Journal of Physiology (1993), **460**, *pp.* 657–673
With 6 figures
Printed in Great Britain

INCIDENCE OF NON-DRIVING EXCITATION OF Ia AFFERENTS DURING RAMP FREQUENCY STIMULATION OF STATIC γ-AXONS IN CAT HINDLIMBS

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(*Received 9 December 1991*)

SUMMARY

1. The aim of this investigation was to identify static γ-axons which do not drive any Ia afferents at any stimulus frequency in any spindle which they supply, and to determine their occurrence in various hindlimb muscles (peroneus tertius, brevis, longus and tenuissimus).

2. Ia responses to static γ stimulation were classified as 'non-driven' when the discharge did not follow the stimulation frequency, or its subharmonics, at any time during a linear increase in stimulus frequency up to 150 Hz lasting 2–3 s, and when tested at two muscle lengths – except in the tenuissimus muscle. In almost all experiments, cross-correlograms were used in addition to evaluate the percentage of these 'non-driven' responses in which a time-locking of discharge to stimulus pulses was obscured by irregularity of the Ia discharge.

3. In 104 spindles, out of 347 responses to stimulation of single static γ-axons 332 (93%) could be characterized, and of these, 57% (183) were of the non-driven type. The mean number of static γ effects characterized per spindle was 4·1 (fourteen experiments). In the large majority of spindles (79%, 82 out of 104) at least one response was of the non-driven type.

4. Of the static γ-axons studied 16% were called 'non driving' ('ndr' γ_s-axons) because they elicited non-driven effects, and since they had the same qualitative effect consistently in all spindles whose discharge was modulated by stimulating them they were called specific 'ndr' axons. If axons with non-driven effects, but acting on one spindle were included in the 'non-driving' category the proportion was 23%. Of spindles tested 63% were innervated by at least one 'ndr' axon.

5. Absence of Ia driving during ramp frequency stimulation of γ_s-axons has been equated with selective bag_2 contraction. All the non-driven responses identified in this study cannot be attributed to exclusive bag_2 involvement because the total number of 'ndr' responses was too high. In fact, in the isolated spindle preparation bag_2 and chain co-contraction were shown to elicit non-driven responses, so chain contraction is not detected reliably in all experimental conditions. Possibly chain

fibre contraction is sometimes too weak to dominate the response, or can be of a non-driving character.

6. Observation of the type of contracting fibres in the isolated spindle preparation also showed that when a γ_d (dynamic)-axon supplying a bag_1 fibre was stimulated together with a γ_s-axon supplying a bag_2 fibre in the same spindle, at constant low frequency, the enhancement of dynamic sensitivity due to the γ_d activation was not occluded by the static effect of bag_2 contraction. These co-operative effects of bag fibre contraction could survive, even when some co-contracting chains were inducing time-locking of the Ia discharge. Therefore, a role of non-driving γ_s-axons may be to give a type of static action which assists, rather than obliterates, dynamic effects.

7. These findings are discussed in relation to recent observations on the control of bag_2 fibres.

INTRODUCTION

Since 1972 it has been clear that static γ-axons may not innervate the same type of intrafusal fibre, either bag or chain, in all the spindles they supply (Barker, Emonet-Dénand, Laporte, Proske & Stacey, 1973), but this non-selectivity requires explanation for, by comparison, dynamic γ- or β-axons are almost entirely selective to bag_1 fibres (Bessou & Pagès, 1975; Barker, Emonet-Dénand, Harker, Jami & Laporte, 1976, 1977; Boyd, Gladden, McWilliam & Ward, 1977).

Support for the idea that the CNS can activate bag_2 fibres, to some extent independently of chains, first came from observations of isolated spindles during stimulation of the CNS (Gladden & McWilliam, 1977; Gladden, 1981). Recently, localized CNS stimulation was found to inhibit spontaneous activity of γ-motoneurones activating chain fibres, while at the same time exciting those supplying bag_2 fibres (Dickson & Gladden, 1990; Asgari-Khozankalaei & Gladden, 1990*b*). While these experiments imply that the CNS has independent control of these two types of intrafusal fibre, more reliance could be placed on their significance if it should be proven conclusively: firstly, that a given static γ-motoneurone does in fact almost exclusively innervate the same type of fibre, chain or bag_2, in all the spindles that it supplies, and secondly, that such selective fusimotor units occur frequently in large limb muscles with a more obvious functional role in movements of the hindlimb than tenuissimus.

For Boyd the diagnostic test of a static γ-axon innervating the bag_2 fibre alone, in a particular spindle, was that its stimulation did not cause 'driving' of the Ia afferent discharge, that is, time-locking of the afferent response to the stimulus pulse (Boyd & Ward, 1982; Boyd, 1986). By 'driving' Boyd meant that each afferent spike followed every stimulus pulse (1:1 driving), or every second or third (1:2 or 1:3 driving) (Boyd *et al.* 1977). The driving phenomenon, identified from isolated spindle experiments as a sign of chain involvement (Bessou & Pagès, 1975; Boyd *et al.* 1977), became the basis of a test developed by Boyd & Ward (1982) to discriminate readily those axons which did not drive at any frequency between 1 and 150 Hz. In 1985, both on the basis of the Ia responses to the ramp frequency stimulation test and on the type of the intrafusal fibres involved, identified by direct observation, Boyd postulated that static γ-motoneurones might fall into two groups, termed provisionally 'static bag' and 'static chain' γ-motoneurones. The muscle he utilized

was the tenuissimus, more precisely the central portion just proximal and distal to the nerve entry.

The present study was based on the premise that bag_2 contraction does not cause driving when tested with ramp frequency stimulation (Boyd, 1986). The aim was to determine whether there were any γ_s-axons which failed to drive any of the primary endings they activated in spindles of some large limb muscles (peroneal muscles) and also of tenuissimus for comparison.

In addition, complementary experiments were carried out on the lower part of the tenuissimus muscle, in the popliteal region, where living spindles were isolated to identify the types of intrafusal fibres involved in the genesis of known static fusimotor effects.

