J. Anat. (1975), **119**, 3, pp. 557–568
With 8 figures
Printed in Great Britain

Sensory innervation of baboon muscle spindles*

K. P. FOX, T. H. KOEZE AND M. SWASH

Section of Neurological Sciences, The London Hospital, London, E1 1BB

(*Accepted* 13 *November* 1974)

INTRODUCTION

The innervation of human muscle spindles is very complex (Swash & Fox, 1972). Primary sensory endings can usually be recognized without difficulty, but the extent of the primary innervation on the nuclear chain fibres remains uncertain. In addition, it has proved particularly difficult to distinguish secondary sensory endings from the various motor endings found intermingled with them on the poles of the intrafusal muscle fibres, and this problem has led to difficulties, both in normal spindles and in spindles studied in various neuromuscular diseases (Swash & Fox, 1974).

It appeared to us that these problems of histological interpretation could best be resolved by study of silver-impregnated, de-efferentated muscle spindles. For this purpose we have used the baboon (*Papio papio*), since we have found that the pattern of innervation of normal spindles in this species closely resembles that of human spindles. In de-efferentated baboon spindles we have studied the number, histological type and location of primary and secondary sensory endings, and have measured the diameters of their afferent axons.

Histological study of the sensory innervation of baboon spindles is of additional interest because the histological data can be considered in relation to Koeze's (1973) suggestion that the conduction velocity of spindle afferent nerve fibres can be used as a reliable criterion for distinguishing primary and secondary afferents. In an attempt to test this suggestion we have compared the axon diameters of our histologically identified primary and secondary afferent fibres, using suitable conversion factors, with the conduction velocity demarcation criteria reported by Koeze (1968, 1973). In addition, comparison of these two sets of data has enabled us to check the validity of the conversion factors themselves by direct calculation (see Friede, 1972).

A brief abstract of some of our findings has already been published (Fox, Koeze & Swash, 1974).

MATERIALS AND METHODS

Three young baboons, weighing 5·6 to 7·2 kg were studied. Anaesthesia was induced and maintained as in previous experiments (Koeze, 1973). The lumbosacral ventral roots were exposed by laminectomy and the L 4, 5, 6, 7 and S 1 roots were

* Requests for reprints of this paper should be addressed to M. Swash.

cut intradurally, on one side, at or near the intervertebral foramina. Post-operatively there was a pronounced flaccid paralysis, which was followed by atrophy of the paralysed muscles. Because the articular facets were removed some scoliosis developed post-operatively, but this did not cause any obvious disability. Power and facility of the normally innervated limb were unaffected and there was no demonstrable sensory impairment on either side.

Thirty-eight to 93 days after de-efferentation the lateral popliteal nerve in the normal limb was exposed below the knee and the threshold stimulus required to produce a small contraction in the peroneal muscle group was noted. The lateral popliteal nerve in the de-efferentated limb was then similarly exposed and stimulated at 100 times this threshold voltage. There was no visible contraction in the paralysed muscles and no evoked electromyographic activity could be detected.

The de-efferentated peroneal, posterior tibial and gastrocnemius muscles, and the normal muscles from the opposite side, were dissected out and prepared for study, using the de Castro silver nitrate block impregnation method, as modified by Barker & Ip (1963). These muscles were then teased under a dissecting microscope.

Fifty-two teased, de-efferentated spindles were considered sufficiently complete and well-impregnated for study. These were drawn, photographed and described, using the classification proposed by Boyd (1962). The number and location of primary and secondary sensory endings were noted and the diameters of their afferent nerve fibres were measured. The measurements were made at the point of entry of these fibres into the spindle capsule, or just below their bifurcation if this occurred outside the capsule, as occasionally happened in the case of primary afferent fibres. The pre-bifurcation swelling was avoided. The measurements were made with an eye piece micrometer by two of us, using a 'best diameter' criterion. No motor end plates were found in the teased de-efferentated muscles, either on extrafusal or intrafusal muscle fibres.

