J. Physiol. (1976), **262**, *pp.* 91–117
With 2 plates and 13 text-figures
Printed in Great Britain

CENTRAL TERMINATIONS OF MUSCLE AFFERENTS ON MOTONEURONES IN THE CAT SPINAL CORD

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(*Received 19 February 1976*)

SUMMARY

1. Intraspinal branches of primary afferent axons in the cat lumbar cord have been revealed by filling then with cobalt, followed by precipitation as cobalt sulphide and silver intensification.

2. Primary afferent collaterals which reached the ventral horn were myelinated and had axon diameters of around 2 μm. Up to four (mean 1) side branches occurred at nodes within the ventral horn. The finest branches were less than 1 μm in diameter.

3. The finest branches formed synaptic boutons on nerve cells. The total observed association between one afferent and a motoneurone (synaptic structure) consisted of up to six boutons (mean 1·85). The boutons constituting a synaptic structure were usually located close together on the soma or dendrites, but sometimes were spread along more than 60 μm of dendrite. It was assumed that these monosynaptic structures on motoneurones were formed by muscle spindle afferents.

4. There was no correlation between the total contact area of a synaptic structure and its location on a motoneurone.

5. A computer model of the motoneurone was used to investigate the effect of multiple synaptic contact on electrophysiological estimates of synaptic location. It was concluded that the observed spread of boutons making up a synaptic structure would not significantly affect distance allocation of distal synapses but could lead to some proximal dendritic structures being incorrectly classified as somatic.

INTRODUCTION

The monosynaptic pathway from muscle spindle primary endings to spinal motoneurones has for long been the focus of physiological study (see Mathews, 1972). The neurophysiological data, which is particularly

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complete for the adult cat is not, however, matched by similarly precise descriptions of the morphology of the pathway. There are three important deficiencies in the available anatomical picture.

1. The gross morphology, branching pattern and extent of myelination of the primary afferent collaterals which reach the ventral horn is largely unknown in the adult cat. Golgi studies have revealed much of this detail in foetal and very young animals (see Réthelyi & Szentágothai, 1973) but it is known that considerable growth, synaptic re-organization and functional change occurs in the spinal cord during post-natal maturation (Skoglund, 1960; Conradi & Ronnevi, 1975). The general course of primary afferents can be deduced by staining degenerating fibres after dorsal rhizotomy in the adult (Sprague, 1958) but individual fibres are rarely well defined by this technique.

2. The number of synaptic contacts made by a single primary afferent collateral on a motoneurone is unknown. Early light microscopists described synaptic boutons on the motoneurone surface (for references see Conradi, 1969). Identification of the primary afferent component of these boutons by experimental degeneration has been a subject of controversy and recent conclusions (Illis, 1967) are at variance with results obtained by electron microscopy. Furthermore, the preterminal axon is rarely revealed in such preparations.

3. There is only incomplete information available on the distribution of monosynaptic terminals on the motoneurone dendritic tree. Primary afferent synapses on motoneurones have been identified by electron microscopy (Conradi, 1969; McLaughlin, 1972*a*, *b*; Kuno, Muñoz-Martinez & Randić, 1973; Bodian, 1975). The relative number of such terminals on the motoneurone soma and proximal dendrites has been ascertained but the preterminal axons cannot normally be followed for more than a few microns in such material. If, as will be shown below, many primary afferents make multiple contacts with motoneurones then the mean synaptic location can only be obtained if all the contacts and their preterminal axon are visualized. Paradoxically, the most complete information on synaptic location has emerged from physiological studies (Jack, Miller, Porter & Redman, 1970, 1971; Iansek & Redman 1973*b*). However, these electrophysiological methods rely upon assumptions concerning the precise manner of termination of the afferents. The present study was instigated primarily in order to examine these assumptions.

It can be seen that resolution of these problems requires complete staining of one or a small number of identified afferents including the terminal branches and synaptic endings combined with differential staining of motoneurones.