A preliminary report has been published (Dickson, Emonet-Dénand, Gladden, Petit, Rowlerson & Sutherland, 1991) on part of this work and on the relation between the ability of static γ-axons to drive Ia afferents and the type of intrafusal fibres innervated (Dickson, Emonet-Dénand, Gladden & Petit, 1991).

METHODS

All experiments were carried out on adult cats, deeply anaesthetized with sodium pentobarbitone, 40 mg/kg, intraperitoneally with intravenous supplements. In thirteen experiments the γ innervation in the whole muscle (peroneus tertius and tenuissimus), or part of the muscle innervated by a nerve branch (peroneus brevis), was studied. The aim of each of these experiments was to investigate the effects of as many γ-axons as possible on up to ten Ia afferents.

The methods used were essentially the same as in earlier experiments (Emonet-Dénand, Laporte, Matthews & Petit, 1977). The lumbar cord was exposed with a laminectomy, and one hindlimb was extensively denervated, leaving intact the innervation of one of the muscles studied: peroneus brevis, tertius, longus and tenuissimus muscles (two, six, one and five experiments respectively). The distal end of the muscle was connected to a servo-controlled electromagnetic puller.

Axons in split ventral root filaments were considered to be functionally single if an all-or-none potential was recorded from the muscle nerve at threshold stimulation of the ventral root, and if no additional potentials were observed at 10× threshold. Afferents activated by γ-axons were considered to belong to a primary sensory ending if the conduction velocity, calculated from the latency and conduction distance, was higher than 70 m/s and/or if they possessed a high phasic sensitivity during small muscle length changes. The other afferent fibres whose conduction velocity was slower than 70 m/s were considered as originating from secondary endings. Functionally single static γ-axons in ventral root filaments were identified by their conduction velocity (< 50 m/s), and when their stimulation caused no change in muscle tension.

Ramp stimulation

Static γ-axons were stimulated using a ramp change in frequency increasing linearly from 5 to 150 Hz, lasting about 2·5 s; stimulation was then maintained at 150 Hz for a further 0·5 s. In the peroneal muscles, the test was repeated at two lengths, at, or close to, the physiological maximum and minimum. In tenuissimus, the static supply was tested at a single muscle length, because the mechanical condition of this muscle is such that stretch applied to the distal end is never transmitted effectively through to the proximal end. Inevitably, distally located spindles would receive a much greater stretch than those more proximally placed. Only the distal part of the tenuissimus was dissected free in order to keep the blood supply intact. At the end of each experiment on tenuissimus the origin of all afferents recorded was established by gently prodding the muscle with a glass probe.

Cross-correlograms

To detect time-locking between stimulus pulses and afferent potentials, where this was not obvious from visual inspection, cross-correlograms were constructed by counting the number of spikes falling into bins of 1 ms for 20 ms following each stimulus pulse (see insets in Fig. 1). The

values in each bin were normalized by dividing them by the total for the twenty bins, so that if time-locking did not occur all the values approximated to 0·05. When driving was present a complex pattern emerged because in addition to a clear peak with a latency equal to the time around the γ-loop (around 14–16 ms for tenuissimus Ia afferents), spikes would also fall into shorter latency bins at those high frequencies during the ramp for which the interstimulus pulse time was less than the γ-loop time.

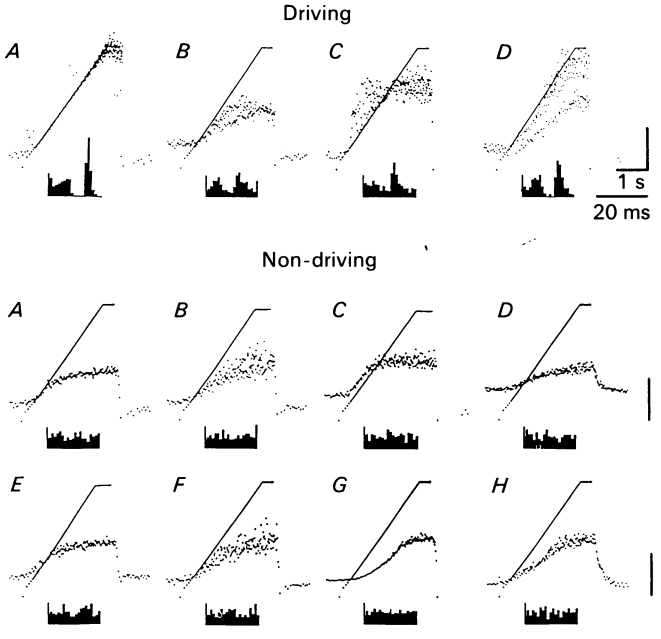

Fig. 1. Identification of non-driving and driving effects of γ_s-axons acting on Ia afferents in the tenuissimus muscle using the ramp frequency stimulation test. Upper traces: responses of four Ia afferents activated by a 'driving' type of γ_s-axons. Lower traces: eight responses to a 'non-driving' type. Calibration 50 impulses/s. Cross-correlograms: twenty bins normalized; calibration at start of each histogram: 0·1. All bin values would fluctuate around 0·05 if no time relation exists.

For further analysis the duration of the ramp stimulation was divided into three equal parts, and in each period a cross-correlogram between stimulation and the response of the primary ending was performed. The three cross-correlograms were then summed to get the cross-correlogram of the whole response. During each period the number of spikes is relatively low, so a statistical test could only be applied on the summed cross-correlogram. The χ^2 test was applied to compare the probability distribution observed in the cross-correlogram and a uniform distribution. A peak was considered as significant with a probability $P < 0{\cdot}001$. It should be noted that in our conditions, a peak observed in the cross-correlogram performed during the first period of stimulation was not detected (except in one case) in the summed cross-correlogram because the number of spikes is low during this period. As a consequence, the summed cross-correlogram was mainly influenced by high frequencies of stimulation.

