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THE EFFECT OF SUCCINYLCHOLINE ON CAT GASTROCNEMIUS MUSCLE SPINDLE AFFERENTS OF DIFFERENT TYPES

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SUMMARY

1. A population of 269 gastrocnemius muscle spindle afferents have been studied in anaesthetized cats for the effects of succinylcholine (SCh) on their response to ramp and hold stretches repeated every 6 s. The effectiveness and reliability of the SCh test was improved by prior stimulation of the muscle at 10 Hz for 30 s to increase the blood flow.

2. Responses have been assessed from averaged cycle histograms before and after a single I.V. dose of SCh of 200 μg kg^{-1}. As for previous studies of jaw muscle spindles the basic measurements were initial frequency (IF), peak frequency (PF) and static index (SI), the frequency 0·5 s after the end of the ramp of stretch. Dynamic difference (DD = PF − IF), dynamic index (DI = PF − SI) and static difference (SD = SI − IF) were derived from these and increases caused by SCh indicated by the prefix Δ.

3. ΔDD and ΔIF were each distributed bimodally and since they were uncorrelated formed the basis for a four-way classification. Since ΔDD can be attributed to activation of bag$_1$ (b_1) intrafusal fibres and ΔIF to bag$_2$ (b_2) fibres, while all afferents receive input from chain (c) fibres it is proposed as with the jaw spindles that the classes correspond to predominant influence from b_1 c, b_1 b_2 c, b_2 c and c intrafusal fibres.

4. The proportion of units in the different groups were similar to those in the jaw muscles except for there being very few b_1 c type in gastrocnemius.

5. Conduction velocity was bimodally distributed with the best dividing line at 63·2 m s^{-1}. The b_1 b_2 c units were all, save one, in the fast group, while the b_2 c units were equally divided between fast and slow.

6. Mean control values for DD did not differ between the b_1 b_2 c and the b_2 c groups, which is taken to indicate that the b_1 fibre does not contribute significantly to the dynamic stretch response of spindles with no intrafusal contraction.

7. The results emphasize the importance of recognizing that some apparently primary afferents lack b_1 fibre influence, while many secondaries have marked b_2 fibre influence.

8. The importance of the SCh classification is discussed in relation to the identification of fusimotor effects on spindle discharge and in relation to studies of central connectivity.

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INTRODUCTION

The accepted practice of classifying muscle spindle afferents as belonging to primary or secondary endings according to conduction velocity arose principally from studies of cat hindlimb muscles. Problems with its application to other situations such as the jaw and neck muscles have led to the use of succinylcholine (SCh) to activate the intrafusal fibres. Originally the enhancement of dynamic sensitivity of primary afferents through contraction of bag_1 (b_1) fibres was the feature of chief interest (Rack & Westbury, 1966; Cody, Lee & Taylor, 1972) but later it was realized that the effect of contraction of bag_2 (b_2) fibres could also be estimated (Dutia, 1980; Price & Dutia, 1987; Price & Dutia, 1989). On this basis a detailed re-examination of jaw muscle spindle afferents has been carried out (Taylor & Durbaba, 1990) and reported in full in the preceding paper (Taylor, Durbaba & Rodgers, 1992). It was concluded that these spindle afferents could be classified into four subgroups according to the separate effects of SCh in increasing the dynamic response and the bias during repeated ramp and hold muscle stretches. Dynamic response was best measured by the dynamic difference (DD) which is the increment in firing frequency during the stretch, while bias was measured by the initial frequency (IF) just before stretch commenced. The increments in these measures caused by SCh were designated ΔDD and ΔIF. The four subgroups were thought to correspond to predominant influences from four of the possible combinations of b_1, b_2 and c intrafusal fibres, namely b_1c, b_1b_2c, b_2c and c.

This method of classification has potential for giving functional insights independent of those concerned with the presumed morphology of the endings. Its wider application, however, requires its relation to other factors to be established, notably sensory ending morphology, axon conduction velocity and patterns of central projection. The present study applies the methods developed for the jaw muscles to the gastrocnemius muscles, which have previously been very widely studied, and so permits direct comparison with classification based on conduction velocity.

METHODS

Twenty-three adult cats (six male, seventeen female) in the weight range 2–3·8 kg were prepared under halothane–air anaesthesia. Cannulae were placed in the trachea, two forelimb veins and the right femoral artery. The left hindlimb was extensively denervated save for the medial and lateral gastrocnemius (MG and LG) muscle nerves. Soleus nerve was usually also cut. Dorsal nerve roots from L5 to S1 were exposed by a laminectomy and the animal secured in a spinal frame. A paraffin-filled leg pool was formed and MG and LG nerves placed on separate pairs of compliant silver hooks for recording or stimulating in continuity. The combined gastrocnemius tendon was separated and attached to an electromagnetic servo puller. On mounting the animal in the frame, anaesthesia was changed to a continuous I.V. infusion of Saffan (1 mg kg^{-1} ml^{-1}) made up in 5% glucose, given at a rate of 0·3 ml min^{-1}. Rectal temperature was monitored and controlled at 37–38 °C by a heating blanket, while the leg pool was kept at 35–38 °C by a second feedback-controlled heating pad and radiant heat. The bladder was drained through a urethral catheter or by suprapubic puncture. On some occasions I.V. Dextran was administered to keep mean femoral arterial blood pressure above 100 mmHg, but usually the continuous I.V. infusion of anaesthetic in 5% glucose was sufficient to maintain blood pressure and urine flow. A respiratory pump was connected at the time when SCh was administered and sometimes for longer periods. Blood gases and cations were monitored from time to time in arterial samples. Antibiotic (flucloxicillin, Beecham, 30 mg kg^{-1}) was administered I.M.

Dorsal rootlets were cut close to the cord and searched for muscle afferents while stretching the muscle sinusoidally ± 1 mm amplitude at 1 Hz. Stretch receptors were isolated as single units and their muscle of origin and conduction velocity (CV) determined by backward spike-triggered averaging from MG and LG nerves. They were characterized as spindles or tendon organs by twitch of the appropriate muscle. A specially constructed multi-electrode and six-channel amplifier and discriminator permitted up to six single units to be recorded simultaneously. This allowed the number of doses of SCh given to be reduced and so to speed up the collection of a large body of data.

