intrafusal fibres : Habenular nuclei and MesADC - Dbl ; periaqueductal grey material - Sb2 (chain fibres - no regions found).

The second group, Schwarz, Sontag and Wand, in 1984 looked at the effect of activation of cells in the substantia nigra - either by electrical stimulation or by drug-mediated disinhibition of the striato-nigral pathway. Both of these removed a tonic static output to Ia afferents of hindlimb flexor muscles.

In 1985, Wand and Schwarz repeated this experiment, looking at II afferents. In contrast to the Ias these were unaffected. De-efferentation showed that there was a tonic drive to the II

which could have been inhibited, but was not. Thus they showed that was separate central control (or gating) on τ -motoneurones supplying Sb2 and those supplying chains. Although the experiments of Wand and Schwarz, and of Gladden have recently generated excitement amongst those concerned with τ -motoneurone sub-types, there is also a rather more neglected body of evidence from earlier work (described above). The third pathway of Shimazu, Hongo and Kubota (1962b) affected Ia and II differently under different sets of circumstances, Appelberg (1962b) proposed inhibition of chains as the explanation most compatible with his results, and - even earlier - Granit and Holmgren (1955) described a fast path from the mesencephalic tegmentum, stimulation of which drove both τ s and Ias : hard to see as anything except selective chain fibre activation.

THE AIM OF THE PROJECT.

This project set out to assess the feasibility of using Appelberg's MesADC as an experimental tool for recruiting Dbl fibres in the tenuissimus muscle - both in the exposed spindle preparation and, for comparison, in a dorsal roots preparation. It was hoped that there might also be neighbouring areas specifically recruiting Sb2 or chain fibres.

After accumulating a fair number of overwhelmingly static results, the aim was changed to testing the validity of the MesADC, so far as tenuissimus is concerned. At some unnoticed point, this changed to testing the validity of the MesADC per se, and trying to assess the nature of the static effects on tenuissimus.

MATERIALS AND METHODS

Two types of experiment were carried out : first, in an exteriorised tenuissimus muscle, direct observation of intrafusal fibre movements was made during stimulation in the mesencephalon and telencephalon ; second, the response of muscle afferents to a ramp stretch was monitored and recorded and any change in them was examined during stimulation in the same regions

The exposed spindle preparation has the advantage that when, as is almost always the case, intrafusal fibres can be identified as Db1, Sb2, or chain fibres, then there is no doubt as to which are being recruited or inhibited, no matter how complex the picture. When looking at the unknown effects of central stimulation, this has obvious merits. It is also a more sensitive indicator of τ activity than is afferent activity, allowing one to visualise effects which may not influence the afferents. Its disadvantage is that it is limited to the spindles which one can expose. To date, this has only been done in the central portion of tenuissimus and a small muscle of the foot (Boyd, Gladden, McWilliam & Ward, 1977). This means that it is difficult to generalise about function on the basis of results, especially since the functional purpose of tenuissimus has not been determined.

The comparative strengths of the afferent response recordings lie where the exposed preparation's weaknesses are. One is not restricted to specific spindles, and is able to build up a more comprehensive picture. Also, one can - in theory, if not in practice - look at many spindles in one experiment.

Its disadvantage is that one has to try to infer the τ activity from changes in the afferents' responses - less direct and less certain.

We felt that a combination of both approaches would yield data of the most value. In addition, the afferent responses could be directly compared with the results of previous workers, providing an interface with them for the exposed spindle responses. To further this end, we decided to look not only at tenuissimus afferents, but also at those in Flexor Digitorum Longus (FDL) and Peroneus Tertius (P.Ter) : the two muscles used by Appelberg and Emonet-Dénand (1965).

Much of the preparatory work for the two experiments was identical, but some was unique to one or other. The sections of the methods are presented in order of execution, but each one is indicated as being : "common"; "exposed"; or "roots".

Thirty-nine cats of either sex in the weight range of 2.0 to 3.8 Kg were used for the project.

A : PREPARATORY SURGERY

Anaesthesia (Common)

In all the exposed spindle experiments and in some of the dorsal roots experiments, barbiturate anaesthesia was used. In this case anaesthesia was induced by intra-peritoneal injection of sodium pentobarbitone (Sagatal; May & Baker) at a dose of 45mg/kg. Anaesthesia was thereafter maintained intravenously by 0.1ml doses.

In most of the dorsal roots experiments, anaesthesia was induced by intramuscular injection of ketamine/xylazine (Vetalar ; Parke-Davis / Rompun ; Bayer). Following this, anaesthesia was maintained by a variety of means : inhalation of 80% N₂O/ 20% O₂, mixed with 1-2% Halothane ; 80% N₂O/ 20% O₂, mixed with 0-1% Halothane, supplemented by small intravenous doses of alphaxolone/alphadolone (Saffan ; Glaxovet) ; 80% N₂O/ 20% O₂, supplemented by small doses of alphaxolone/alphadolone ; or 100% O₂, mixed with ca. 3% Halothane. Also, in the very early experiments, α-chloralose was tried (40mg/kg), with very little success.

Except during induction with ketamine, the level of anaesthesia was gauged by the following : Guedel's signs (pupil size, respiration - thoracic or abdominal) (Lewis, 1970) ; flexion-withdrawal reflex ; blood pressure and heart rate. During brain stem stimulation, less credence was given to pupil size due to the proximity of the oculomotor centres

to the stimulation. Blood pressure varied greatly from experiment to experiment, but within any particular one was a reliable indicator.

It was assumed that any peripheral effects the anaesthetics used had on spindles were negligible.

Shaving (Common)

Once anaesthetized, the cat was prepared for surgery by shaving the forelimb, hindlimbs (both medial and lateral aspects), back, throat region and top of the head with fur clippers.

Tracheostomy (Common)

A pretracheal midline incision was made through the skin from the suprasternal notch to the hyoid bone. The skin was retracted and the superficial muscles covering the trachea were separated by blunt dissection to expose the trachea. Any overlying connective tissue and the sides of the trachea was cleared by blunt dissection. A linen thread was passed round the trachea using an aneurysm needle. The thread was then tied in a half knot round the trachea. The trachea was lifted by the thread and a cut made half way through it between two cartilaginous rings cranial to the thread. A small longitudinal cut was given to the caudal ring in order to ease insertion of cannula into the trachea. The caudal edge of the incision was gripped with Spencer-Wells forceps

and a glass cannula of appropriate size was then slipped into the trachea and tied in position with the thread. The incision was then closed with Michel suture clips.

Intravenous Cannulation (Common)

An intravenous cannula was inserted into a superficial branch of the cephalic vein of the right forelimb. Three way taps were attached to the end of the cannula to allow syringes to be connected. This allowed the infusion of supplementary doses of intravenous anaesthetic when required. Any nutritive solution (Dextran or a mixture of D-Glucose and sodium bicarbonate-"strong soup") was given by the same means.

Hindlimb Denervation: Femoral and Obturator Nerves (Common)

With the animal still in the supine position it was convenient to carry out denervation of the medial aspect of the left thigh at this stage. An incision, about 4 cm long, was made from the ileopectineal arch along the line of the femoral neurovascular bundle. The femoral nerve was exposed. A thin cotton roll soaked in Lidocaine (2%) was looped round the femoral where it emerges from the dorsal ileopectineal arch, to minimize any possible central effects via afferents on cutting the nerve. The cotton roll was removed after a minute or so, and the femoral nerve was cut.

The obturator nerve gives off a superficial branch called the gracilis nerve which lies in between the two adductors - femoris and brevis - before becoming superficial and ultimately going to gracilis muscle. Following this nerve centrally while retracting the muscles surrounding it, enables one to approach the deep branches of the obturator nerve which lie next to the pubis. After the superficial and the three deep branches of the obturator nerve had been exposed, they were cut, taking care not to rupture any blood vessels. The incision was then closed with sutures, rather than clips which might obstruct flow in the femoral artery during the course of the experiment.

Cannulation of the Right Femoral Artery (Common)

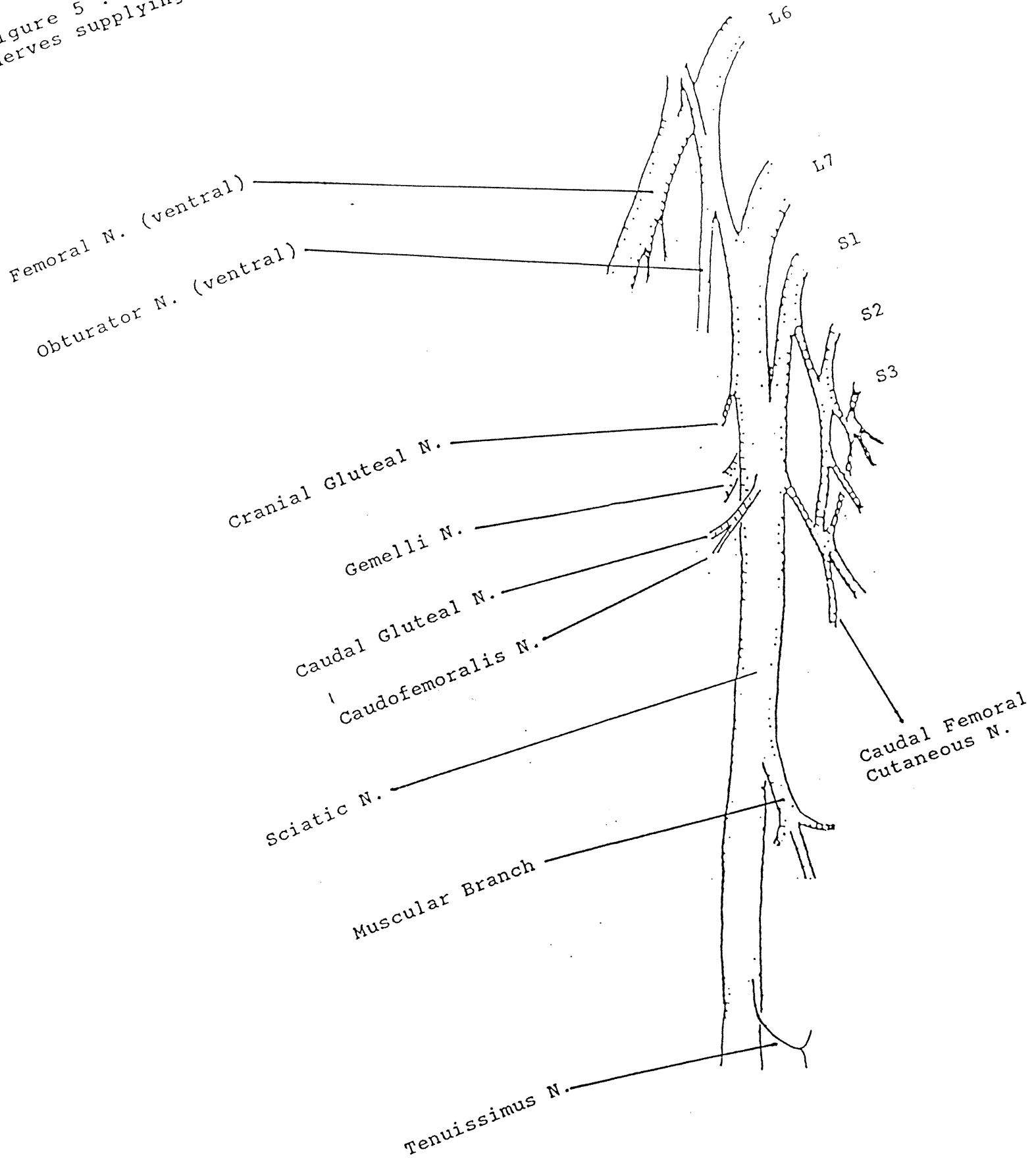
The right femoral artery was cannulated by separating it from the femoral nerve and vein over the upper half of the thigh. Two pieces of linen thread were passed under the artery each one at the upper and lower ends of the freed artery. The lower end thread was then ligated and the upper one half tied. A pair of Spencer-Wells forceps were clamped on the artery just above the upper ligature. The artery was opened between the ligatures and a white (or red) luer nylon cannula (Portex) was inserted. The upper ligature was made firm but not tight around the cannula, and the Spencer-Wells forceps removed. The cannula was then advanced into the artery until the tip was estimated to be at or near

the bifurcation of the aorta. The upper ligature was then fully tightened. The skin incision was sutured and the animal was turned over to lie prone on the operating table.

Laminectomy (Roots)

The purpose of the laminectomy was to expose the spinal cord and its roots without damaging them and with the minimum loss of blood. A midline skin incision was made along the vertebral column from L4 to the sacrum. The skin on both sides was freed from the underlying fascia by blunt dissection. With the skin flaps pulled back, cutaneous nerves emerging from the body wall and running to the skin on the left and right sides of the animal were located and cut. Two parallel incisions of the same length as the skin incision were made on either side of the dorsal processes of the lumbar vertebrae. The longissimus dorsi muscles on both sides were separated from lumbar multifidus muscles of the vertebral column, and held aside by retracting hooks. The multifidus muscles were then cleared from the vertebral column and removed and the longissimus dorsi muscles were denervated. To begin laminectomy, the joint between S1 and L7 was opened by gripping the L7 spinous process in Spencer-Wells forceps and lifting the vertebral column as much as possible. Lifting the vertebral column ensured that the spinal cord remained on the floor of the vertebral canal, out of the way of the bone nibblers. One jaw of a large pair of nibblers was inserted into the joint and the

Figure 5 : The Lumbo-sacral Plexus, showing the nerves supplying the muscles of the hip.



bone forming the wall of L7 was cut away which exposed the dorsal longitudinal ligament. Cutting the dorsal longitudinal ligament by using a pointed pair of scissors allowed the use of a small bone nibblers to remove the rest of the vertebra. Each vertebra from L7 to L5 was removed in a similar fashion. Any jagged edges of bone were trimmed with a small pair of bone nibblers. The cord was then covered with a moist swab (or cotton roll) and the incision closed with Michel suture clips.

Hindlimb Denervation: Sciatic Nerve (Roots)

With the cat in the prone position an incision was made from just below the base of the tail over the posterior aspect of the left thigh to 2 cm below popliteal fossa. By blunt dissection with scissors, the lateral edge of biceps femoris was freed along its length and the muscle was reflected medially to expose the thick sciatic nerve. At this point it was usually possible to see the thin tenuissimus muscle as it crossed the fat pad in the popliteal region. The sciatic nerve was exposed by blunt dissection between gluteus maximus and biceps femoris. The gluteal muscles were lifted using forceps to expose the sciatic nerve as far centrally as possible (see fig. 5). Tenuissimus (abductor cruris caudalis) muscle originates from the tip of the transverse process of the second caudal vertebra and inserts with biceps femoris into one third of the dorsal border of the tibia along its lateral margin. It is a weak abductor and extensor of the

thigh and a weak flexor of the shank. It is innervated by a branch of the sciatic nerve and sometimes a second nerve higher up in the sciatic and receives its arterial supply from branches of the caudal gluteal, deep femoral and popliteal arteries.

The first nerves cut were the caudal and cranial gluteal nerves which branch off the sciatic trunk before it emerges from the great sciatic notch. Then the caudal femoral cutaneous, caudofemoralis and gemelli nerves were cut, the last two supplying the caudofemoralis and gemelli muscles respectively. Finally in the hip, the muscular branch going to the semimembranosus/semiotendinosus and biceps femoris group of muscle was cut.

In the experiments using tenuissimus, its nerve was then identified and the sciatic was cut 2 cm below the branch. Local anaesthetic (Lidocaine; 2%) was applied to the sciatic nerve before cutting to minimize any effects on the cardiovascular and pulmonary systems. If the muscle under investigation was FDL or P Ter then the branch to tenuissimus was cut and the sciatic left intact. The innervation in the lower leg was reduced to the single muscle in question.

As a final check, the laminectomy incision was opened and the L7 and S1 ventral roots stimulated. A successful denervation would result in only the muscle of interest twitching. Occasionally some tail and upper hip muscles twitched as well. If so, attempts were made to find and cut these intact nerves, leaving only the desired nerve intact. The caudal end of the tenuissimus, or tendon of P Ter or FDL, was freed and

a thread tied to it as caudally as possible for the purpose of attaching the muscle to the puller later on. The skin incision was closed with Michel suture clips.

Fixing to the Experimental Frame (Common)

Before moving the animal to the experimental frame, a knitting needle was inserted between the interspinous ligament 5 cm cranial to the laminectomy. A second was passed underneath the pelvic bone, through the body cavity to support the hip bone in frame. Then, the animal was transferred to the frame.

Cats were mounted in a conventional stereotaxic frame (Narishige). About 1 minute prior to insertion of the ear-bars, the ears were infused with local anaesthetic (Lidocaine; 3%), again to minimise unwanted repercussions.

B : FINE SURGERY AND PREPARATION FOR EXPERIMENT.

Temperature and Blood Pressure Control (Common)

To prevent a steady fall in the body temperature the animal was heated from above by two infrared lamps, one of which was thermostatically controlled and was set to switch in if the temperature was below $36\pm 1^{\circ}\text{C}$. The temperature was monitored throughout the experiment with a rectal thermistor, which was also the control source for the thermostat.

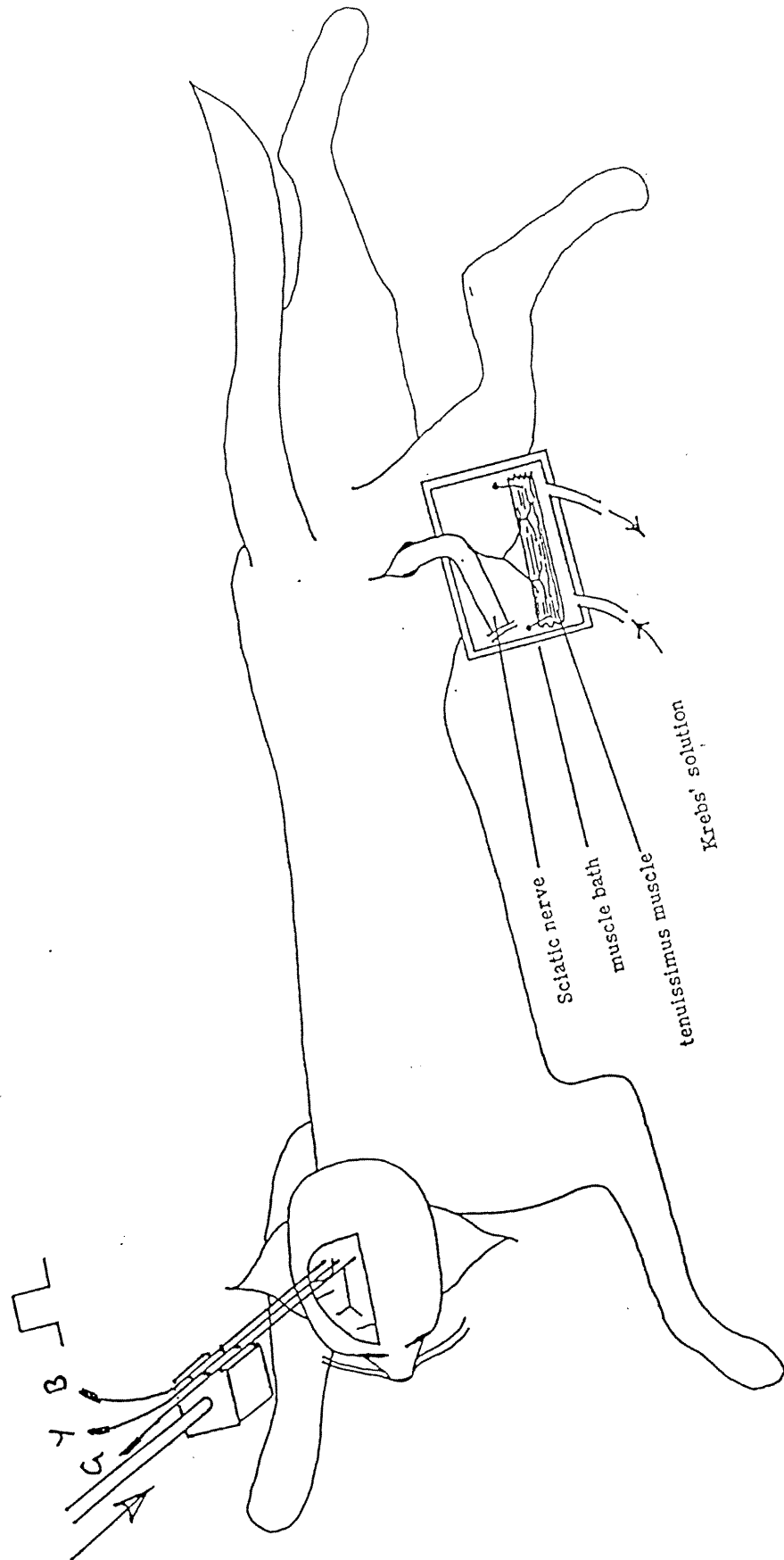
The cannula in the right femoral artery was flushed with heparinized saline and then connected to a pressure transducer (Elcomatic EM752) which was in turn connected to a pressure monitor. The pressure monitor device had an alarm tone which sounded at pressures below 70 mmHg.

Isolation of Muscle Spindles in Tenuissimus Muscle (Exposed)

In the series of experiments in which the spindles of tenuissimus muscle were to be observed visually, the spindles had to be "isolated" or "exposed". The arduous process of exposing muscle spindles by careful removal of single extrafusil fibres was carried out by Dr. M. H. Gladden.

Once the previous procedures were carried out, the cat was turned over to a prone position. An incision was made from just below the base of the tail over the posterior aspect of the thigh to popliteal fossa. Lateral edge of biceps femoris was freed along its edge by blunt dissection. The biceps

Figure 6 : Rudimentary set-up for the exposed spindle experiments.



femoris was reflected medially to expose the sciatic nerve and tenuissimus muscle. The muscle dissected free from surrounding tissue and reflected into a glass bottomed bath containing Krebs solution (in mM: NaCl, 119; KCl, 4.7; KH_2PO_4 , 1.2; NaHCO_3 , 24.8; CaCl_2 , 2.5; MgSO_4 , 1.2 and glucose, 1 g/l; equilibrated with a 95% O_2 , 5% CO_2 gas mixture). The sciatic nerve was cut below the tenuissimus nerve, and it was also necessary to cut the nerves to biceps femoris, semitendinosus and semimembranosus to free the muscle with the sciatic nerve pedicle so that it could be exteriorised. The bath was mounted on a microscope stage and the muscle was illuminated from below.

The basics of the exposed spindle preparation are illustrated in fig. 6.

The tenuissimus muscle normally receives its blood supply from branches of the gluteal, deep femoral and popliteal arteries. Where the muscle was dissected free and reflected into the bath these vascular supplies were severed. However, a certain amount of vascular re-routing usually took place and the small blood vessels serving the sciatic nerve and the tenuissimus muscle nerve provided an adequate blood supply to the portion of muscle in the bath.

Once the tenuissimus muscle was in the bath, a spindle fluid space was located under a binocular dissecting microscope (Wild-Heerbrugg). In most tenuissimus muscles the fluid spaces could not be seen at this stage. The extrafusal fibres of the tenuissimus muscle were removed until the fluid spaces of muscle spindles became clearly visible and

the intrafusal fibres could then be directly observed using a high power light microscope or/and could be displayed on a TV monitor (Phillips) and at the same time recorded on videotape (recorder - UVCSR, model CR-8200E). Usually three muscle spindles were dissected.

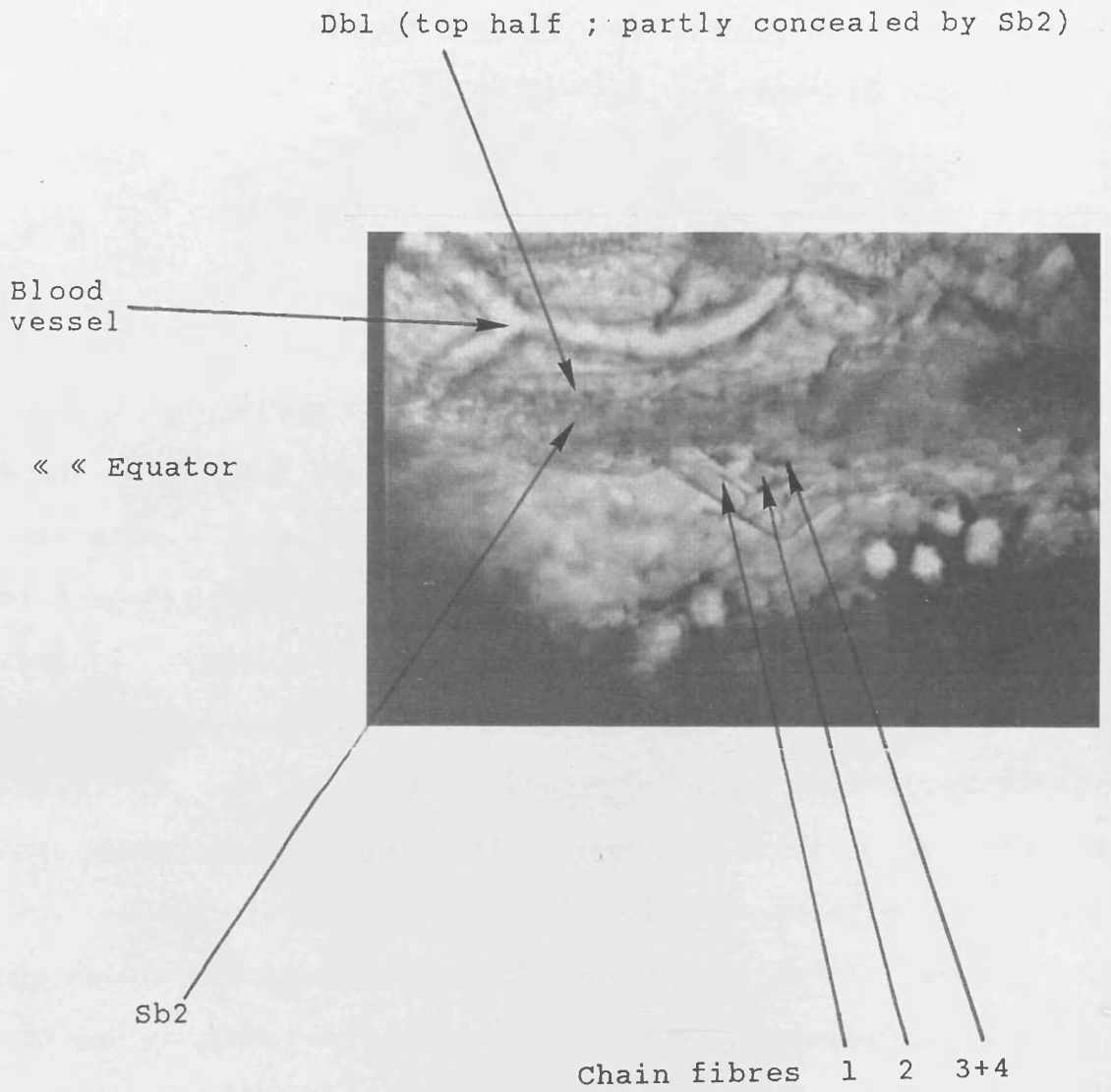
Identification of Intrafusal Fibres (Exposed)

A video still from one of the exposed spindle experiments (14/9/89) is shown in fig 7. This is a reasonably typical field of view at a fairly low magnification, showing a short length of the intrafusal bundle to the right of the equator. Nuclear chain fibres could easily be distinguished from nuclear bag fibres by their smaller diameter. It was sometimes possible to distinguish between Db1 and Sb2 fibres by their mechanical behavior (Boyd et al., 1977). If it was not possible to tell them apart during the experiment, the bag fibres had to be identified histologically later.

Also, Gladden (1976) showed that the elastic tissue round Sb2 fibre has considerably more elastic tissue round it in the polar region than does the Db1 fibre, which sometimes provides another way to distinguish the two nuclear bag fibres.

At the end of the experiment, the two bag fibres could be distinguished by the fact that the Db1 is more sensitive to application of suxamethonium (Scoline) When the muscle bath

Figure 7 : Video still of Tenuissimus spindle.



Expt : 14/9/89, Spindle 1

was infused with increasing concentrations of Scoline, the Db1 showed a contraction at about 5µg/l, with the Sb2 contracting at about 10µg/l (Boyd,1985c).

Differences in the diameters of the Db1 and Sb2 fibres are too inconsistent to be a reliable means to tell them apart.

Hindlimb Arrangement (Roots)

The hindlimb had to be arranged to allow easy access to the tenuissimus muscle for stimulating electrodes and the muscle puller. Surgical forceps were attached to the free edge of the biceps femoris, reflecting it back, and then taped to the surrounding apparatus.

A flexible flat plastic tape bearing two fine recording wires on its upper surface was passed underneath a short length of the tenuissimus muscle to record the EMG. Contact was only superficial, and the tenuissimus muscle was free to move.

The Spinal Pool (Roots)

The laminectomy incision was re-opened. An oval-shaped pool was formed by attaching a strip of X-ray film to the skin flaps of the laminectomy incision, using Michel suture clips. Liquid paraffin at 37°C was then poured into this pool, covering the spinal cord.

Retraction of the Dura and Exposure of the Spinal Cord (Roots)

With the aid of a dissecting microscope, an incision was made in the dura along the full length of the exposed spinal cord, care being taken not to rupture any of the small superficial blood vessels of the spinal cord. The left hand flap of the

incised dura was then retracted and cut transversely in 2-3 places along its length to facilitate exposure of the dorsal roots.