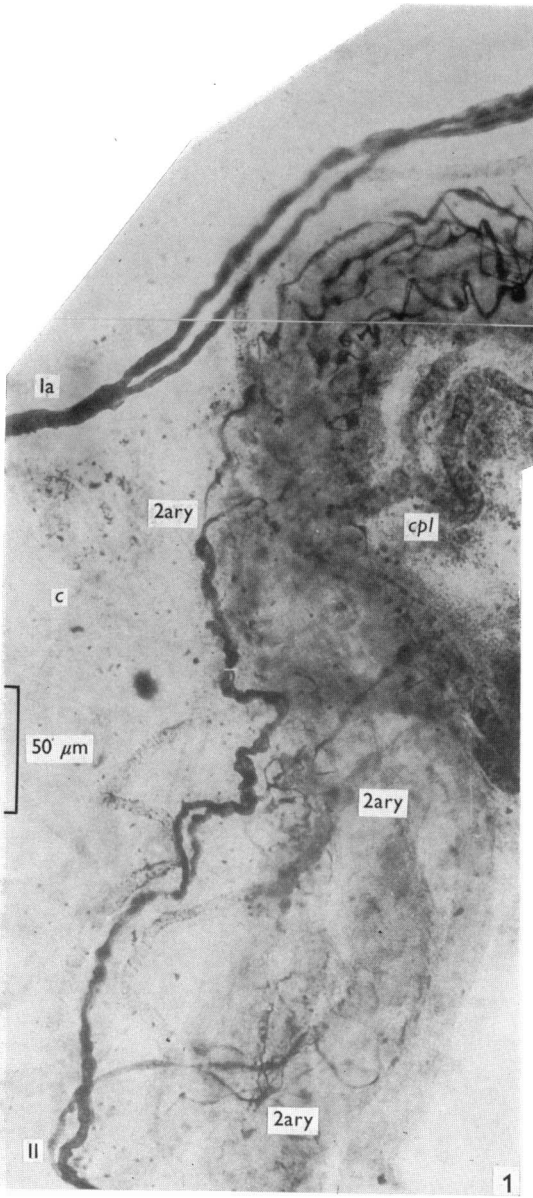
The lateral popliteal nerves from the normal and de-efferentated limbs were examined in transverse and longitudinal section, using the method of Fernand & Young (1951). In the nerves from the de-efferentated limbs there was a marked loss of large and small nerve fibres.

RESULTS

Primary innervation

The 52 spindles received 55 primary afferent nerve fibres, whose mean axon diameter was 5·5 μm (range: 4·5–7·1 μm). Each spindle, however, contained only one primary sensory ending, which was invariably located in the mid-equatorial region where the periaxial space was typically expanded. The slight excess in the number of primary afferent axons may have been due to branching occurring at a more proximal site, beyond the limits of the dissection (see Eccles & Sherrington, 1930).

The heavily myelinated primary afferent nerve fibre usually bifurcated in the periaxial space before becoming further subdivided into three to seven terminal branches (Fig. 1). These branches were more thinly myelinated. On the surface of the intrafusal muscle fibres these branches themselves subdivided to form a complex



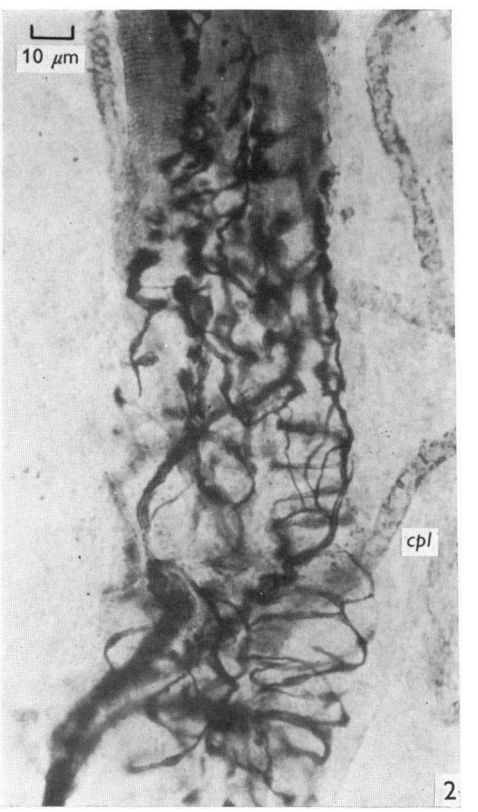


Fig. 1. Barker & Ip silver impregnation. The primary afferent nerve fibre (Ia) bifurcates in the periaxial space before branching again to form the primary sensory ending itself (1ary). Several secondary sensory endings (2ary) are supplied by two secondary afferent nerve fibres (II). The juxta-equatorial secondary sensory ending is almost contiguous with the most distal part of the primary ending. *c*, capsule; *cpl*. capillary.

Fig. 2. Barker and Ip silver impregnation. The terminal branches of the primary afferent nerve fibre can be followed. The primary sensory ending consists of a complex network of thin axons. Sensory enlargements are prominent in the distal parts of the ending. *cpl*, capillary.

Table 1. *Numbers and diameters of primary and secondary afferent axons in 52 spindles*

	No. of axons	Total %	Mean axon diameter (μm)	Range (μm)	S.D.
Primary	55	—	5·5	4·5–7·1	0·07
Secondary	81	—	3·3	1·5–5·2	0·88
S1 Secondary	57	70	3·6	2·2–5·2	0·80
S2 Secondary	20	25	2·7	1·5–4·8	0·67
S3 Secondary	3	4	2·0	—	—
S4 Secondary	1	—	—	—	—