Isolated spindles

In four experiments the effect of stimulating single γ-axons was directly observed in spindles isolated in the tenuissimus muscle. Single static γ-axons were first isolated in ventral root filaments, and characterized by the ramp stimulus frequency test and by tonic stimulation at 100 Hz during standard ramp-and-hold stretches. To locate spindles supplied by these axons, small stretches were applied along the muscle, until the Ia afferent discharge changed similarly in

response to both proximally and distally directed pulls. In each experiment one or two spindles were isolated in order to observe the contraction of intrafusal fibres during stimulation of static γ-axons. Unlike any spindles previously isolated from the tenuissimus muscle in Glasgow, the location of all five spindles isolated was at, or distal to, the popliteal vascular pedicle; formerly all spindles isolated were within 5 cm of the nerve entry. All the six static γ-axons studied innervated chain and bag_2 fibres or solely bag_2 fibres. Chain fibres were recognized by their smaller diameters. Bag_2 fibres were distinguished from bag_1 fibres by their greater amplitude of contraction (the bag_1 fibres being activated in these experiments by stimulating dynamic γ- and β-axons, two of each type).

RESULTS

Single functional γ-axons were isolated by ventral root splitting to characterize the static fusimotor supply of muscle spindles in the peroneus brevis, tertius, longus and the tenuissimus muscles (two, six, one and five experiments respectively). A total of 178 axons were found to excite at least one primary ending (431 effects; mean number of effects per spindle, 4·31). They were classified as dynamic or static axons, according to the criteria of Matthews (1962) and Appelberg, Bessou & Laporte (1966) on the behaviour of the Ia responses to a large amplitude ramp-and-hold stretch of the muscle during tonic γ stimulation at 100 Hz (see also Emonet-Dénand *et al.* 1977). A hundred and forty-one γ-axons were found to exert static effects.

Type of responses elicited from individual spindles during γ_s *stimulation*

The Ia afferent responses to static γ stimulation ($n = 347$) were further subdivided into 'driven' or 'non-driven' according to the character of the discharge during ramp frequency stimulation, ranging from 5 to 150 Hz, at two muscle lengths (except in the tenuissimus preparation, see Methods).

All static responses, regular or irregular, in which, on visual analysis of the records, there were no signs of time-locking to stimulus frequencies, 1:1 or subharmonically, at either muscle length were classified as 'non-driven'. Approximately half (56·5%) of the characterized effects were of this type (179 'ndr' and 138 'dr' responses). For technical reasons, twenty-five effects were not classified.

Both 'non-driven' and 'driven' responses could be elicited from a large proportion of spindles (69·5%) found to be innervated by more than one static γ-axon (Fig. 2, filled area). However, there were some spindles innervated by at least two axons (Fig. 2, categories 2–8) whose responses took the same form, either 'driving' only (ten spindles, horizontal hatched area) or 'non-driving' only (seventeen spindles, stippled area); these spindles were mainly amongst those with a low incidence of static actions. However, four spindles in which driven responses were never elicited were found to be innervated by a substantial number of γ_s-axons: five in one case, six in another, and seven in two cases (Fig. 2 upper and lower histogram, categories 5–7 stippled area).

Character of the static response elicited in a fusimotor unit

Static γ-axons were termed 'non-driving' ('ndr' γ_s) when their stimulation elicited non-driven responses only from all Ia afferents tested. The best example of such an 'ndr' γ_s-axon is illustrated in the lower part of Fig. 1. In the case of this axon analysis of all cross-correlograms of the Ia responses (see below) did not reveal any obvious time-locking between stimulus and responses.

Similarly, static 'driving' axons ('dr' γ_s) were those whose stimulation provoked exclusively driving responses from all spindles activated. A third group of axons had different effects from spindle to spindle (γ_s-axons with mixed effects, 'mx' γ_s).

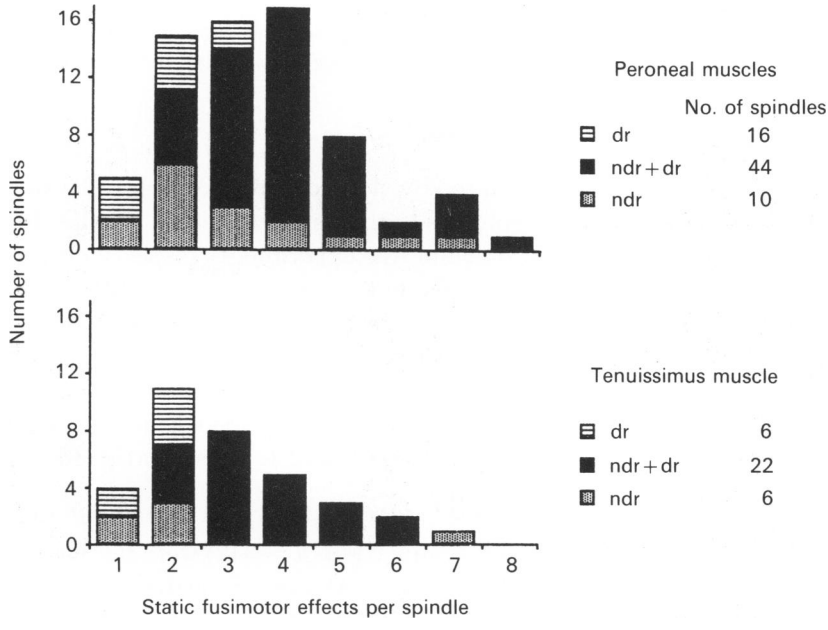

Fig. 2. The number and category of the responses elicited from peroneal (nine experiments) and tenuissimus (four experiments) spindles. Stippled areas: all responses to stimulation of individual γ_s-axons were non-driving (ndr); horizontal hatched areas: all responses driving (dr); filled areas: both driving and non-driving responses elicited in the same spindle by γ_s stimulation. Note that both types of responses, driving or non-driving, occurred in the large majority of spindles. Nevertheless, only one type of effect was obtained from some Ia afferents even though as many as seven γ_s-axons could modulate the discharge.