Setting-Up the Puller (Roots)

The thread on the distal end of the tenuissimus muscle was tightly connected to the puller which was driven by a power amplifier (TPO, 25) which was controlled by a wave-form generator (Servomex Control), triggered by the Digitimer

Preparation of Afferents (Roots)

The first stage involved cutting the dorsal roots L7 and S1 as close to their entry into the spinal cord as possible. L6 was also cut to remove any reflex activity from that source. A single pair of silver/silver chloride electrodes were lowered into the spinal pool and a pair of stimulating electrodes were placed on the peripheral tenuissimus nerve. A stimulus pulse (usually of 0.02 msec duration) was generated through an isolated stimulator (Devices Sales) which was controlled by a digitimer (D4030 Device Sales). The digitimer also provided trigger and gated pulses for : the muscle puller; the Neurolog pulse generator; and also the trigger pulse for the oscilloscope (Tektronix).

The nerve to the muscle being studied was stimulated, and evoked action potentials were looked for in sub-divided filaments of L7 and S1 dorsal roots. Individual natural rootlets of the dorsal roots were teased free and placed in

turn on bipolar recording electrodes in the spinal pool. The electrical activity of each rootlet was amplified (x1000) and displayed on one beam of the Tektronix 502A oscilloscope. The digitimer triggered the oscilloscope every 500 msec, 1 msec before the stimulus was applied to the muscle nerve. The oscilloscope sweep speed was set initially to 1 msec/division and rootlets which showed regular evoked potentials 1 to 5 msec following the muscle nerve shock were isolated. These rootlets were then subdivided to give filaments which contained only active afferents from tenuissimus muscle. Occasionally some of these "single fibre" filaments contained other active fibres which were "separated" from the 1a fibre by use of the window discriminator. When a "single fibre" filament was isolated, a tiny piece of coloured silk thread was tied to the end of it as a marker.

Afferents from tenuissimus primary sensory endings were distinguished from others on the basis of conduction velocity (>75 m/sec). A rough estimate of the conduction velocity of a fibre could be made during the experiment by the fact that the conduction distance from the spinal roots to tenuissimus was approximately 12 ± 1 cm.

Primary afferents from flexor digitorum longus (FDL) and peroneus tertius muscle spindles were distinguished from 1b tendon organ afferents by inducing a twitch in the parental muscle. During muscle twitch, 1a afferents fall silent. Also, 1b afferents tend to have no resting discharge at low levels of tension.

Action potentials picked up by recording electrodes were amplified x1000 by a pre-amplifier, the output of which was sent to a distribution board, and from there to oscilloscope, window discriminator, and to a tape recorder channel (see fig. 8).

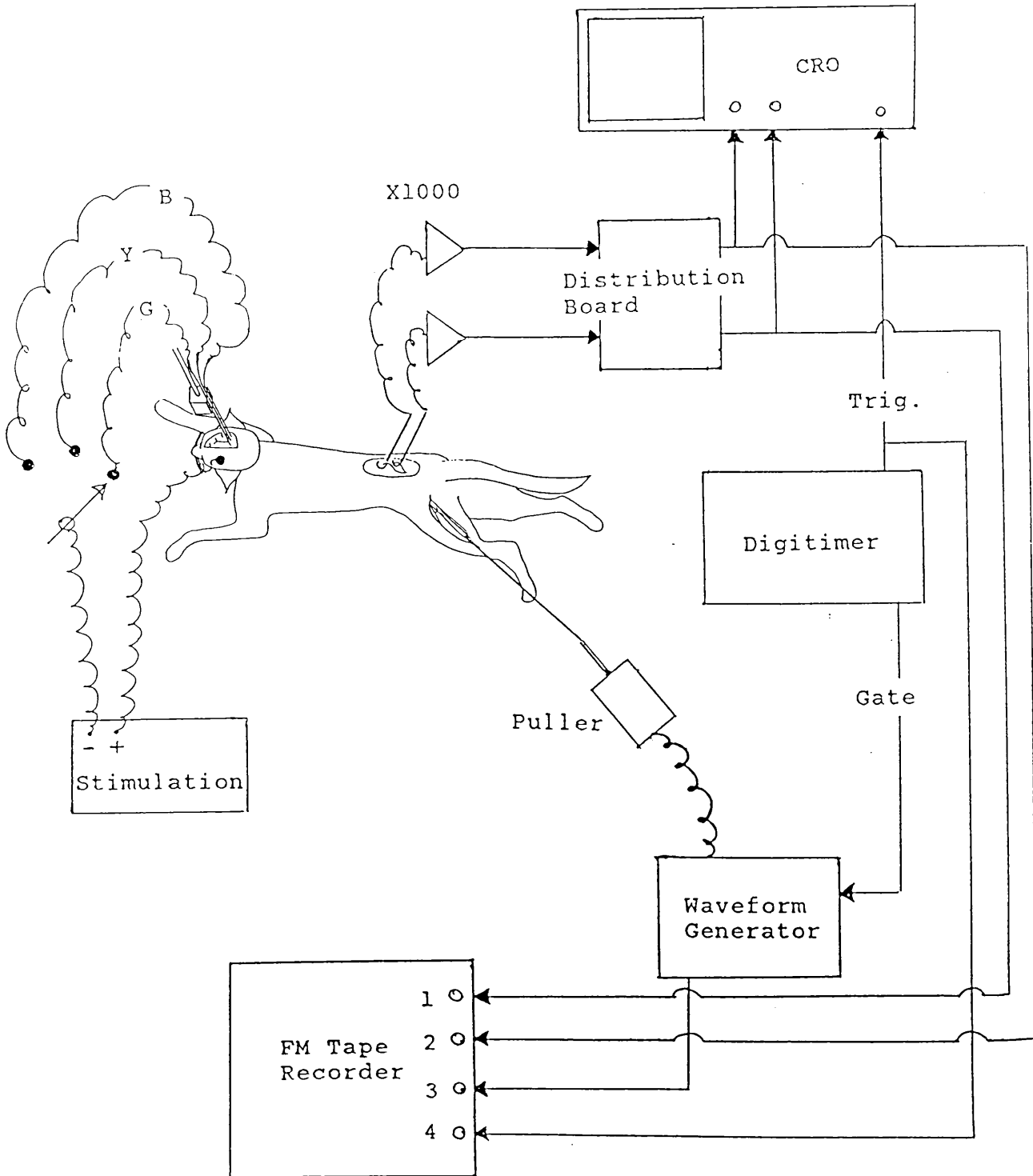
Head Pool (Common)

After finding as many tenuissimus afferents as possible, or exposing 2 or 3 spindles, the surface of the brain was exposed. To accomplish this, with the head of the animal in the head holder, a midline incision was made on the top of the head. The skin flaps were then retracted, revealing the temporalis muscle. The right temporalis muscle was dissected free of its origin, scraped from the skull using a scalpel, and retracted.

Using a trephine, a 1 cm diameter hole was trephined in the skull parietal bone just lateral to the sagittal suture. The disc of bone was removed, and the area of the hole enlarged using bone nibblers. Bone wax was applied over the cut ends of the bone to prevent bleeding, air embolism, and paraffin embolism.

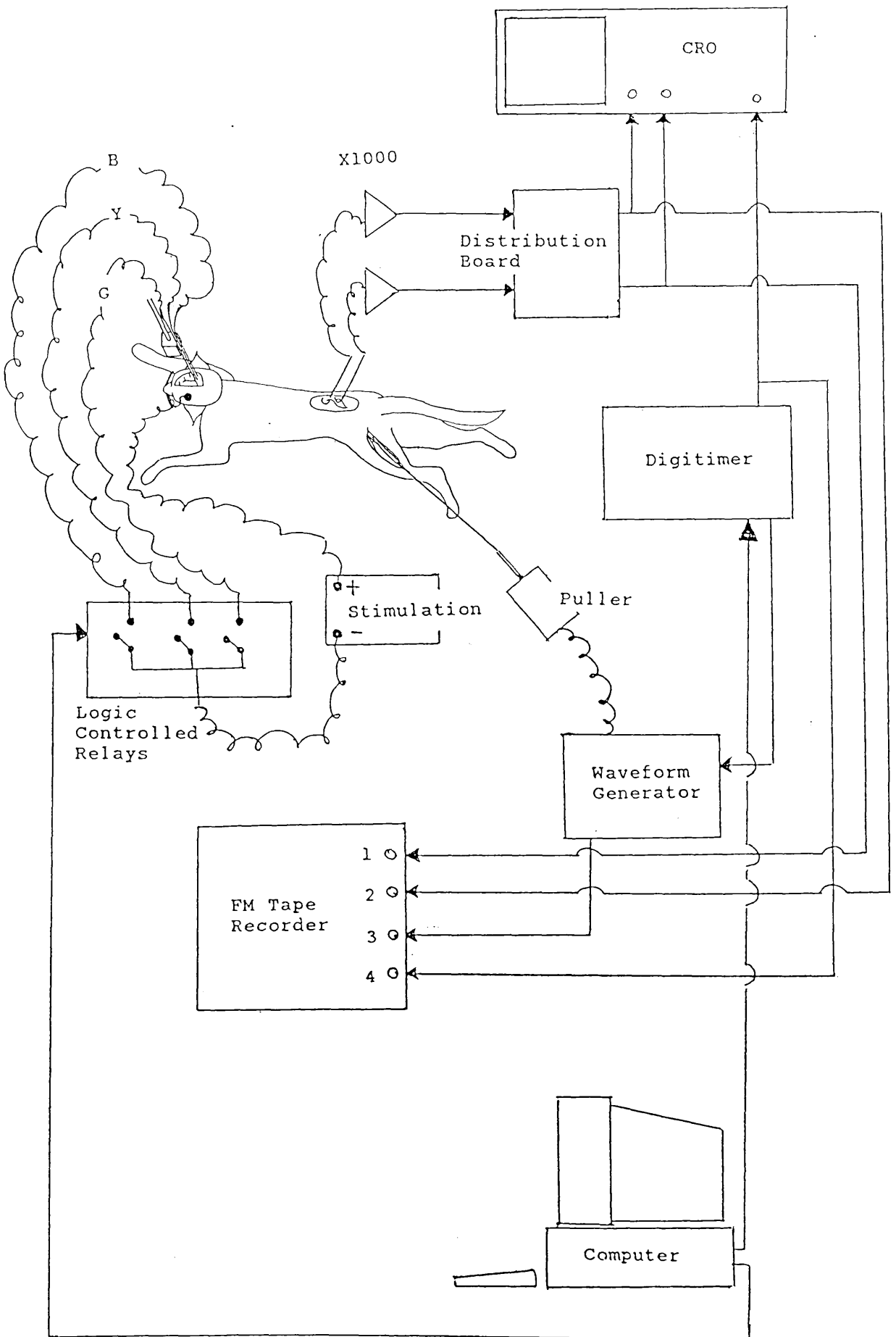
A metal loop of 10 cm diameter was mounted above the skull. The retracted skin was tied round the metal loop using surgical thread. Liquid paraffin at 37°C was introduced to this pool before any attempts were made to remove the dura.

Figure 8 : Experimental set-up in earlier dorsal root experiments.



A slightly-bent syringe needle was used to lift the dura to avoid inadvertant damage to the surface of the cortex. The cut dura was then retracted and pia-covered cortex exposed.

Figure 9 : Experimental set-up in later dorsal roots experiments.



C) EXPERIMENT : STIMULATION AND DATA GATHERING.

Fig. 8 shows the arrangement of equipment used in the earlier dorsal root experiments, and fig. 9 that for the later ones.

Electrode mounting and Insertion. (Common)

Stimulation was delivered to the chosen areas by means of stereotaxically placed microelectrodes. These were guided by micromanipulators attached to the stereotaxic head clamp. The co-ordinates for the micromanipulators were chosen according to a stereotaxic atlas (Berman, 1968). Sometimes only a single microelectrode was mounted on the micromanipulator, but in the majority of cases there were three. These were arranged mediolaterally, and referred to as Green (G), Yellow (Y), and Blue (B), running from medial to lateral. In earlier experiments they were 1mm apart. In later experiments due to concern about tissue compression, this gap was increased to 1.5mm.

Plastic-insulated micro-electrodes were used, with impedances of 5 or 12 M Ω at 1kHz, and exposed tips of approximately 0.1mm. Most often stainless steel electrodes were used, but sometimes tungsten ones were. Tungsten ones are more rigid, but cannot be used for place marking. When stainless steel ones were used, then at some interesting sites, and at the top and bottom of penetrations, iron ions were deposited by

passing anodal current through the microelectrodes. The Fe^{3+} ions thus deposited could be visualised in the tissue after histological reaction (see Histology).

Central Stimulation (Common)

Stimulation was provided by a constant current stimulator (Neurolog NL 510 and NL800). Within limits, this will adjust the delivered voltage in the face of changing resistance (in tissues or electrode tips) so as to maintain the same stimulation current. The actual current delivered was confirmed by the value of a voltage drop across a $1\text{k}\Omega$ series resistance. In all the exposed experiments and the earlier roots experiments, the stimuli were delivered to a hand controlled switching box. From this the stimulus train could be either switched off, or delivered to any one of the 3 microelectrodes. In later roots experiments (see fig 9), stimulation was switched between the different microelectrodes by a bank of logic-gated relays. These were controlled by an IBM computer which was synchronised with the digitimer. The microelectrodes always delivered cathodal stimulation. Stimulus pulse duration was always 0.1ms. stimulus intensity was varied to suit conditions and, if possible, to elicit effect at the minimum necessary stimulus strength. Several frequencies of stimulation were used. In the earlier experiments 500Hz was used as recommended by Appelberg (1981). In later experiments ca. 300Hz, as most

frequently used by Appelberg and co-workers was used. In some other experiments the slower, but still unnatural, frequency of 200Hz was used.

Data Storage (Roots and Exposed)

A 4-channel FM tape recorder (3960 instrumentation recorder, Hewlett-Packard) was used to store the data for further analysis. The first two channels were usually fed the afferents' inputs while the third channel took the length (ramp) signal and the fourth the synchronization pulse.

In the isolated muscle spindle experiments, a U-matic videorecorder was used to store pictures of movements for later observation.

Analysis of Data (Roots)

An IBM Personal Computer fitted with a digital input/output interface card was used to collect the data. The spike-trains to be analyzed were fed into Neurolog NL 200 Spike-Trigger units (either during the experiment or later) and the gate pulses from these units were fed into the digital interface card. The data could then be printed out as instantaneous frequency plots.

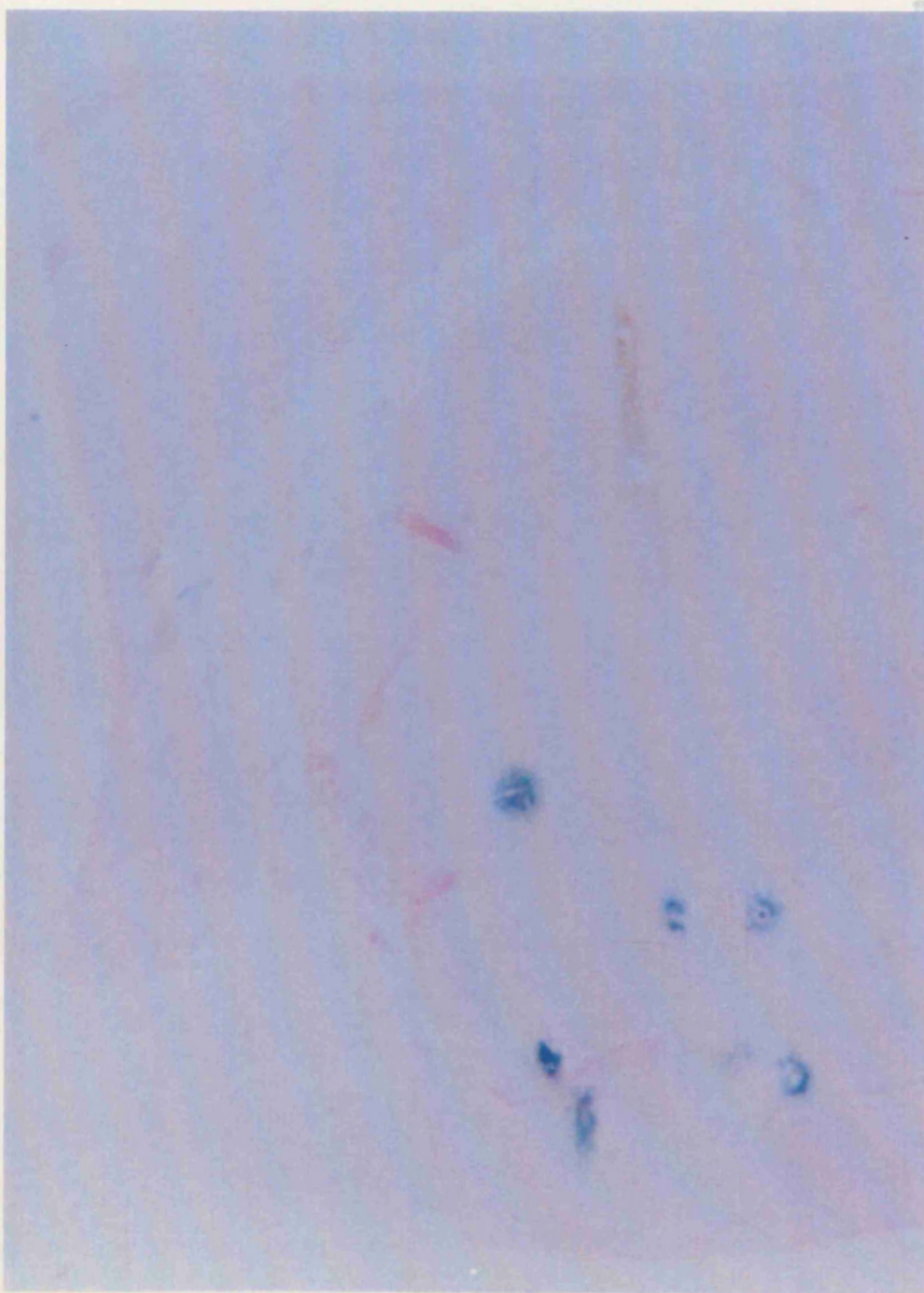
Miscellaneous Points (Roots)

To standardise conditions to some extent, with the animal under deep anaesthesia (no spontaneous τ firing), the initial length of the muscle was set so that the Ia afferents were just firing spontaneously. Thereafter, variation in the background firing of afferents could be taken as being due to variation in τ activity - either spontaneous or induced.

Once the series of stretches began, at least two passive ones were allowed to pass before any stimulation began. This was to remove any additional variations caused by spindle after effects. These occur when spindle length is changed after a period at fixed length. They are absent, or at least constant, during a series of stretches. The physiology underlying after-effects is still not settled, and recent contributions to the two opposing views can be found in Gregory, Morgan & Proske (1987), and Emonet-Dénand et al. (1985).

From fears similar to those which made us increase the inter-electrode spacing, whenever we carried out multiple penetrations we would work from rostral to caudal, so as to avoid obstruction of descending activity by any possible damage done by previous penetrations.

Figure 10 : Brain-stem section showing Fe^{3+} deposits and a 'tear' caused by electrode insertion.



D : HISTOLOGY

As mentioned before, when stainless steel electrodes were used, iron ions were deposited as markers. A small anodal current was passed for 20-30 seconds. At the end of the experiment, Formal saline was injected into the carotid arteries and then a slice of the brain in the frontal plane, containing all of the electrode tracks was removed and placed in formal saline for fixation. Usually, the electrodes were left in situ.

The tissue was dehydrated in a series of alcohols, then embedded in paraffin wax. The block was serially sectioned at 10 μ m, and the sections stained by the Perl's Prussian Blue Method (Disbrey & Rack, 1970) - which reacts with Fe³⁺, to form ferric ferrocyanide, colouring the tissue blue. The sections were then counter stained with Neutral Red to allow the structures of the brain-stem to be identified.

The stain for Fe³⁺ took with varying degrees of success. Sometimes it appeared in only one or two tracks, sometimes in none, and only once did it appear strongly in all. Sometimes a track left a "tear" in the tissue. This is presumed to be where blood had flowed into the track and then clotted. Otherwise, the tissue closed up, leaving no trace of the tracks, regardless of whether or not the electrodes were left in place during fixation.

Both of these being the case, histology could not routinely be used as evidence of electrode position. So, the stereotaxic co-ordinates were used and, happily, such histological clues as there were gave reasonably good confirmation of these. One of the rare occasions where the sections showed iron staining in all 3 tracks and a tear is shown in fig. 10.

RESULTS

In all there were 39 experiments (27 dorsal roots type / 12 exposed spindle type). In 19 of these (15 dorsal roots / 4 exposed spindle) no effects were seen from CNS stimulation, but in 20 (12 roots / 8 exposed) there were indications of changes in fusimotor activity. Possible reasons for a lack of effect are dealt with in the discussion section.

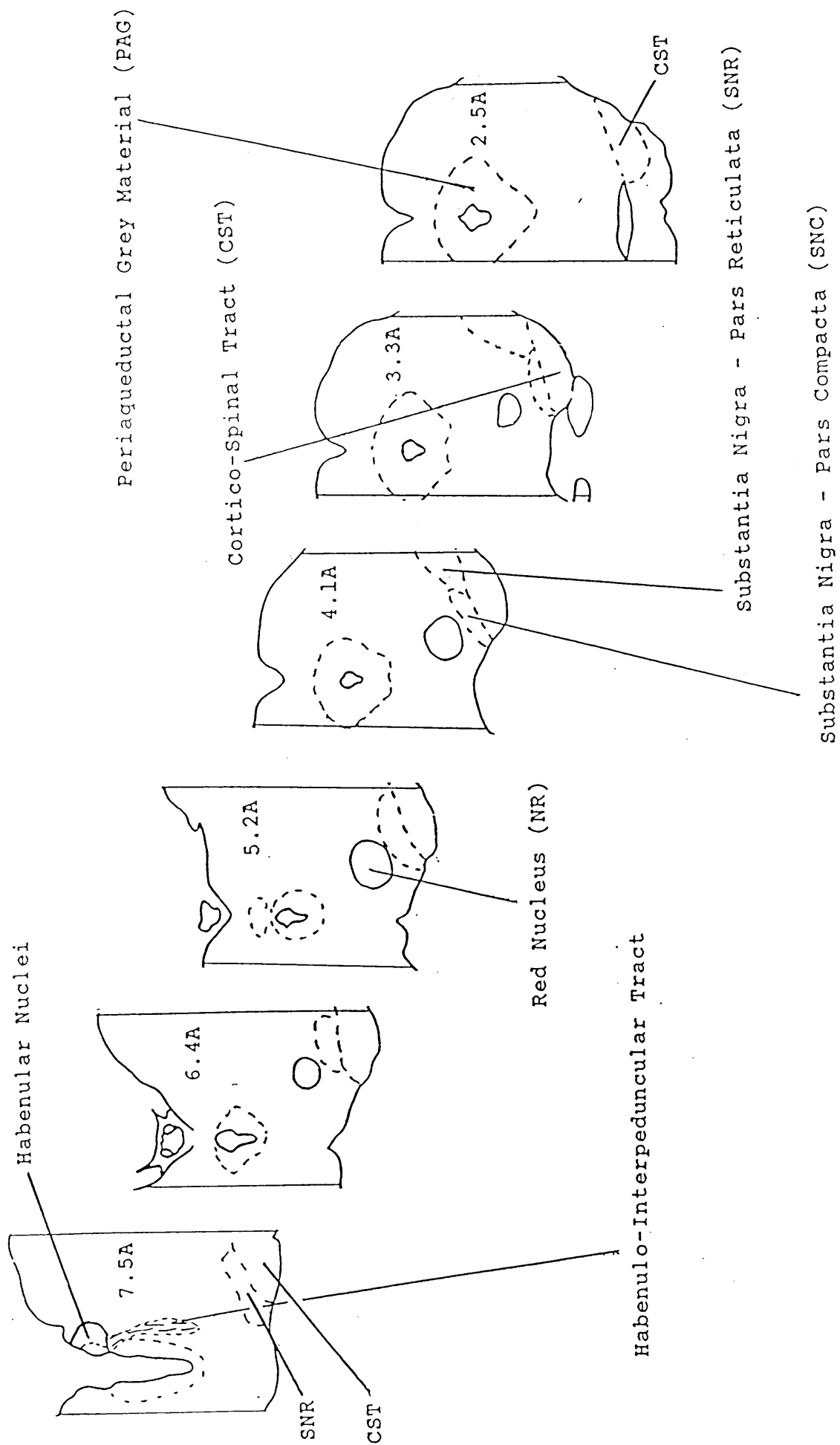
Of the two types of experiment, all of the exposed spindle experiments were on the tenuissimus muscle, and all but two of the roots experiments were also on that muscle. One of the other roots experiments was on Peroneus Tertius (P.Ter) and the remaining one was on Flexor Digitorum Longus (FDL).

In most experiments, only the best or most interesting spindle out of those exposed was selected for examination, but in two, 2 were, and in one, 3 were. Generally, the effects seen in all spindles in one experiment were the same or similar. The strength of this similarity is also addressed in the text.

Within any one experiment, the required stimulus strengths were usually within a fairly small range, though different experiments differed greatly in the values of their ranges. Typical values for each experiment are indicated in the figures, and the thresholds of any individual stimulation sites which were outstandingly different from the typical values are indicated.

On some occasions, threshold mapping was carried out, to locate the point of lowest threshold within the plane of the three electrode tracks.

Figure 11 : Standardised set of brain-stem sections used as the basis for figs. 13-24 and figs 37-45.



Sagatal (barbiturate) was the anaesthetic used in all of the exposed spindle experiments. In the dorsal roots experiments, several anaesthetic regimes were used (see methods for detail: most commonly N₂O/O₂ mixed with 0-1% Halothane, supplemented intravenously by small doses of Saffan (alphaxolone/alphadolone)). To allow assessment of this variable, each reference to a dorsal root experiment is accompanied by the anaesthetic regime(s) used.

Key to Figures

For efficiency of compilation, to aid cross-comparison, and because the histology was not routinely available for confirmation (see methods: histology), a standard set of diagrams of brain stem frontal sections was made, approximately 1mm apart. The stereotaxic position of each track - supported in most cases by histology - was projected onto the nearest of these sections. Figure 11 shows the sections, their distance anterior to the stereotaxic zero plane, and their major anatomical features. These standard sections are used for figs 13-24 and also figs 37-45. Figure 12 is a key to the symbols used on the sections in figs. 13-24 (the exposed spindle experiments). In figs. 37-45 (the dorsal roots experiments), where only one category of effect is illustrated, solid black circles are used for all points. The stimulation sites tested in each experiment are indicated by vertical black bars along the course of the electrode tracks. Any effects seen are marked by symbols, and the unadorned bars indicate a negative result.

Figure 12 : Key to symbols used in figs. 13-24.



Static Bag2 Fibre Excitation



Static Bag2 Fibre Inhibition



Dynamic Bag1 Fibre Excitation



Dynamic Bag1 Fibre Inhibition



Chain Fibre Excitation

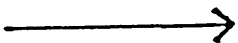


Chain Fibre Inhibition

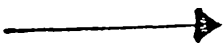


Extrafusal Excitation

Two symbols - Combined Effects



Stimulus strength for an individual point



Stimulus strength for a small group of points

Where two different symbols are placed side to side on the same track, this indicates a mixed effect. On some occasions, the two components of the effect had the same threshold, and on others they were slightly different. Whether or not this was the case is remarked on in the accompanying commentary. On some occasions, two different effects were obtained at different times. If so, this is explained in the text, and indicated in the figures by two symbols apart, separated by a slash.

EXPOSED SPINDLE EXPERIMENTS.

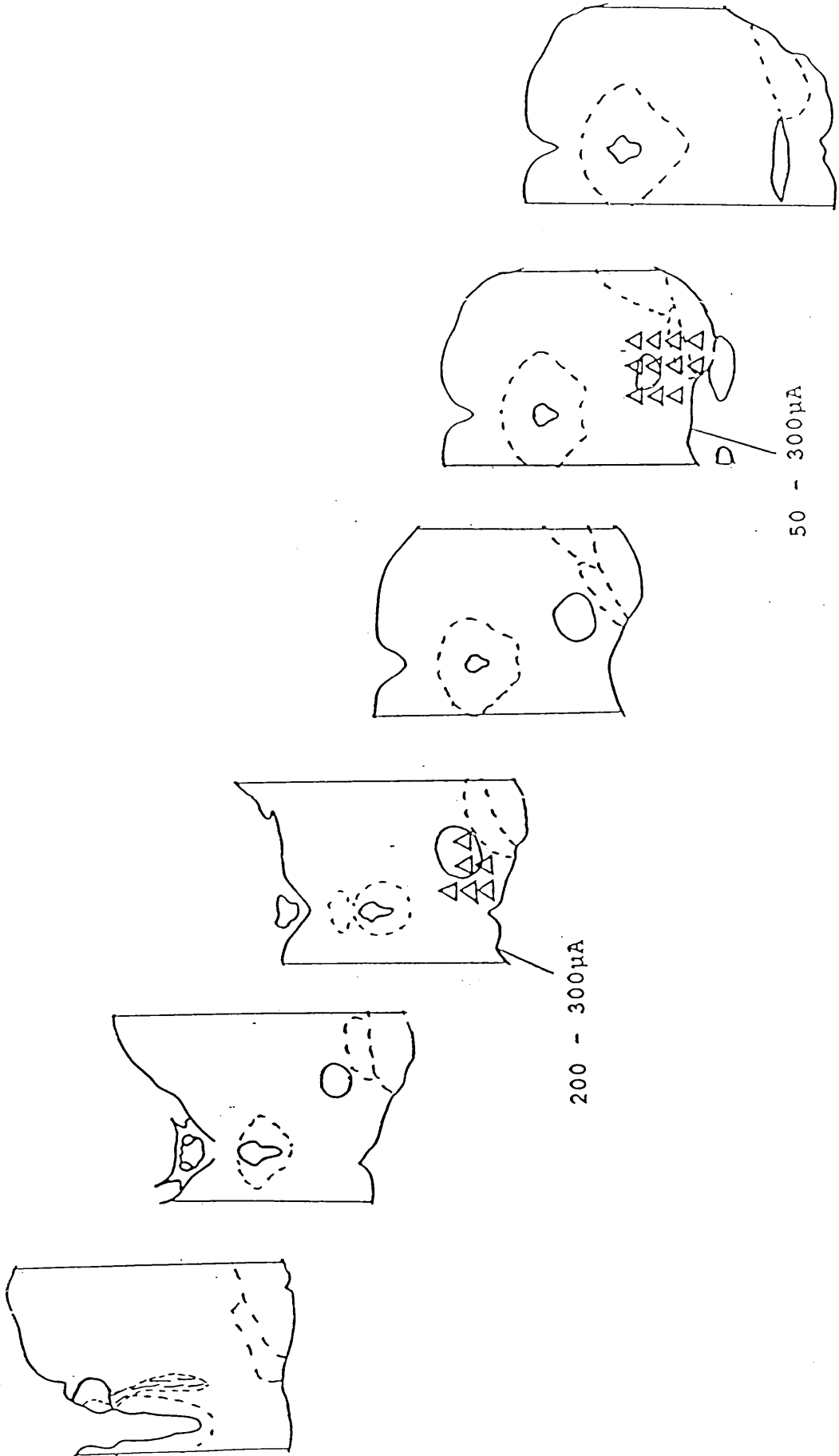
There were eight exposed spindle experiments which yielded results.