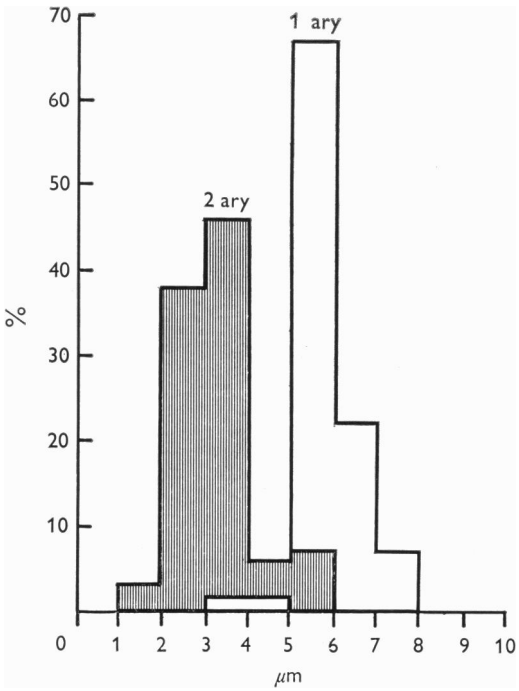


Fig. 3. Histogram of the axon diameters of afferent fibres supplying 52 primary and 81 secondary sensory endings in 52 spindles.

network of apparently unmyelinated spiral loops or clasps which were distributed to all the intrafusal muscle fibres (Fig. 2). Fine, unmyelinated sensory endings could usually be identified within the central part of the network. In the juxta-equatorial parts of the ending, flat, spade-like, or ring expansions were common; the spiral network itself rarely extended to this part of the ending (Fig. 2). The nuclear bag component of the primary sensory ending was always conspicuous and easily recognized. The nuclear chain primary sensory innervation was of the same general form but was less complex than that found on the adjacent nuclear bag fibres.

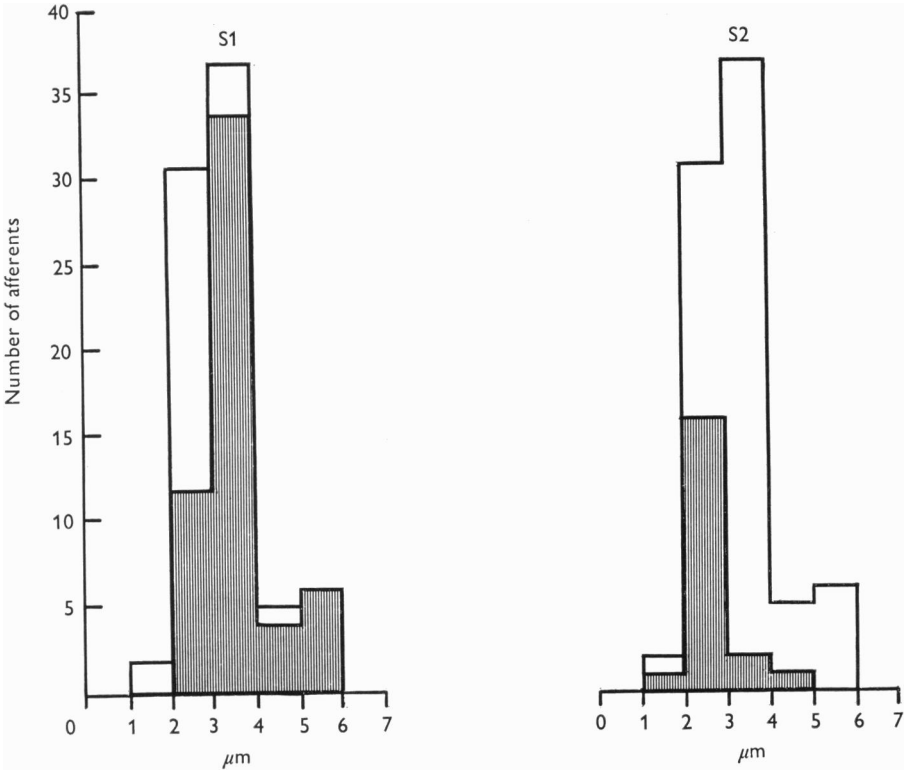


Fig. 4. Histograms of the axon diameters of 57 afferent nerve fibres supplying secondary endings located in the S1 position, and of 20 afferent fibres supplying endings found in the S2 position. The unshaded columns represent the whole population of secondary afferent fibres.

Secondary innervation

Eighty-one secondary endings were found in 45 spindles. In seven spindles (13 %) no secondary sensory innervation could be identified. Each secondary ending was innervated by a single, afferent nerve fibre. The 81 afferent fibres varied in diameter from 1·5 to 5·2 μm (mean 3·3 μm). The difference in distribution of axon diameters of primary and secondary afferent fibres is shown in Table 1 and, as a histogram, in Figure 3. Simple inspection revealed a trend for the thicker secondary afferent fibres to supply the most equatorial secondary endings, and the thinner the most polar endings, and this was confirmed by the measurements of axon diameter shown, as a histogram, in Figure 4 and in Table 1.