In experiments described here cobalt has been introduced into the

central branches of afferent fibres by electrophoresis through their cut axons in filaments of dorsal root. Cobalt has been precipitated intracellularly as a dark brown sulphide and then further intensified with silver. The result is similar in appearance to a Golgi preparation. Motoneurones have been stained with thionine. This technique by no means fulfils all the requirements outlined in the previous paragraph but has permitted some progress to be made.

METHODS

Histology

Adult cats weighing 2·5–3·5 kg of either sex were used. Most of the animals were prepared for physiological investigations and were only used for the present purposes if the former were not feasible. Some animals were anaesthetized with Nembutal and others with Fluothane plus nitrous oxide followed by decerebration. The lumbar and sacral spinal cord was exposed by laminectomy and flooded with liquid paraffin at 37° C. The dura was slit and S1, L7 and L6 dorsal roots on one side were cut distally and reflected away from the cord.

Cobalt was introduced into a small number of afferent axons by iontophoresis (Iles & Mulloney, 1971). Small filaments (about 5% of the total) were split from each cut dorsal root and the ends placed in small baths containing 140 mM cobaltous chloride solution which had been introduced below the surface of the paraffin pool. The filaments were trimmed to a length which minimized the distance which cobalt ions had to move to reach the intraspinal branches but was sufficient to avoid contact between the cobalt solution and cord surface. A current of 5–50 μA was passed for 3–7 hr from each bath towards the spinal cord. The cathode was a large silver plate sewn on to the muscles of the back. Some other workers using this technique (Prior & Fuller, 1973; Mason, 1975) have performed iontophoresis or simple diffusion on tissues excised from the animal and placed in a refrigerator. This variation has been tried but was found to lead to loss of the fine structural detail which was of interest in the present investigation.

After iontophoresis the lumbar and sacral cord was removed and placed in chilled artificial cat c.s.f. (Coppin, 1973) to which a few drops of ammonium sulphide had been added (Pitman, Tweedle & Cohen, 1972). After 20–25 min the tissue was fixed in chilled buffered 4% formaldehyde plus glutaraldehyde or 2% glutaraldehyde alone (Karnovsky, 1965; Tyrer & Bell, 1974). Frozen sections 90 μm thick were cut 6–16 hr later and attached to slides with gelatin. There was less than 15% change in gross dimensions using this technique but differential shrinkage cannot be ruled out.

In some early experiments the tissue was Nissl stained and examined without further treatment (Iles, 1973). In these preparations the afferents were only weakly filled with cobalt sulphide and difficult to follow. A considerable improvement has now been achieved by intensification of the precipitate with silver using the Timm sulphide–silver method. The procedure used was that perfected for work on invertebrate material by Tyrer & Bell (1974) but using 5% silver nitrate solution 1:9 with stock developer (C. Mason, personal communication). After intensification slides were passed through alcohol and xylene and mounted.

At a later date the material was counterstained and re-examined. Cover-slips were removed by immersion in xylene contained in a deep jar (sections exposed to air tended to bleach). Progressive hydration was followed by staining in 0·2% thionine and re-mounting.

It should be stressed that although cobalt iontophoresis is now used routinely in studies of invertebrate nervous systems it is still an extremely capricious method in the mammal. This may be due to the longer distances over which cobalt is required to move but other factors cannot be ruled out. The intensification technique is similarly not completely reliable. As a consequence of these two factors most of the results illustrated in the present paper are from segment L7 of a single animal but sufficient results were obtained from three others to show that those illustrated are typical. Comparable results have recently been obtained for the frog spinal cord (Székely, 1976).

The material was examined with a conventional light microscope. Linear and circular eyepiece graticules were used for most measurement but axon diameters were estimated with a Watson image shearing eyepiece. Axon trajectories were plotted by projecting the image on to large sheets of paper at 750 × magnification. Fine details of synaptic structures were traced from photographs.