Interesting effects were seen in most experiments, which tended to have an individual character. No common pattern of response was seen throughout all experiments.

The effects on the different intrafusal fibres are presented first, then the results obtained are presented individually for each experiment.

Identification of excitatory effects was straightforward. Inhibition could manifest itself in two ways. Either any pre-existing spontaneous activity in the intrafusal fibres could be reduced, or the intrafusal fibres could show a 'rebound' excitation at the end of stimulation.

Figure 13 : Stimulation sites in the brain-stem shown to affect dynamic bagl intrafusal fibres.

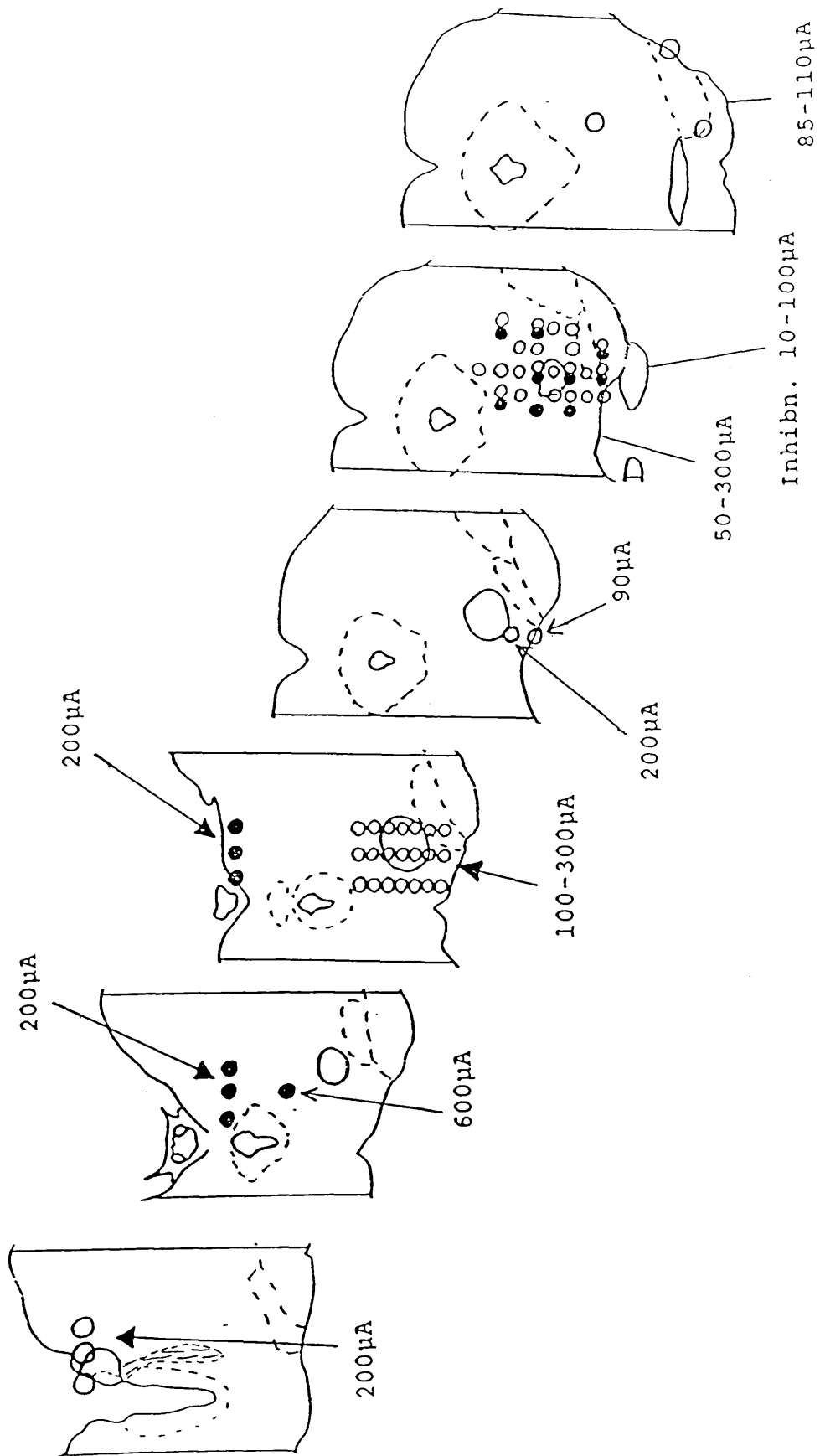


Exposed spindle experiments - Recruitment of Intrafusal fibre types.

Dynamic Bag 1

Recruitment of Dbl fibres was seen in 3 out of the 8 exposed spindle experiments. The points from these are plotted in fig. 13, with some indication of their thresholds. The points are approximately clustered around the NR, but although those with the lowest thresholds are situated at the caudal part, these are situated more ventrally, and do not correspond well with Appelberg and Jeneskog's described area for dynamic activation (Appelberg & Jeneskog, 1972).

Figure 14 : Stimulation sites in the brain-stem shown to affect static bag2 intrafusal fibres.



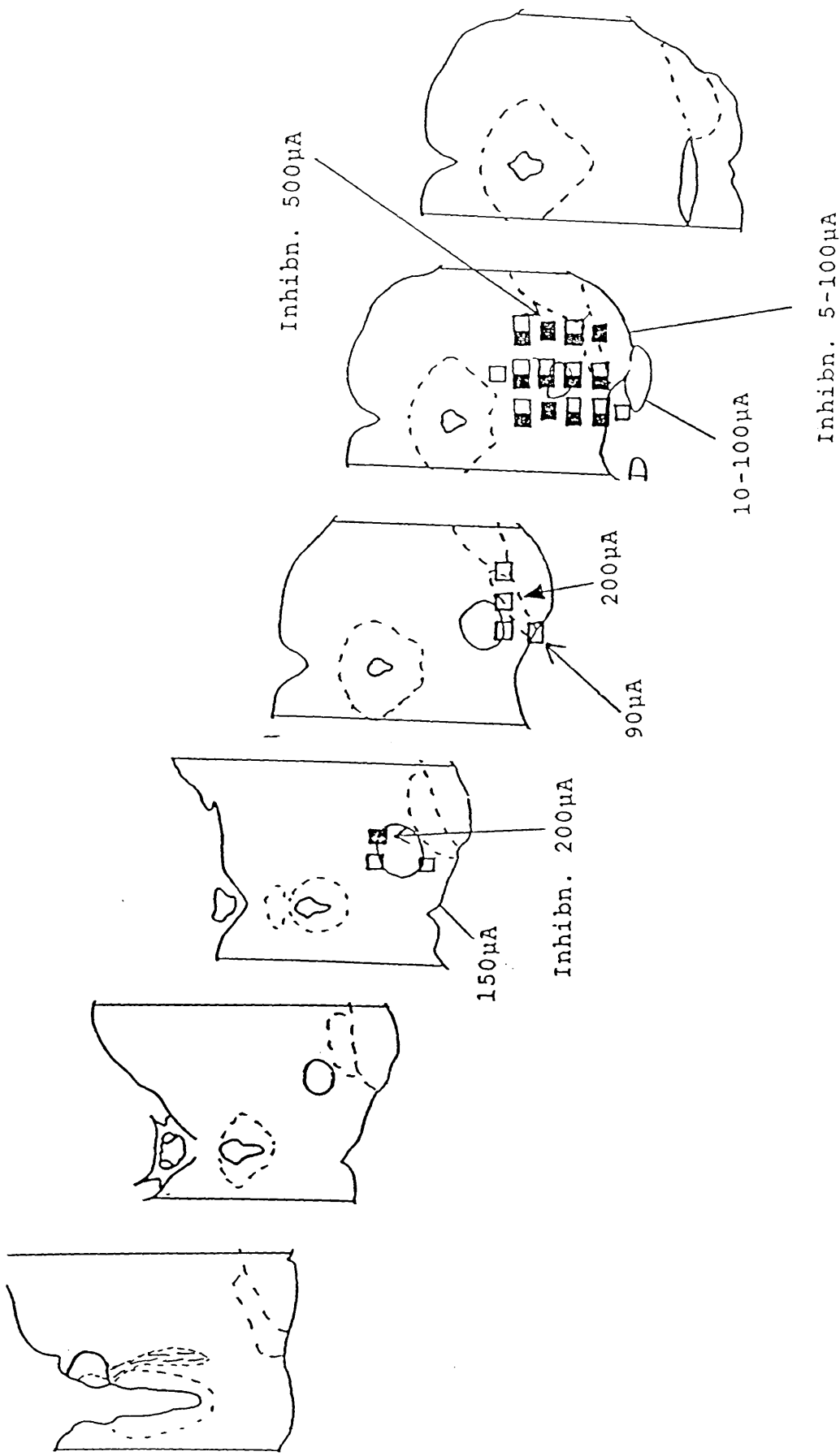
Static Bag 2

Of the three intrafusal fibres, Sb2 were recruited from the widest area and most commonly : every successful experiment showed effects on Sb2. These sites are shown in Fig. 14. Comparison of figs. 13, 14, and 15(chain fibres) shows that only Sb2 were recruited from the habenulae and the habenulo-interpeduncular tract (in 2 experiments out of 8), and these at higher than usual stimulus strengths. High threshold can mean : a) that the relevant structure is relatively difficult to stimulate (e.g. large cell bodies); b) that stimulation has to spread to another, effective region; or c) that a wider area has to be activated for the summed descending activity to be effective. Since effects were obtained from a fairly wide area, extending into the 'uncharted waters' of the reticular formation nuclei, I find the latter two of these explanations more appealing. Having said that, the only excitatory effects were found exactly at the habenulae, while a distance away - in the same experiment - inhibitory effects were seen.

From the rostral and caudal NR, and the areas surrounding, excitatory effects on Sb2 were seen. Generally, those from the caudal part were of lower threshold. Also, inhibitory effects were seen from the caudal part only, tending to be lower threshold than excitatory effects (but see below: chain fibres). Although both kinds of effect could be recruited from a wide area in both regions, thresholds were lower in the ventral part of the NR, and ventral to the NR.

In the small number of penetrations tried more caudally, again Sb2 fibres were the only ones recruited. Two of these lie within the boundaries of the CST, with another more dorsally in the central tegmental field of the reticular formation.

Figure 15 : Stimulation sites in the brain-stem shown to affect chain intrafusal fibres.

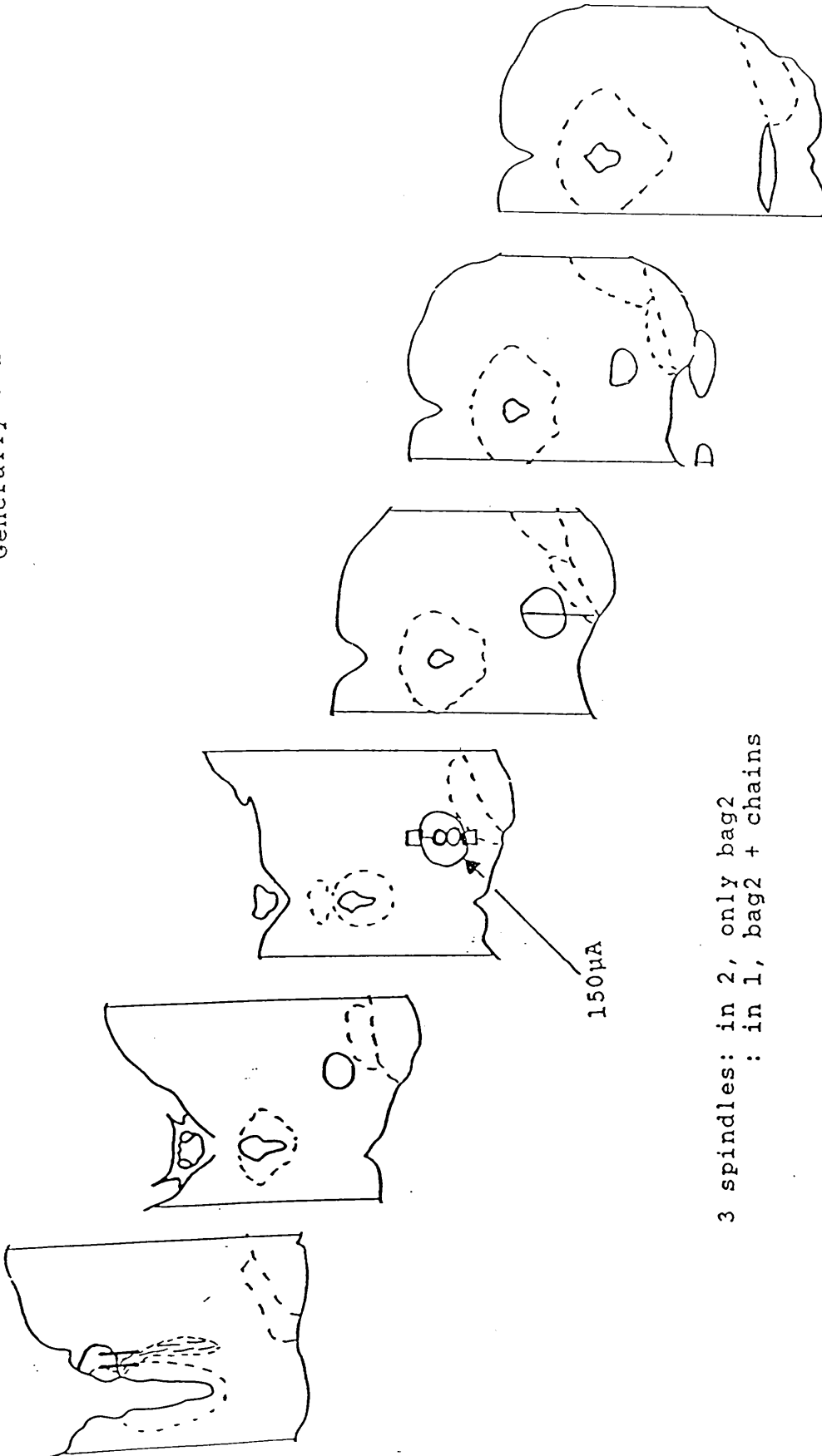


Chain Fibres

Chain fibre excitatory and inhibitory effects were seen in and around the NR (fig. 15). Rostrally, the effective points made quite a tight cluster, but caudally spread out to cover a wide area. Again, the stimulus strengths are lower caudally and lower in the ventral parts. This wider area for recruitment suggests that the effective area has a lower threshold caudally, and is therefore accessible by stimulus spread from any given strength of stimulation over a wider area. Where inhibitory effects are seen, these tend to be at a lower stimulus strength than excitatory effects from the same spot. This is true also for the Sb2 fibres. However, since most excitatory and inhibitory effects come from different experiments, circumstances are different, and no firm conclusions can be drawn. Furthermore, in experiments where both excitation and inhibition are evoked, there is no apparent difference in threshold.

Figure 16 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 23/2/89.

Generally : 150 - 500µA



3 spindles: in 2, only bag2
: in 1, bag2 + chains

150µA

Exposed spindle experiments - Individual experiments

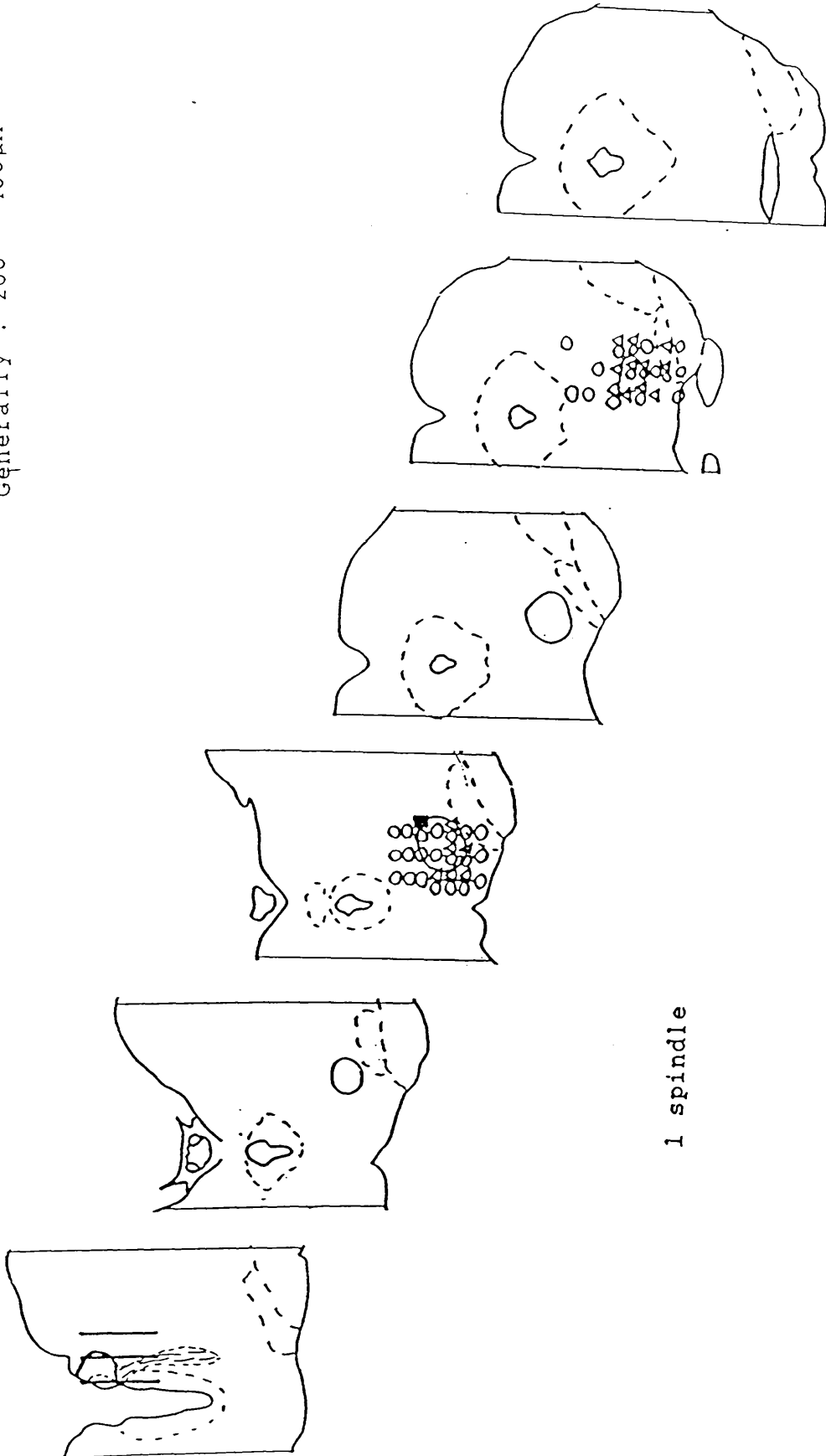
Experiment of 23/2/89 (Fig. 16)

In this experiment, 4 single track penetrations were tried. In one, in the forward portion of the NR, effects were obtained. All 3 spindles exposed were affected, at similar thresholds. In two, only chain fibres were excited, and in the third, both chains and the Sb2 were.

There was no spontaneous intrafusal activity in this experiment.

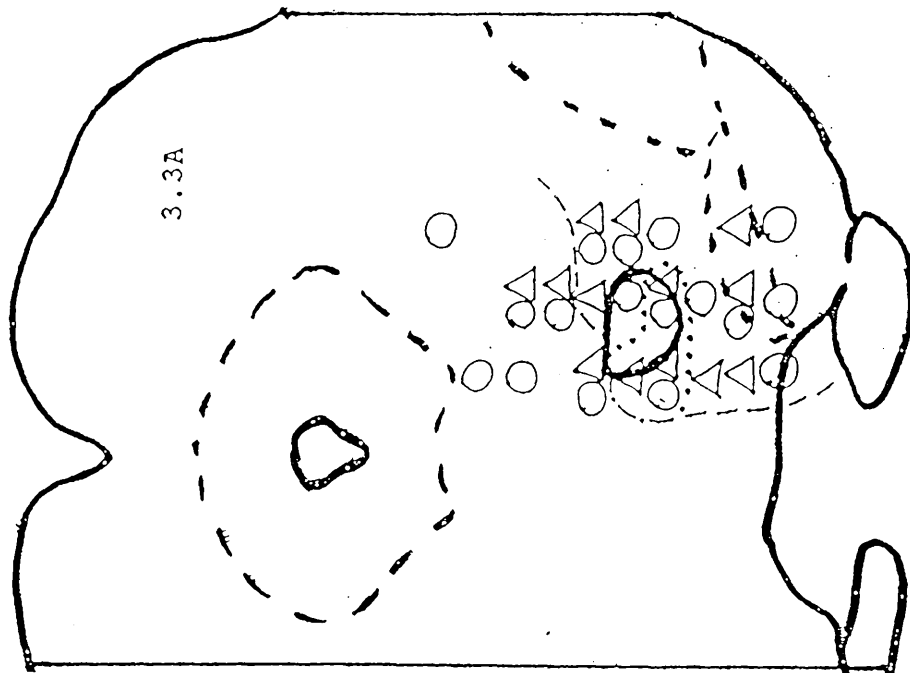
Figure 17 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 2/3/89.

Generally : 200 - 400µA

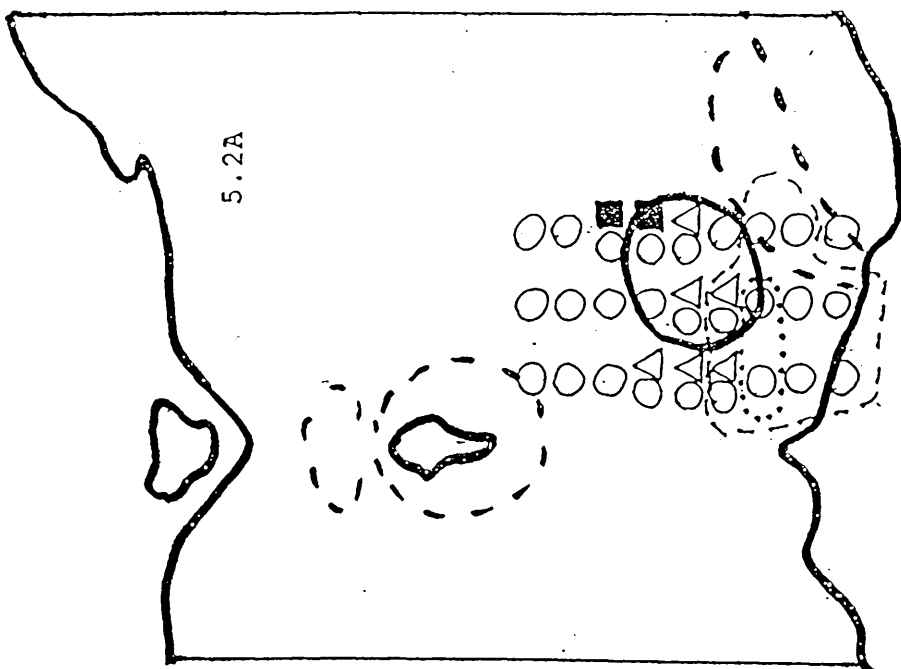


1 spindle

Figure 18 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 2/3/89.



Unbordered: Threshold (T) = 100 - 300µA
 - - - - - T ≤ 100µA
 T ≤ 50µA



Unbordered: Threshold (T) = 300µA - 0.5mA
 - - - - - T ≤ 200µA
 T ≤ 100µA

Experiment of 2/3/89 (Figs. 17 and 18)

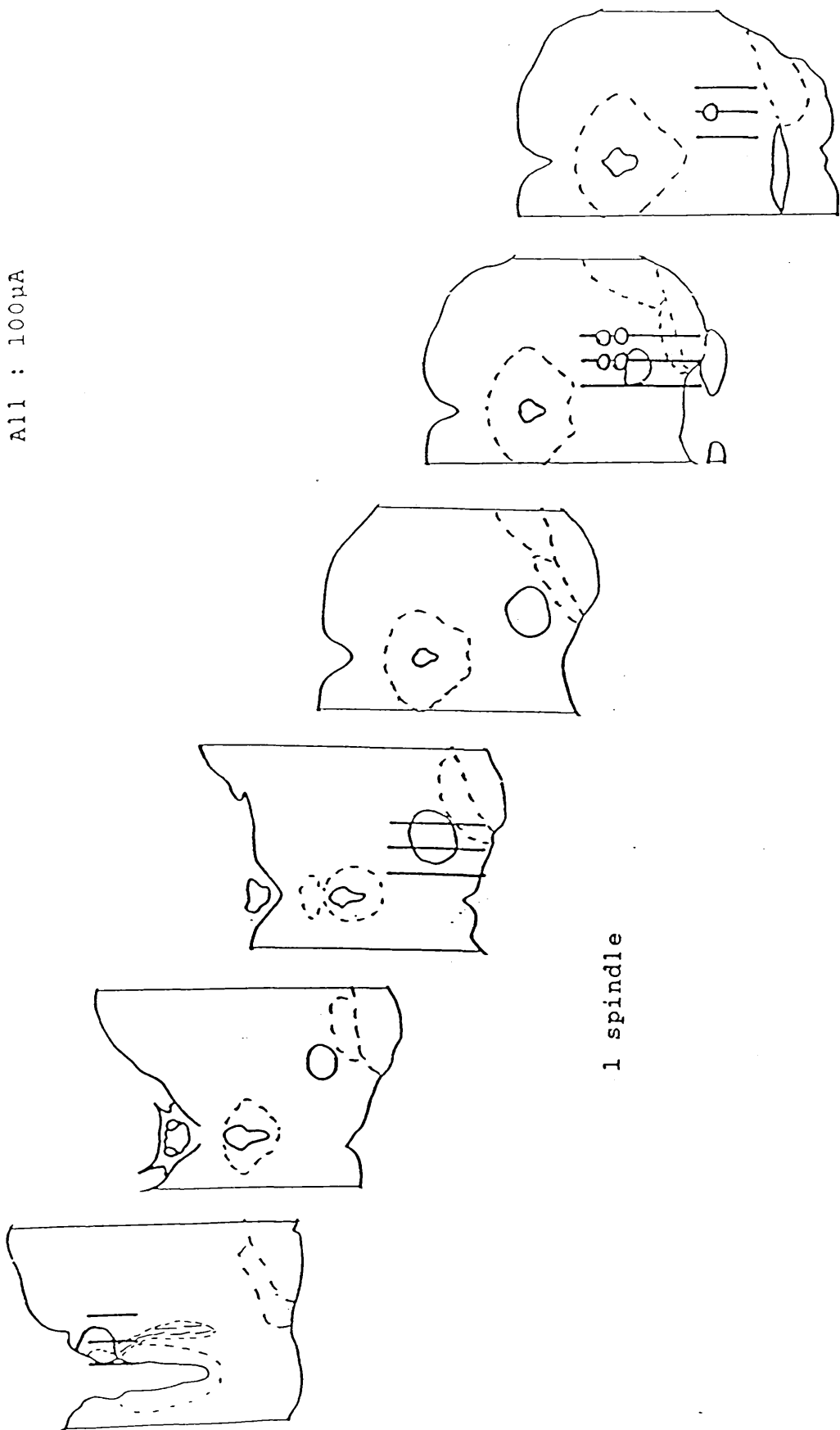
3 triplet penetrations were made during this experiment : one in the habenulae and habenulo-interpeduncular tract; and two spanning the rostral and caudal NR, respectively.

In most penetrations of the NR, the electrodes were aimed generally towards the caudal part or the rostral part. As it happens, these usually projected best onto either the 3.3A section or the 5.2A section, leaving the 4.1A section blank. However, the tracks were usually slightly anterior of 3.3A, or posterior to 5.2A, and the pattern of stimulations was not as unbalanced as it appears to be.