Early in the study it was suspected that delivery of SCh to the muscle after I.V. injection might be slow and variable because of the low muscle blood flow in the preparation. A procedure was therefore adopted of giving a train of stimuli to the muscle nerve (2 × threshold) at 10 Hz for 30 s prior to each recording. This was shown by use of a laser Doppler meter to enhance blood flow 7-fold falling to 5-fold 1 min later.

Muscle stretches had the same time course as for previous studies on jaw muscles (rising phase 1 s, plateau 2·5 s, falling phase 1 s, repeated every 6 s) but with 5 mm amplitude. The amplitude of stretch was intended to be comparable with that used in the previous paper for jaw muscles. Some measurements available for the latter (Taylor, 1981) indicate that the stretch used for jaw muscles (8·5 deg opening) would correspond to length changes of 8–14% of the resting length of anterior temporalis or deep masseter, which contain most of the spindles. For gastrocnemius, 5 mm stretch represents approximately 5–10% of resting muscle length according to whether muscle plus tendon length or purely muscle length are taken (see Appenteng, Prochazka, Proske & Wand, 1982). Resting muscle length was adjusted to just remove slack at the minimum length.

Data were recorded with a CED 1401 interface (Cambridge Electronic Design Ltd, UK) and TI'KO 386 computer running the Spike 2 package. This allowed continuous display of the instantaneous frequency responses of up to six units together with muscle stretch and arterial blood pressure. Each recording consisted typically of a control period of ten cycles. This was followed by five further control cycles after muscle nerve stimulation. The dose of SCh of 200 μg kg^{-1} in 1 ml saline was then given I.V. during one cycle and recording continued for a further 4·5 min. Each data file generally occupied 10^5 bytes stored on hard disk and could be recalled for further processing as follows. Cycle histograms with 50 ms bin widths were constructed for the control period and for the fifth to ninth cycles after SCh administration finished. Initial frequency (IF) was the mean frequency in the 0·5 s preceding stretch. Peak frequency (PF) was the maximum frequency at the end of the dynamic stretch, and static index (SI) the frequency 0·5 s later. Derived from these were dynamic difference (DD) = PF − IF, dynamic index (DI) = PF − SI and static difference (SD) = SI − IF. The time course of responses was plotted by estimating the above values for each cycle. In some cases the single I.V. SCh injection was replaced by an I.V. infusion at the rate of 100 μg kg^{-1} min^{-1} and maintained for 5 min.

RESULTS

Effects of muscle stimulation and time course of SCh effect

Responses were analysed for 269 muscle spindle afferents (MG = 205, LG = 64) recorded in twenty-three animals. Of those designated as LG, ten were gathered from experiments in which soleus nerve was left intact so that some units up to this maximum number could have originated in soleus muscle. As stated above in the Methods, a regime of stimulation was introduced in order to increase muscle blood flow. The effects of this were examined for forty-five units in seventeen of the animals by comparing the time course of the effects of SCh on the response variables before and after the 30 s period of stimulation at 10 Hz. The mean pre- and post-stimulation time course for IF, PF and SI are shown in Fig. 1*A* and *B* respectively and the differences, due to stimulation, in *C*. Control values are not significantly affected, but the SCh effects on all these measures are greatly enhanced. When the differences due to stimulation are normalized with respect to maximum values (Fig. 1*D*) it is evident that the time course is very similar for the three measures. Changes in blood flow observed by laser Doppler in one experiment are superimposed on this plot and add

some support to the idea that it is the increase in blood flow caused by exercise which is responsible for this enhanced SCh effect. It seems likely that muscle blood flow was low and that its increase with stimulation permitted a much more effective delivery of SCh to the muscle spindles. As a consequence of these observations all data on SCh

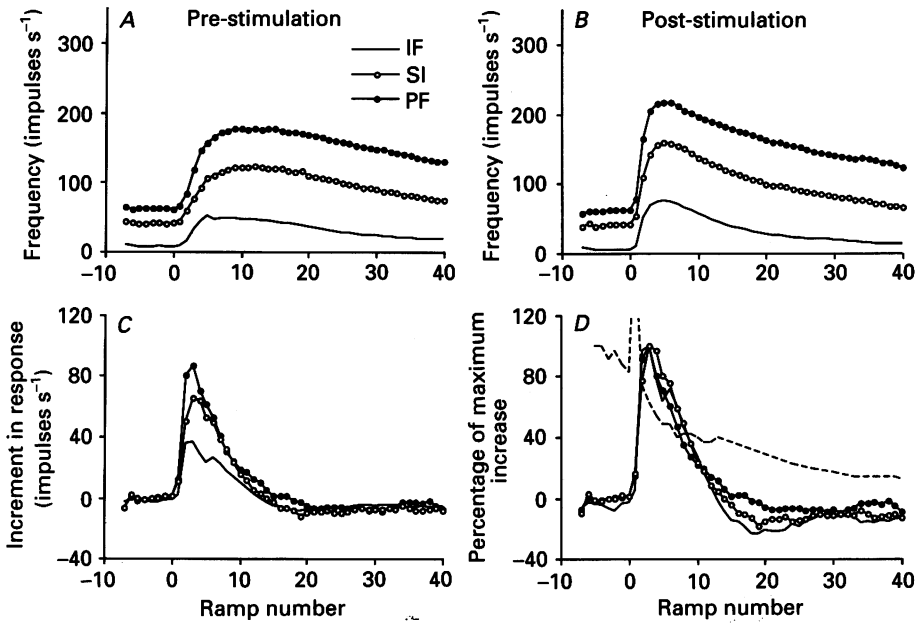

Fig. 1. Mean time course of effect of SCh upon the response to ramps of forty-five afferent units. Time is represented in ramps, repeated every 6 s, with SCh administered at ramp 0. In *A* the response to SCh is seen in the absence of previous muscle stimulation. In *B* the muscles have been stimulated for 30 s immediately prior to recording. In *C* the time course of the increment in response produced as a result of stimulation is displayed. In *D* the increment in muscle blood flow and response due to stimulation has been normalized (dashed line). IF, PF and SI were normalized with respect to their maximum increment, whilst blood flow was normalized with respect to the flow increment immediately after stimulation. Resting blood flow is at 0%.

effects reported below are taken from measurements made after the period of stimulation.