RESULTS

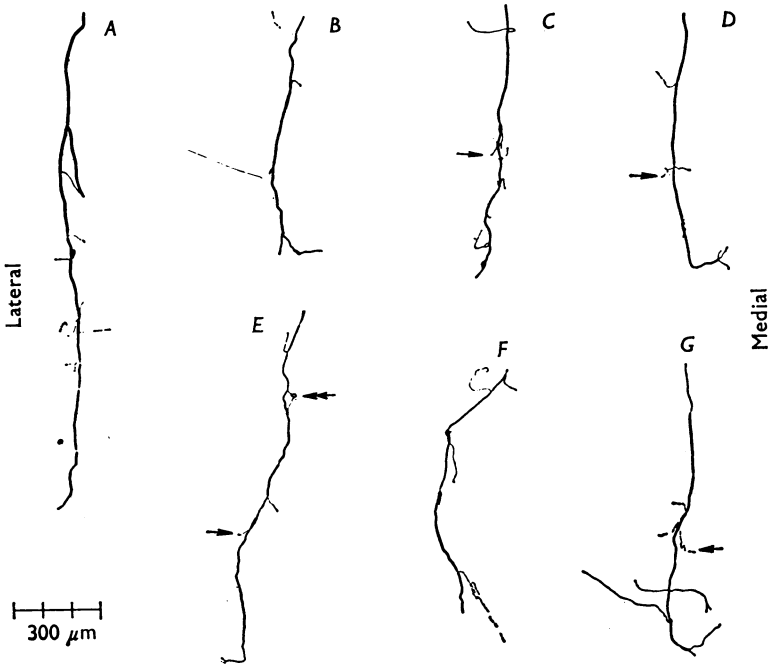
General disposition of afferent fibres

As was anticipated from classical descriptions (see Réthelyi & Szentágothai, 1973) afferent axons bifurcated on entering the cord sending branches rostral and caudal in the dorsal funiculus. Ventrally directed major collaterals branched periodically from these longitudinal axons (see also Llinás, 1973, his Fig. 4). These collaterals formed micro-bundles (Scheibel & Scheibel, 1969) that were especially obvious in sagittal sections.

In transverse sections of segment L7 afferents from L7 dorsal root were seen to enter the dorsal horn through its dorsal aspect and penetrate to lamina IV (Rexed, 1952). Afferents finally reaching the ventral horn pursued a direct course, generally curving slightly laterally towards the motoneurone cell columns. These will be referred to as major collaterals. A distinct medial division of thinner fibres was seen in some sections. These fibres crossed the intermediate region and followed the medial border of the ventral horn (cf. Sprague & Ha, 1964). No contacts with large cells were observed in the present material (cf. Réthelyi, 1968) but a presumed synapse with a medio-ventral motoneurone was found in an earlier study (Iles, 1973, fig. 1*b*). No contralateral afferent branches were found in L7.

The major collaterals in the ventral horn branched in the transverse plane but showed very little longitudinal spread. Seven examples are illustrated in Text-fig. 1. The collaterals were clearly myelinated, being periodically interrupted by structures from which branches often arose and which were regarded as nodes of Ranvier. In preparations which had not been intensified by the Timm method these nodes appeared denser than the internodal axon (Iles, 1973). This may have resulted from accumulation of cobalt ions at the nodes or a more rapid influx of sulphide ions at these points (Bennett in discussion of Llinás, 1973). In silver-intensified material

the complete axon was uniformly stained but there was a distinct contraction in diameter at each node. This appearance would be expected to result if the silver precipitate filled the axon, since at central nodes the axon is probably constricted (Hess & Young, 1952). The extreme thinning observed in the present material may be partly artifactual but a very similar result is obtained by staining with Protargol (Bodian, 1951).