TABLE 1. Incidence of static and dynamic γ-axons in the peroneal and tenuissimus muscles, and of each category of static γ-axons

			γ-axons						
			γ_s ndr		γ_s dr			γ_s not	
Muscles	Ia	Mean/ spindle	ndr1	ndrx	dr1	drx	γ_s mx	char.	γ_d
P. brevis (2)	13	4·92	4	4 (9)	12	4 (10)	4 (7+4)	3 (6)	5 (12)
P. tertius (6)	51	4·43	3	11 (29)	5	7 (14)	35 (66+44)	4 (12)	27 (53)
(P. longus (1))	6	3·50	1	2 (4)	5	1 (2)	—	2 (6)	2 (3)
Tenuissimus (4)	34	3·53	—	9 (35)	5	7 (19)	12 (21+23)	1 (1)	3 (16)
Total	104	4·14	8	17 (77)	22	12 (45)	39 (94+71)	9 (25)	34 (84)

The numbers of experiments are shown in parentheses against each muscle; in all other columns, numbers not in parentheses show the numbers of axons, either Ia or γ as indicated; parentheses (where present) indicate the number of effects elicited. ndr: non-driving axons; dr: driving axons; ndr 1 and dr1: axons affecting one spindle only; ndrx and drx: axons with the same effect on more than one spindle; mx: axons with both driving and non-driving effects (relative proportions indicated within parentheses, ndr+dr); not char. indicates γ_s-axons whose effects could not be characterized for technical reasons.

Out of ninety-six γ_s-axons whose effects could be characterized twenty-nine supplied more than one spindle, and had either a non-driving or driving effect in all spindles. Of these seventeen axons were identified as 'ndr' (seventy-seven afferent responses). The number of axons of the different categories in each muscle, and in total, are indicated in Table 1 (ndr; dr; mx columns).

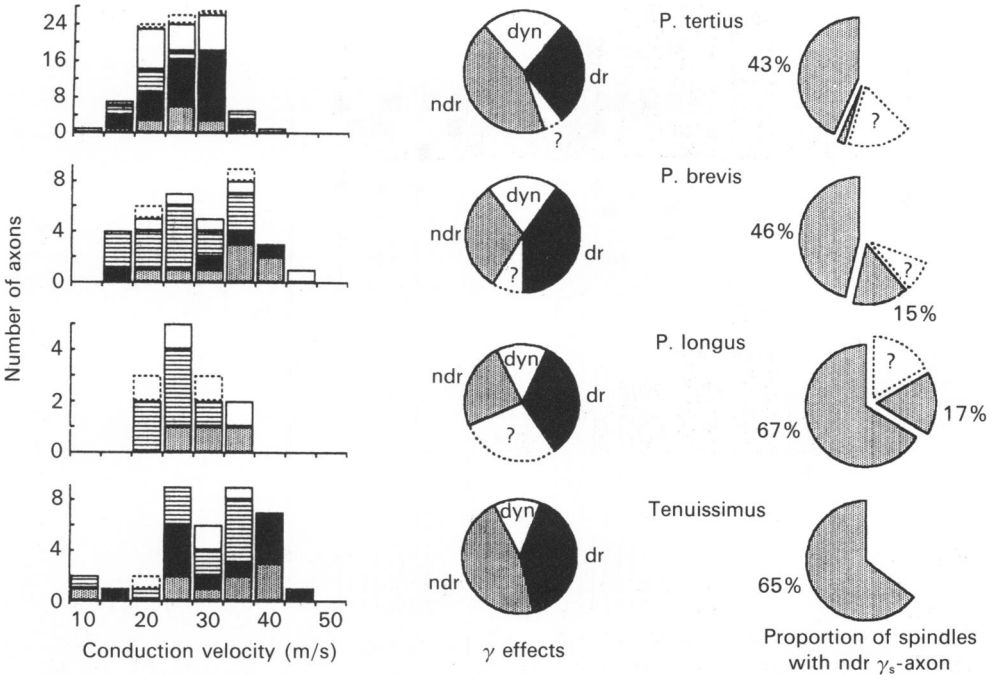

Fig. 3. Type of fusimotor effects (middle and right column) elicited by the stimulation of fusimotor axons (left histograms) in each type of muscle studied. First column: conduction velocity of static γ-axons in the peroneal and tenuissimus muscles divided according to the type of effects they elicited. Stippled: non-driving axons; filled: axons with mixed effects, driving and non-driving; horizontal hatched: driving axons; open: dynamic; dashed lines: non-characterized. For simplicity, non-driving or driving axons acting on one spindle only are included with those acting on more than one spindle; they are separated in Table 1. Middle column: the type of effects elicited from Ia afferents irrespective of the type of γ_s-axon inducing them (whether non-driving or driving only, or causing both effects). dyn, dynamic effects. Last column: the proportion of spindles innervated by γ_s-axons giving non-driving effects only ('ndr' axons; stippled). The smaller segment, where present, represents those spindles innervated by 'ndr' axons which innervated one spindle only. Open areas indicate those spindles in which 'ndr' innervation cannot be excluded due to failure in the characterization of their static supply.

Stimulation of some γ_s-axons affected only a single Ia afferent. These axons were particularly numerous in peroneus brevis and longus (see Fig. 3, last column). Probably some γ_s-axons do supply only one spindle. If stimulation of a γ_s-axon elicited a non-driven or driven response from a single Ia afferent it was allocated to the 'ndr' or 'dr' groups. However, these γ_s-axons were kept numerically separate so that the effect of their inclusion in the aggregate results can be appreciated.