The time course of the response to SCh was more rapid for the hindlimb muscle than previously observed for the jaw muscles (Durbaba, Rodgers & Taylor, 1991), which suggests that the increase in blood flow caused by stimulation does significantly improve the delivery of the drug to the muscle spindles. The effects of SCh were therefore measured from the cycle histogram derived from cycles 5 to 9 after the I.V. injection rather than from cycles 10 to 14 as in the previous report for jaw muscles (Taylor *et al.* 1992).

Population properties

Figure 2 presents histograms of the distributions of the basic measures IF, PF and SI and the derived measures DD, DI and SD. The controls show no evidence of separation into subpopulations. Many units have very little or no resting discharge

(IF). The other distributions are roughly symmetrical except for DI which is skewed to the right. Figure 2 also shows the distributions in the period 30–60 s after SCh injection. The distributions are generally greatly broadened to the right and a suggestion of bimodality is seen in all except SI. For IF it is particularly clear that

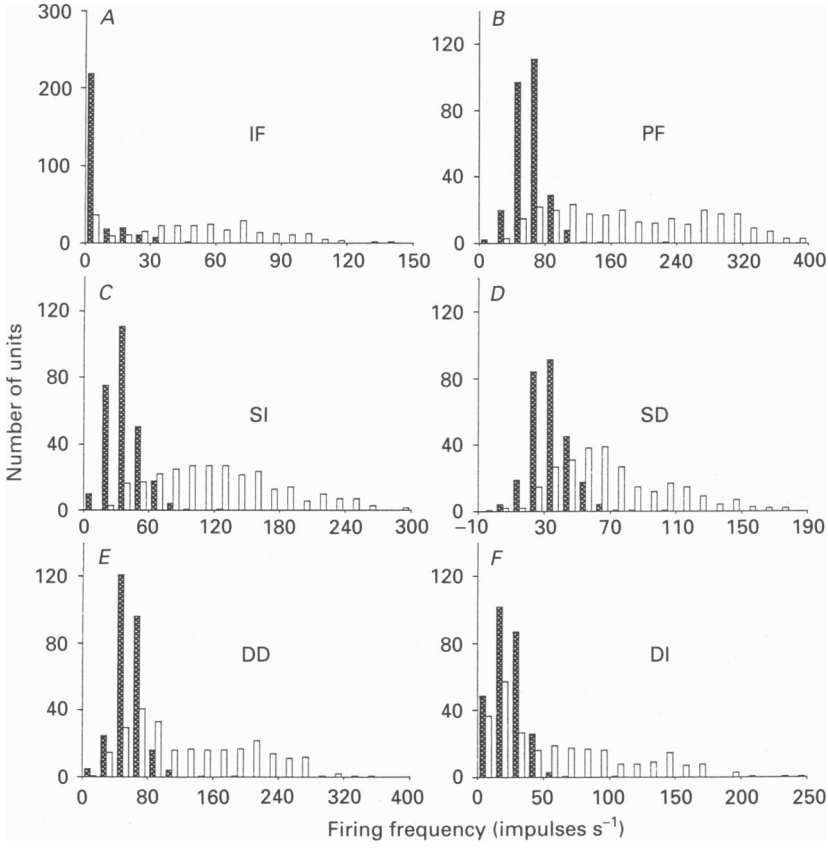

Fig. 2. Frequency histograms of the measures of spindle afferent responses to ramp stretches for 269 units. *A*, *B* and *C* indicate the basic measures IF, PF and SI respectively, while *D*, *E* and *F* show the derived measures SD, DD and DI respectively. Filled columns are control values and open columns 30 s after 200 μg kg^{-1} SCh.

one group of units shows virtually no change, while the rest show a wide range of increases.

The changes are best seen in the distributions of increments caused by SCh in Fig. 3 and symbolized by the prefix Δ. A tendency to bimodality of distribution is again obvious in all save ΔSI and ΔSD. As detailed in the previous paper (Taylor *et al.* 1992) it is to be expected that SCh-induced contraction of b_1 fibres will enhance dynamic stretch sensitivity which is best represented by ΔDD. The effect of b_2 fibre contraction will be most marked on bias, which is measured by ΔIF (see Boyd, 1985). It is noteworthy that it is the distributions of these two measures of the effect of SCh which become most markedly bimodal (ΔPF being the sum of ΔIF and ΔDD is also bimodal). This appearance is much clearer in the distribution of the logarithmically

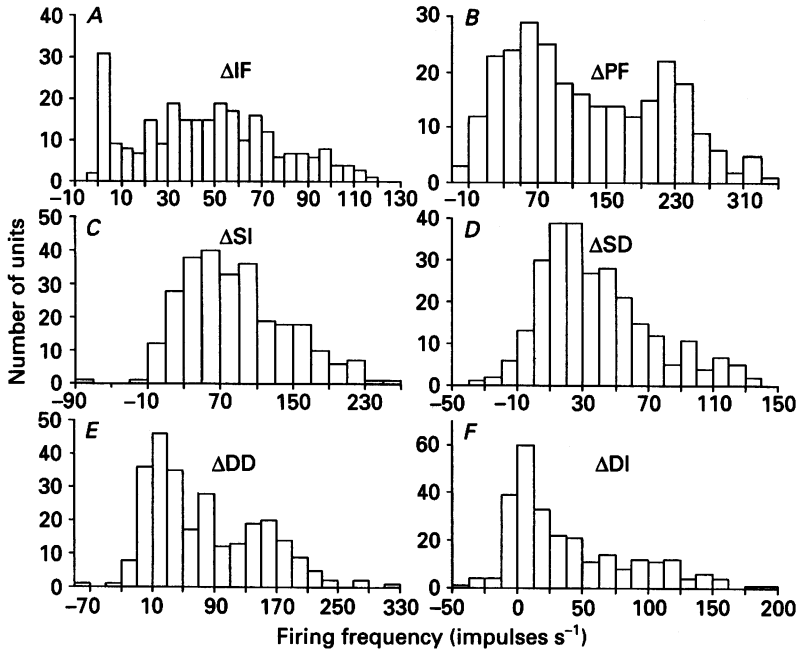

Fig. 3. Distributions of the increments in the measures of the ramp stretch responses caused by SCh.