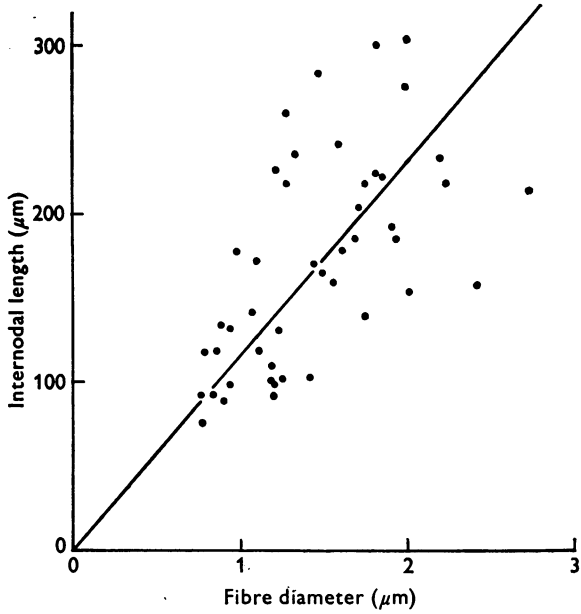


Text-fig. 1. Major afferent collaterals with branches in the ventral horn of segment L7. Drawn from transverse sections of cobalt filled and intensified preparations. Note constriction of the axons at nodes where branching frequently occurs (the axon diameters have been exaggerated to permit photographic reproduction). Single arrows in *C*, *D*, *E* and *G*: fine branches which were found to form synaptic structures on motoneurones, Double arrow in *E*: location of synaptic structure (S31) formed on a presumed 1a inhibitory interneurone (illustrated in Text-fig. 7 and Pl. 2).

Afferent axon diameters

In order to interpret measurements of the diameters of axons further evidence that the silver precipitate filled only the axon (diameter d) and did not impregnate the myelin sheath (diameter D) was sought. An attempt was made to resolve these alternatives by examining the relation between measured fibre diameter (x) and internodal length (l). Data for forty-six internodes of major collaterals in the ventral horn is presented in

Text-fig. 2. The fitted regression line has a slope (l/x) of 155. Hursh (1939) has provided a similar plot based on material from cat peroneal nerve stained with osmium (a myelin stain). His relationship is linear for small fibres with a slope (l/D) of ninety-four (an essentially similar result has been given by Lubinska, 1960). If the l/D relation of peripheral nerve continues to apply to fibres within the spinal cord then the ratio x/D can be seen to be



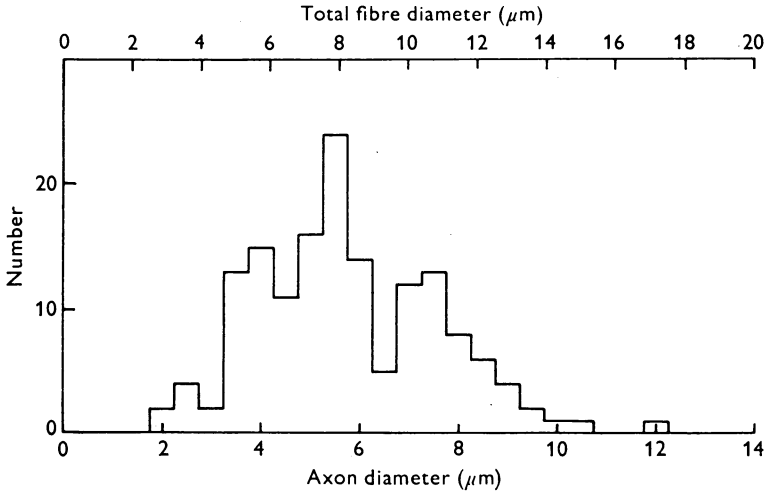
Text-fig. 2. Relation between measured fibre diameter (μm) and internodal length (μm) for forty-six internodes of major afferent collaterals reaching the ventral horn. The regression line has been drawn through the origin (diameter is regarded as the dependent variable).