If γ-axons acting on only one spindle are included, 'non-driving' γ_s-axons were identified in all muscles studied ($n = 34$: 26+8 in Table 1). They represent 25·95% of the static γ population of axons (20·24% of the overall γ_s and γ_d population). But the number of 'ndr' axons was not consistent in each muscle studied. It varied

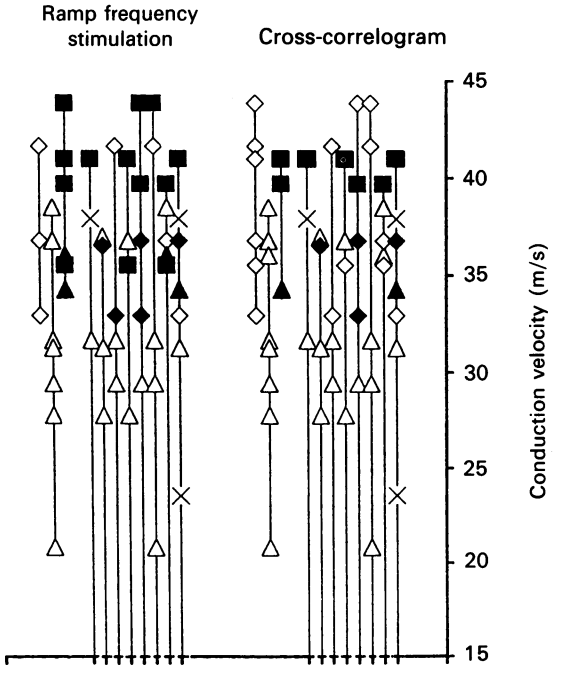

Fig. 4. Comparison of the characterization of static γ-axons in one peroneus brevis muscle by direct visual inspection of the responses to the ramp frequency stimulation test (left) and by consideration of the cross-correlogram of the response (right). Each vertical line crossing the abscissa represents a spindle, whilst the others link together all the axons belonging to the same group (diamonds: axons which had an inconsistent or mixed effect, either driving or non-driving, on all spindles innervated; open triangles: axons which elicited driving; filled symbols: axons whose stimulation exerted a 'ndr' effect on one (▲) or more (■) Ia afferents. For each spindle (vertical lines crossing the abscissa), the filled symbols represent ndr effects elicited by either 'ndr' axons (▲ and ■) or mixed axons (◆). The open symbols represent driving effects either elicited by mixed (◇) or driving (△) axons. The crosses indicate the static effects which were not characterized as driving or non-driving. Note that the proportion of 'ndr' effects as well as the number of axons identified as 'ndr' are smaller when the cross-correlogram is considered (right, cross-correlogram).

considerably between individual animals, for example in peroneus tertius, from one axon activating one spindle up to five axons which elicited fourteen non-driven effects and involved almost all of the spindles encountered.

Overall, the static 'ndr' axons were found to innervate more than 50% of the spindles studied (66 out of 104 spindles). The relative proportion of these spindles in the experimental muscles are indicated in Fig. 3, right column.

In one experiment on peroneus brevis (Fig. 4) γ_s-axons with slower conduction velocities drove when stimulated. This trend was not obvious in any other

experiment, but the mean conduction velocities were higher for 'ndr' axons than 'dr' axons in the tenuissimus muscle also (Fig. 3). Some axons with mixed effects were among the fastest conducting γ_s-axons (filled area of the histograms in Fig. 3).

Analysis of cross-correlograms of 'ndr' responses

Whether or not the 'ndr' axons, as identified by the ramp frequency stimulation test, exerted their action via an exclusive or almost exclusive bag_2 effector is not clear for the percentage of 'ndr' effects observed in this study seems too high to be attributed to selective bag_2 contraction. Even if the bag_2 fibre by itself never drives the primary ending when stimulated with a ramp frequency while chain fibres do, co-contracting chain fibres might induce time-locking of the Ia discharge to the stimulus. Such time-locking, whether restricted to a limited range of frequencies, or hidden within an extremely erratic discharge, can be detected by plotting a cross-correlogram from the Ia response during a ramp frequency stimulation test (Fig. 1).

Analysis of the cross-correlogram of 172 'ndr' responses revealed a definite or possible time-locking on forty-five occasions (26%). In one third of these forty-five a peak appeared in the cross-correlogram at one length only, either at short or at long length. The proportion of such instances was highest in peroneus brevis compared with peroneus tertius. The effect of using the ramp frequency stimulation test alone is illustrated in Fig. 4 for one peroneus brevis experiment. However, note that although analysis of the cross-correlogram can be used in this way to detect possible chain participation in the genesis of the static response it cannot exclude bag_2 contraction. It also cannot be excluded that driving might have occurred at muscle lengths other than those which were tested (see Methods).

Another test, recently developed by M. Dickson & J. Petit (unpublished observations), may in fact differentiate between instances when bag_2 fibres contract alone from when they contract together with chain fibres. Random stimulation helps to differentiate slow and fast components in the Ia response which might reasonably be attributed to the co-contraction of slow and fast intrafusal elements, probably bag_2 and chain fibres respectively. In tenuissimus, the comparison of the γ_s–Ia relation during a ramp frequency stimulation and random stimulation did not always match; perhaps fast intrafusal contractions and driving of the Ia discharge are not always linked.

The type of intrafusal fibres involved in the non-driving response

Chain fibre contraction was not always associated with overt driving if there was co-contraction of bag_2 fibres. Non-selective γ_s-axons gave responses with no driving, an irregular discharge, or obvious driving. In Fig. 5*A*, upper trace, the γ_s-axon stimulated supplied the bag_2 fibre at both spindle poles, and several chain fibres at one pole. There was no driving at constant frequency, or during ramp frequency stimulation (not shown).

In Fig. 5*A*, middle trace, the γ_s-axon innervated the bag_2 fibre and a single chain fibre, both at one pole only. The discharge was highly irregular during stimulation at 100 Hz. The lowest trace in Fig. 5*A* shows that several chain fibres, contracting with the bag_2 fibre at one pole, can dominate the response, to give driving.