Most (70 %) of the secondary endings studied were located in the S1 position. Twenty-one of these 57 endings were situated on spindle poles which were also innervated by secondary endings located in the S2, S3 or S4 positions, and the remaining 36 were situated on spindle poles which did not receive additional secondary sensory innervation. The former groups of S1 secondary endings were supplied by afferent axons of 4·0 μm mean diameter (S.D. $\pm$ 0·75), and the latter by afferent axons

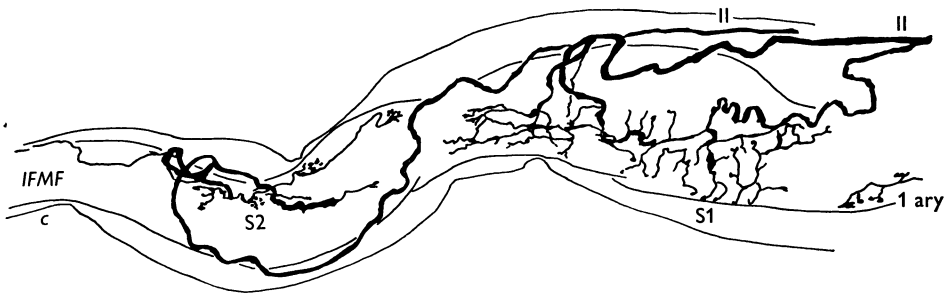


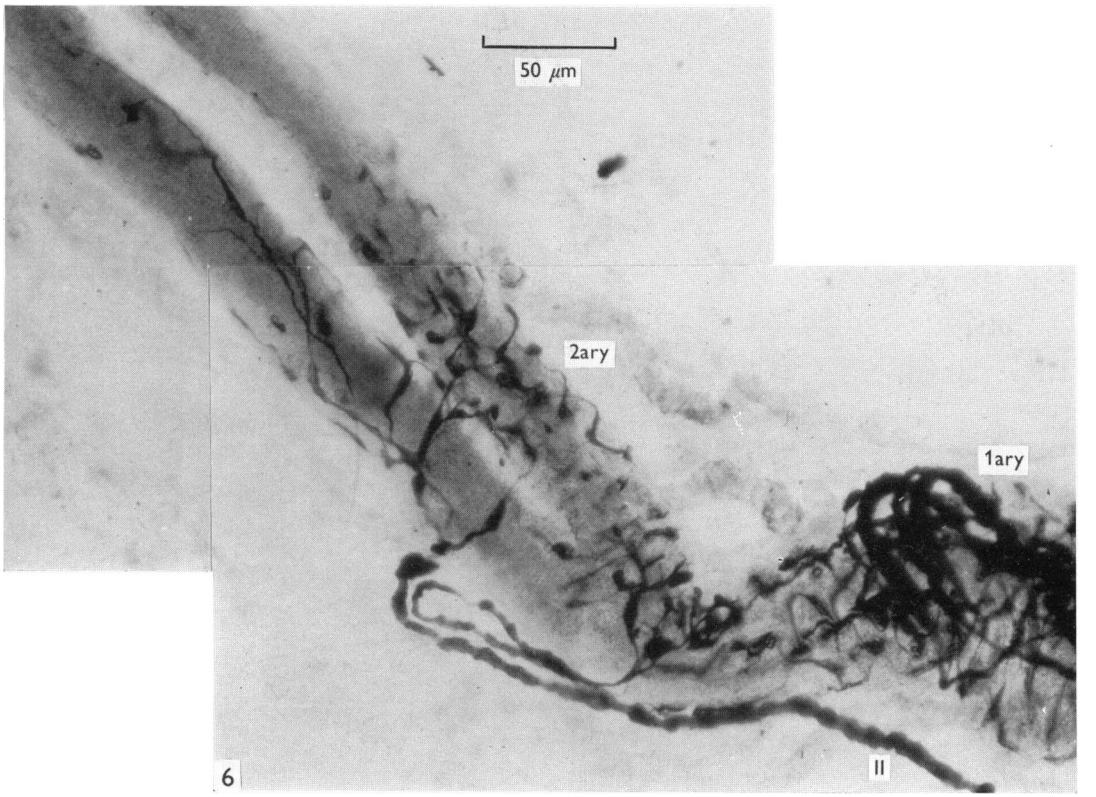
Fig. 5. This drawing shows the morphological differences between juxta-equatorial (S1) and mid-polar (S2) secondary endings. The tendency for juxta-equatorial endings to resemble the network of the primary sensory ending, and for this ending to be innervated by an afferent fibre of larger diameter should be noted. 1ary, primary; *IFMF*, intrafusal muscle bundle; II, secondary afferent fibre; *c*, capsule.

of 3·4 μm mean diameter (S.D. $\pm$ 0·77). These differences in mean axon diameter were statistically significant (one tailed $P < 0{\cdot}04$) when tested by the Mann–Whitney U test (Siegel, 1956).