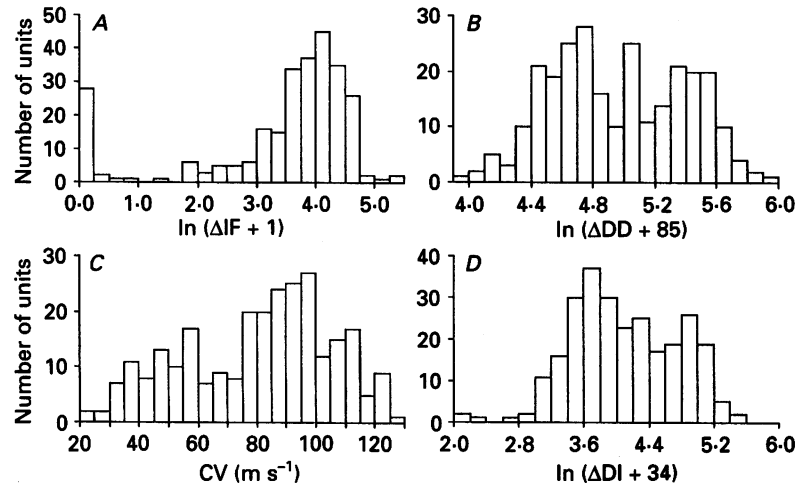

Fig. 4. Distributions of logarithmically transformed values of ΔIF, ΔDD, CV and ΔDI. In the case of ΔIF, ΔDD and ΔDI a constant has been added before transformation (as indicated), to avoid negative values.

transformed values of ΔIF and ΔDD in Fig. 4. This figure (*C*) also shows the distribution of values of conduction velocity (CV) with the expected bimodal appearance dividing in the region of 70 m s^{-1}. The most obvious interpretation of Fig. 4*A* and *B* is that ΔDD is mainly dependent on the strength of influence of b_1

fibres upon each afferent and ΔIF upon b_2 influence. The bimodality of the distributions may then be taken to indicate subpopulations defined by the stretch of b_1 and b_2 influence, perhaps the presence or absence of such influence. A means of objectively separating normal subpopulations described by Hald (1952) has been

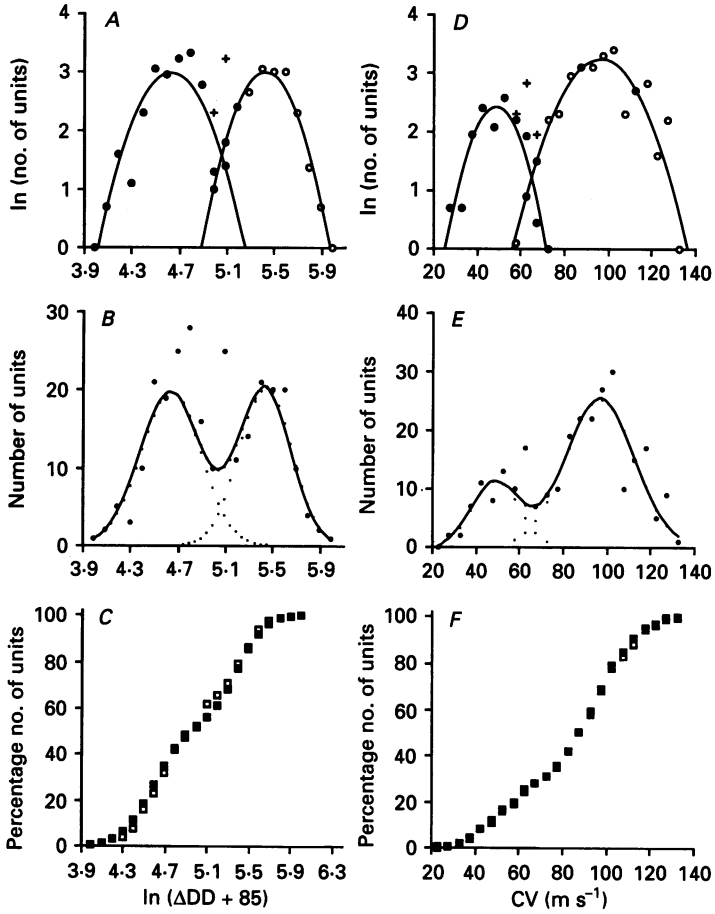

Fig. 5. Steps in the process of dividing the distributions of ΔDD (*A*, *B* and *C*) and of CV (*D*, *E* and *F*) each into two normal subpopulations. In *A* the ordinates of the distribution (see Fig. 4*B*) have been logarithmically transformed and fitted with a parabola for abscissa values from 5·19 to 5·99 (○). This was extrapolated to the left and subtracted from the original data leaving points fitted by a second parabola (●). Original data in the region of overlap are shown by crosses. In *B* the ordinates of the parabolas have been transformed back into linear values and plotted separately (dotted line) and summed (continuous line); ● original data values. In *C* the cumulative sums (normalized with respect to the total) of the observed distributions (□) are compared with those of the fitted distributions (■). The process is repeated for CV in *D*, *E* and *F*, except that the first parabola was fitted for abscissa values from 72·5 to 132·5.

explained in the previous paper (Taylor *et al.* 1992) and is applied to the data for ΔDD and CV in Fig. 5. The separation into two subgroups for ΔIF in Fig. 4 is so obvious as not to require further analysis. The best separation is taken to be at a

value of ΔIF of 4 impulses s^{-1} (ln (ΔIF + 1) = 1·6). From Fig. 5 the best value of ΔDD for separation is 70·8 impulses s^{-1} and for CV is 63·2 m s^{-1}. The value of DI is usually taken as a good measure of dynamic sensitivity, however, the distribution of its change with SCh (ΔDI in Fig. 4) is not so obviously bimodal as is ΔDD. This might