Regularity of discharge is thought to be a sign of bag_2 fibre contraction (see for

example Emonet-Dénand *et al.* 1977). This is illustrated in Fig. 5*B* where there are two examples of the bag_2 fibre contracting at one pole only. However, note that contribution of chain fibres to the afferent response does not necessarily result in a greater irregularity than can be seen when the bag_2 contracts alone (compare the top

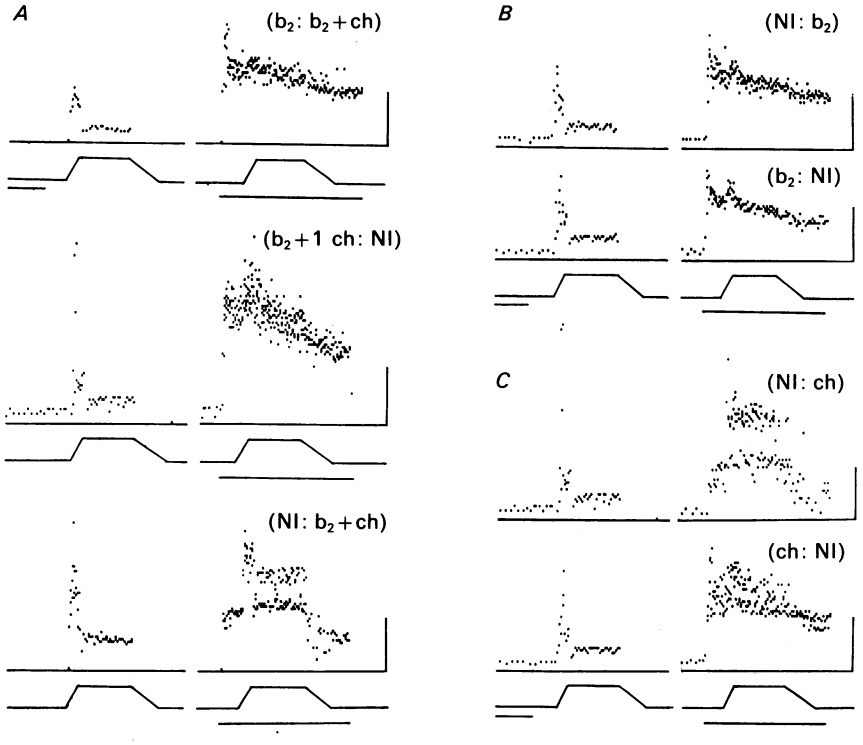

Fig. 5. Effects of γ_s-axons non-selectively innervating both bag_2 and chain fibres (*A*), and selectively innervating either bag_2 fibres (*B*), or chain fibres (*C*), in the tenuissimus muscle. Left: passive Ia responses to a standard ramp-and-hold stretch (time bar, 1 s); right: with tonic stimulation of γ_s-axons at 100 Hz for the duration of the continuous line below the ramp (frequency calibration 50 impulses/s). Type of intrafusal fibres innervated shown in parentheses, the innervation of each spindle pole being separated by colon. b_2: bag_2; ch: chain fibres; NI: pole not innervated.

trace in Fig. 5*A* with the top trace in 5*B*). Nevertheless, the most regular response was obtained when the bag_2 fibre contracted alone (Fig. 5*B*, lower trace).

The traces in Fig. 5*C* are examples of effects caused by several chain fibres contracting at one pole. Chain fibres contracting by themselves can induce irregularity (see the initial part of Fig. 5*C*, lower trace), as well as obvious driving throughout the period of stimulation (Fig. 5*C*, upper trace).

The mean frequencies achieved during bag_2–chain co-contraction did not bear any simple relation to the number of fibres contracting, nor to whether one or both poles contracted. Thus, contraction of both poles of a bag_2 fibre, together with several chains at the pole where the bag_2 contraction was stronger, (Fig. 5*A*, top trace), resulted in a lower mean frequency throughout the stimulation than co-contraction

of a single pole of the bag_2 and a chain fibre (Fig. 5*A*, middle trace). All these contracting fibres were observed in two spindles only a few centimetres apart in the muscle, so that the mechanical conditions were likely to be the same for both during recording.

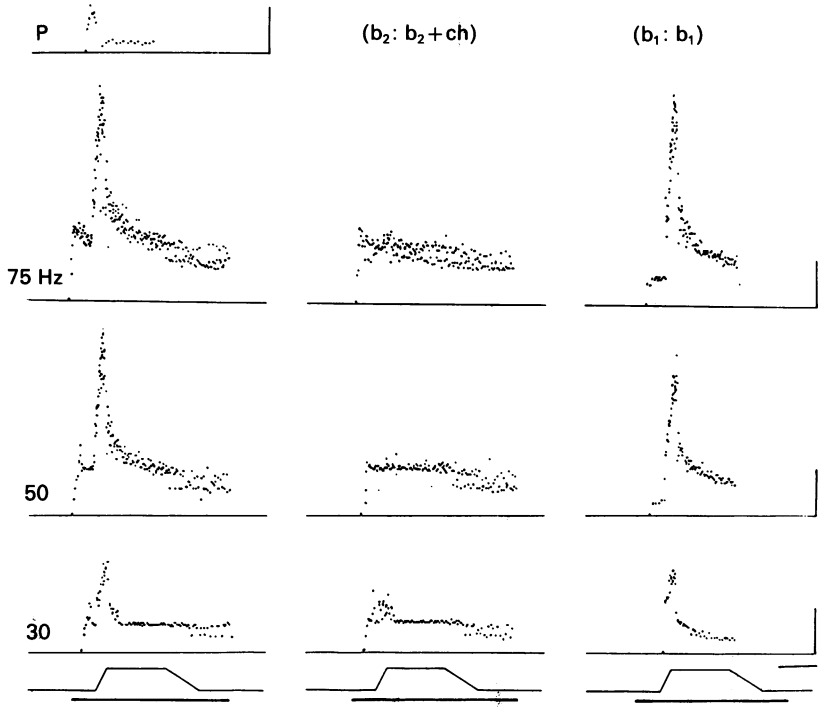

Fig. 6. Static γ innervation which allowed enhanced dynamic effects to be expressed. Left: $\gamma_s+\gamma_d$; centre: γ_s alone; right: γ_d alone. Tonic stimulation for the duration of the continuous line below the ramp at 75 Hz (top), 50 Hz (middle) and 30 Hz (right). Inset: passive Ia response (P). Types of intrafusal fibres observed to be innervated shown above. b_1: bag_1; other abbreviations and calibrations as in Fig. 5.