The afferent nerve fibres innervating secondary sensory endings followed a characteristically variable, looped course after entering the periaxial space (Fig. 5). These afferent fibres usually formed two to five terminal branches, each of which wandered across the surface of several intrafusal muscle fibres before forming an ending. Some secondary sensory endings, especially those located in the S1 position close to the primary sensory ending, had a spiral, network form which was very similar to that of a primary ending. However, this network was usually more loosely arranged, and beaded terminal sensory enlargements were usually more prominent, in these juxta-equatorial secondary sensory endings than in primary endings (Figs. 5 and 6). The most equatorial parts of secondary endings in the S1 position were sometimes so closely intermingled with the polar terminals of the primary sensory ending that it was difficult to separate them (Fig. 6).

Secondary endings located in the more polar parts of a spindle were different. These endings usually consisted of several discrete clusters of fine, straight, unmyelinated axons arising from a single myelinated branch of the secondary afferent nerve fibre (Figs. 5, 7). Each of these clusters of terminal branches was only 50–100 μm in length. Discrete, terminal sensory enlargements were not usually a prominent feature of these endings (Fig. 7).

All the secondary afferent nerve fibres studied supplied endings distributed on both nuclear bag and nuclear chain intrafusal muscle fibres. The nuclear chain secondary innervation, however, was usually the more prominent, particularly in the case of endings located in the S1 position, when the secondary innervation was often limited to the nuclear chain fibres.



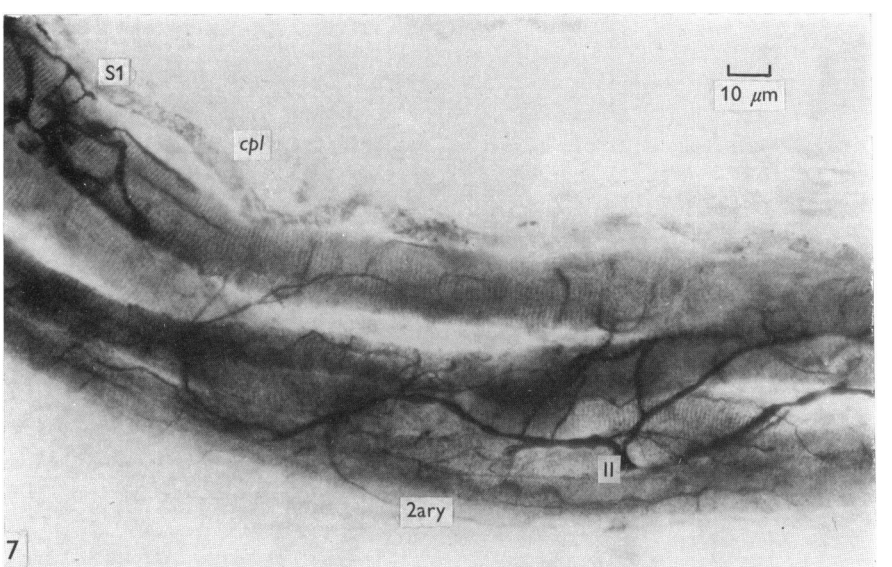


Fig. 6. Barker & Ip silver impregnation. A juxta-equatorial secondary sensory ending, consisting of several discrete clusters of sensory terminals (2ary), arises from the two major branches of a secondary afferent nerve fibre (II). Part of the primary sensory ending can be seen (1ary).

Fig. 7. Barker & Ip silver impregnation. This mid-polar secondary ending (2ary) consists of a spray of widely distributed, fine, branching axons arising from a thin, secondary afferent nerve fibre (II). Part of the juxta-equatorial secondary sensory ending, consisting of a spray of sensory terminals, can also be seen (S1). *cpl*, capillary.