94/115 = 0·82, which is close to the expected ratio d/D for small fibres in the central nervous system (Waxman & Bennett, 1972). It is thus likely that the present technique visualizes the axon only.

The l/d relation of Text-fig. 2 has considerably more scatter than corresponding results for peripheral nerve but is comparable with data from the rabbit central nervous system (Hess & Young, 1952). This may partly result from error in measurement (diameter was estimated from two points in each internode) but may also reflect an influence of branching on internodal length.

In invertebrates it has been found that during axonal iontophoresis of cobalt or Procion Yellow dye large axons tend to fill more rapidly and for longer distances than smaller ones (Iles, 1972, and unpublished). The diameters of filled afferents in the present material were measured in the

L7 dorsal root just at the point of entry into the spinal cord. The diameter distribution is illustrated in Text-fig. 3. The cats used in this work were 9–12 months old so this distribution may be compared with the histogram for all myelinated fibres in this root of a 1-year cat given by Skoglund & Romero (1965, their fig. 7). These authors found myelinated axons with external diameters ranging from 2 to 20 μm. Strict comparison with the present sample requires that an assumption be made about myelin thickness of fibres in this dorsal root (see legend to Text-fig. 3). However, the general conclusion may be drawn that most sizes of myelinated afferent can be filled by cobalt to the point of root entry. The present description concerns those fibres from this group which terminate in the ventral horn.



Text-fig. 3. Histogram of the diameters of filled afferent axons measured in the L7 dorsal root at the point of entry into the spinal cord. The abscissa labelled total fibre diameter has been constructed assuming a ratio of axon to total fibre diameter of 0·7. This must be treated as an approximation (see Coppin, 1973).

The largest major collaterals reaching the ventral horn had axon diameters of around 2·5 μm at the level of the central canal and all showed a slight but progressive reduction in diameter towards their distal termination. There is thus a considerable reduction in diameter from dorsal root group I axons to the largest major collaterals. Sometimes branching into two equally sized axons occurred within the ventral horn (Text-fig. 1*A*) and a few definitely myelinated branches of intermediate diameter ($\sim$ 1 μm) were found. The majority of branches were, however, very fine ($<$ 1 μm), somewhat varicose in appearance, and themselves branched. Up to four daughter branches, not necessarily all of the same diameter, were seen to

arise from a node. It was not possible to be certain whether the finest branches, which could often be followed for 500 μm, were myelinated or not.

Synaptic contacts with neurones

Some of the varicosities on the finest afferent branches in the ventral horn were larger ($\sim$ 3·5 μm diameter) and of a more smooth appearance than the rest. In many cases these swellings were closely applied to the surface of a neurone and had all the features associated with synaptic knobs (Auerbach, 1898). In the following description each individual contact between an afferent fibre and neurone will be referred to as a bouton (Cajal, 1909) and the term synaptic structure (Sprague & Ha, 1964) will be applied to the boutons and preterminal axon constituting the total observed association between one afferent and a neurone.