For clarity, the two runs giving results are shown enlarged in fig. 18. Only one spindle was examined and in it, all three types of intrafusal fibres were affected. The chain fibres were spontaneously active and in them there was a relatively high threshold inhibition in the approximate location of the dorsolateral NR. Excitatory effects on Sb2 were seen in all 6 electrode tracks, often in combination with Dbl excitation. Dbl excitation was seen in amongst the Sb2 from a rather more restricted area. Most often it accompanied Sb2 excitation, but this was not always the case. However, no clear cut area for selective dynamic recruitment emerges. In the cases where both bag fibres were recruited, generally the Sb2 was recruited at marginally lower stimulus strengths. The areas of lowest threshold for one or both bag fibres centre approximately on the NR or, in the rostral track - the NR and the tributary fibres of the oculomotor nerve.

Figure 19 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 7/3/89.

All : 100µA



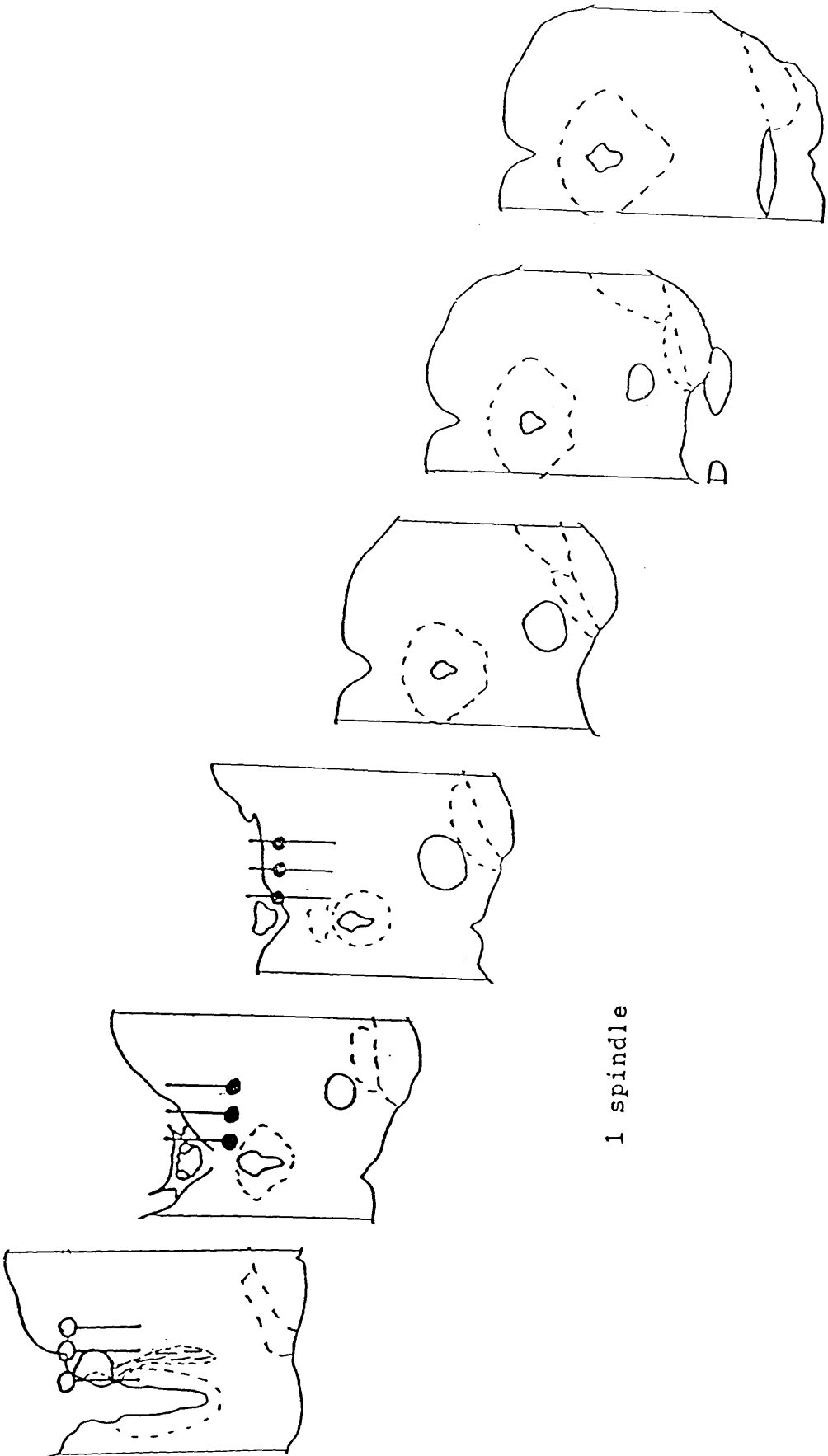
1 spindle

Experiment of 7/3/89 (Fig. 19)

In this experiment no spontaneous activity was present. Four triplet penetrations were carried out, and from 3 out of the 12 electrode tracks, excitation of Sb2 only occurred in one spindle at 100 μ A. The effective areas correspond to the dorsolateral extreme of the NR, and the central tegmental field of the reticular formation.

Figure 20 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 18/5/89.

All : 200µA



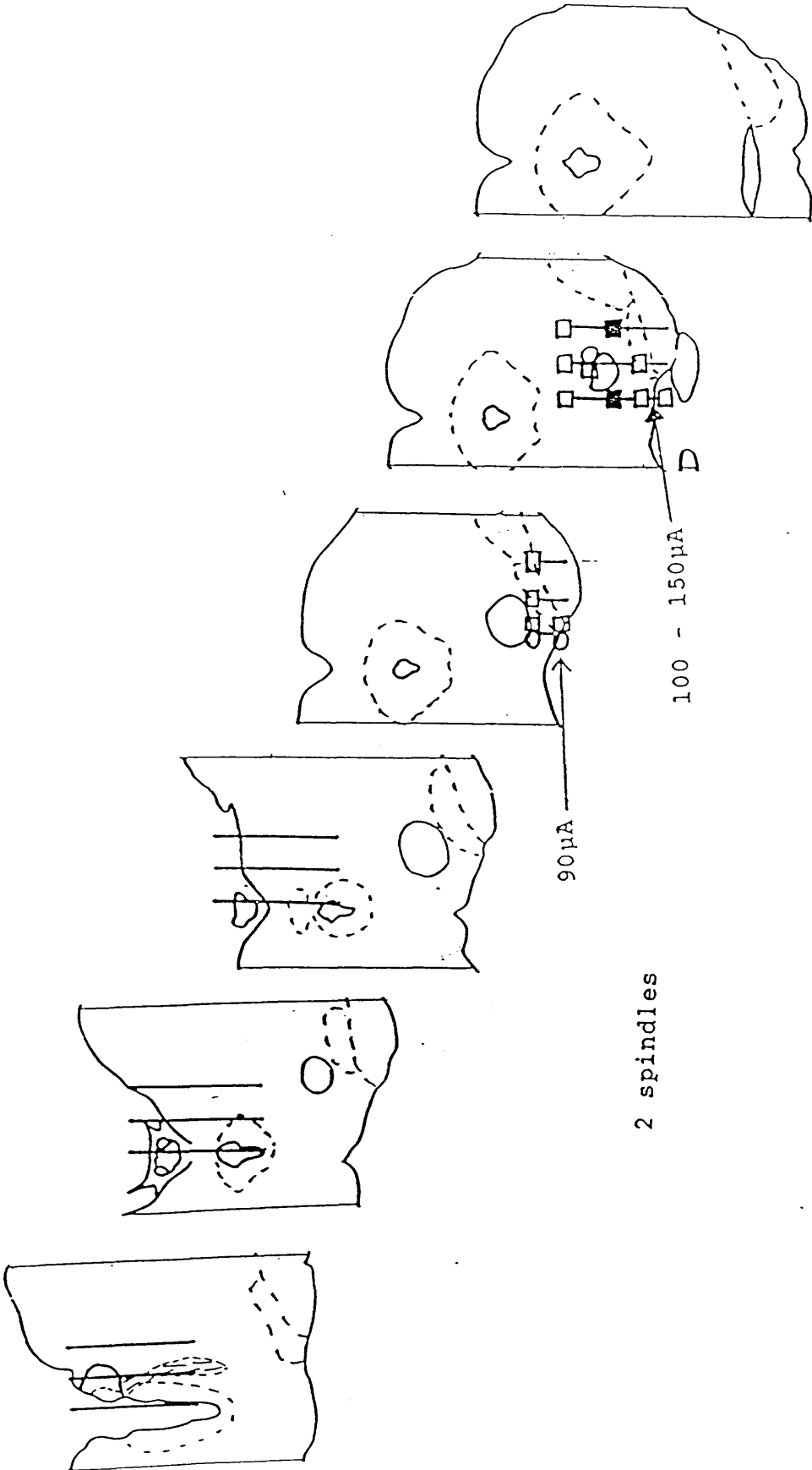
1 spindle

Experiment of 18/5/89 (Fig. 20)

The habenulae and the regions ventral and caudal to them were investigated in this experiment, by three penetrations of the triple electrode. In the habenulae themselves, or slightly dorsal to them and extending laterally to the mediodorsal nucleus of the thalamus, recruitment of Sb2 was seen at 200 μ A. In the other tracks - which correspond to the medial pretectal area - inhibition of Sb2 was seen, also at 200 μ A. This manifested itself as a relaxation of an existing contraction. The medial pretectal area is primarily concerned with the control of pupil size in response to light level, but some of its efferent fibres run in the tectospinal tract (Warwick & Williams, 1973).

Figure 21 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 8/6/89.

Generally : 300 - 500µA



Experiment of 8/6/89 (Fig. 21)

Five triplet penetrations were carried out in this experiment : three around the habenulae; one through the caudal NR; and one in the Substantia Nigra - Pars Compacta (SNC). Two spindles were examined, and in neither of these were any effects seen from the habenulae, even at high stimulus strengths (but see below). In the caudal NR however, both excitation and inhibition of chain fibre spontaneous activity were seen in both spindles. In the 3 most ventral sites the thresholds were lowest. At these 3 points, and generally throughout the whole experiment, the minimum stimulation necessary to affect spindle 1 was consistently lower than that required for spindle 2.

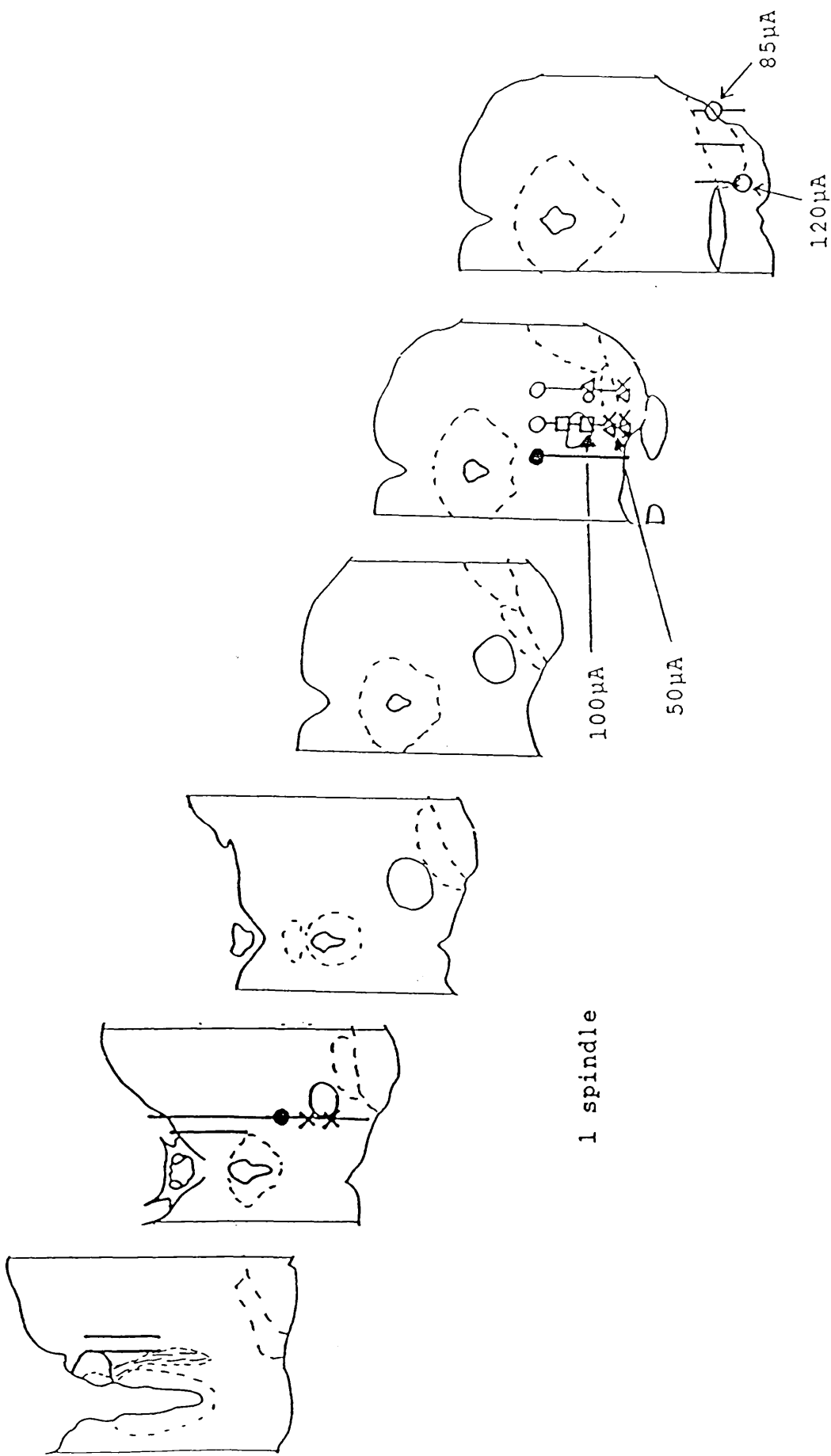
Further forward, in the tracks in the SNC, again both spindles were affected; and again spindle 1 effects had a lower threshold than those of spindle 2. From all sites, chain fibres were excited. In addition, from the most medial track Sb2 fibres were also recruited in both spindles. However, in each spindle, the manner of Sb2 recruitment was different. The effects seen were most consistent with 3 τ -motoneurons being separately recruited. One apparently innervated both the chains and Sb2 of spindle 2, while the other two separately innervated the chains and the Sb2 of spindle 1. The lowest threshold effect was excitation of chain fibres in spindle 1. Next, the chains and Sb2 of spindle 2 were recruited together. Then, at higher stimulus strength again, the Sb2 of spindle 1 was recruited in addition.

In both fruitful penetrations, the lowest thresholds for effects were at the most ventral points. The structure with which both sets of results correspond best is the ventral tegmental area of Tsai (function unknown).

The lack of effects in this experiment from electrical stimulation in the habenulae conceals a result of a different kind. The inevitable compression of tissues in this region stifled the ongoing spontaneous activity. This is dealt with properly in a separate section (see Exposed spindle experiments - Effect of compression on the habenulae).

Figure 22 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 15/6/89.

Generally : 300 - 400µA



Experiment of 15/6/89 (Fig. 22)

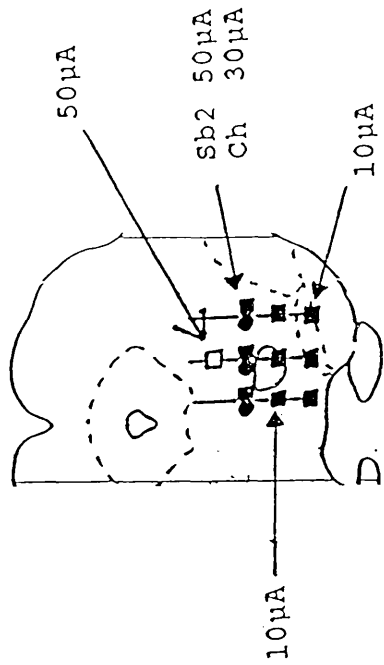
Four single penetrations were made in the rostral part of the brain stem, and two triple ones were made through the tegmentum and substantia nigra of the caudal part.

In the rostral tracks, the only intrafusal effect seen was inhibition of the Sb2, at the fairly high stimulus strength of 400 μ A. The inhibition itself was not immediately apparent, but was inferred from the 'rebound' activity following the cessation of stimulation. Further down this track, extrafusal fibres became recruited, obscuring any intrafusal effects. However much concomitant extrafusal activity may complicate the afferent discharge, in the exposed spindle any but the very smallest amount causes defocussing of the image, making work completely impossible.

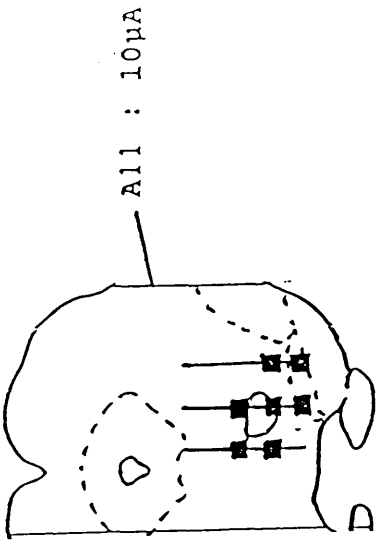
In the most caudal set of tracks, Sb2 recruitment was seen from parts of the CST at fairly low thresholds (85 and 120 μ A). In the region of the caudal NR, chain fibre excitation was seen at 100 μ A. Dorsal to this, effects on the Sb2 were seen at a higher stimulus strength. Laterally, the effects were excitatory on the Sb2, and stimulation at the most medial point caused inhibition to it. Lateral to the NR, combined recruitment of Sb2 and Db1 was seen, with Sb2 at a slightly lower threshold. Ventral to the NR, in the approximate locality of the SNC and retrorubral nucleus, recruitment of Db1 was again seen. This time, it was always accompanied by extrafusal contraction. Intrafusal movements were not obscured because the extrafusal contraction was only the bare minimum mentioned above. A slight movement of the

whole spindle was the only indication of it. Since this and Dbl excitation appeared at the same stimulus strength, we reasoned that this was a dynamic- β . Assuming this to be true, then either the Dbl recruitment seen more dorsally was from a separate (τ) fusimotor neurone, or the slight extrafusally induced movement was obscured by the Sb2 contraction also present.

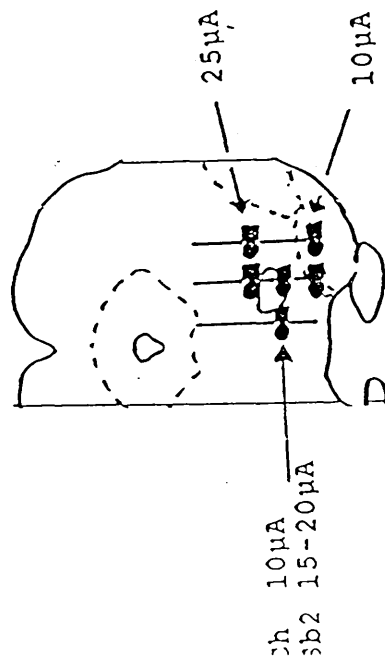
Figure 23 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 31/8/89.



Spindle 2



Spindle 1



Spindle 3

Experiment of 31/8/89 (Fig. 23)

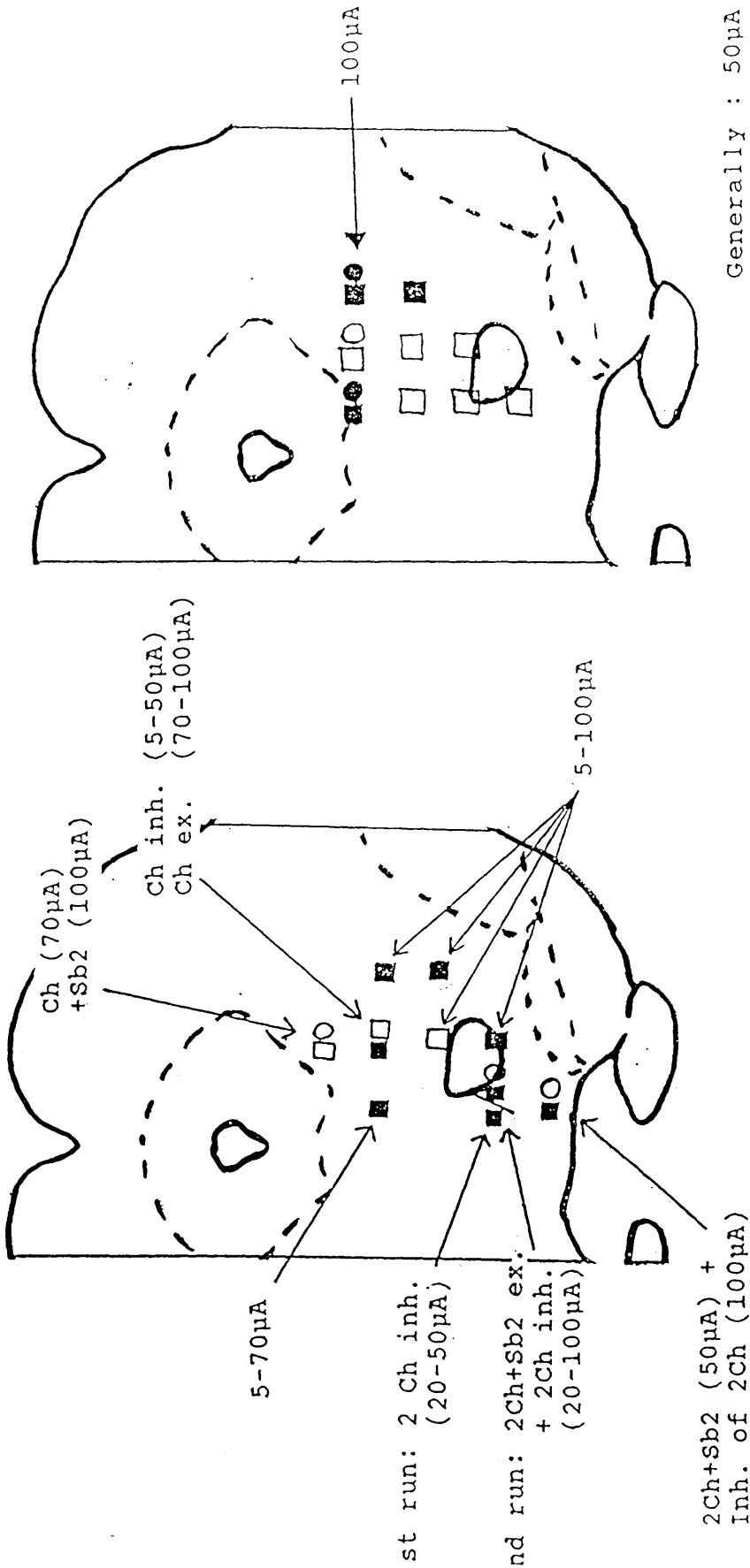
In this experiment, effects were observed on 3 different spindles from the same set of stimulation sites. Only one penetration was made by the triple electrode; through the caudal NR.

In all 3 spindles there was spontaneous chain fibre activity. In 2 (spindle 2 + spindle 3) there was also spontaneous Sb2 activity.

In one spindle (spindle 1 in fig. 23) inhibition of chain fibres was seen from a wide area, at a very low stimulus strength (10 μ A). In another spindle (spindle 3) chain fibre inhibition was seen from a more restricted region at 10 μ A, but could be recruited from as large an area as spindle 1 with a higher stimulus strength. In addition, the Sb2 was also inhibited, sometimes at a higher stimulus strength again, sometimes at apparently the same one.

In the remaining spindle (spindle 2), again chain fibres were inhibited from all points in the lower tegmentum and substantia nigra at 10 μ A. Slightly dorsal to the NR, chain fibres were again inhibited at a higher stimulus strength. At a higher stimulus strength again, Sb2 was also recruited from these points. At one point more dorsally - in the central tegmental field of the reticular formation - chain fibres were excited at a stimulus strength of 50 μ A.

Figure 24 : Effects on intrafusal fibres during brain-stem stimulation seen in experiment of 14/9/89.



Spindle 1

Spindle 2

Experiment of 14/9/89 (Fig. 24)

Again in this experiment, the effects on more than one spindle from stimulation sites in only one penetration were examined in detail. Two very interesting phenomena were found in spindle 1 (see below).

In spindle 2 effects on chain fibres were seen from a wide area of the tegmentum, including the NR, the central tegmental field (FTC) of the reticular formation, and the medial longitudinal bundle (MLB). In the lower points (in the NR and neighbouring parts of the FTC) excitation was universal, at a threshold of 50 μ A. More dorsally there was instead inhibition in the most medial and most lateral tracks, also at 50 μ A. At the most dorsal point of each track (in the MLB and the lateral parts of the FTC) the threshold increased to 100 μ A and the stimulus now also affected Sb2, in the same way as the chain fibres. Namely : excitation in the middle point and inhibition in the most medial and most lateral. The MLB is largely a conduit for connections between different nuclei of the brainstem, including the vestibular nuclei.

The effects in spindle 1 were approximately the same as those in spindle 2, with two additional interesting features. At the bottom of the most medial track, it was noticed that stimulation had opposite effects on the Sb2 and the chain fibres; inhibiting the chains and exciting the Sb2. Also at this point, it was noticed that of the four chain fibres in the spindle, the 2 less prominent ones (see fig. 3) were affected in the same direction as the Sb2. On withdrawing the

electrodes, it was found that at the more dorsal position, it was now also possible to excite Sb2 and two chains in addition to the more obvious inhibition of the other two chains seen from this site on the descent. Assuming that the Sb2 and the two chains affected similarly were supplied by the same τ - reasonable, since they had apparently the same threshold - then this τ was not seen to be recruited on the first (downward) occasion. Since on the second time the same minimum stimulus strength was used (20 μ A), this requires some explanation. Either, during the time elapsed between the first and second times, the anaesthesia had lightened, lessening the stimulus required; or, starting at a higher stimulus strength and expecting to see something, the observer was now able to discern the more subtle movement of the Sb2 at the lower stimulus strengths. Unfortunately, no comments on either of these points was recorded at the time. Different sub-types of static τ have been proposed (Boyd, 1986; Gladden, unpublished work) after accumulation of anatomical and experimental evidence. This result is best explained by the central stimulation exerting different effects on 2 of these types.

Excitation of Sb2 accompanied by inhibition of chain fibres was also seen in two other experiments. In one of these, this was recorded on video tape. Tragically, the results for the third experiment were accidentally destroyed.

Near the top of the middle track, chain fibres were observed to be inhibited at fairly low stimulus strengths (5-25 μ A), and the same ones excited at higher strengths (70-100 μ A). This was seen on both the downward track and on the withdrawal. Since the second occasion was a considerable time after the first, it is unlikely that the level of anaesthesia was the same, and possible changes in this parameter cannot be used to dismiss this as an isolated result. It must, therefore, be a 'genuine' effect of the stimulation. Given this, there are 2 likely explanations. Either the greater stimulus spreads to another, excitatory area, which is more potent than the inhibitory one ; or 2 components of the same region which exert different effects have different susceptibilities to stimulation (e.g. a high threshold cell body, and a lower threshold pre-synaptic terminal), as in Baldissera, Lundberg & Udo (1971).

Exposed spindle experiments - effect of compression on the Habenulae.

In one exposed spindle experiment (8/6/89), during the actual act of penetrating the habenulae, it was observed that the pre-existing spontaneous activity in the chain fibres fell silent, and no effects were seen during stimulation there. The electrodes were then moved to the NR, and during stimulation there, spontaneous chain fibre activity resumed. On subsequent penetration of the Substantia Nigra, spontaneous activity of the chain fibres was unaffected, but on re-penetration of the Habenulae, it again fell silent. Although interesting in its own right, this raised the possibility that effects from electrical stimulation in the habenular area were being counteracted by simultaneous effects from compression. Accordingly, in the next experiment, single electrode penetrations were made, and subsequently the inter-electrode spacing in the grid was increased from 1mm to 1.5mm. Actually, the use of a single electrode did not improve matters noticeably, and was discarded.

DORSAL ROOT AFFERENT RESPONSES.