Possible role of 'ndr' static excitation of spindles

If the 'ndr' axons always excite the bag_2 fibres, whether alone or with chains, in each spindle innervated, a functional role for 'ndr' excitation may be associated with properties specific to bag_2 fibres.

Since bag_1 and bag_2 fibres are known to be co-excited during central or reflex activation of isolated spindles (Gladden & McWilliam, 1977; Gladden, 1981; Asgari-Khozankalaei & Gladden, 1990*a*; Dickson & Gladden, 1990) we tested the effect of bag_2 contraction on the enhanced dynamic sensitivity of spindles induced by γ_d or β_d excitation. In each case the identity of the contracting fibres was checked by isolating the spindles.

When bag_1 and bag_2 fibres contracted together, during simultaneous stimulation of dynamic (γ- or β-) and static γ-axons, the enhanced response to stretch (indicated by the increase in dynamic index) was not significantly reduced (see Fig. 6). This occurred whether the bag_2 fibre contracted alone or together with chain fibres, when

some driving was present (as in Fig. 6), and even when the bag_1 and bag_2 contraction occurred in opposite poles.

In soleus muscles under specific conditions – low frequency of stimulation and short muscle lengths – it is known that static excitation of spindles may elicit dynamic 'paradoxical' effects and 'after-effects', revealed by small amplitude stretches superimposed on large movements (Baumann, Emonet-Dénand & Hulliger, 1982, 1983; Hulliger, Emonet-Dénand & Baumann, 1985). These effects are particularly powerful when tonic static excitation causes regular and weak Ia excitation, a sign of bag_2 involvement (see previous section on the isolated spindle).

Similar observations were obtained with 'ndr' γ_s-axons when tested in preliminary experiments. 'Ndr' axons were observed to elicit after-effects and paradoxical effects in the peroneus brevis muscle.

DISCUSSION

The distribution of non-driving γ_s-axons

Frequency ramp stimulation of certain static γ-axons evoked the same non-driving effect in all the spindles which the axon supplied. Although in the present work these 'specific non-driving γ_s-axons' were numerically few, only seventeen of the ninety-eight characterized, they excited over 50% of the Ia afferents tested. When considering signs of very weak time-locking between the input and output frequencies in the cross-correlograms, the number of γ_s-axons which could be regarded as clearly specifically non-driving was reduced, but a significant proportion of them still remained (44%).

In our experimental conditions the proportion of specific non-driving axons varied between muscles (see the last column in Fig. 3). This may be analogous to the well-known variation in the relative proportion of static to dynamic γ-axons between muscles. There was also some indication that categories of γ_s-axons vary in a particular muscle between individuals, and even between different parts of the same muscle.

Another striking illustration of qualitative differences in γ_s supply was the finding that the majority of γ_s-axons found in peroneus longus, as well as in one experiment on peroneus brevis, supplied only one spindle, that is, the fusimotor units were small in these muscles. Brown & Butler (1975) also found that almost all γ_s-axons in peroneus longus supplied single spindles. The small size of fusimotor units may be an indicator of the involvement of a muscle in finely modulated motor control, although relative spindle abundance is more commonly considered with this (see Banks & Stacey, 1988).

In two experiments on peroneus tertius no specific non-driving axons were found, although there were axons which elicited non-driving effects but innervated only one spindle. Also, since not all axons known to be γ_s could be tested it cannot be excluded that some of these may have been specific non-driving axons.

The present observations on non-driving axons fit with those of Emonet-Dénand *et al.* (1977) on static γ-axons in peroneus brevis; these authors also found static γ-axons which did not drive Ia afferent responses in any of the spindles they were shown to innervate (10/25 γ_s-axons in Fig. 14 of their paper). These ten γ_s-axons excited two to four primary sensory endings and in each case at least one response

was regular. Emonet-Dénand *et al.* (1977) suggested that regularity of response could indicate static bag contraction. We confirm this from direct observation of isolated spindles, but further, we found that bag_2 fibres contracting alone could give a moderately irregular Ia response.

Static effects and change in muscle length

When the ramp frequency stimulation test was applied at two muscle lengths driving sometimes occurred at short but not long muscle length after stimulating γ-axons, and vice versa for others. One possible reason for driving to appear at long, but not at short, length is that the chain fibres were slack at short length, so that their contraction might not be transmitted effectively to the primary sensory ending. It is well known that chain fibres are the first fibre type to fall slack when spindles are shortened. Driving at short length only is less easily explained; possibly the contracting chain fibres were unloaded by a more powerful bag_2 contraction that was more effective when the intrafusal bundle became tauter at the longer length. Interestingly, in the large limb muscles, when overt driving was not present in the response to the test a peak sometimes occurred in the cross-correlogram (indicating time-locking) at one length and not the other, either short or long. This could be taken to imply that even changing muscle length may not provoke overt driving (but see below).

Changes in muscle length also influence non-driving effects. In preliminary experiments on peroneus brevis non-driving axons were found to give the same type of 'dynamic paradoxical effects' described for soleus γ_s-axons by Hulliger *et al.* (1985). These effects are seen exclusively at short and intermediate muscle lengths.

Chain fibre participation in non-driving effects

According to Boyd (Boyd, 1986; see also Gladden, 1991) the type of intrafusal fibres contracting in response to γ_s stimulation could be predicted by the pattern of the Ia response to the ramp frequency test. In fact, Boyd must have encountered at least one instance of driving caused by bag_2 contraction since in 1981 he wrote that bag_2 fibres occasionally caused driving (Boyd, 1981), although later (Boyd, 1986) this was not mentioned. It was anticipated, therefore, that the test would underestimate bag_2 innervation. However, the reverse was encountered; some Ia afferents were not driven by stimulating any γ_s-axon, even though the number of γ_s-axons modulating the discharge was as high as seven in one case; so far as the authors are aware no spindle has even been encountered in the cat which had seven axons supplying bag_2 fibres and none innervating chain fibres.