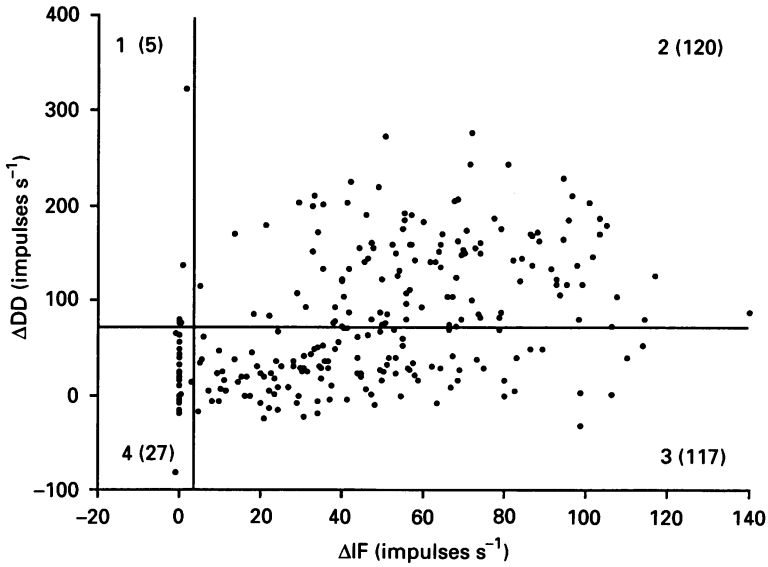

Fig. 6. Scatter diagram of values of ΔDD against ΔIF for all 269 spindle afferent units. The position of the vertical line divides ΔIF values at 4 impulses s^{-1} and of the horizontal line divides ΔDD values at 70·8 impulses s^{-1} as indicated by Fig. 5. The four quadrants so defined are designated 1–4 as shown, and the size of each population is shown in parentheses.

have been predicted however since DI = PF − SI = DD − SD and SD is not a simple reflection of either b_1 or b_2 effects.

The scatter plot of Fig. 6 shows all the data for ΔIF and ΔDD. There is evidently no correlation between these two measures. This is to be expected if they do indeed measure different features of the spindle afferents properties which vary independently. It is consistent with the view that the effects of contraction of the b_1 and b_2 fibres by SCh can be separately estimated by the values of ΔDD and ΔIF respectively. From a statistical point of view also it can be argued that since there is evidence for bimodal distribution of ΔIF and ΔDD (Figs 4 and 5) and these two measures vary independently, then four distinct regions can be defined on the scatter plot of Fig. 6. All afferents may be taken to have some influence from chain fibres so that the quadrants of Fig. 6 numbered 1–4 indicate regions of predominant influence of b_1 c, b_1 b_2 c, b_2 c and c intrafusal fibres respectively. The numbers of units in the different groups are indicated in Fig. 6, their percentage and mean values for different properties are given in Table 1.

The repeatability of the effects of SCh was checked in four b_1 b_2 c units, each with five doses of 200 μg kg^{-1} SCh, which were interleaved at 30 min intervals with other test doses of between 50 and 600 μg kg^{-1}. The mean values observed for ΔDD were:

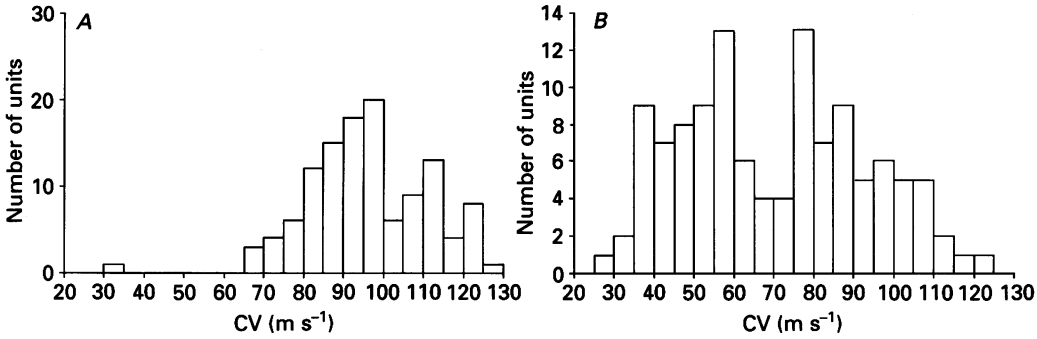

Fig. 7. Frequency histograms of CV for the $b_1 b_2 c$ group (*A*) and the $b_2 c$ group (*B*).

TABLE 1. Numbers and proportions of the five different afferents types in the gastrocnemius muscles

	Afferent type					
	1	2	3 a	3 b	4	Total
Mean ΔDD	138	141	22	24	7	—
S.E.M.	37·4	4·3	3·0	2·9	5·9	—
Mean ΔIF	0·6	64	56	32	0·1	—
S.E.M.	0·3	2·3	5·2	2·6	0·1	—
Muscle of origin						
MG	4	84	52	41	24	205
(%)	2·0	41·0	25·3	20·0	11·7	100
LG	1	36	13	11	3	64
(%)	1·5	56·3	20·3	17·2	4·7	100
Total	5	120	65	52	27	269
(%)	1·9	44·6	24·2	19·3	10·0	100

Abbreviations: MG, medial gastrocnemius; LG, lateral gastrocnemius.

148, 155, 131, 153 and 167 impulses s^{-1} and for ΔIF were: 78, 64, 53, 58 and 54 impulses s^{-1}. It is evident that, given this period for recovery, the responses are repeatable and the spindles do not become significantly desensitized.

The mean values for ΔIF in $b_1 b_2 c$ units in MG and LG were 63·5 and 65·6 impulses s^{-1} respectively and for ΔDD 141·1 and 134·8 impulses s^{-1} respectively. The differences were not significant. Testing the whole population of units by analysis of variance showed no significant contribution to the variance in ΔIF or ΔDD due to muscle of origin.