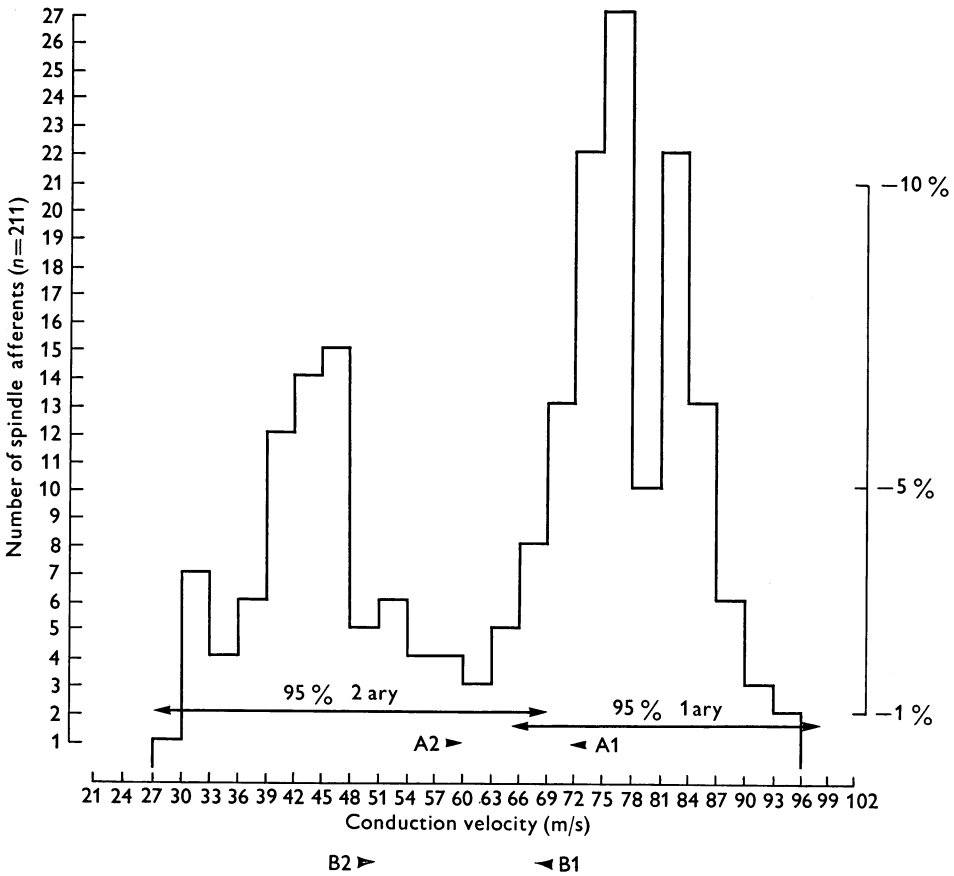


Fig. 8. Histogram of the conduction velocities of 211 baboon spindle afferent fibres, taken from data reported by Koeze (1968, 1973). The 95 % ranges of the axon diameters of afferent fibres supplying histologically identified primary and secondary endings, converted to conduction velocities as described in the text, are shown by horizontal bars (95 % 1ary; and 95 % 2ary respectively). The demarcation criteria for separating primary and secondary afferent fibres suggested by Koeze (1973) are indicated by the two arrowheads marked B1 (primary afferents) and B2 (secondary afferents). Our modified demarcation criteria are indicated by the arrowheads marked A1 (primary afferents) and A2 (secondary afferents).

DISCUSSION

In our preparations of de-efferentated baboon spindles primary sensory endings were morphologically distinct from secondary endings. Primary endings were usually supplied by a single, large diameter afferent nerve fibre which branched at a distance from the ending. The nuclear chain component of the ending was often small and poorly developed in relation to the complexly branching, spiral network found on the nuclear bag fibres. All the spindles received a primary innervation, and 87 % also received secondary sensory innervation. The ratio of secondary to primary endings was 1·55:1·0. In a similar study of cat muscle spindles, Boyd (1962) found a

ratio of 1·5:1·0. It therefore seems unlikely that any substantial number of secondary endings was missed in our preparations.

There were striking morphological differences between juxta-equatorial and polar secondary endings. The juxta-equatorial endings were usually similar in form to primary endings, but the more polar secondary endings were usually arranged as a fine spray of attenuated branches, rather than as a network, and rarely formed terminal sensory enlargements. These polar endings were supplied by afferent nerve fibres of thinner axon diameter than the juxta-equatorial secondary endings. The juxta-equatorial secondary ending of spindle poles receiving more than one secondary ending was supplied by a significantly thicker afferent nerve fibre than similar endings in spindle poles receiving only a single secondary ending. Juxta-equatorial secondary endings tended to be restricted to nuclear chain muscle fibres, whereas polar secondary endings were found as a rule on all the intrafusal muscle fibres.

These differences are consistent with the observations of Ruffini (1898) and Boyd (1962), who, using the gold chloride method, observed that some secondary sensory endings in cat spindles, particularly those located in the S1 position (Boyd, 1962), had an annulo-spiral form. Barker & Ip (1960), also using the gold chloride technique, thought that most secondary endings in the cat were of this type, but our studies show that, in the baboon, the more polar endings are not annulo-spiral. Boyd (1962) also commented, in his description of cat spindles, that secondary sensory endings located in the S1 position seemed to be supplied by afferent fibres of larger diameter than the more typically flower-spray (Ruffini, 1898) secondary endings found in the polar regions. Our own work provides a statistical basis for this impression (Table 1 and Fig. 4).

In silver preparations of normal human spindles it is difficult to distinguish the fine, terminal branches of the polar secondary sensory endings from those of motor endings of gamma-trail type, since these two different endings are usually found intermingled on the same intrafusal muscle fibres (Swash & Fox, 1972). Our observations in these de-efferentated baboon spindles illustrate this difficulty, but the stellate branching form of these secondary endings should be sufficiently characteristic to enable them to be separated from most gamma-trail motor endings. Secondary endings located on the juxta-equatorial parts of the intrafusal fibres usually contain prominent sensory enlargements and are, therefore, more easily recognizable, although they may be contiguous with the primary ending.