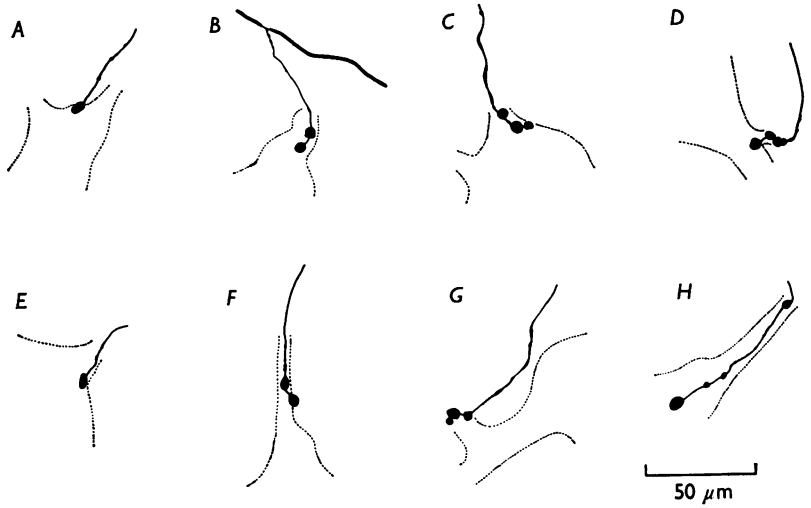
Seventy-nine associations between afferents and neurones were studied on a total of sixty-one cells in the ventral horn. Some typical examples of these synaptic structures are illustrated (Text-figs. 4, 11; Pl. 1). All but two of the neurones on which these synaptic structures were found were large cells in lamina IX. These have been classified as motoneurones (the exceptions are described below). Many other afferents had similar arrangements of large boutons where no post-synaptic cell was visible. It is assumed that these boutons are associated with longitudinally running dendrites (Laruelle, 1937; Scheibel & Scheibel, 1970) of motoneurones with somata in adjacent sections.

Transverse sections were cut 90 μm thick. This distance is only 10% of the average dendritic length of a motoneurone (Barrett & Crill, 1974). Even in the transverse plane the method of counter-staining used rarely permitted dendrites to be traced for more than 100 μm. In a few cases boutons were associated with counterstained objects of circular profile which may have been the proximal parts of longitudinal dendrites.

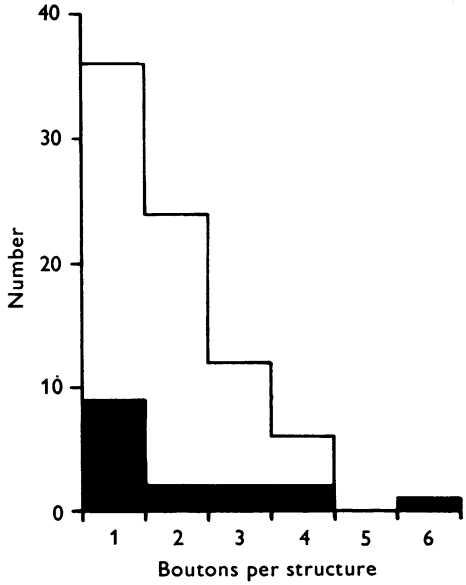
The synaptic structures on motoneurones sometimes consisted of a single bouton but were frequently multiple up to a maximum of six boutons (mean value 1·85; Text-fig. 5). There was generally variation in the size of boutons making up a single synaptic structure. Almost invariably one bouton was terminal and the rest arranged *en passage* along the same preterminal axon. Such boutons were usually located close together but sometimes were spread along over 60 μm of motoneurone dendrite.

Synaptic location on motoneurones

Twenty per cent of the observed synaptic structures were located on motoneurone somata and the rest were distributed along dendrites. When measuring dendritic location an imaginary ellipsoid within the soma was regarded as the soma surface and distances were measured from this. Where