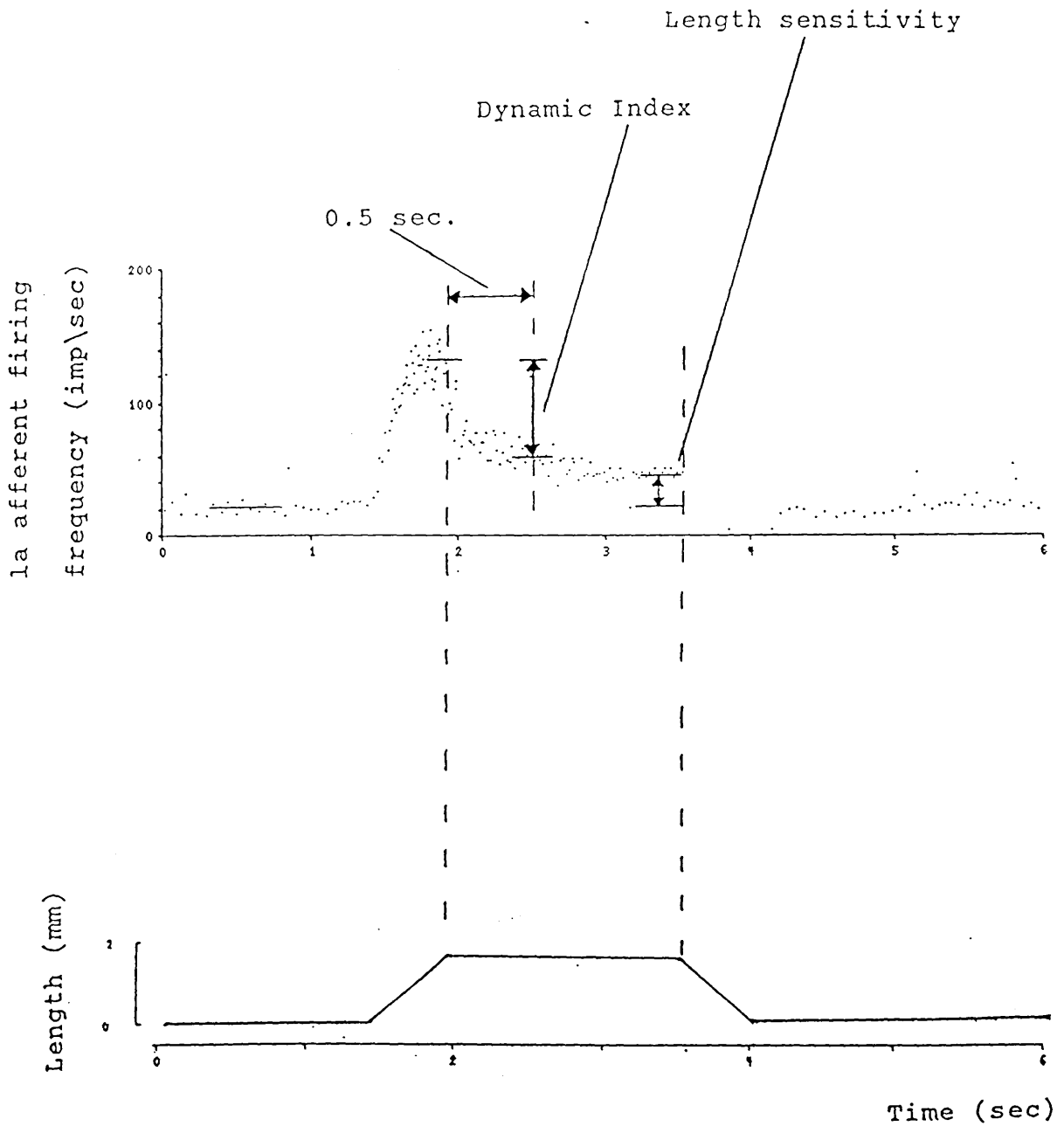
Twenty-one Ia afferents were recorded from in the 12 experiments where effects were seen. The effects seen in the different experiments are best considered individually. Although responses were fairly consistent within each experiment, different experiments produced different effects.

It was found that although all effects would be broadly classified as static, there were two features both falling into this category which varied with a fair degree of independence in the extent to which they were affected by central stimulation. Best shown by example, they were :

- 'Variability' (e.g. figs. 27 and 33 particularly) - a tendency for the Ia discharge to increase erratically, obscuring and occluding the existing responses to stretch. It has the appearance of responses to stretch becoming submerged in the fusimotor evoked activity.

- 'Baseline' (e.g. figs. 26 and 33 particularly) - a smoothly changing continuous increase in the ongoing Ia afferent discharge which adds to the existing response. On most occasions (e.g. fig. 33), there is summation between the 'baseline' effect and the responses to stretch ; on others,

Figure 25 : Ia afferent response to muscle stretch, showing measurements of the dynamic index and the length sensitivity.



(e.g. fig 26) it occludes the lower parts of the response but summates with the larger ones. Concurrent responses have the appearance of riding on the back of the 'baseline' effect.

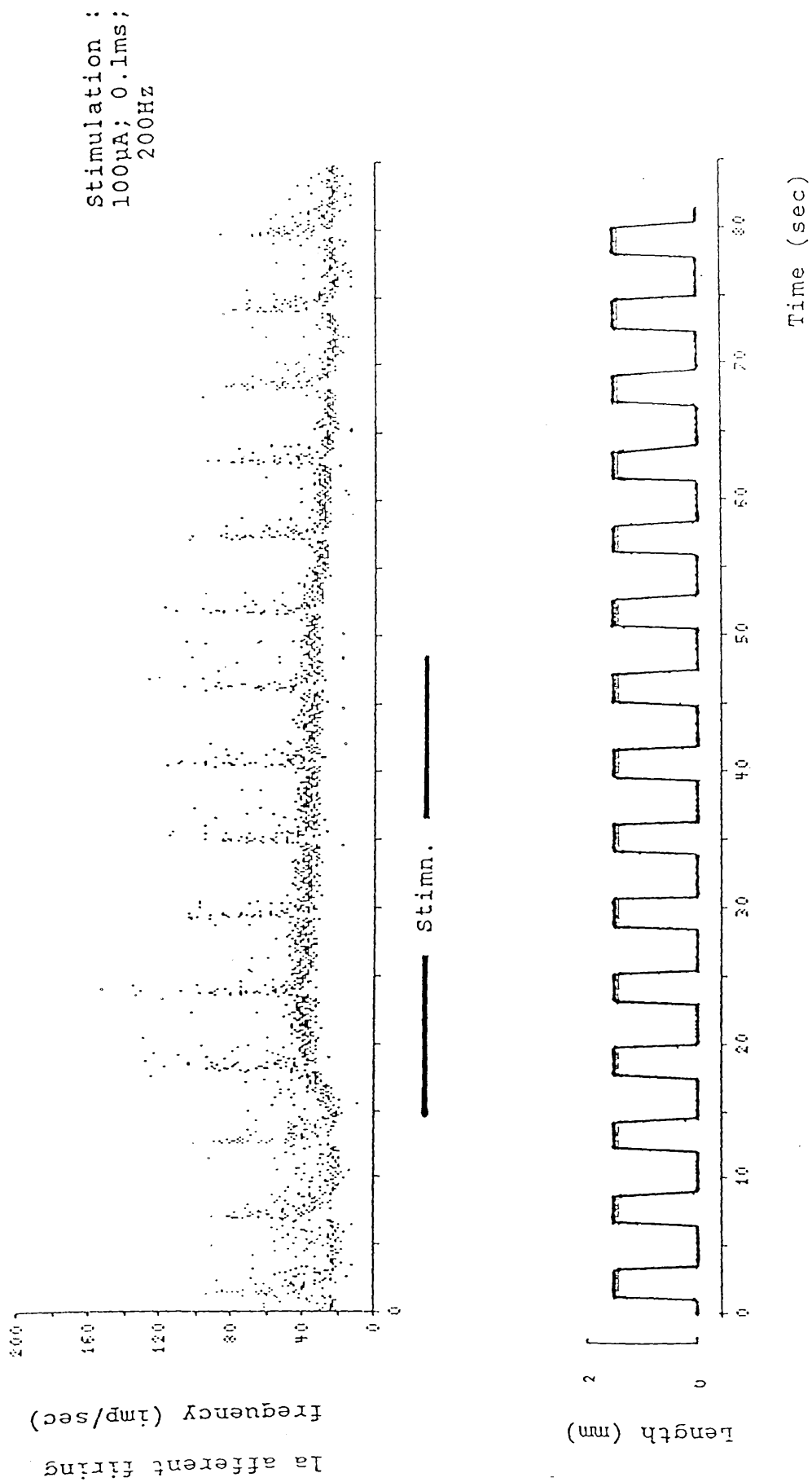
When it was informative and feasible to do so, two features were measured in individual responses whose time-scale was expanded to allow measurement (e.g. fig. 32 and fig. 25). These features were the dynamic index and the length sensitivity (fig. 25). On some occasions a distinction was made between the fast and the slow components of the dynamic index.

However, in most of the experiments on the tenuissimus it was not possible to quantify these, and the effects were best assessed simply by their overall appearance.

In these expanded responses, the initial bursts and any changes in them were also appraised. However, no significant changes in this feature were seen, probably because of continual τ excitation (Jansen & Matthews, 1962a).

To turn to the actual responses : as mentioned above, there were two effects which seemed to be somewhat independently variable. Both could be either excited or inhibited, during a relative lack of effect on the other. However, it was never seen that one was excited while the other was inhibited. Their independence only extended to a differing degree of the prevailing effect.

Figure 26 : Illustration of predominantly 'baseline' effect.



Tenuissimus muscle.

As in the exposed spindle experiments, there was very little evidence of any recruitment of any τ -d motoneurons. In fact, judged solely by the dynamic index, there was none whatsoever. All effects seen were either entirely or predominantly static in nature ; and these were of several different kinds.

Figure 26 : Predominantly 'Baseline' Effect

In figure A a clear 'baseline' effect can be seen. Following commencement of stimulation, the background activity rises slowly for about 9 -10 seconds. Thereafter, it declines from about 40 impulses per second (imp/sec) to about 25 imp/sec irrespective of whether or not there is any stimulation, taking about 1 minute to do so. This adaptation may take place either in the spindle (e.g. Boyd, 1981) or centrally. The only way to choose between these two possibilities would be if one had been able to record the efferent activity. The maximum discharge of each response runs approximately parallel to the background discharge, so there would appear to be summation between the response to stretching and the effect on the background. However, the rest of the ramp response - the adapted response at constant length - which is reasonably apparent in the 3 responses preceding stimulation is not evident in the responses during background elevation, so some occlusion has occurred.

Responses like that of fig. 26 were seen on the following no. of occasions, in the following experiments :

4/4/89 (12 occasions): (N₂O/O₂+0-1% Halo.+ Saffan)

The locations of these are illustrated in fig. 37

Figure 27 : Illustration of predominantly 'variability' effect.

Stimulation :
50 μ A; 0.1ms;
500Hz

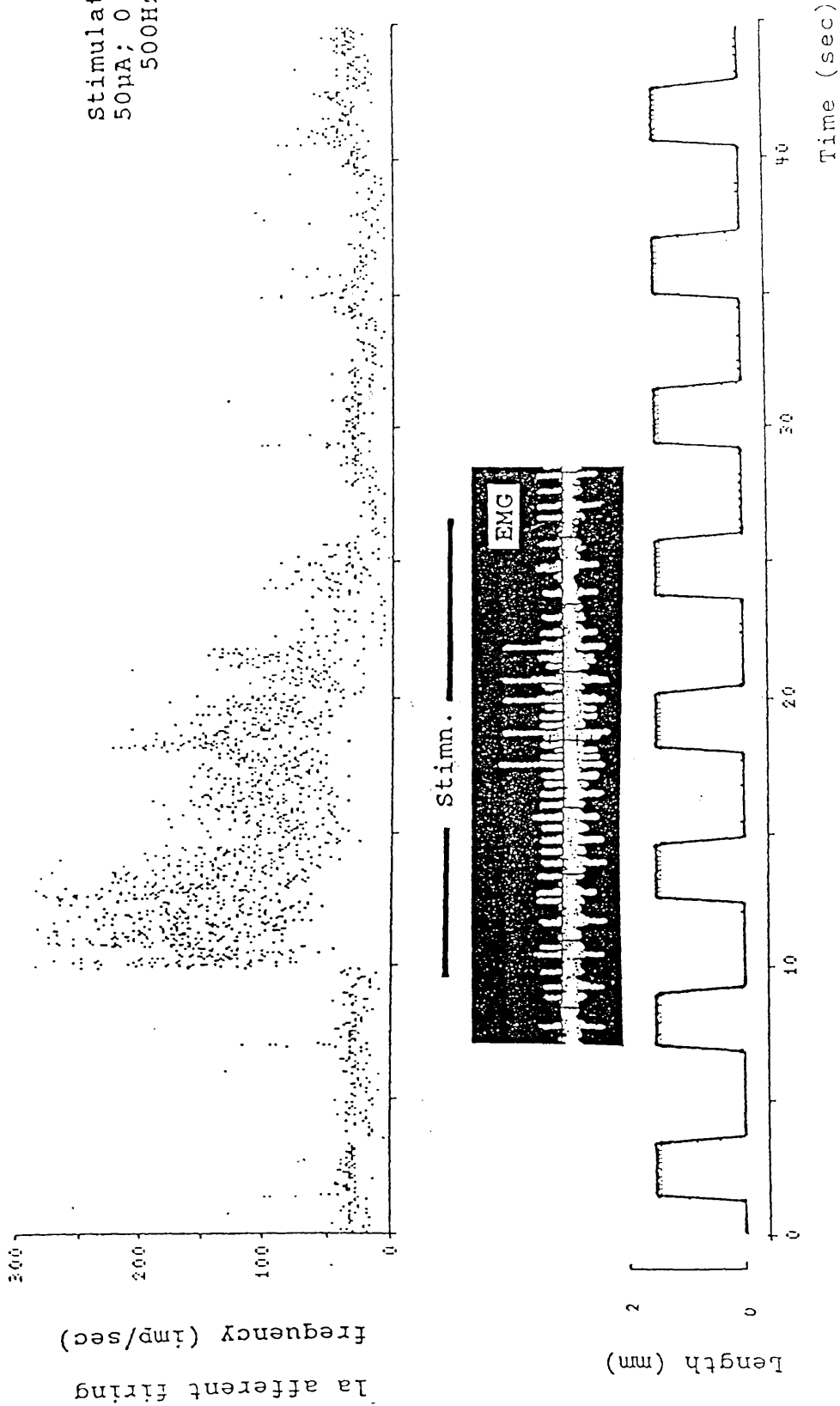


Figure 27 : Predominantly 'Variability' Effect

Figure 27 shows a strong 'variability' response. There is already a marked degree of fusimotor activity of this nature before stimulation. Nevertheless, once stimulation begins, there is a dramatic rise to between 250 and 300 imp/sec. As stimulation proceeds, this decays back to and below the previous level. Evidently then, a prolonged stimulation not only habituates, but actually reduces the afferent discharge. There was some extrafusal activity during stimulation, but only a small amount and not enough to unload the spindle (EMG was recorded routinely in all experiments. This is one of the very rare positive instances). Although it is an imponderable, I do not believe that the amount of extrafusal activity recorded was enough to account for any substantial contribution to the response by an 'in-series' contraction. There is certainly no obvious relationship between EMG spikes and changes in the afferent response. Whether contributing to the response or reducing it by unloading, it is likely that the extrafusal activity is responsible for some of the erratic nature of the response.

The strength of the response would suggest involvement of more than one τ , since no single static τ is capable of exciting an afferent to this extent alone. (Boyd, 1981; Hunt & Kuffler, 1951).

It is possible that the increase in discharge around 18sec. is an enhanced response to stretch, indicating some τ -d activity, but the matter cannot be decided from this one feature. There is no prominent response to the stretches before or after that one.

The onset of the response is almost immediate, although it starts to decay almost straight away. The descending pathway must therefore be rapid, although because the stimulation was controlled by a hand switch, it is not possible to give an exact figure. Again, without knowing the efferent activity it is not possible to say whether the decay is due to central or peripheral adaptation.

Responses like fig. 27 were seen in the following experiments, the following no. of times.

24/10/89 (3 occasions): (N_2O/O_2 +0-1% Halo.+ Saffan)

13/6/89 (21 occasions): (N_2O/O_2 + Saffan)

4/4/89 (4 occasions): (N_2O/O_2 +0-1% Halo.+ Saffan)

The locations of these are illustrated in fig. 38.

Figure 28 : Illustration of increase in both 'baseline' and 'variability' effects.

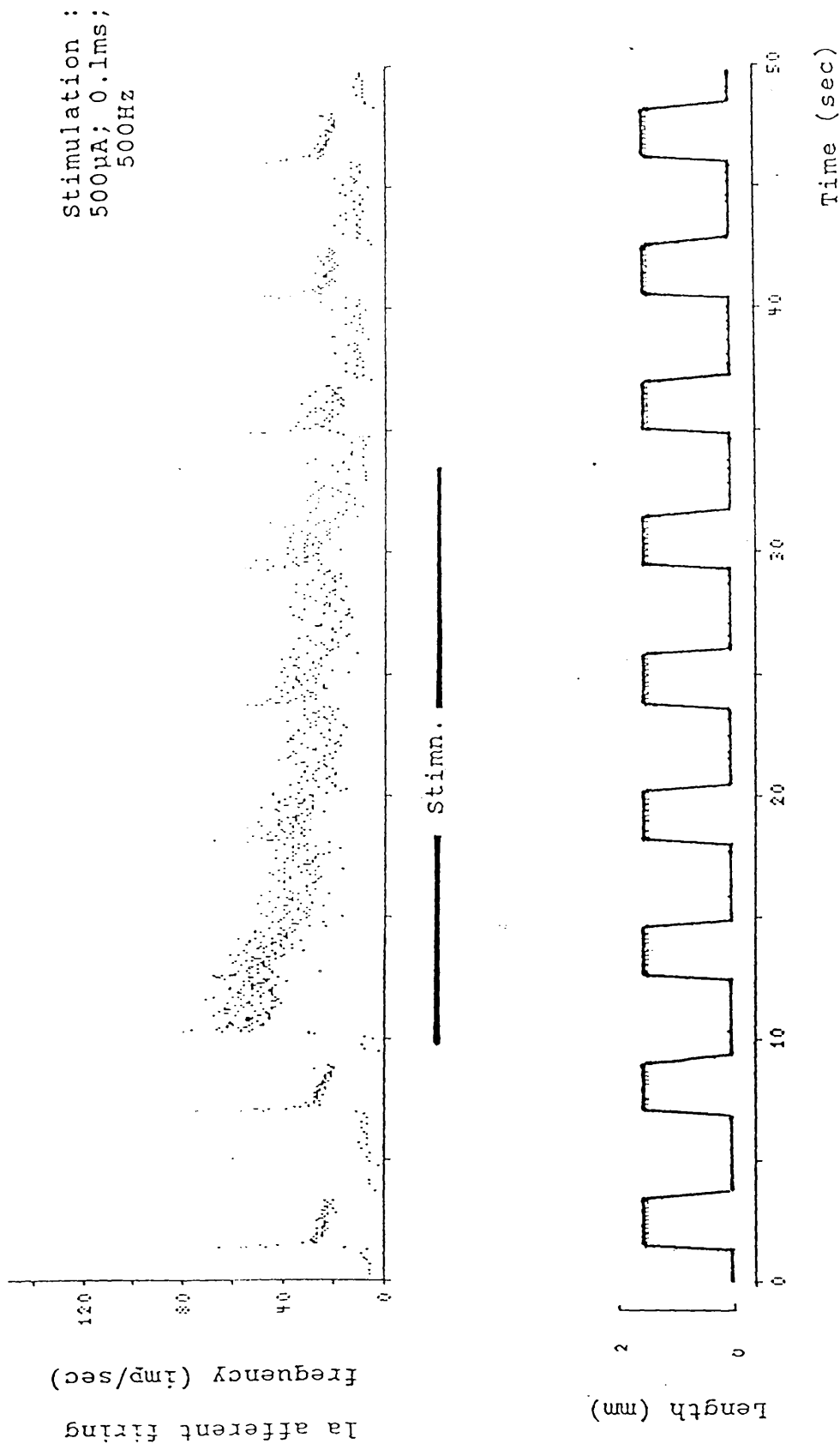


Figure 28 : Increase in both 'Baseline' and 'Variability' Effects

Figure 28 shows a different mixture of the two static effects with, as an estimate, roughly equal proportions of the two. The response rises rapidly and then tails off throughout stimulation over about 23 seconds. The scatter is much less here than in fig. 27, although the effect on the 'baseline' is about equal, since the minimum response to which the firing falls, even during shortening, is raised by about the same amount. In this case the 'submerging' of the response can be seen clearly, with it re-appearing as the excitation decays (there is some persistence of the excitation after stimulation ceases). It is interesting to note that although the stretch responses reappear, the formerly conspicuous initial burst is absent.

Stimulation sites giving effects like those in fig. 28 were found in the following experiments, the indicated no. of times, and these sites are shown in fig. 39.

20/6/89 (3 occasions): (N_2O/O_2 +1-2% Halo.; O_2 +1-2% Halo.)

24/8/89 (2 occasions): (N_2O/O_2 +0-1% Halo.+ Saffan)

12/4/89 (6 occasions): (Sagatal)

Figure 29 : Illustration of inhibition of both 'baseline' and 'variability' effects.

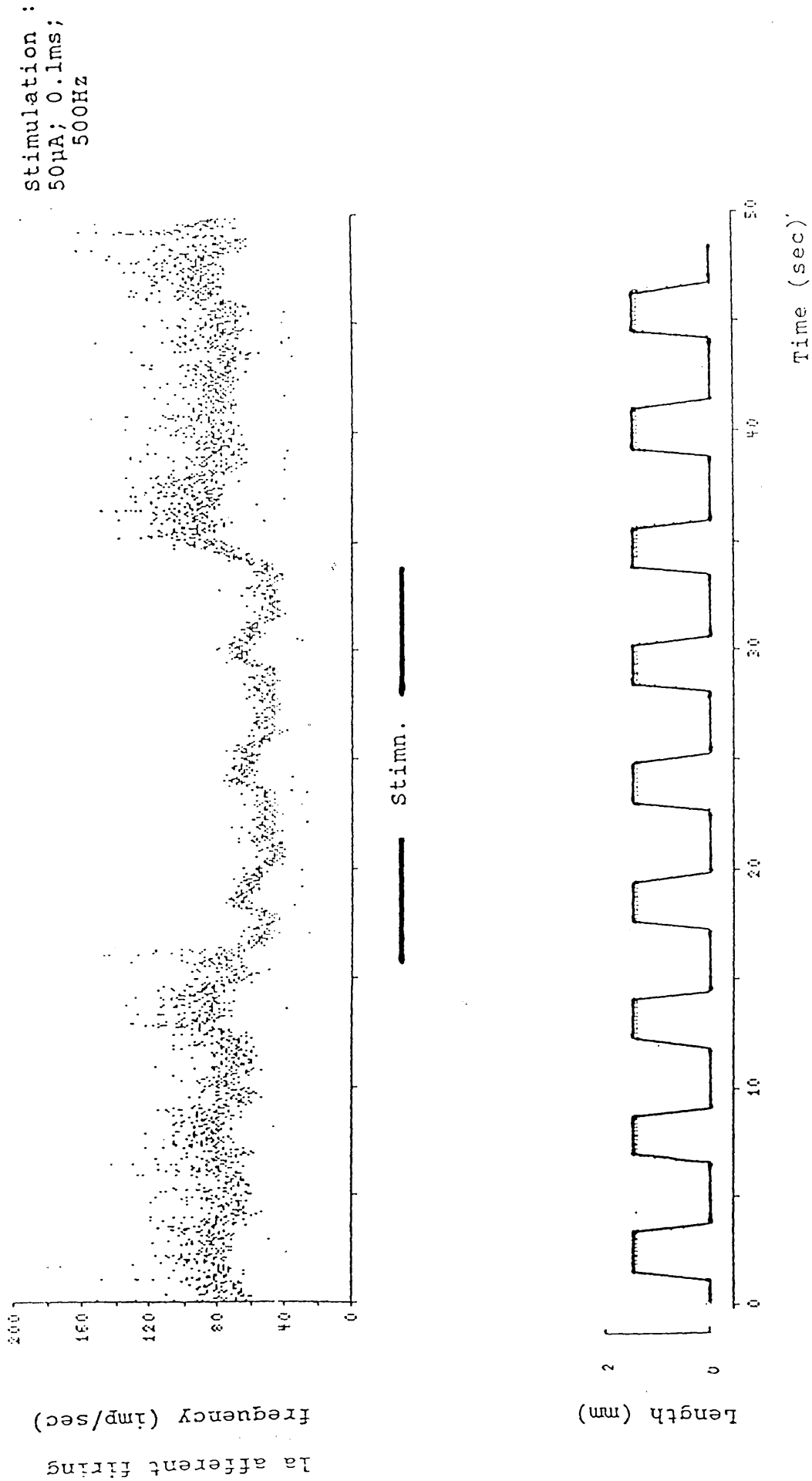


Figure 29 : Inhibition of both 'Baseline' and 'Variability' Effects

Figure 29 shows static inhibition of both the 'baseline' and of the 'variability'. As stimulation begins, the discharge drops from its initially high level and the responses to stretch become much more apparent. The discharge both at long and short lengths becomes lower, although - as far as can be judged by eye - the length sensitivity is not significantly changed. After stimulation stops, the discharge increases over about 1 second. Taking an approximation of the new level of firing, it is higher at this point than prior to stimulation, so there appears to have been some rebound excitation to the inhibition.

Even though inhibition has taken place there is still τ drive to the spindle. The initial length was set for each experiment (see methods) so as to just have an ongoing discharge. Given this, then during stimulation the response at short lengths is obviously under fusimotor influence.

Effects like figure 29 were only seen in one experiment:

8/5/89 (6 occasions): (N_2O/O_2 +0-1% Halo.+ Saffan)

and the stimulation sites are shown in fig. 40.

Figure 30 : Illustration of inhibition of 'variability'.

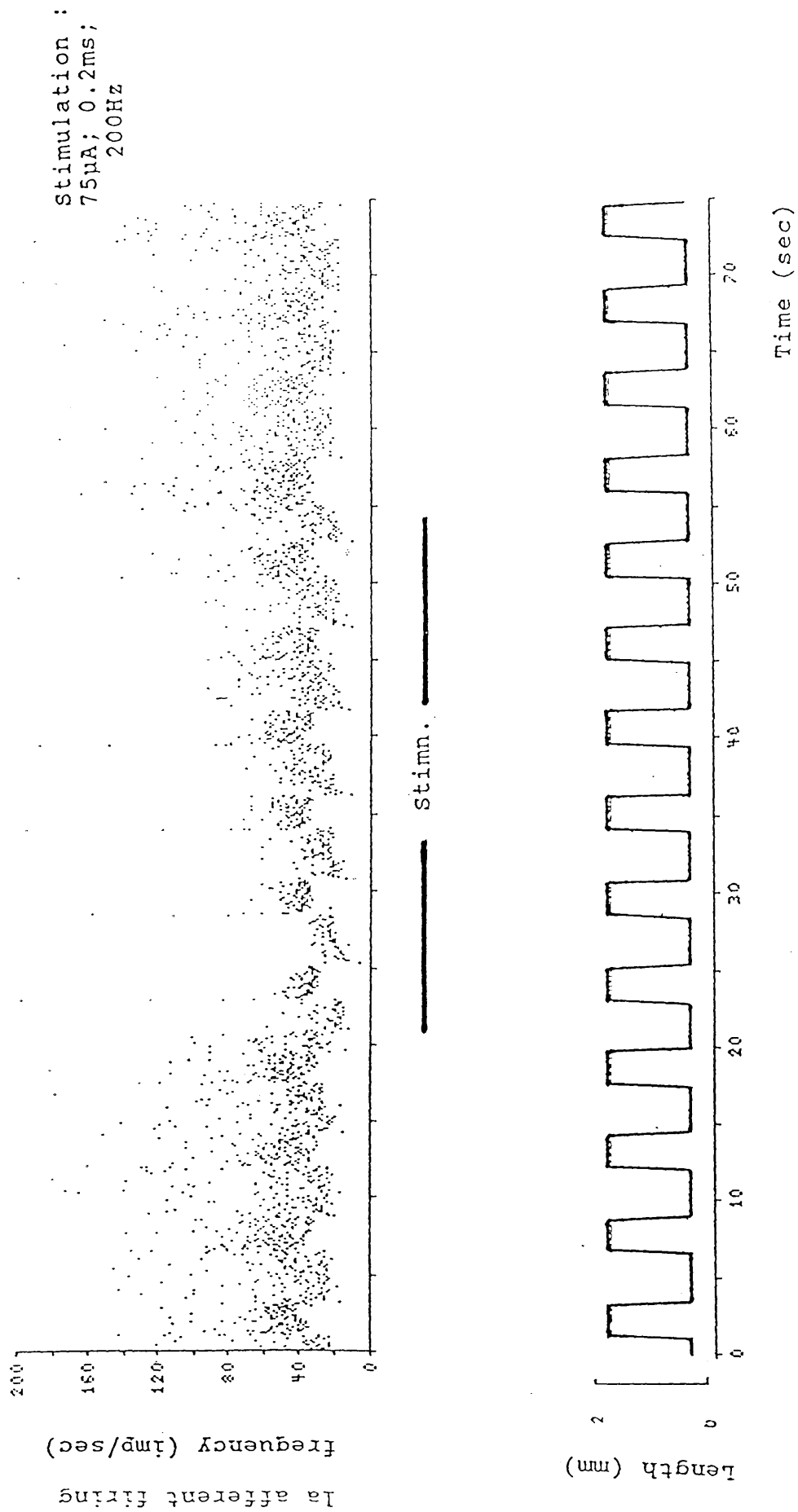


Figure 30 : Inhibition of 'Variability'

Figure 30 is passingly similar to fig. 29. However, a comparison of the two shows that in fig. 30 the 'baseline' is less affected during stimulation. Although the scatter is noticeably reduced, the minimum discharge is not reduced to the same extent as in fig. 29. However, it should be noted that the ongoing spontaneous activity was higher in fig. 29, so it may be that a greater reduction to the same amount of inhibition would be expected in this case. Nevertheless, it shows a difference in degree of effect on the 2 static features, and merits consideration for that. One other difference is that whilst the effect in fig. 29 is fairly constant, in fig. 30 spontaneous activity is beginning to encroach again by the end of the stimulation. Again there is a difference in the ongoing activity which obstructs any definite discrimination between figs 29 and 30, but one would have expected the stronger inclination to spontaneous activity in fig. 29 to be less vulnerable to inhibition. Like in fig. 29, although the ongoing τ activity is reduced, it is not abolished completely. There is also a slight suggestion of τ -d activity during the first few responses during stimulation. The post release undershoot is larger. Effects like those in fig. 30 were found on the following occasions:

12/4/89 (6 occasions) (Sagatal)

and the sites for these are shown in fig. 41

Figure 31 : Illustration of increased 'baseline' and 'variability' with probable dynamic admixture.