The significance of these histological differences between primary and secondary sensory endings, and between the several groups of secondary sensory endings themselves, is uncertain, but this problem could be clarified if it were possible to compare our histological data with those derived from physiological work. We have attempted such a comparison by using our histological data to test the suggestion put forward by Koeze (1973) that, in the baboon, spindle afferent nerve fibres with conduction velocities greater than 68 m/sec can be classified as primary afferents and those with conduction velocities less than 50 m/sec can be classified as secondary afferents. By using suitable conversion factors these suggested demarcation criteria can be compared with the calculated conduction velocities of the fibres supplying the histologically identified primary and secondary sensory endings found in our silver preparations. (N.B., the baboons used in the two studies were of similar body weight.)

The mean conduction velocities can be calculated from the mean axon diameters of the two groups of afferent fibres by using established conversion factors. First, the total sheath thickness of these myelinated axons can be estimated from the mean axon diameters, using a conversion factor of 1·43 to 1·67 (Friede, 1972). We have chosen the middle value: 1·55. The calculated myelin sheath diameter must then be corrected for shrinkage, due to fixation, using Stacey's factor: 1·41 (Stacey, 1969). The conduction velocity itself can then be calculated from the corrected myelin sheath diameter using Hursch's factor of 6 (Hursch, 1939). The conduction velocity of the spindle afferents whose axon diameters we have measured in our silver preparations can therefore be calculated by multiplying the mean axon diameters by $1{\cdot}55 \times 1{\cdot}41 \times 6 = 13{\cdot}1$.

This theoretical conversion factor can be compared with that obtained by direct calculation using the published physiological data (Koeze, 1968, 1973), and our measurements. Fig. 8 is a histogram of 211 baboon spindle afferent conduction velocities collected by Koeze (1968, 1973) in the course of other experiments. The mode of the primary afferent conduction velocities was 76 m/sec and that for secondary afferents was 45 m/sec. Division of these modal values by the mean axon diameters of our histologically identified primary and secondary afferent fibres yields a conversion factor of 13·8 for primary afferents and 13·6 for secondary afferents. These calculated conversion factors are similar to the theoretical factor of 13·1 predicted from other studies. This suggests that it is reasonable to compare the two sets of data in this way.

This result also suggests that Hursch's factor of 6 is adequate for conversion of myelin sheath diameters to conduction velocity in both primary and secondary afferent fibres. Others (McLeod & Wray 1967; Boyd & Davey, 1968), have suggested that the conversion factor varies with fibre size, but our data do not support this contention. It might be suggested that error could have been introduced into our calculations because the mean axon diameters were measured at, or near, the muscle spindle capsules, whereas the conduction velocities were estimated more proximally, between the popliteal fossa and the dorsal roots. For example, subdivision of these fibres into several smaller branches might have occurred between the roots and the spindles, leading to disparity between the axon diameter and conduction velocity measurements (see Eccles & Sherrington, 1930). Such an error is ruled out, however, by the similarity of the directly calculated conversion factors for primary (13·8) and secondary (13·6) afferents, both to each other and to the theoretically determined conversion factor (13·1).

The similarity of these directly calculated conversion factors (13·8 and 13·6) to the theoretically predicted conversion factor (13·1) led us to use the fibre diameter measurements of the histologically identified afferent axons to test the demarcation criteria for separating primary and secondary afferent fibres based on conduction velocity estimations, which have been put forward by Koeze (1973). Koeze (1973) suggested that afferent fibres of conduction velocity greater than 68 m/sec should be classified as primary afferents, and fibres of conduction velocity less than 50 m/sec should be classified as secondary afferents.

The histograms of the primary and secondary afferent axon diameters (Fig. 3) clearly indicate that the measurements in each group are not normally distributed;

indeed, in each group of measurements a chi-squared test for normality (Snedecar & Cochran, 1967) showed that the probability that these data were normally distributed was less than 0·005. We have therefore employed non-parametric statistical methods, and have used the 95% range for each group of measurements, rather than the standard deviation, in our calculations.

Figure 8 is a histogram of the conduction velocities of 211 baboon spindle afferent fibres reported by Koeze in earlier work (Koeze, 1968, 1973). The 95% ranges of the primary and secondary afferent axon diameter measurements shown in the two histograms in Fig. 3 can be converted to conduction velocity ranges, using the appropriate, directly calculated conversion factors (viz. 13·6 and 13·8) and these converted 95% ranges are shown as horizontal bars in Fig. 8. Comparison of these ranges with the demarcation criteria put forward by Koeze (1973), which are also shown in Fig. 8 (arrowheads marked B1 and B2), suggests that the latter may be too conservative. An upper value of 60 m/sec for secondary afferent fibres, rather than the 50 m/sec criterion of Koeze (1973), would exclude all but a very few primary afferents. On the other hand, the suggested criterion of a lower limit of 68 m/sec for primary afferents might include about 8% of the secondary afferent population. If this lower limit were raised to 72 m/sec almost all secondary afferents would be excluded from the primary afferent population. Afferent fibres whose conduction velocities were between 60 and 72 m/sec would remain unclassified by these criteria.

We suggest that these modified conduction velocity demarcation criteria (marked A1 and A2 in Fig. 8) should be adopted in future work on baboon spindle afferent fibres.