Relationship to conduction velocity

The distribution of CV (Fig. 4*C*) indicates 196 units (72·9 %) with CV $\geqslant$ 63·2 m s^{-1} and 73 with CV < 63·2 m s^{-1} (27·1 %). The importance of CV in the classification is best seen in the $b_1 b_2 c$ and the $b_2 c$ groups. Figure 7*A* shows the distribution of CV for the $b_1 b_2 c$ group as defined by the SCh test. All save one of these units had CV $\geqslant$ 63·2 m s^{-1}. If, when using CV as the sole criterion for classification, the dividing line had been set at 80 m s^{-1} then 15 out of 119 units (12·6 %) with $b_1 b_2 c$ properties

(thought to be characteristic of primary afferents) would have been missed. Within the class of 117 b_2 c units (Fig. 7*B*), 65 (55·6%) had CV $\geqslant$ 63·2 and 52 (44·4%) had CV $<$ 63·2 m s^{-1}. Again taking the 80 m s^{-1} boundary for securely defining primary afferents, forty-two units (44% of the total of ninety-five afferents with

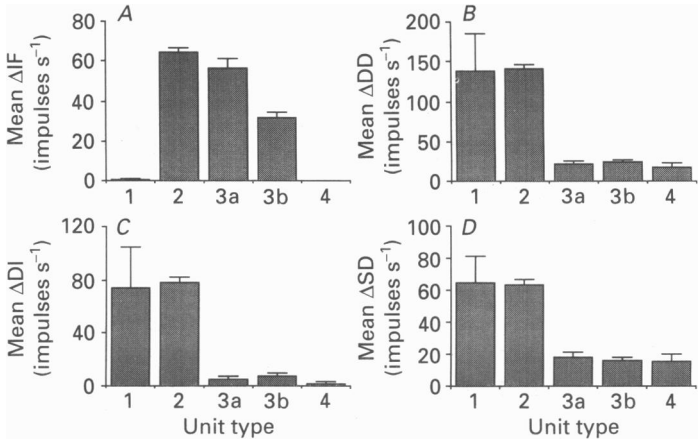

Fig. 8. Mean values of measured properties for each of the five types of unit as defined by Figs 4, 5 and 7*B*. *A*, *B*, *C* and *D* show the relative effects of SCh on IF, DD, DI and SD respectively. Bars indicate S.E.M.

CV $\geqslant$ 80 m s^{-1}) would have been designated as primary though they lacked the capacity to show significant b_1 effects. Taking the usually accepted upper boundary of 60 m s^{-1} for safely defining secondary afferents, forty-seven (69% of the sixty-eight afferents with CV $\leqslant$ 60 m s^{-1}) accepted as secondaries would have had significant b_2 influence. The division of the b_2 c group into two subgroups by conduction velocity at 63·2 m s^{-1} is so clear that in what follows the subgroups will be designated as 3 a for the fast group and 3 b for the slow group. Of the twenty-seven units classified by SCh as c type, five (18·5%) had CV $>$ 80 m s^{-1}. Finally, in the small group of five units classified with SCh as b_1 c, two had CV $<$ 60 m s^{-1} and three had CV $>$ 80 m s^{-1}.

Properties of the different afferent types in the passive and activated states

The properties of the different groups as defined above are summarized by bar graphs of mean values in Figs 8 and 9. The mean values for ΔIF, ΔDD and ΔDI which constitute the basis for the classification are shown in Fig. 8*A–C* and ΔSD in *D*. The very low values of ΔIF in types 1 and 4 are conspicuous and attest to the complete lack of any effect of SCh on bias in these groups. It is interesting to note that ΔIF is higher in type 3 a than in 3 b. Thus higher conduction velocity appears to be associated with higher sensitivity to b_2 fibre contraction. The uniformly low values of ΔDD in types 3 a, 3 b and 4 help to confirm that ΔDD is essentially independent of ΔIF. The smaller changes in DD with SCh in these three types does not appear to depend on conduction velocity or on the presence of b_2 fibre influence as defined by ΔIF. The mean values of ΔDI (Fig. 8*C*) are interesting because they are

not inescapable consequences of the method of classification. DI is given by DD−SD. Thus the differences between the high values of ΔDI for types 1 and 2 on the one hand and low values for type 3 a, 3 b and 4 on the other depend on the similar status of these types with respect to ΔDD and ΔSD (Fig. 8*D*). It appears that ΔSD is a function of b_1 influence rather than of b_2 influence.

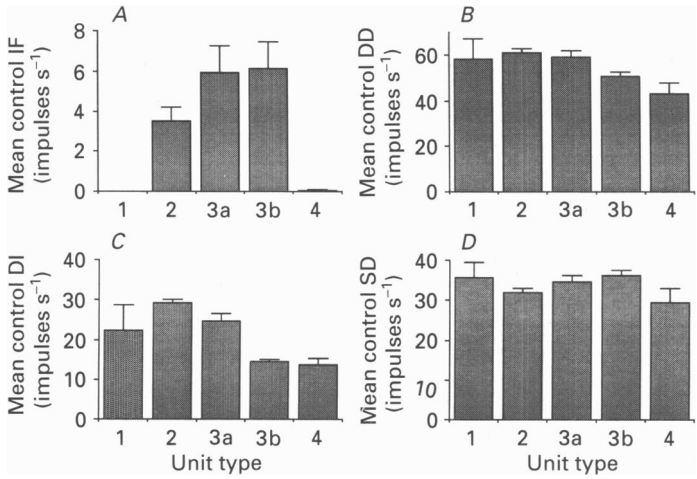

Fig. 9. Mean values of measured properties for each of the five types of unit as defined by Figs 4, 5 and 7*B*. *A*, *B*, *C* and *D* show the passive state values of IF, DD, DI and SD respectively. Bars indicate S.E.M.

Figure 9*A–D* shows the corresponding mean control values. An interesting feature is that control IF is very close to zero for types 1 and 4 (Fig. 9*A*). As these are the afferents deduced to lack b_2 influence it appears that IF under passive conditions (no intrafusal contraction) might depend on endings on b_2 fibres. Under passive conditions DD (Fig. 9*B*) varies only a little between the different unit types, though there is a tendency for types 3 b and 4 to be lower than the rest. This may be in some way linked to afferent CV rather than to the presence of b_1 influence.

Mean values of control DI are clearly lower in types 3 b and 4 than in the rest, and this is presumably a reflection of DD difference because SD does not vary significantly amongst the types (Fig. 9*D*).