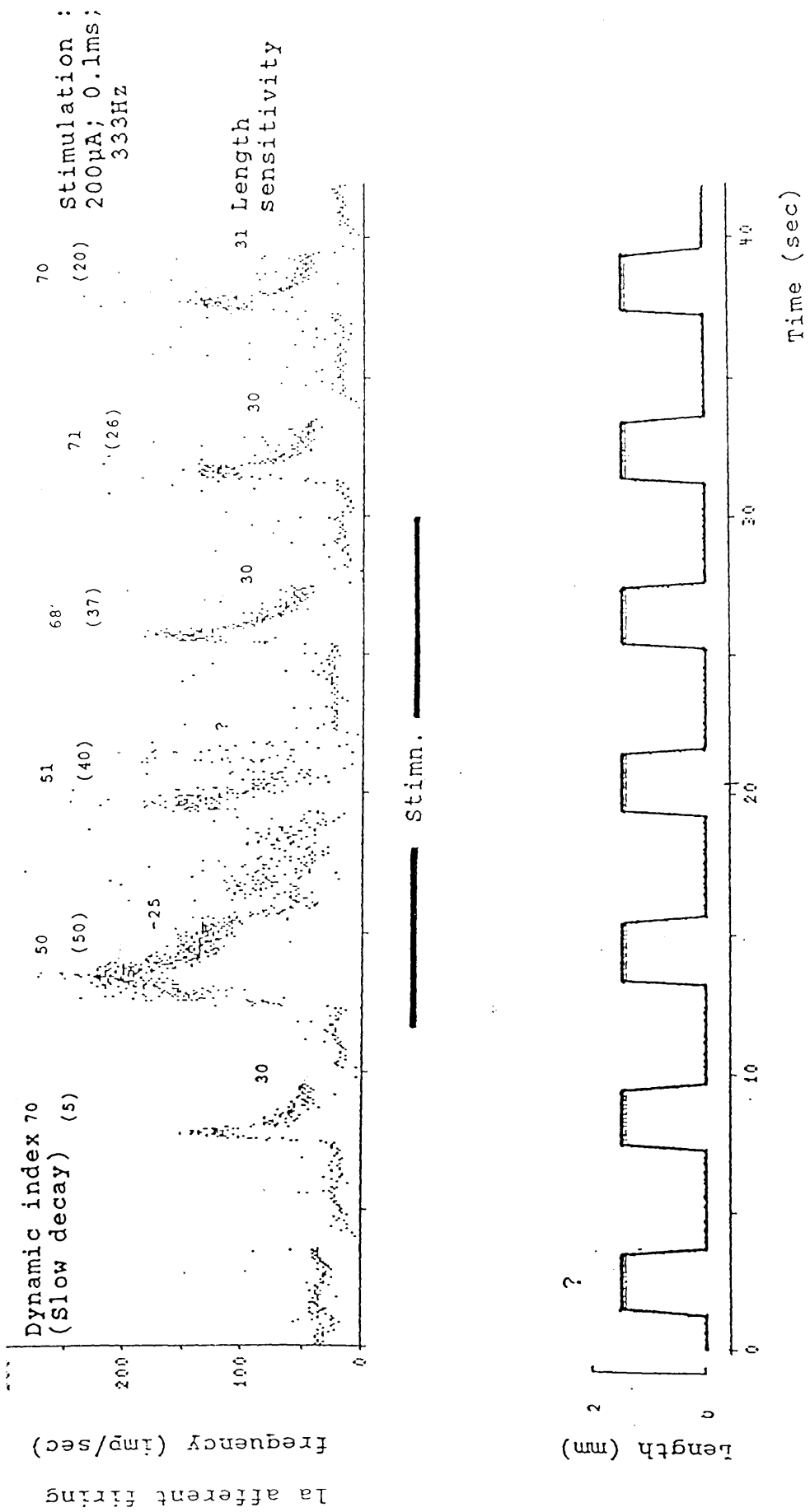


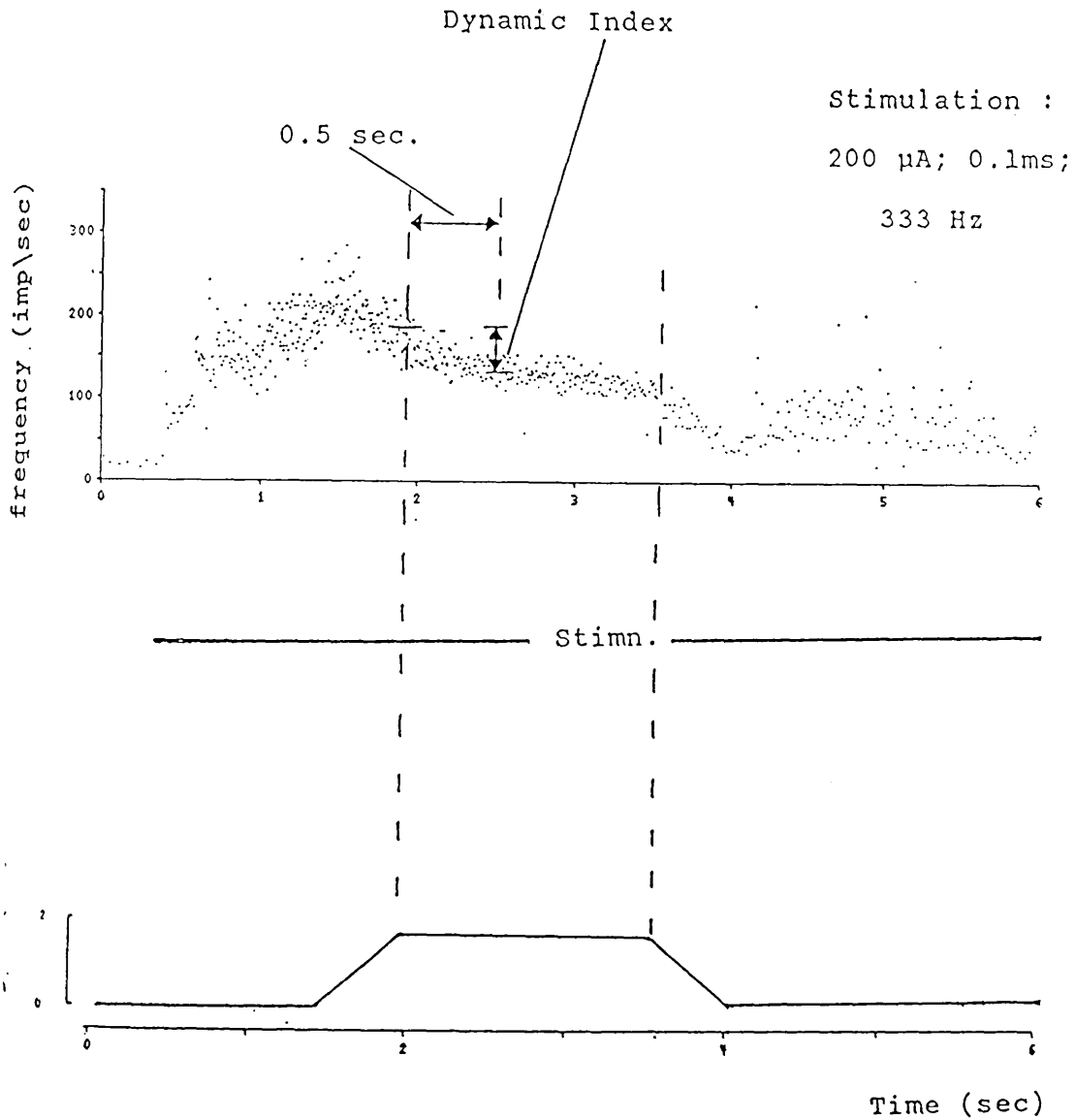
Figure 31 : 'Baseline', 'Variability' and probable Dynamic Admixture

The responses seen in one experiment were rather different from the rest of the effects on tenuissimus afferents. In fig 31 a strong excitatory effect can be seen to act on the afferent, followed by what appears to be an enhanced response to the ramp stretch with a strong dynamic component. The maximum discharge during stretching is much higher and falls by a much greater amount on the plateau of the ramp than the pre-stimulation responses. The response to the succeeding stretch is obscured by some scattering, but seems to be affected similarly to the first one, though less so. The next response again, likewise. After stimulation stops, the maximum discharge drops to its former level.

In fact, measurement of the dynamic index shows that this was actually reduced during stimulation, and returns to pre-stimulation values immediately afterwards. Fig 32 shows an expanded plot of the first length-ramp response during stimulation, and the dynamic index. However, there are two features of the responses which suggest that, despite the reduction in dynamic index, there is a τ -d influence on the spindle which probably increases during stimulation.

The first is that if one splits the dynamic index into its two components - the rapid fall and the slow decay - then the figure for the slow decay (shown in brackets below the dynamic index in fig 31) increases during stimulation. The second is a profound drop in firing frequency during the shortening phase of the length ramp. The presence (and

Figure 32 : First response to stretch during stimulation in figure 31.



magnitude) of slow decay and silencing of the afferent during shortening were found by Emonet-Dénand et al (1977) to be the surest indicators of τ -d activity during mixed fusimotor activity. Admittedly, there is no actual silencing during shortening in the first two responses, and this does not happen until the third. However, such a sharp drop would also indicate the increased length modulation of τ -d activity (shown, for example, for 1Hz sine waves by Hulliger, Matthews & Noth, 1977) , and there is also the unknown factor of how much other (static) fusimotor activity is also present.

There is clearly other fusimotor activity present, and this is static in nature. The highly erratic discharge during the first and second stimulated responses indicates a 'variability' effect, and the increase in the minimum discharges between stretches suggests a 'baseline' effect. Also, during the first stretch after stimulation begins (see fig. 32) the response decays, even though the muscle is being stretched, suggesting static activity. Indeed, if the responses to length were less pronounced, one can imagine this would be similar to fig 27 or fig 28.

One feature of the whole record which seems strange is the bursting discharge in the response towards the end of the second length ramp during stimulation. It is hard to see why there should be a sudden flurry of strong fusimotor activity to produce this.

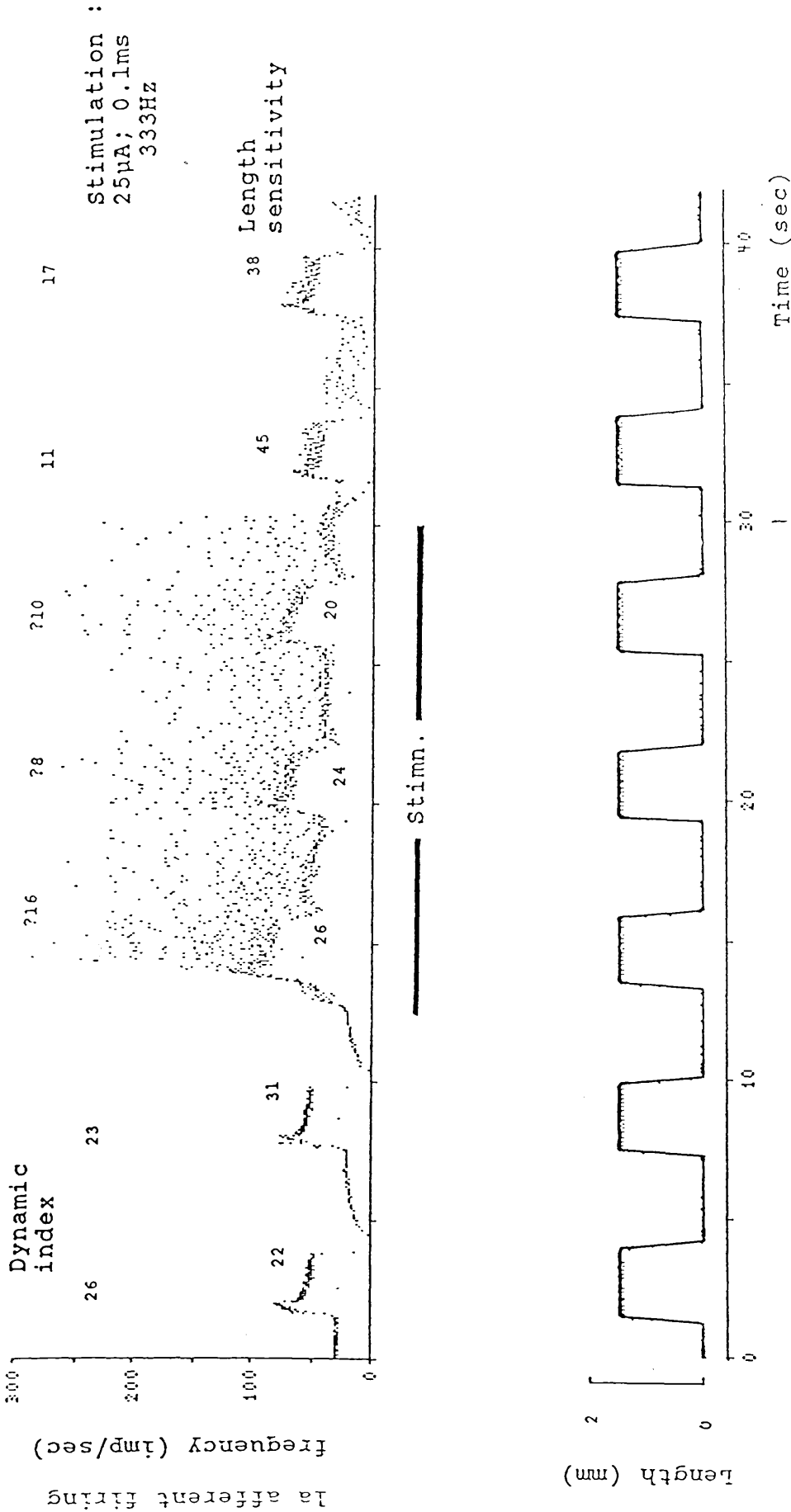
It must be stressed that this was the only tenuissimus afferent recorded from which showed any sign of an increase in dynamic activity. The responses before and after show a

prevailing level of τ -d activity, and this was also the only occasion on which this was encountered. It may be that if τ -d are already active spontaneously, then they are available to excitation by brain stem stimulation. It is not possible to draw any firm conclusions on the basis of only one finding, but the connection between these two singular events is an appealing one.

Due to a temporary fault (a stray connection between the thermostat and the Digitimer), there was a failure in the control signal, and a peculiar stretch was given at what would have been the very first of the series. This is why there is no afferent response at that place.

Effects like these were only found in one experiment (24/10/89, on 3 occasions): (N_2O/O_2 +0-1% Halo.+ Saffan), and the effective sites are shown in fig. 42.

Figure 33 : Example of a response to brain-stem stimulation in a Peroneus Tertius Ia afferent.



Peroneus Tertius (Fig. 33)

One successful experiment (29/8/89): (N_2O/O_2 + 0-1% Halo.+ Sagatal) was completed using a Peroneus Tertius muscle. This muscle was chosen because it was the one used by Appelberg & Emonet-Dénand (1965) as being representative of flexors. It has not been used by Appelberg's group for any published work since then.

The effect of mid-brain stimulation can be seen in fig 33. The overall impression is one of an increase in static activity with a maintained dynamic component. There is considerable scatter; the response during short length is elevated, and the afferent does not fall silent upon release. However, a closer look at the dynamic indices reveals that - as gauged by this means - dynamic activity is actually reduced.

The first response during stimulation appears to adapt by a similar amount to the pre-stimulation responses. However, the maximum discharge in the response occurs before the end of the stretch, and so is not measured in the dynamic index. Whether or not this high discharge during stretch should be taken as a sign of dynamic activity is moot, but in any case there is no sign of any actual increase in τ -d activity. Also, the two following responses show a reduced dynamic influence. It seems possible that in the absence of any length changes, the evoked fusimotor activity would have driven the afferent discharge to an initially high level, decaying slowly thereafter. There is some evidence for this

in the progress of the response between stretches. It seems more likely that the high discharge at the first stretch is due to fusimotor activity more than stretch, though this obviously contributes.

In this set of responses it was also possible to make a reasonable measure of the length sensitivity. It can be seen that during stimulation this is not changed to any great extent from pre-stimulation values. However, after stimulation ceases it is considerably increased. In the case of the first post-stimulation response this appears to be largely due to a drop in background activity following the cessation of stimulation. However, in the second post-stimulation response it is still increased.

The effects of stimulation linger for a while. Although there is an abrupt end to the high scattering of the discharge, after a short drop, the responses still have considerable 'variability', and their dynamic indices are reduced.

Fig 43 shows the sites giving Fig 33-like effects.

Flexor Digitorum Longus (Fig. 34)

In one experiment (15/11/89): (N_2O/O_2 + 0-1% Halo.+ Saffan), the effects of mid-brain stimulation were tested on FDL. A typical result from this is shown in fig 34. During stimulation there is an increase in the dynamic index from an already fairly high level. This increase is reflected in both the rapid fall and the slow decay of the adaptation. The increased effect on the slow adaptation persists for about 40sec after stimulation stops.

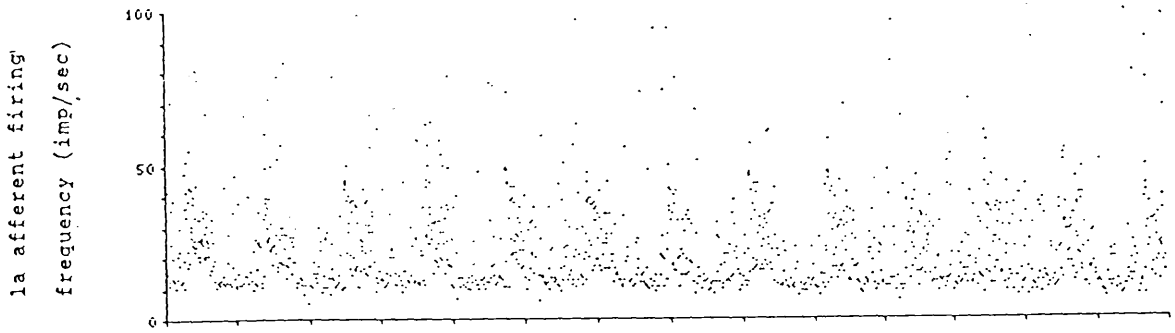
Comparing before and after stimulation, the length sensitivity is greater afterwards. During the stimulation there is an increase in the 'variability' static activity. This results in a measured increase in length sensitivity in the first response during stimulation, and a negative one in the second. This static effect does not persist after stimulation stops.

In some stimulations, what was apparently driving of the I_a appeared when the muscle was at short lengths, and was interrupted by the responses to stretch.

The sites where effects like that of fig 34 were obtained are shown in fig 44.

Figure 35 : Effect of different levels of anaesthesia on the length-ramp responses of a Ia afferent from a spindle with efferent innervation intact.

Lighter anaesthesia



Deeper anaesthesia

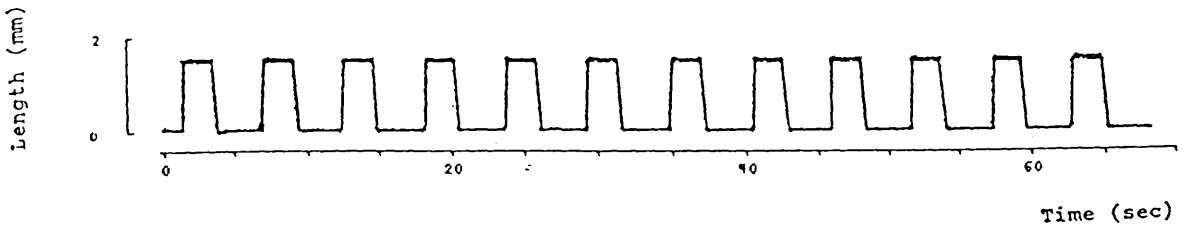
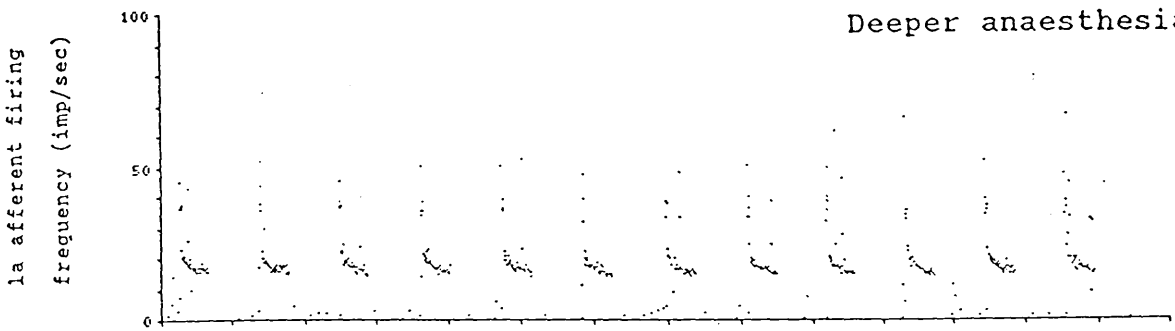
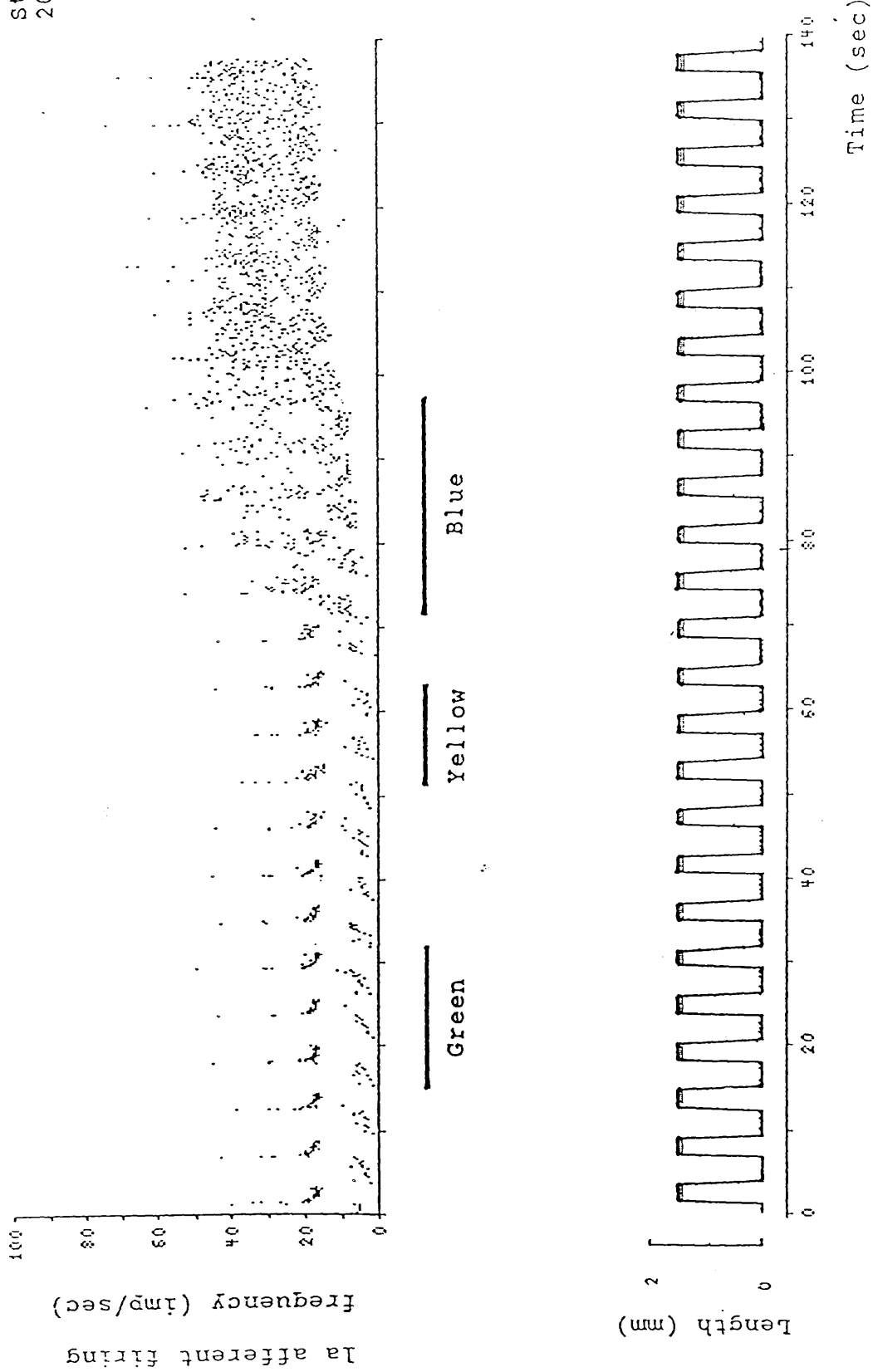


Figure 36 : Effect of stimulation in the lateral mesencephalon on the length responses of a Ia afferent.

Stimulation :
200 μ A; 0.1ms;
333Hz



Miscellaneous Effects (Tenuissimus)

Figure 35 shows the spontaneous discharge of the same afferent at different levels of anaesthesia. There is no stimulation during either. During the lighter level, the discharge is much more erratic, and the minimum firing is higher, never falling to zero. It is evident that as anaesthesia (N_2O/O_2 + 0-1% Halo.+ Saffan) lightens, the static τ tend to increase their firing. Both the 'variability' and the 'baseline' components increase. It was shown by Gladden & McWilliam (1977a) that the spontaneous movements of the Sb2 were the first to appear in tenuissimus spindles with lightening anaesthesia, and then the chain fibres. The spontaneous movements of the Db1 were the most susceptible to anaesthesia.

In figure 36, stimulation only by the most lateral microelectrode effects a dramatic change in the afferent's discharge. This is the same afferent as shown in fig 35. There is an increase in the 'variability' static effect, obscuring the length responses. The effect increases throughout the stimulation and also increases after stimulation ceases. Unfortunately, the recording stopped after another 30 or so seconds, but it was observed that the discharge continued like this until more anaesthetic was given. The impression was that the level of anaesthesia had jumped from one level to a lighter one. The timing of this may just be coincidence, but given that this electrode is the

most laterally placed one (see fig 45), it is credible that stimulus spread to the reticular activating system has 'reset' the level of alertness of the animal, necessitating more anaesthesia.

Figure 37 : Stimulation sites producing effects in la afferents like those of fig. 26.

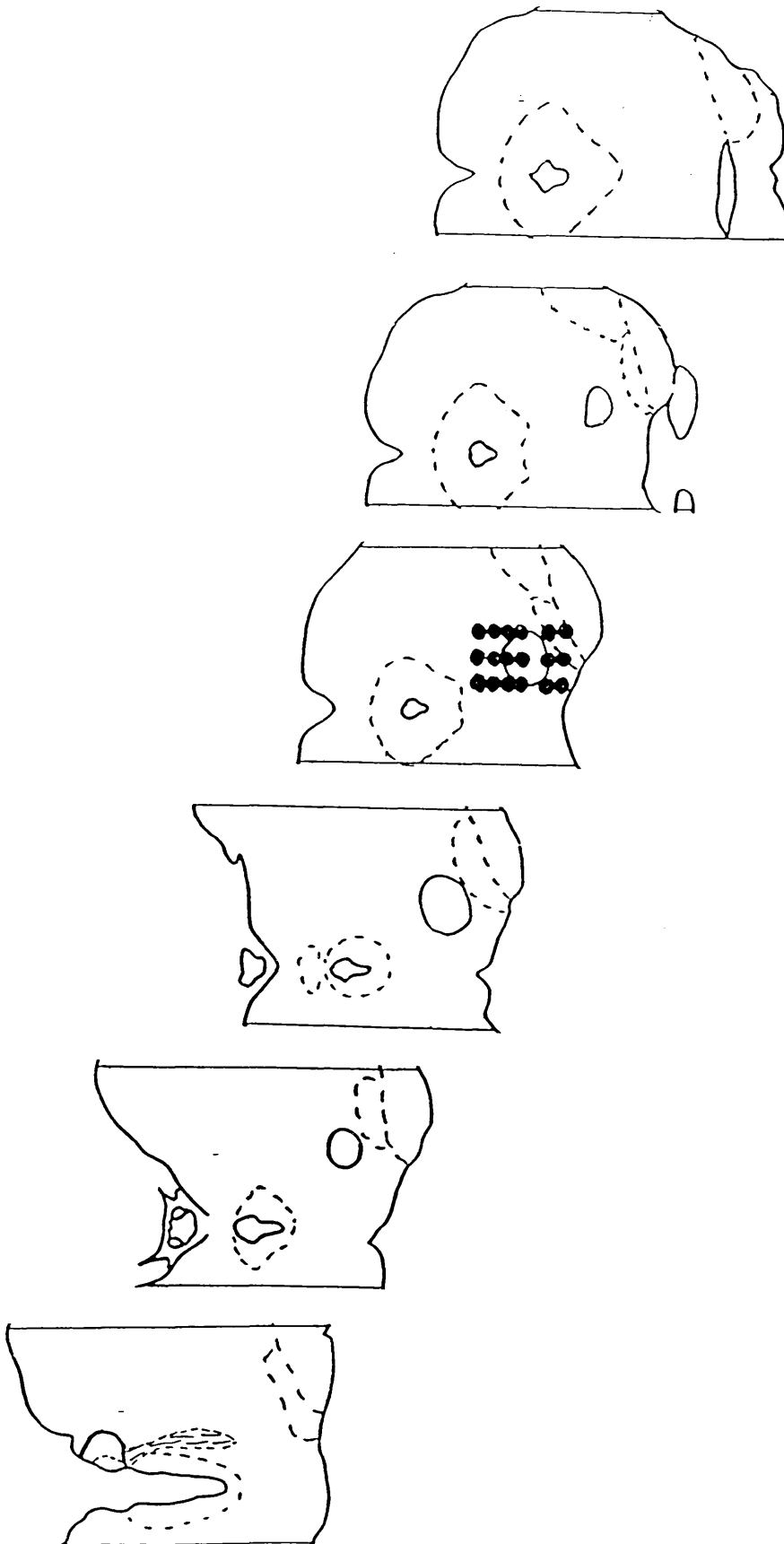


Figure 38 : Stimulation sites producing effects in la afferents like those of fig. 27.