SUMMARY

The number and distribution of primary and secondary sensory endings has been studied in 52 de-efferentated baboon muscle spindles and the axon diameters of the afferent fibres innervating these endings have been measured.

Each spindle contained a single primary sensory ending; most of these endings were supplied by a single afferent nerve fibre. Each primary sensory ending consisted of a multi-branched network distributed on both nuclear bag and nuclear chain fibres. Beaded sensory terminals were prominent in the central part of the ending. Eighty-one secondary endings were found in 45 spindles (87% of the number of spindles examined). Of these endings, 70% were found in the S1 position, 25% in the S2 position and 4% in the S3 location. The afferent axons supplying the most equatorial of these endings were of thicker mean diameter than those supplying the most polar endings. In addition, the juxta-equatorial secondary endings were similar in form, although less regularly organized than the primary endings. The more polar secondary endings rarely formed terminal sensory enlargements and usually took the form of a fine spray of unmyelinated branches.

A non-parametric statistical comparison of physiological and anatomical data in baboon spindles has suggested that the demarcation criteria for separation of primary and secondary spindle afferents, using conduction velocity, should be modified. It is suggested that afferent fibres of conduction velocity less than 60 m/sec should be classified as secondary afferents, and fibres of conduction velocity greater than 72 m/sec should be classified as primary afferents.

K. P. F. and M. S. acknowledge the generous support of The Wellcome Trust and The London Hospital Research Fund, and T. H. K. gratefully acknowledges the support of the National Fund for Crippling Diseases. A preliminary account of some of this work was presented at the Second Muscle Spindle Conference, held by the Anatomical Society of Great Britain and Northern Ireland, at the University of Durham in April 1974.

REFERENCES

BARKER, D. (1967). The innervation of mammalian skeletal muscle. In *Myotatic, Kinesthetic and Vestibular Mechanisms* (Ciba Symposium: Ed. A. V. S. de Reuck and J. Knight), pp. 3–15. London: Churchill.

BARKER, D. & IP, M. C. (1960). The primary and secondary endings of the mammalian muscle spindle. *Journal of Physiology* **158**, 8–108.

BARKER, D. & IP, M. C. (1963). A silver method for demonstrating the innervation of mammalian muscle spindles in teased preparations. *Journal of Physiology* **169**, 73–74 *P*.

BOYD, I. A. (1962). The structure and innervation of the nuclear bag muscle fibre system and the nuclear chain muscle fibre system in mammalian muscle spindles. *Philosophical Transactions of the Royal Society of London, Series* B **245**, 81–136.

BOYD, I. A. & DAVEY, M. R. (1968). In *Composition of Peripheral Nerves*, pp. 24–27. London: Livingstone.

ECCLES, J. C. & SHERRINGTON, C. S. (1930). Numbers and contraction values of individual motor units examined in some muscles of the limb. *Proceedings of The Royal Society, Series* B **106**, 326–357.

FERNAND, V. S. V. & YOUNG, J. Z. (1951–2). The sizes of the nerve fibres of muscle nerves. *Proceedings of The Royal Society, Series* B **139**, 38–58.

FOX, K. P., KOEZE, T. H. & SWASH, M. (1974). Sensory innervation of primate muscle spindles. *Journal of Anatomy* **119**, 185.

FRIEDE, R. A. (1972). Control of myelin formation by axon caliber (with a model of the control mechanism). *Journal of Comparative Neurology* **144**, 233–252.

HURSCH, J. B. (1939). Conduction velocity and diameter of nerve fibres. *American Journal of Physiology* **127**, 131–139.

KOEZE, T. H. (1968). The response to stretch of muscle spindle afferents of baboon's tibialis anticus and the effect of fusimotor stimulation. *Journal of Physiology* **197**, 107–112.

KOEZE, T. H. (1973). Muscle spindle afferent studies in the baboon. *Journal of Physiology* **229**, 297–317.

MCLEOD, J. G. & WRAY, S. H. (1967). Conduction velocity and fibre diameter of the median and ulnar nerves of the baboon. *Journal of Neurology, Neurosurgery and Psychiatry* **30**, 240–247.

RUFFINI, A. (1898). On the minute anatomy of the neuromuscular spindles of the cat, and on their physiological significance. *Journal of Physiology* **23**, 190–208.

SIEGEL, S. (1956). In *Non-parametric Statistics for the Behavioral Sciences*. London: McGraw-Hill.

SNEDECAR, G. W. & COCHRAN, W. G. (1967). In *Statistical Methods*. Iowa City: University of Iowa Press.

STACEY, M. J. (1969). Free nerve endings in skeletal muscle of the cat. *Journal of Anatomy* **105**, 231–254.

SWASH, M. & FOX, K. P. (1972). Muscle spindle innervation in man. *Journal of Anatomy* **112**, 61–80.

SWASH, M. & FOX, K. P. (1974). The pathology of the human muscle spindle: effect of denervation. *Journal of the neurological Sciences* **22**, 1–24.