The completeness of activation of b_1 and b_2 fibres by SCh

In the original work of Rack & Westbury (1966) the majority of the data were gathered using single I.V. doses of 200 μg kg^{-1} SCh as in the present study. This was shown to have a near maximal effect on dynamic response by the discharge frequency in the shortened condition (IF) and the static sensitivity (SD) could be increased further by increasing the dosage to 500 μg kg^{-1}. The recent study on jaw muscles (Taylor *et al.* 1992) employed 200 μg kg^{-1} and the present work used the same dosage in order to produce a coherent body of data. Higher doses were also generally avoided because of reported irreversible damage caused by high doses combined with muscle stretch (Rack & Westbury, 1966).

DISCUSSION

The basic purpose of this present study was to determine whether the system of functional classification using SCh elaborated for the jaw-closer spindles (Taylor & Durbaba, 1990; Taylor *et al.* 1992) could be used for other muscle groups. By studying gastrocnemius muscle in particular, for which so much related data already exists, it was expected to be able to gain additional insights into the meaning of this classification in relation to the usual one based on conduction velocity. As with jaw-muscle spindles, the gastrocnemius afferents showed bimodality of distribution of values of ΔIF and ΔDD. The separation into two subgroups was very complete for ΔIF, while for ΔDD fitting of two normal distributions indicated a small degree of overlap of the subgroups.

The scatter diagram of ΔDD against ΔIF (Fig. 6) is generally similar to that obtained for jaw muscle spindles (except for the very few b_1c types in the gastrocnemius). The lack of correlation of these measures is consistent with the idea that they measure different features of spindle properties which can vary independently. These features seem most likely to be the strengths of the influence of b_1 and of b_2 intrafusal fibres upon each afferent, which in turn might reasonably be equated with the extent of termination of each afferent on b_1 and b_2 fibres respectively. We have at the moment no way of testing this proposal morphologically but a number of lines of thought indicate its consistency. First, all save one of the units classified as b_1b_2c would have been classified as primaries on the basis of CV alone. Most (81·5%) of the afferents in the c group would have been classified as secondaries. Secondly, approximately 50% of the group diagnosed as b_2c had CV in the primary range. Thus, of the 196 afferents in the primary conduction velocity range, sixty-five (33·2%) have no evidence of significant b_1 fibre influence. The main group of primary afferents with no b_1 termination comprises those innervating the b_2c capsules of tandem spindles. The frequency of these in cat hindlimb muscles is known, from morphological studies, to range from 11 to 28·6% (Banks, Barker & Stacey, 1982, see also Swett & Eldred, 1960), though there is also the possibility that a small number of primaries to normal capsules may also lack significant b_1 terminals. A considerable number of secondary endings are known to contact b_1 and b_2 fibres as well as c fibres. Consequently, the occurrence of SCh responses indicating b_2c secondary types is to be expected. However, it is impossible to predict the functional strength of such morphologically recognized contacts.

There are some features of the methods used here which may be of particular importance in relation to the results of previously published work using SCh. It was noted in the previous paper (Taylor *et al.* 1992) that the effects of SCh given by close intra-arterial (I.A.) infusion were very variable and often weaker and more delayed than when a single I.V. dose was given. This was thought to be due to difficulties in ensuring that the drug went directly to the muscle in question and not via the general circulation. It was for this reason that I.V. dosage was preferred in the present work because a single dose would then be expected to mix with the circulating blood volume and quickly achieve a reasonably standardized concentration. The size and reliability of the response also seems to have been improved by preliminary muscle stimulation designed to produce dilatation of the muscle vessels and increase in blood

flow. It is well known that resting muscle has only a low blood flow and it is likely to have been even further reduced in the conditions of surgical shock prevailing. The possibility that the lack of signs of b_1 fibre activation in a substantial group of units might be due to an inadequate concentration of SCh reaching some spindles can be discounted for several reasons. While recording from six units simultaneously it was commonly possible to see all types of response with a single dose. The effects on the responsive units were very rapid in onset and were repeatable. Furthermore, since b_2 fibres are less sensitive than b_1 fibres to SCh, inadequate activation would be expected to give a shortage of units with b_2 effects, rather than as observed a surprisingly high incidence of low b_1 effects.

Assessing stretch responses from cycle histograms rather than from the usual instantaneous frequency plots was used to give greater statistical reliability to the measurements and to avoid the problems of subjective measurement of frequency plots which are strongly influenced by random variation of interspike intervals. Peak frequency particularly is liable to be overestimated and this is crucial to the measurement of dynamic response. The disadvantages of the cycle histogram method are that brief, transient effects such as the initial burst and the brief drop in frequency at the end of dynamic stretch (the deceleration response of Cheney & Preston, 1976) are likely to be missed. Also the histograms obscure the changes in regularity of firing which can be helpful in interpreting intrafusal effects (see Price & Dutia, 1987). These features have not been considered at present, but the method of digital data storage used will permit further analysis from these points of view in the future.

It has been argued that SCh has a direct effect on the spindle nerve endings as distinct from its effects via the intrafusal muscle fibres (Dutia, 1980; Price & Dutia, 1987). The fact that in this present large sample a significant number (twenty-four units) of afferents were essentially unaffected (less than 4 impulses s^{-1} increase in IF) supports the view that the secondary nerve endings themselves are not significantly depolarized by SCh at the concentrations used here. The increments in IF above 5 impulses s^{-1} are smoothly distributed (Fig. 4*A*) and could be accounted for by varying amounts of contribution from bag_2 fibre contraction. This would certainly be the present interpretation of increases in IF seen for some presumed secondary afferents illustrated in studies of neck muscle afferents (Price & Dutia, 1987, Fig. 2*B*). In earlier work on soleus spindles also (Dutia, 1977) the biassing action on secondaries was accompanied by filling in of the silence caused by the release of each stretch. This is generally believed to be an effect of static fusimotor action (Emonet-Dénand, Laporte, Matthews & Petit, 1977), in this case due to b_2 fibre contraction. The presumption that the effect was direct and not mediated via b_2 fibre was based on the lack of a large late increase in static sensitivity (SD). However, Boyd (1981) has shown that b_2 contraction has no more than minimal effect on length sensitivity of secondary endings.