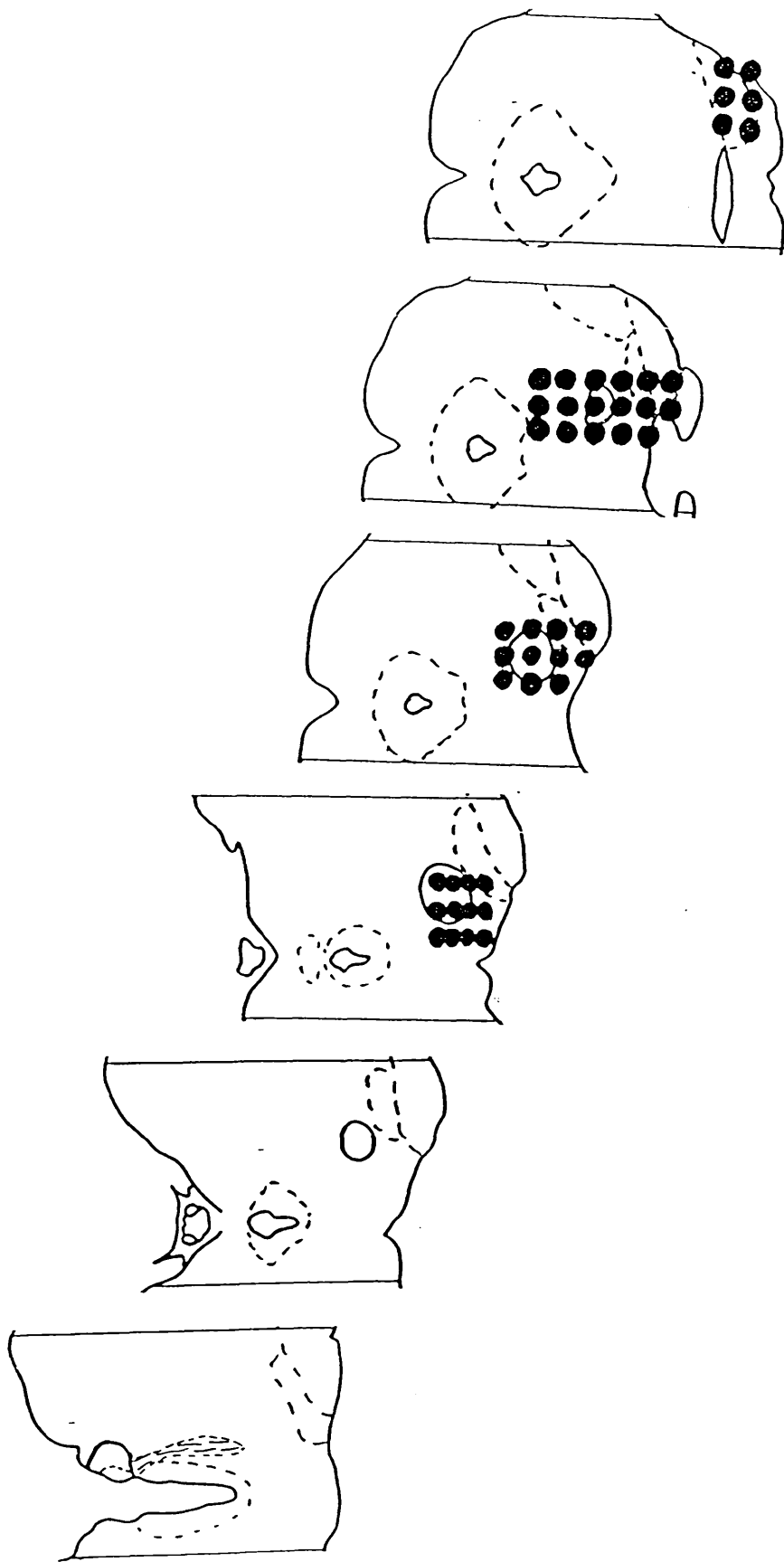


Figure 39 : Stimulation sites producing effects in Ia afferents like those of fig. 28.

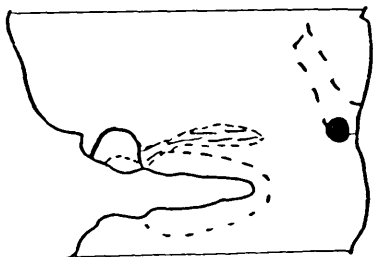
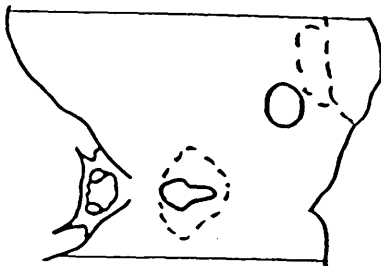
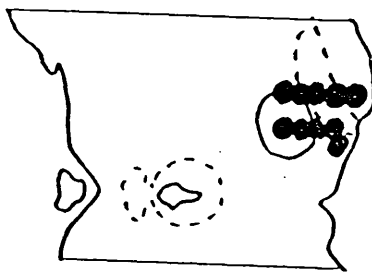
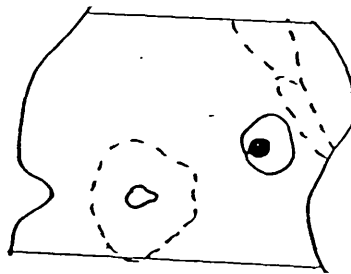
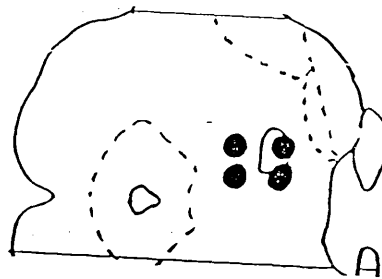
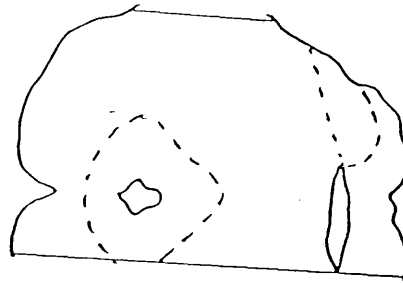


Figure 40 : Stimulation sites producing effects in
1a afferents like those of fig. 29.

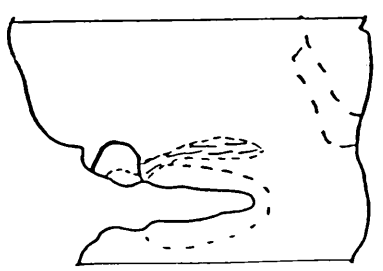
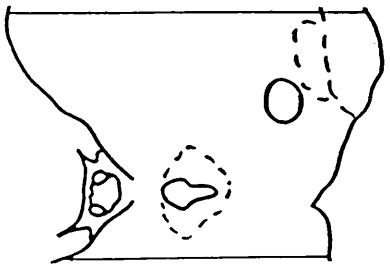
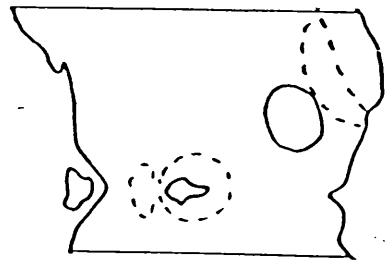
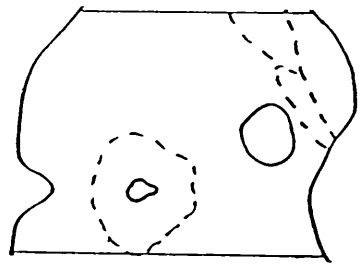
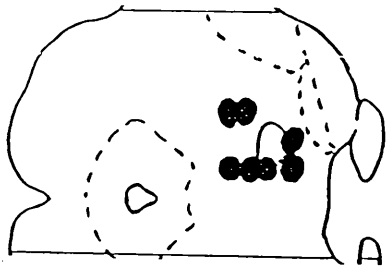
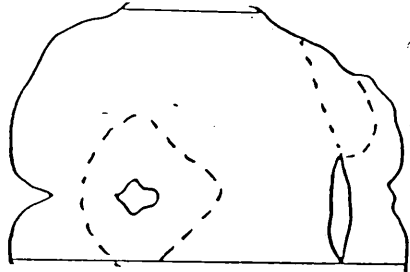


Figure 41 : Stimulation sites producing effects in la afferents like those of fig. 30.

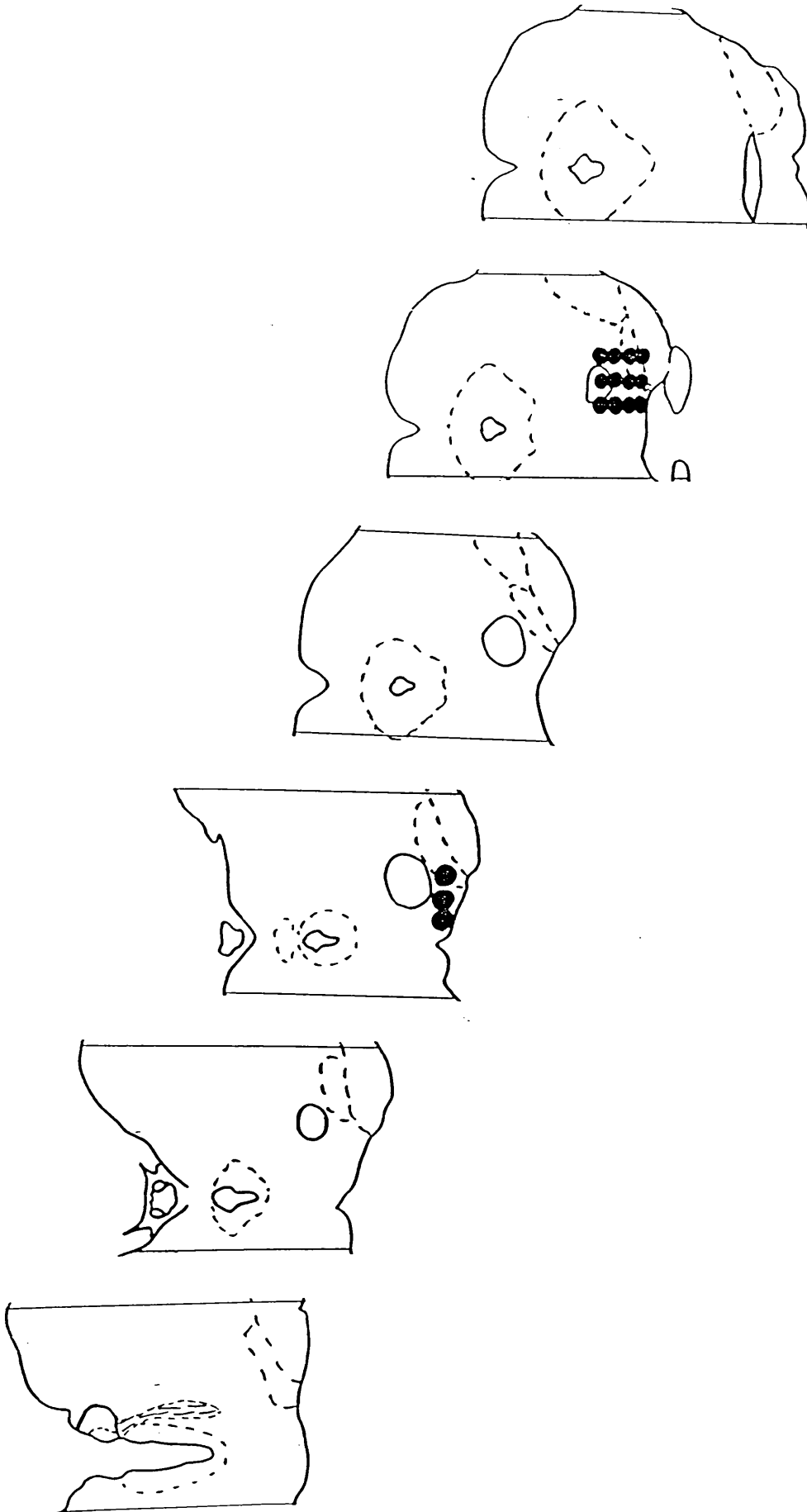


Figure 42 : Stimulation sites producing effects in la afferents like those of fig. 31.

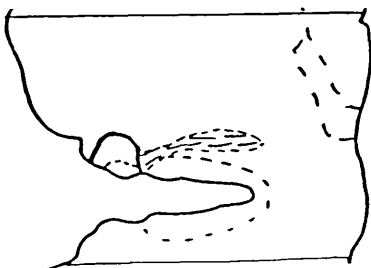
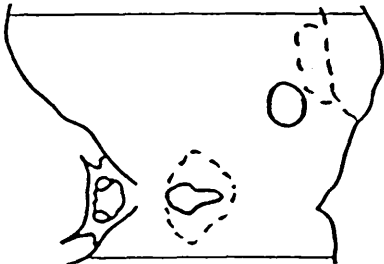
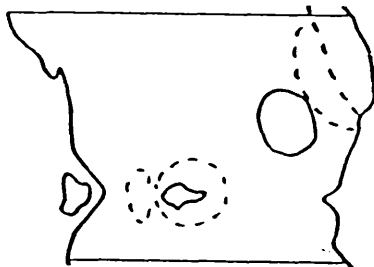
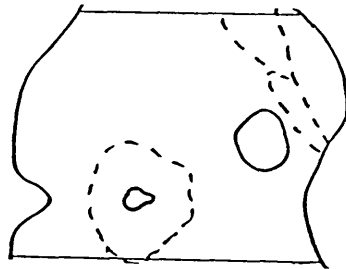
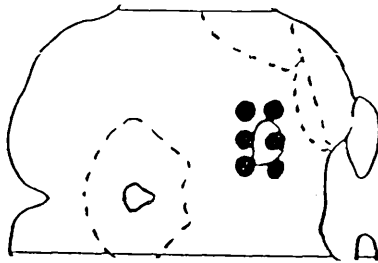
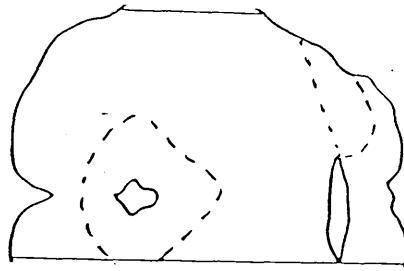


Figure 43 : Stimulation sites producing effects in la afferents like those of fig. 33.

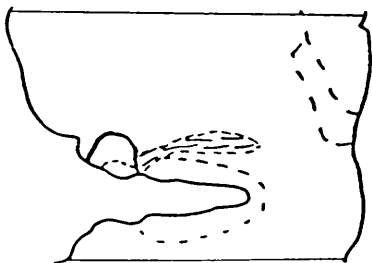
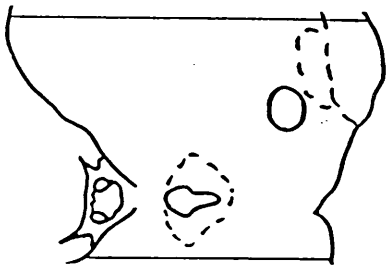
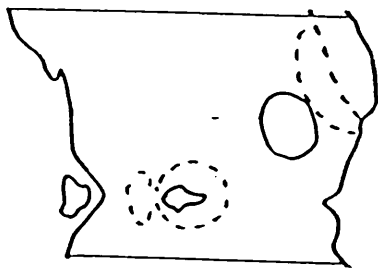
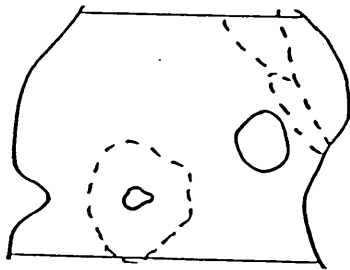
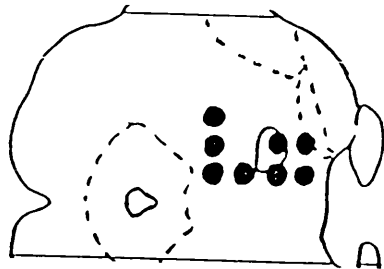
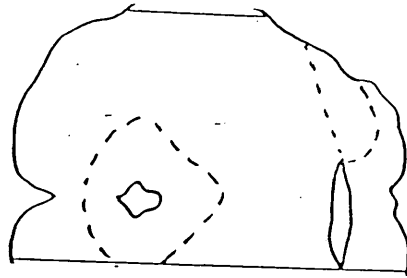


Figure 44 : Stimulation sites producing effects in la afferents like those of fig. 34.

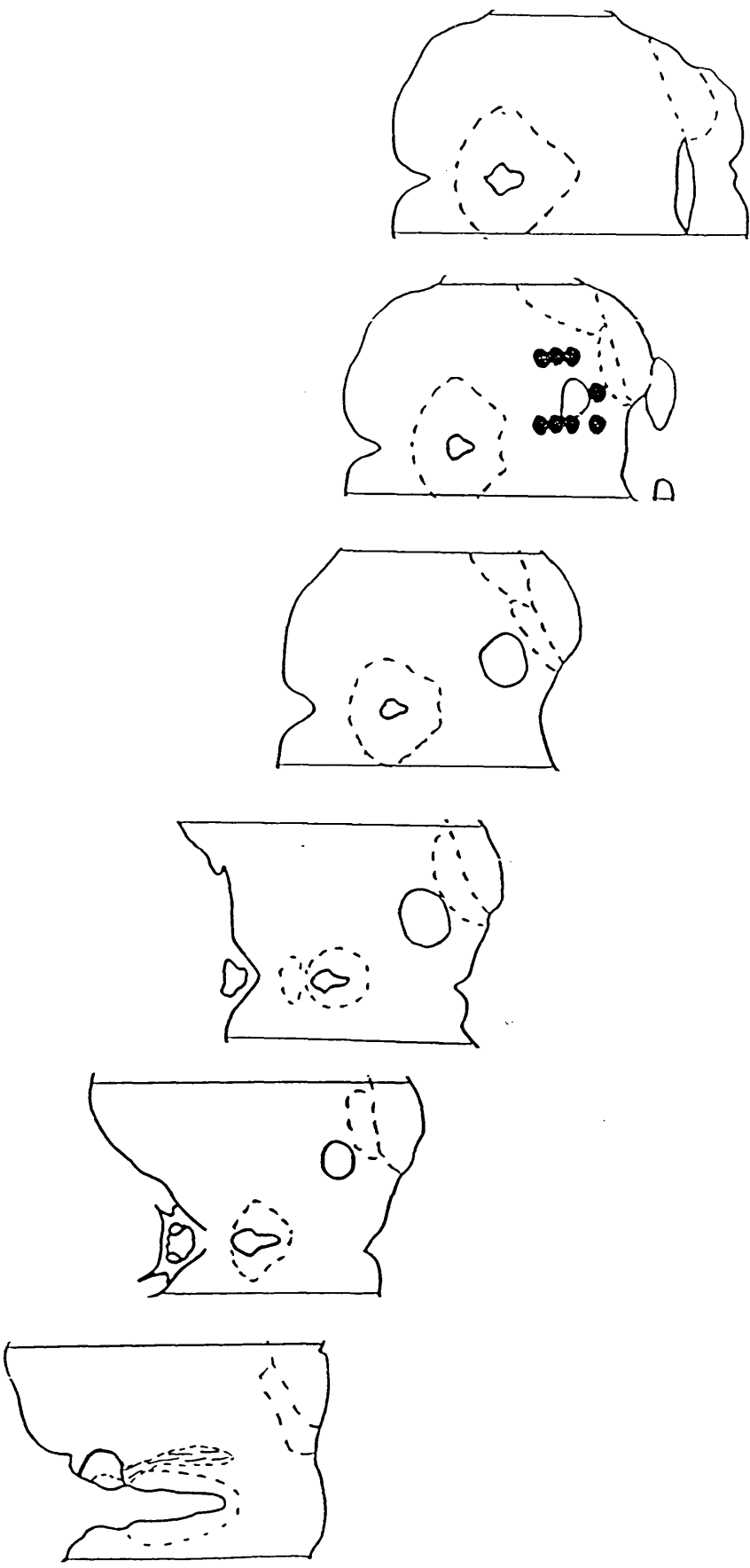
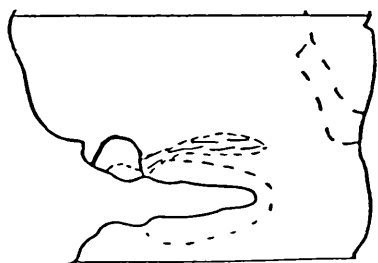
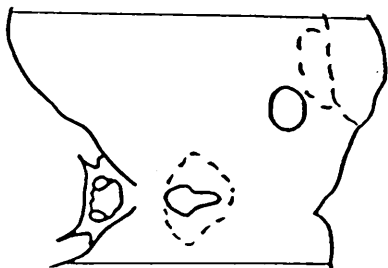
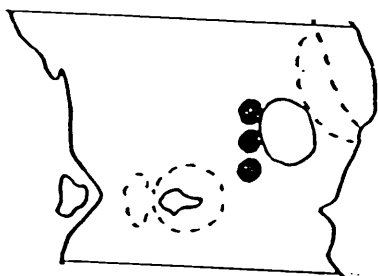
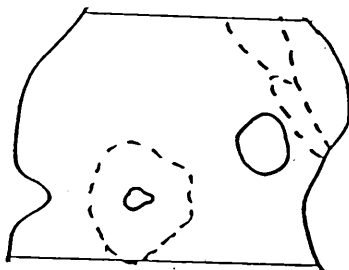
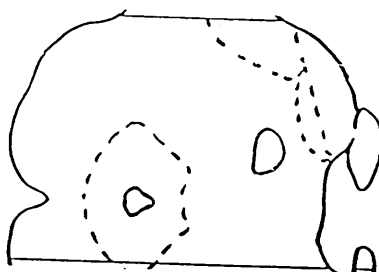
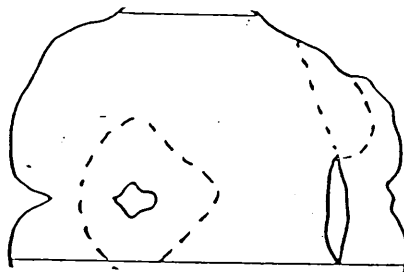


Figure 45 : Stimulation sites producing effects in la afferents like those of fig. 36.



from a much wider area - the greatest extent of any category of effect - and it cannot be guaranteed that anaesthesia was the same throughout the experiment in question. Although it would certainly be at a similar level on the two occasions, it is an impossible quality to quantify, and small differences can have surprisingly large effects.

In the experiment of 12/4/89 (Sagatal), two very different effects were seen. From the rostral tracks excitation was seen - of both 'baseline' and 'variability'. At similar stimulus strengths, from the caudal tracks inhibition - mainly of 'variability' was seen. Although this is intriguing, again it cannot be said that anaesthesia (or any other relevant parameter) was at the same level.

In the experiment of 24/10/89 (N₂O/O₂ + 0-1% Halo.+ Saffan), similar fig. 27-like ('variability' and 'baseline' : predominantly 'variability') and fig. 31-like effects ('variability' and 'baseline' with probable dynamic admixture) were seen from different parts of the same track. In the more dorsal parts the fig. 31 like effects were seen. These effects were thought to be like those of fig 27, but with a τ -d admixture. It is interesting that the points giving Fig 31-like effects (i.e. with some additional dynamic component) correspond fairly well with the description of Appelberg's MesADC. However, they do not overlie well the areas giving Dbl recruitment in the exposed spindle experiments.

In figure 34, there is no doubt that τ -d were recruited. The

points giving these effects also correspond fairly well with Appelberg's area. However, only one penetration was made in that experiment, so it cannot be said that similar effects were not obtained from other areas. Also, this effect seemed to 'burn out'. A second burst of stimulation, even at a considerable time later (3 or 4 mins.), and at apparently lighter levels of anaesthesia, was ineffective. Because of this it was not possible to pinpoint the area of lowest threshold.

COMPARISON OF SITES RECRUITING KNOWN INTRAFUSAL FIBRES AND THOSE RECRUITING DIFFERENT CATEGORIES OF AFFERENT EFFECTS.

In the dorsal root experiments, as in the exposed spindle experiments, the thresholds for effects are lowest at the level of the caudal NR. Likewise, ventrally the necessary stimulus strengths tend to be lower, although when the threshold was mapped to a degree, the very lowest ones for the afferent experiments were not the most ventral, but those on the ventral border of, and just ventral to, the NR. One conspicuous difference between the two sets of experiments was in the effects of stimulation in the habenular region. In all the afferent experiments, there is a complete absence of effect, whereas in the exposed spindle experiments, influences on Sb2 were seen. This has to be qualified on three counts : firstly, it was only in two exposed spindle experiments that these were seen and only at one point in one of these ; secondly, the effects seen were mostly inhibition, which may be less evident in the afferent responses ; thirdly, in the exposed spindle experiments, the stimulus strengths required in the habenulae were higher than those in the other areas tested.

Like the inhibitory effects on the Sb2 and chains, inhibitory effects in the afferent recordings (figs 29 and 30) were most common from the caudal NR and even rarer elsewhere than the excitatory ones, compared to the exposed spindle experiments.

DISCUSSION

COMPARISON OF EFFECTS SEEN WITH PREVIOUS WORK.

The MesADC system: Appelberg and co-workers.

We did not find any evidence to support the selective recruitment of dynamic fusimotor neurones in the tenuissimus muscle from anywhere around the red nucleus. This does not support the concept of a descending system selectively recruiting dynamic τ in hind-limb muscles as described by Jeneskog (1974) and Appelberg (1981), based on the results of Appelberg and Jeneskog (1972) and Appelberg and Emonet-Dénand (1965). This allows for two possibilities : either that there is some systematic difference in the techniques used which has resulted in different effects; or that tenuissimus and FDL are controlled differently from the brain-stem.

Regarding the first possibility, this is not likely to be the case. In order to achieve a dynamic effect we mimiced the methods used by Appelberg's group closely. With time, some were shed as superfluous in the absence of any dynamic activation. For example, we did not record the cerebellar potentials, as we felt that the time was better spent searching the mesencephalon. If we had seen dynamic activation, then we would have wanted to ascertain its origins by this means. Similarly, we did not record the potentials descending in the dorsolateral funiculus of the spinal cord. Some of the other methods we changed because we felt that it improved matters. There is the possibility that Saffan (alphaxolone/alphadolone) has a particular

pharmacological effect which would block the descending dynamic control, but it is unlikely, since we also used the gaseous anaesthetics only , and also barbiturates. Also, Appelberg and co-workers have used several different types of anaesthetic.

With regard to the second possibility, I find this more likely. The function of tenuissimus is not properly known and its connections with the brain-stem may be unusual and/or tenuous. In this light, it is worth noting that in the only result we obtained from FDL - the muscle favoured by Appelberg and co-workers - we obtained dynamic effects. This could be taken as a distinction between tenuissimus and other muscles were it not for our results from PTer. The whole case for Appelberg's generalisation of effects (to flexors and extensors) rests on the finding of Appelberg and Emonet-Dénand (1965) that during stimulation in an area approximating the NR, dynamic effects were recruited in both an extensor (FDL) and a flexor (PTer). In our only experiment, we found static effects in PTer. It is possible that in this one experiment, we missed the appropriate area, though I would not believe that this could be true through the whole series of experiments on tenuissimus.

Other work on brain-stem stimulation.

In most work on central control of τ , the investigators have looked at the responses in ventral root filaments, and it is only since the distinction between τ -s and τ -d was made that afferent responses have been used for this purpose to any great extent. Hence, most early work is of limited value for those wishing to study recruitment of different types of intrafusal fibres.

Granit and Kaada (1952) and Shimazu, Hongo and Kubota (1962a) found a diffuse pattern of excitation during brain-stem stimulation which lingered after stimulation had ceased. The latter group linked the appearance of this particular pattern to 'light' anaesthesia, or 'moderate' anaesthesia combined with a low stimulus strength. We have seen similar effects in some experiments, but not as a general rule.

Vedel and Mouillac-Baudevin (1969a) also saw changes in effect as the level of anaesthesia was changed. At their 'light' level (approximately equivalent to ours), they saw static effects in soleus spindles during stimulation in the mesencephalic reticular formation. At a deeper level of anaesthesia they recruited dynamic ones. It is thus conceivable that if we had deepened anaesthesia slightly, we would have seen dynamic effects, though I do not think this would have been the case. There were many occasions when the anaesthetic level was deeper, and at these times we had to wait for effects to re-emerge, and when they did, these were the same as those seen before - usually static. No

intervening dynamic responses were seen. In any case the two studies are not directly comparable, since Vedel and Mouillac-Baudevin stimulated the reticular formation only, whereas we stimulated in this region only incidentally; and they were studying the effects on soleus, while we used tenuissimus spindles.

Taylor and Donga saw dynamic effects on spindles in triceps surae and jaw muscles from stimulation sites around the NR. Since their results are in broad agreement (though not on all points) with those of Appelberg's group, then at first appearance, they disagree with ours. Triceps surae is a third muscle, which is affected in the same way as FDL (predominantly dynamic fusimotor recruitment). This tends to marginalise tenuissimus, and adds weight to the idea that tenuissimus is an exception in the control of hindlimb muscles. However, like FDL, triceps surae is an extensor, so the slight doubt about the generality of the effect as extending to extensors and flexors is not decided by this study. It would be interesting to test a wide range of muscles, and correlate their function (not only flexor or extensor) with effects seen. Tenuissimus may lie at or near one end of a broad spectrum of effects.