It appears that the ramp frequency stimulation test is not reliable in detecting chain contraction under all experimental conditions, and we were able to obtain direct confirmation of this in one instance. It may be reasonable to regard the ramp stimulation test, and analysis of the cross-correlogram of the response to the test as filters. This is illustrated in Fig. 4 which compares the classification of sixteen γ_s-axons analysed in both ways in the same experiment.

There are two possible sources for time-locking of the Ia discharge with stimulus pulses without overt driving during ramp stimulation, the bag_2 fibres themselves, and 'weak' chain contractions. Possibly the bag_2 fibres that can drive are those

which support propagated action potentials, rather than junctional potentials (Barker, Bessou, Jankowska, Pagès & Stacey, 1978; Gladden, 1981). However, so far as the authors are aware, driving caused by bag_2 fibres is confined to low frequencies (< 50 Hz), and the statistical test for significant time-locking in the cross-correlograms was less sensitive at these frequencies (see Methods). Chain contraction, which is stronger at high frequencies, was more likely to be detected.

Weak fusimotor effects involving chain fibres are not necessarily related to the numbers of contracting fibres, as is illustrated in Fig. 5*A*. On the other hand, a single chain fibre can evoke driving with a ramp frequency test. This was confirmed both by observation of the contracting fibre, and by histological reconstruction of the motor supply (I. A. Boyd, M. H. Gladden & F. I. Sutherland, unpublished observations). There could be a gradation in the strength of contraction of individual chain fibres, or possibly chain fibres may be capable of distinct forms of contraction, related perhaps to the type of static γ-axon innervating them. Barker *et al.* (1978) recorded action potentials from some chain fibres and junctional potentials from others. Chain fibre mechanical properties are not homogeneous either, since some do not twitch when axons supplying them are stimulated at 1 Hz (Boyd, 1976). In addition, Rowlerson (in Dickson *et al.* 1991) has found that myosin type can vary along the length of chain fibres in some spindles. Weak chain contraction may have been responsible for Banks' observation (1991), that the biasing effect of some static γ-axons – that is an increase in the rate of discharge of primary endings without driving – can be produced either by the bag_2 acting alone or in combination with chain fibres.

If the cross-correlogram does detect chain participation, however weak, it is not surprising few 'non-driving' axons survive so rigourous a test; even γ_d-axons sometimes supply single chain fibres in addition to bag_1 fibres. Interestingly, these chain fibres do not cause overt driving, as it is generally accepted that driving is not associated with dynamic effects (Emonet-Dénand *et al.* 1977).

Possible function of non-driving γ_s-axons

Although a group of 'specific non-driving' γ_s-axons has been identified, it remains to be seen whether the CNS controls them as a distinct entity. Separate recruitment would presumably give the CNS access to static effects dominated by the properties of bag_2 fibres and, as Dickson *et al.* (1991) pointed out, these effects ought to be principally channelled along group Ia rather than spindle group II axons because secondaries have much more extensive terminations on chain than on bag_2 fibres.

It would be appropriate if bag_2 contraction did not obliterate dynamic action, since central stimulation frequently co-excites bag_2 with bag_1 fibres (at least under experimental conditions where it is possible to verify this). Strong static effects can be occlusive when combined with dynamic effects (Emonet-Dénand *et al.* 1977). Figure 6 demonstrates that a dynamic effect can persist during bag_2 fibre contraction. This occurred when bag_2 fibres alone were contracting with the bag_1 fibres, but chain fibre contraction in addition did not necessarily obliterate the dynamic effect. Thus, non-driving axons could be involved in combined static/dynamic γ activation in circumstances when assistance rather than occlusion is required. Figure 6 illustrates, in addition, that low grade static Ia excitation combined with dynamic γ

excitation can polarize the Ia response, making it unidirectional; lengthening is signalled, not shortening.

Boyd (1985) suggested that the phenomenon of driving gives the CNS the possibility of calibrating the muscle length signal it receives from spindle secondaries by feeding back through the Ia afferents the 'aggregate static fusimotor outflow to the secondaries'. However, driving is unlikely to provide the CNS with a simple feedback because the conditions governing its expression are so complex. Activation of many γ_s-motoneurones would give mixed effects, driving in some spindles and not in others (Table 1). Driving may be length dependent, and, in addition, extrafusal twitches can 'drive' Ia afferent discharges.

Driving excitation of spindles abolishes the Ia muscle length signal whether the axons whose stimulation evokes the driving innervate chain fibres alone, or bag_2 and chain fibres together. How Ia endings behave during muscle length changes when most of the chain fibres are co-activated with the bag_1 fibre remains to be determined (cf. Fig. 6).

Stimulation in the area of the red nucleus in lightly anaesthetized cats can simultaneously recruit γ_s-mc neurones which innervate bag_2 fibres and inhibit γ_s-motoneurones innervating chain fibres in tenuissimus spindles (Dickson & Gladden, 1990, 1991). However, only a proportion of the total γ_s-motoneurone population could be influenced in this manner. In those experiments the γ_s-motoneurones were identified as such by the type of intrafusal fibre which they were observed to innervate in dissected exteriorized spindles (that is, bag_2 and/or chain fibres, rather than bag_1 fibres which are innervated by γ_d- or β_d-motoneurones). Since the γ_s-axons in the present study were divided into categories by their effect on the Ia response, not by the fibre type innervated, it is not possible to relate the categories of γ_s-axons in the two sets of experiments. If such a correlation were to be attempted it would be desirable to use a muscle with a more clearly understood function than the tenuissimus.

The authors are very grateful to Professors Y. Laporte, P. B. C. Matthews and C. C. Hunt for their helpful comments on a draft of the manuscript, to Mrs D. Lan-Couton and Miss J. Wilson for their technical support, and to Dr J. Celichowski for his assistance. This work was supported by the MRC, the Wellcome Trust and by travel grants from the Royal Society/CNRS European Science Exchange Program to F. Emonet-Dénand, and from the Rushton Fund to M. Dickson.

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