The existence of b_2c type afferents as clearly demonstrated by Price & Dutia (1987, 1989) for neck muscles and for MG has been fully confirmed. A difference of emphasis, however, is justified by the present observations that the conduction velocities of this type range all the way from the lowest (29 m s^{-1}) to the highest (122 m s^{-1}). It seems very likely that those afferents of the type with CV < 62 m s^{-1}

are actually from secondary endings subject to significant b_2 effects. The fast group (CV > 62 m s^{-1}) – presumably from primary endings – are not limited to the lower part of this conduction velocity range. Direct measurements of afferents supplying b_2 c capsules of tandem spindles in hindlimb muscles (Banks *et al.* 1982) and in neck muscles (Richmond, Stacey, Bakker & Bakker, 1985) have found them to be smaller than the primary afferents of normal capsules. This does not necessarily mean that their afferents remain smaller throughout their course. It is also possible that some primary afferents from complete capsules do not innervate b_1 fibres and so would be included in the b_2 c primary group (Banks *et al.* 1982). Given that a large number of gastrocnemius b_2 c type afferents are in the secondary CV range, it is not possible to say how many of the b_2 c type found in the jaw muscles might have been primary or secondary. One indication, however, may be based on the control values of DI. In the gastrocnemius, mean control DI values for the b_1 b_2 c group and for the b_2 c(a) group were similar and significantly higher than those for the b_2 c(b) and c groups (Fig. 9*C*). For the jaw data, mean control DI values for the b_2 c and c group were similar and very significantly lower than for the b_1 b_2 c group. This implies that many of the jaw b_2 c type afferents are to be considered as belonging to secondary endings with significant terminations on b_2 fibres.

The relative numbers of the different afferent types described here probably do not accurately reflect their true proportions. The present population of spindle afferents is unduly low in the slow conducting fibres, presumably because of the greater difficulty of isolating them than the large fibres in dorsal root filaments. By contrast, in the case of the jaw muscle study it is likely that all types were fairly sampled because recordings were made in that case by microelectrodes from the first order afferent cell bodies, in which small differences in size would probably not affect the chances of recording.

The classical view of secondary endings as being essentially restricted to chain fibres is now seen to be oversimplified from both the morphological and functional evidence. The study by Banks *et al.* (1982) of 351 secondary endings in hindlimb muscles showed 67·8% on b_1b_2 c fibres, 20·8% on b_2 c, 6·3% on c only and 5·1% on b_1 c. It is therefore not surprising that with maximal b_2 activation by SCh many secondary afferents should show an increase in IF characteristic of b_2 fibre influence. By the same token, it is natural to expect some b_1 influence to be seen on many secondary endings also. In fact the present SCh data show a mean ΔDD of 20 impulses s^{-1} for the secondary CV range afferents (columns 3 b and 4 in Fig. 8*B* which contain nearly all of them). This may well be a size of dynamic effect appropriate to the size of the b_1 fibre terminals, as Banks *et al.* (1982) found the proportion of terminal area of a secondary to be 75% on chain, 17% on b_2 and only 8% on b_1 fibres.

A surprising finding is that nine out of twenty-seven of the units classified as c afferents by SCh were in the high CV range typical of primaries. This goes very much against the usually accepted view that all the primary afferents as distinguished by high conduction velocity necessarily have important terminals on b_1 fibres and hence are capable of showing the effects of γ-dynamic activation. Apart from lacking b_1 effects these fast 'c' afferents (mean CV 84·2 m s^{-1}) rather resemble primaries in having distinctly higher control DI values than the slow 'c' afferents (mean CV

40·9 m s^{-1}). The possibility has to be considered that the former may be ordinary primaries in which SCh has not had its proper effect, perhaps through inadequate muscle blood flow. An argument against this is that their control DI at 17·8 impulses s^{-1} is well below that for the $b_1 b_2 c$ group (30 impulses s^{-1}). In future it would be desirable to test such units carefully with increased doses of SCh after vasodilatation or with stimulation of brainstem regions which produce dynamic fusimotor outflow.

Because primary afferents characteristically have large contact areas on b_1 fibres and these are the intrafusal fibres identified with the expression of dynamic fusimotor action, it is natural to suppose that the b_1 fibre contacts will be responsible for the dynamic sensitivity of primary afferents under all conditions. However, it is quite clear that control DD and control DI do not differ significantly between the $b_1 b_2 c$ and fast $b_2 c$ groups (2 and 3a in Fig. 9*C* and *D*). In other words whether a primary terminates significantly on b_1 fibres or not makes no difference to the control dynamic properties. Essentially the same conclusion was reached by Dutia & Price (1990) and most recently by Proske, Gregory & Morgan (1991) using a quite different approach.

The potential relevance of the present results to muscle spindle studies generally is both practical and theoretical. When muscle spindle responses to stretch are recorded to estimate the type and strength of fusimotor outflow, it can now be seen to be very desirable to add testing with SCh to the usual measurement of CV. Only in this way will it be possible to be sure that any given (primary) afferent will have the potentiality for showing dynamic fusimotor effects. Another point is that if attempts are to be made to distinguish separate control of b_2 and c fibres by static fusimotor action (see Wand & Schwarz, 1985; Dickson & Gladden, 1990), then only by testing with SCh will it be possible to know if a given secondary ending is able to respond significantly to b_2 contraction. By recording a number of single spindle afferent fibres simultaneously and testing them all under the standardized conditions as set out above it will generally be possible to have examples of each type. From comparisons of the changes in IF and DD much clearer descriptions of the distinct changes in drive to b_1, b_2 and c intrafusal fibres may be made than has previously been possible. Additionally, in studies of the central connections and functional role of muscle spindle afferents, it seems likely that the suggested classification using SCh may provide important insights to supplement the usual classification as primary or secondary by CV. It may be that the central connections of each afferent are dependent on the relative influence of the different intrafusal muscle fibres as well as on peripheral ending morphology. Some indications to this effect have already emerged in jaw muscle studies (Taylor, Durbaba & Rodgers, 1990) which showed a clear correlation of monosynaptic projection strength with b_2 influence but not with b_1 influence. This question and the relationship of the SCh classification with sensory ending morphology are problems deserving further study.

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