Schwarz et al (1984) and Wand and Schwarz (1985) showed different effects on Ia and II afferents, suggesting a selective inhibition of Sb2 fibres, in spindles of Extensor Digitorum Longus and Tibialis Anterior during activation of cells of the reticular part of the substantia nigra. Although it was not the main thrust of our project, on some occasions

stimulation in both parts of the substantia nigra was tried. A variety of effects was seen from the area, but these did not seem to be confined to its borders. Yet again, the effects seemed to be recruited fairly consistently from a wide area within any one experiment. As well as the qualifying remarks which apply to the other experiments (see above and below), there is also the fact that these experimenters used ketamine as the anaesthetic during the experiment. Ketamine is a 'dissociative anaesthetic' and is believed to exert its anaesthetic action via its property as an antagonist at the NMDA sub-type of amino acid receptors (shown in rat and cat Renshaw cells by Anis, Burton and Lodge, 1982). It is thus fundamentally different from most anaesthetics, which are broadly similar in their mode of action. Specifically, ketamine has some manifest effects on the motor system, amongst which are: - "muscle tone is poor.....and may be increased.....purposeless movements sometimes occur.....occasionally violent and irrational responses to stimuli are observed." (from Marshall and Woolman, 1985). Given this, it would be easier to compare their results with those of others (including ourselves) if they had used a more conventional anaesthetic.

RECRUITMENT OF INTRAFUSAL FIBRE TYPES AND THE TYPES OF
INTRAFUSAL FIBRE WHOSE CONTRACTION IS RESPONSIBLE FOR
CHARACTERISTIC CHANGES IN THE Ia AFFERENT DISCHARGE.

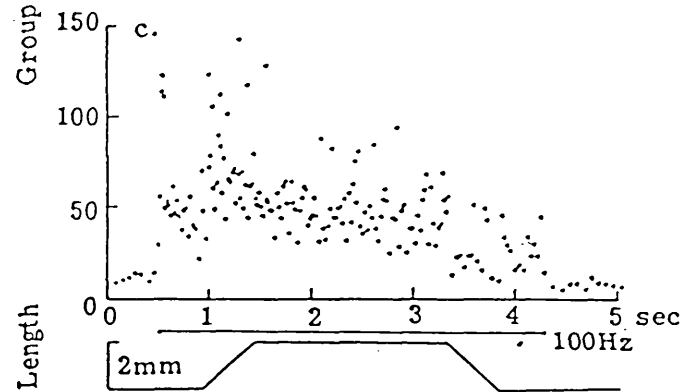
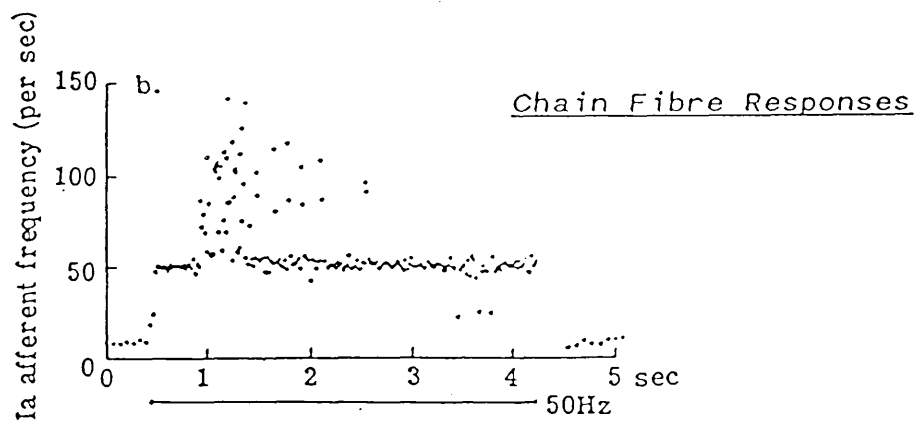
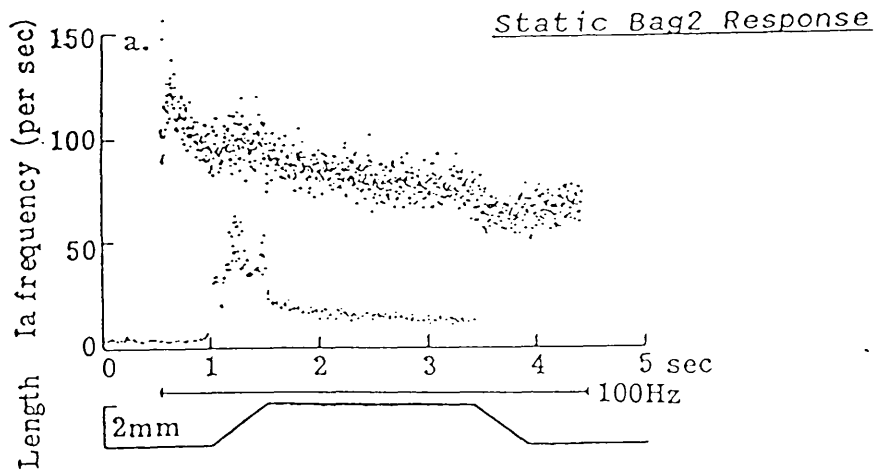
Tenuissimus.

In the tenuissimus muscle, the great majority of effects were static. In both the exposed spindle experiments and the dorsal roots experiments, such small signs of Db1 excitation as were present were always intermingled with recruitment of one or both of the static intrafusal fibres. In short, we found no evidence for any particular area in the parts of the brain tested which recruited Db1 preferentially, and certainly none which did so exclusively.

In the exposed preparation there is no real doubt as to whether the chains or bag fibres were recruited. Most of the time any uncertainty between the two types of bag fibre could be resolved by application of scoline (suxamethonium) at the end of the experiment (Boyd, 1985c), but on most occasions this was only to confirm absolutely the identification.

In the dorsal roots preparation, there is a much greater degree of uncertainty as to which intrafusal fibres are active, and to what relative degrees.

Figure 46 : Effects of stimulation of τ motoneurons to different intrafusal fibres on Ia afferent responses.



All from Boyd, 1981

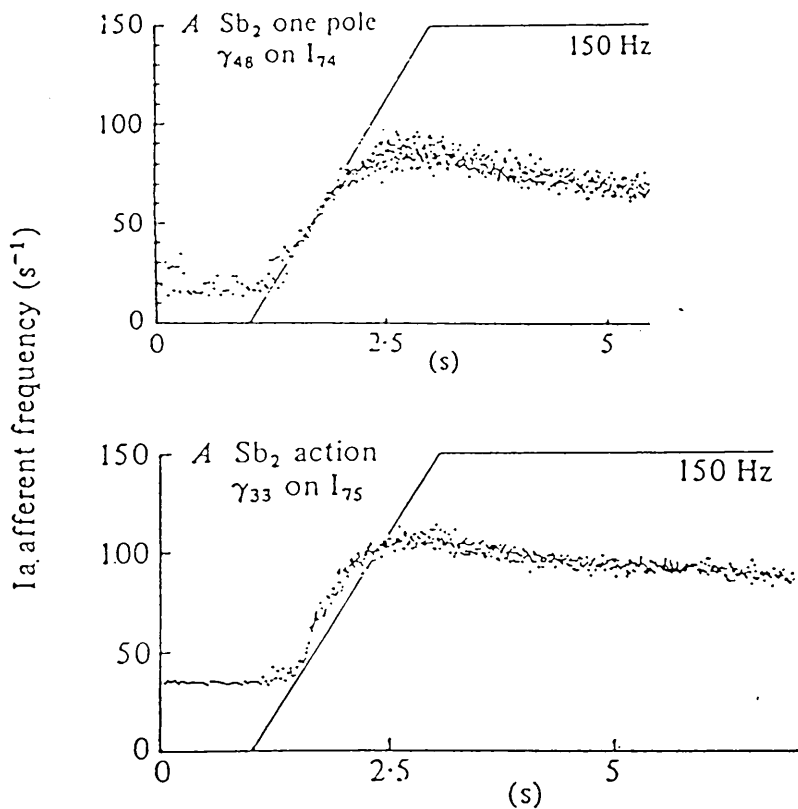
For reasons which should become clear below, I would like to propose that the 'baseline' effects are due (at least mainly) to changes in firing of τ supplying Sb2, and changes in the 'variability' are mainly due to changes in τ supplying chain fibres.

The responses of Ia afferents to stimulation of the different intrafusal fibres are presented in Boyd (1980) and Boyd (1981). In these he stimulated τ -fibres supplying the different intrafusal fibres at fixed frequencies of 75Hz and 100Hz, with a few at 50Hz.

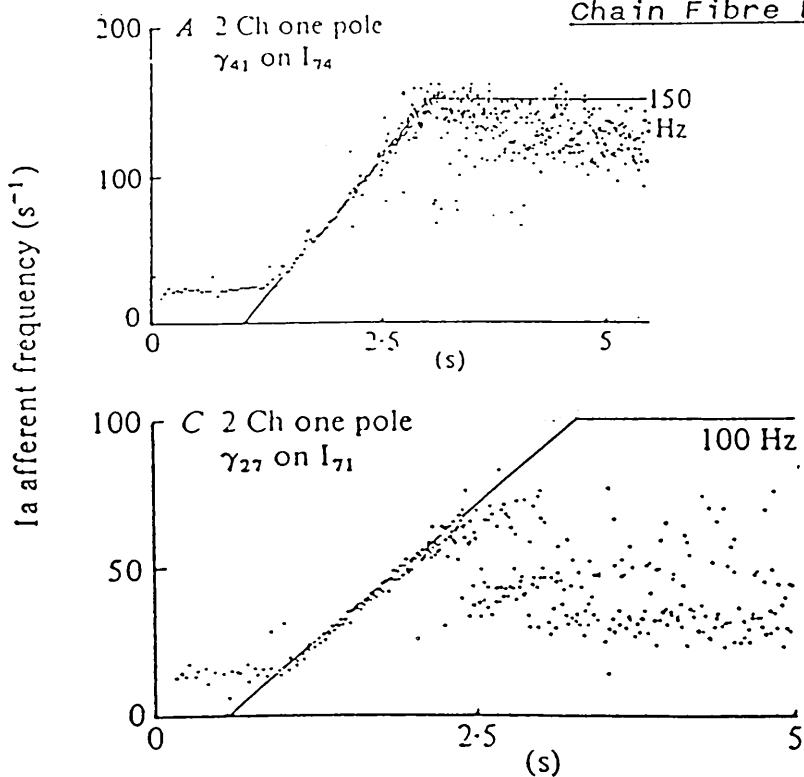
In these it is seen that : the action of the Sb2, stimulated at 100Hz is to increase the ongoing level of Ia discharge (see fig 46). This then decays, which must be due to receptor adaptation, since the intrafusal contraction is maintained. The dynamic index of the Ia is reduced, and the length sensitivity of the afferent is decreased or unchanged. At fairly low frequencies (<50Hz), the chain fibres usually drive the Ia discharge, either 1:1 or at a sub-multiple. If not driving, chain fibres cause the Ia discharge to become very irregular. In either case, the afferent's response to length is obscured, and often the dynamic response is as well. Contraction of the Db1 fibre has the effect of increasing the dynamic response. The effect of Db1 contraction on length sensitivity (after completion of adaptation) is variable (0-200%), but usually leaves it approximately unchanged.

Figure 47 : Effects of 'ramp frequency' stimulation of τ motoneurons to different intrafusal fibres on Ia afferent responses.

Static Bag2 Responses



Chain Fibre Responses



However, the effects on the afferents of the 2 different static intrafusal fibres have only been tested under the restricted conditions mentioned above - where the τ firing rate is at a fixed frequency, and only 1 single τ is active. There is no published work showing the effects of mixing different static τ supplying different intrafusal fibres, and of varying the rates of stimulation independently. In his 1986 paper, Boyd showed the effect of an increasing frequency of stimulation to the different static τ . Something similar to this is likely to happen to τ motoneurons during central stimulation, as the drive to activity filters down through the intervening synapses. The distinguishing features of Sb2 activity and chain fibre activity are (see fig. 47) : at lower stimulation frequencies, the chain fibres will drive the Ia, forcing it to follow the stimulation frequency very closely, while the Sb2 is capable of transiently pushing the Ia frequency higher than the stimulation frequency ; at higher stimulation frequencies chain fibres usually make the Ia afferent's discharge very erratic, while the Sb2 leaves the discharge with a comparatively smooth profile. Without knowing the efferent activity, the only means to distinguish them in our case is the degree of fluctuation of the Ia discharge.

It is a great pity that a II afferent was not available for recording in any experiment which yielded effects. This would have been the simplest and a highly reliable means to distinguish between Sb2 and chain activity. It could also

have been used to assess the amount of chain fibre activity in an accompanying mixed effect on the Ia. The absence of them is due to a lack of surgical finesse on my part, combined with the fact that they are much more difficult to isolate in the dorsal roots than the Ia afferents. Nevertheless, there is still a sufficiently strong body of evidence, based on the criteria of Boyd's work (see above) to implicate the Sb2 as the fibre responsible for the changes in 'baseline' effects on the Ia, and the chain fibres primarily responsible for those on the 'variability' (chains and Sb2 together will also increase the variability).

Also, the fact that in one of our experiments (again, in the single one using FDL), the 'variability' response developed into driving of the Ia supports the hypothesis that the 'variability' response is mediated by the chain fibres. Only the chain fibres drive the Ia, and if one makes the reasonable assumption that the driving effect is a progression from the 'variability', then they must both be served by the same (chain) intrafusal fibres.

I would like to stress that it has been taken into account that the 2 effects do not have an exclusive relationship with the 2 intrafusal fibres. Sb2 activity will also make the discharge more erratic than the passive state, but less so than the chains. It is the degree to which this happens which distinguishes them, with the Sb2 enhanced discharge lying comparatively tightly about the mean level.

With regard to recruitment sites in the brain-stem, the 'baseline' effects being mediated by Sb2, and the 'variability' by chains means that - as in the case of the exposed spindle experiments - the tenuissimus Ia afferents do not show any correlation between different intrafusal fibres and different stimulation sites.

Peroneus Tertius.

The effects on Peroneus Tertius (PTer) were - like the tenuissimus ones - static. Again the 2 components of 'variability' and increased 'baseline' were seen, suggesting recruitment of both types of static intrafusal fibre. Like the tenuissimus ones, this single afferent showed no sign of dynamic fusimotor activity.

Flexor Digitorum Longus.

In the one FDL afferent tested, there were clear signs of increased dynamic activity. However, these were obtained from a fairly wide area which does not correspond particularly well with any of Appelberg's areas.

Only one fruitful FDL experiment was completed, though two experiments were attempted. In the first of these, a preparation of afferents from tenuissimus and FDL was attempted, to allow comparison of the two with a higher degree of confidence than from 2 separate experiments.

Unfortunately, this didn't work, and that experiment became one of the successful tenuissimus ones (24/10/89). In the second, less ambitious attempt, we successfully isolated an FDL afferent.

Since only one afferent from one experiment is available for comparison, no general conclusion can be drawn. However, I think the fact that in the only FDL afferent tested, dynamic effects were seen, and the fact that it is in stark contrast with the consistently static effects seen from tenuissimus and PTer, allows one to attach more significance to this result than one normally could to a single result.

Assuming that the FDL afferent is genuinely representative of all FDL afferents, then the effects of stimulation in the brain-stem on this muscle are markedly different from those on tenuissimus. If one makes a similar set of assumptions for the PTer afferent, then FDL is different from this muscle also.

To conclude, I think that although the fact that the muscle most often investigated by Appelberg and co-workers yielded dynamic effects, and at least one (perhaps two) other muscles did not, is a remarkable occurrence. I think it certainly merits further investigation.

OTHER EFFECTS

Effect of pressure on habenulae.

The compression of the tissues around the habenulae by the microelectrode grid appeared to inhibit spontaneous fusimotor activity. This was not the case for the NR or the SNC, or at least not to the extent of blocking responses to stimulation. Presuming that this is an effect on a descending influence - either interruption of excitatory drive or excitation of an inhibitory system - then it would not appear to travel via the NR or the SNC. To what extent this can account for the relative lack of effects from the habenulae has, unfortunately, to be left as an open question. We relieved the pressure subsequently by either using single electrode penetrations or increasing the inter-electrode spacing of the grid to 1.5mm. However, neither of these produced any dramatic effect on the number of effects from the habenulae. In this context, it is worth noting that our micro-electrodes are more slender than those used by most previous workers. We used commercial plastic (Parylene-C)- coated tungsten or stainless steel micro-electrodes. In the past, the most commonly used type have been concentric micro-electrodes, which are thicker (e.g. Vedel & Mouillac-Baudevin, 1969; Appelberg & Emonet-Dénand, 1965). Some used hypodermic needles as guides (e.g. Granit & Kaada, 1952; Wand & Schwarz, 1985). Since 1973, Appelberg's group have used glass coated tungsten-iridium electrodes (marginally thicker than ours) -

but in a grid of three, 1mm apart. Given all this, the observed compression effects cannot be the cause of any differences between our results and those of others. The compression may partly account for why Taylor & Donga (1989) were the first to observe effects from the habenulae, since they were the first to use single, glass coated tungsten micro-electrodes.

Simultaneous excitation of Static Bag2 and inhibition of chain fibres.

The fact that these two opposite effects were seen on intrafusal fibres simultaneously has important implications for the question of the number of types of static τ there are. Previously, different degrees of effect have been reported on the different static intrafusal fibre types (Gladden, 1981; Gladden & McWilliam, 1977; Wand & Schwarz, 1985). Here, however, we have two types of the putative static τ which are affected oppositely by central stimulation. Assuming that the descending influence is a physiologically meaningful one (even, to some extent, if it is not), then the distinction between different τ -s previously made on anatomical and experimental grounds must also be a valid one in terms of motor function.

VALIDITY AND EVALUATION OF EFFECTS SEEN.

For tenuissimus afferents, there was no pattern linking stimulation site and effects seen. It seemed more that in all the spindles in any one experiment, broadly similar effects could be seen. Different experiments tended to yield different effects. Although in some experiments, more than one effect was seen, no consistent pattern emerged in those cases either.

There is no overall picture for brain-stem recruitment and I think it would be a mistake to try to constrain the results into one. This leaves the question : why are different experiments different? My impression is that the τ in any given experiment are disposed to fire in a particular pattern or order of preference. What then is the basis of this disposition?

The seemingly most likely candidate is the one with the most obvious effect on the nervous system - namely, anaesthesia. This parameter is the one most often cited to account for the differences in effects (e.g. Appelberg & Emonet-Dénand, 1965). Since it has been shown to alter the effects from brain-stem stimulation (e.g. Shimazu, Hongo & Kubota, 1962), and is one of the few points of interference by the experimenter which cannot be made identical from experiment to experiment, it is tempting to attribute the different effects to differences in level of anaesthesia, or to the different regimes of anaesthesia used (see methods -

anaesthesia). Regarding the different types of anaesthetic, there is no evidence to suggest that this has an effect. The different effects seen in the roots experiments were spread across all the different types and mixtures of anaesthetics. In addition, in some experiments more than one regime was tried, with no change in effects obtained. In any case, the same anaesthetic was used all throughout the exposed experiments (Sagatal), and there was as much variation in these experiments as in the dorsal roots ones. Also, the level of anaesthesia was kept approximately the same in different experiments. Admittedly, it would fluctuate within the measured levels - which are imprecise, but this ought to affect all experiments similarly, and should not account for any consistent differences.

Other possible sources of variation could be the temperature of the animal, or of the spinal pool, or the acid/base balance of the animal.

If the pH were to change, then this would alter the ionic environment of the CNS, and may alter differently the excitability of different components between the stimulation point and the fusimotor neurones. However, the animals only rarely had to be artificially ventilated, and one would expect that - even though depressed by anaesthesia - left to its own devices, respiration would be able to cope with any changes in H^+ concentration.

The temperature of the animal was maintained by two radiant lamps, one of which was thermostatically controlled by the rectal temperature. Since the temperature monitor was checked

to be accurate, and since it read the temperature as $37\pm 1^{\circ}\text{C}$, which agreed with measurements by a mercury thermometer, one can only assume that the body temperature was properly maintained.

This leaves the question of the temperature of the spinal cord pool. With the benefit of hindsight, it is obvious that this is not necessarily the same as body temperature. Since the cord lies between the heat source and the temperature monitor, it is likely to be higher. The extent of this will depend on the exact positioning of the rectal thermistor, and the individual anatomy of the animals. These are both likely to vary from experiment to experiment. Also, variation in the depth of the paraffin pool will alter the amount of insulation offered by this source. Alteration in local temperature may alter the interneuronal pathways taken and correspondingly different effects will be seen. Although the cord temperature can only be very slightly different from body temperature, and shifted in the same direction in all experiments, this cannot be ruled out as one source of variation.

To my mind, the possible variation of spinal cord temperature is the parameter most likely to affect the inclination of different τ -systems (assuming such things exist) in the different experiments. It may also combine with the other two mentioned - or even other, unconsidered, variables.

It must be stressed that while the above variables may be sufficient to account for differences between individual experiments, they should not produce any consistent

difference between this and other work. This means that they cannot account for any difference between our results and those of others, only for variation from experiment to experiment.

On reflection, there are several things which could have been done to make interpretation of the results easier and more complete. If carried out successfully, these would also have increased the value of the results as a whole. Some of these were attempted without success. They were :

- to record simultaneously from II and Ia afferents. This allows separate assessment of the degree of recruitment of Sb2 and chains.

- to record the efferent activity going to the muscle. Spikes recorded in the ventral roots would not be likely to be restricted to the particular muscle of interest, but in a small muscle nerve like tenuissimus, it may be possible to monitor individual units of the small τ spikes in the muscle nerve by their height, using a window discriminator. The feasibility of this would depend on the amount of neural traffic. This would allow the effectiveness of the τ on the afferents to be assessed. It would perhaps allow one to see whether or not driving of the Ia was taking place, implying chain fibre recruitment, and also enable one to split any adaptation to stimulation seen in the afferent into central and peripheral components. Also, were it possible to monitor

individual units, it would be interesting to build spike-triggered frequencygrams, in the manner of Bessou, Laporte & Pagès (1968).

Alternatively, if τ -spike discrimination was not feasible, it would have been interesting to cut the upper branch to tenuissimus (Hunt, 1951), and used this to monitor the level of efferent activity. The lower branch could be left intact and, assuming that tenuissimus acts as one functional unit, the afferent (Ia and II) responses from it could be looked at to gauge the overall nature of the τ -motoneurons active.

-to vary the stimulation frequency and, in particular, to lower it. Ideally the stimulation would excite the τ to fire at a rate of about 30-50 imp/sec, allowing the τ to chain fibres to drive the Ia firing frequency, rather than the extremely erratic discharge seen in 'variability'. Due to 'erosion' at synapses, a stimulation rate faster than the desired τ firing frequency would be necessary, but probably the ones we used are still too fast (200Hz, 333Hz, and 500Hz). Obviously, this would be of most value if it were possible to monitor the efferent activity simultaneously.

-to carry out more experiments on FDL and Pter, particularly the former, to build up a reliable picture of the effects of stimulation on these muscles. The one experiment done on FDL is tantalisingly different, and certainly deserves to be investigated further. It would be of most value to record simultaneously from FDL and tenuissimus Ia (and II?)

afferents. If different types of effect could be seen simultaneously in the same experiment, then the difference could be relied upon as being genuine, since all other influences must be the same. This would afford extra weight to the different results gained in the experiments involving only one muscle or the other.

To assume all these could be carried out successfully may seem overambitious. However, the main obstacle to success was always the incompleteness of the denervation in the hip. This meant that there were most on most occasions contaminating afferents in the dorsal root filaments. These have to be removed by splitting, thus increasing the risks to the preparation of damage and dehydration. We have since been shown an improved technique (Emonet-Dénand, personal communication) which allows this to be done more easily and more successfully. This is simply the removal of the piriformis muscle by maceration, instead of retracting it. This would allow us to accomplish some of the above. The matter of recording efferents would, I suspect, be more difficult, and of less value. Appelberg et al. reported that, to the same end, Noth (personal communication in Appelberg et al., 1982) was then working on recording spikes in τ -cell bodies in preparations with the ventral roots in continuity. Since no published work has appeared, it can be assumed that he was not successful.

Experiments where no effects were seen.

Into the evaluation of the effects seen must be taken account the fairly high number of experiments where no effects were observed. There are several practical reasons why this may occur : the anaesthesia being too deep; missing the effective site(s); or too low a stimulus strength. Also, as seen in this project, compression caused by the microelectrodes themselves may counteract any effect from stimulation.

However, while these may account, alone or in combination, for many of the instances of no effects, I do not think that they are sufficient explanation for all of them. The connections between the higher CNS and the tenuissimus seem to be much weaker than is apparently the case for other muscles, judging by the apparent ease with which other workers found effects. The case for this is supported by the strong, easily recruited effects seen by us in FDL and Pter. The absence of effects seen in many experiments may be explained by the weak nature of the connections between tenuissimus and the upper CNS making effects much more vulnerable to the obstructing factors above. Alternatively, the range of individual anatomy in the experimental animals may vary between weak connections from the mesencephalon to the τ -motoneurone pool - in which case effects may be seen, and no connections at all.

CONCLUSION.

As will have become evident, although varied and interesting intrafusal effects can be recruited in the tenuissimus by stimulation in the various regions of the brain-stem tested, there is no strong association between the site of stimulation and the intrafusal fibres recruited. Considered in isolation, the results from tenuissimus can be interpreted in either of two ways. Either to reject outright the notion of an area which recruits dynamic τ -motoneurons, or to qualify it, with tenuissimus (and possibly other muscles) as exceptions to the general nature of its influence. The single dynamic result from FDL suggests that the latter conclusion is the more prudent one. The strange shape of tenuissimus is only a reflection of its peculiarity, and its function is a mystery. It is entirely credible that this muscle could be the one exception in the hind-limb to any given influence from higher motor centres. However, there is also the single result from PTer to consider. The fact that no dynamic effects were obtained in this muscle either, suggests that tenuissimus may not be the only exception, and that the whole concept may be flawed. Its basic weakness is that it has only ever been tested thoroughly in one muscle (FDL). Even though it may consistently recruit dynamic fusimotor fibres in FDL, it may do this not through involvement with a particular fusimotor system, but with a particular task or posture, and may have quite different effects in other muscles.

To conclude, the results from our experiments show that at least in the tenuissimus muscle dynamic fusimotor neurones cannot be consistently recruited from the area of the mesencephalon described by Appelberg (1981). The limited results from the other two muscles tested suggest that while dynamic effects in FDL can be recruited from around this area, this may not be the case in all hindlimb muscles.

RECOMMENDATIONS FOR FUTURE WORK.

Most recommendations have already been alluded to throughout the thesis, but it is worthwhile to collate them all briefly in one section.

I would recommend that any future project pursuing the same aim :

a) obtained II afferents in the dorsal root filaments and monitored activity in these simultaneously to that in the Ia afferents. This would be a straightforward and reliable means to differentiate between effects on Sb2 and effects on chain fibres.

b) used the mixture of 80% N₂O / 20% O₂ + 0-1% Halothane, supplemented by small doses of Saffan intravenously to maintain anaesthesia. It is not possible to quantify, but I gained the strong impression that this combination provided the best features of the different anaesthetic agents. Halothane alone is an unwieldy agent. Changes made took an impractically long time to develop. Also, there seemed to be elements of positive feedback (perhaps through slower respiration allowing greater accumulation of anaesthetic) which further hindered attempts at a gradual progress towards the desired level. Also, halothane alone lowers the blood pressure dramatically. Sometimes this can (and did) develop into a fatal downward spiral of lowered blood pressure leading to lowered venous return, which leads in turn to

lowered cardiac output, which further lowers blood pressure. The positive feature of halothane is that once a stable state is reached, the level of anaesthesia is more or less constant, with only small fluctuations. The N₂O admixture, though not itself an anaesthetic, lessens the amount of halothane required to achieve the same depth of anaesthesia, thus minimising the risk of halothane overdose.

Saffan is a steroidal anaesthetic which is cleared from the blood and CNS by fairly rapid catabolism in the liver, rather than the usual route of a gradual absorption into the poorly vascularised but bulky adipose tissue. This means that : a) Saffan does not eventually saturate the body, and repeated doses can be given; b) that it is short-acting. Because of these 2 properties, the level of anaesthesia can be very precisely controlled, almost from moment to moment. This is also its disadvantage, because the anaesthetic can lighten very rapidly, and hence needs to be monitored very carefully. We found that the above regime allowed the gaseous mixture to set a certain 'plateau' of anaesthesia, with small doses of Saffan used to deepen it to the desired level.

c) made some attempt to monitor the efferent output to the muscle or spindle in question. This would allow some assessment of intrafusal recruitment, depending on how precisely it could be done.

d) attempt to control, or at least monitor, the temperature of the spinal cord pool. To this end a small, thermostatically controlled heating element in the pool would provide more precise control than the overhead radiant lamps alone.

e) removed the piriformis muscle during the surgical procedures. This provides a clearer view of the lumbosacral plexus, and access to it, making denervation in this region easier and more certain.

f) varied the stimulation frequency. As well as possibly allowing any driving of Ia afferents present to emerge, the different types of τ may be differently susceptible to different rates of synaptic input.

g) in the histological procedures, tried leaving the micro-electrodes in situ not only during fixation of the tissues, but also during the subsequent dehydration, and perhaps also during embedding in wax. The latter cannot fail to leave a track, but the consequent gap in the set paraffin wax might make cutting and mounting of sections difficult.

h) investigate Flexor Digitorum Longus and Peroneus Tertius more extensively. Assuming that this proved successful, it would also be interesting to extend the investigation to other muscles.

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