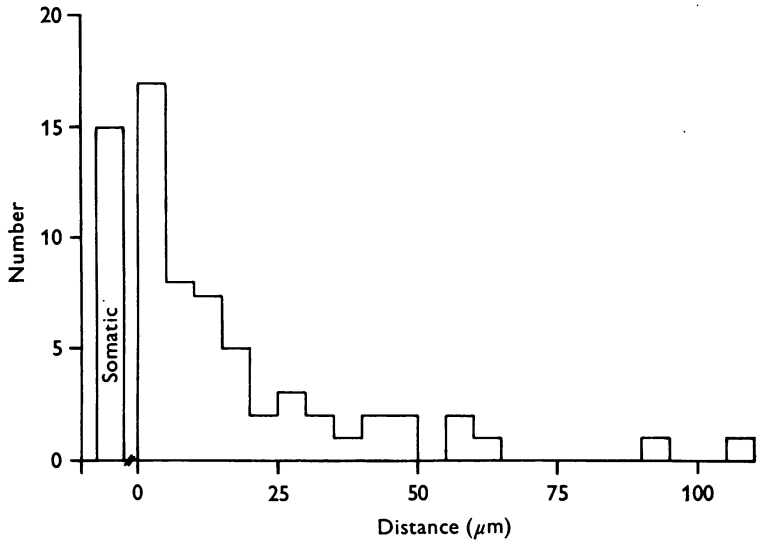


Text-fig. 4. Drawings of synaptic structures formed by fine branches of major afferent collaterals on motoneurones. Outlines of the motoneurones are indicated by interrupted lines. Examples of stuctures with one, two, three and four synaptic boutons are included. Note that in all multiple structures one bouton is terminal and the rest form en passant contacts. The structure illustrated in *B* is formed by the major afferent collateral drawn in Text-fig. 1*E*.



Text-fig. 5. The relative numbers of synaptic structures with different numbers of boutons on motoneurones. Filled columns refer to somatic structures.

a synaptic structure contained more than one contact the mean distance of all the boutons was taken. Synaptic location was only estimated when the nucleus of the post-synaptic cell was present in the section and in the case of dendritic locations when the dendrite was approximately transverse. No correction was made when the dendritic axis was not precisely parallel



Text-fig. 6. The relative numbers of synaptic structures located at different distances (μm) from the motoneurone somata.

to the plane of the section and so the distances measured will tend to be underestimates. This inaccuracy was tolerated since the observed distribution of synaptic structures (Text-fig. 6) is probably significantly distorted by the inability to trace motoneurone dendrites much beyond 100 μm (see Discussion).

Some of the most extensive evidence available on synaptic location for comparison with the present work has been obtained in electrophysiological experiments (Jack *et al.* 1971; Iansek & Redman, 1973*b*). In these investigations location was measured in terms of electronic distance along the dendrite. Electrotonic and physical distance cannot be directly compared in branching systems (Rall, 1959). However, by assuming average values for the electrical parameters of the motoneurone the presently observed distances, x, have been converted to electrotonic distance, X, and re-plotted in Text-fig. 7.

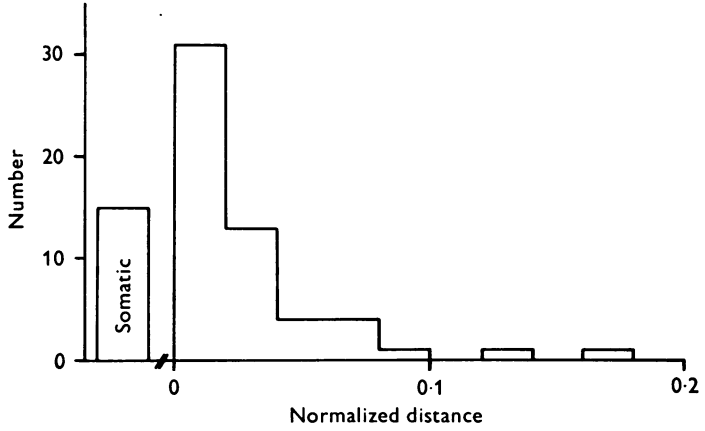
The electrical model of the motoneurone is described in the Appendix. The following values for the electrical parameters of an average motoneurone were chosen: specific membrane resistivity, $R_m = 2700\ \Omega\ \text{cm}^2$; axoplasm resistivity,

$R_i = 70\ \Omega$ cm (Lux, Schubert & Kreutzberg, 1970; Barrett & Crill, 1974). The diameter, d, of the parent dendrite, and where necessary of its branches, was measured and the dendritic characteristic lengths calculated from

$$\lambda = \sqrt{\left(\frac{R_m}{R_i}\frac{d}{4}\right)} \quad \text{(Rall, 1970).}$$

Physical distances were then normalized by the characteristic length,

$$X = x/\lambda.$$



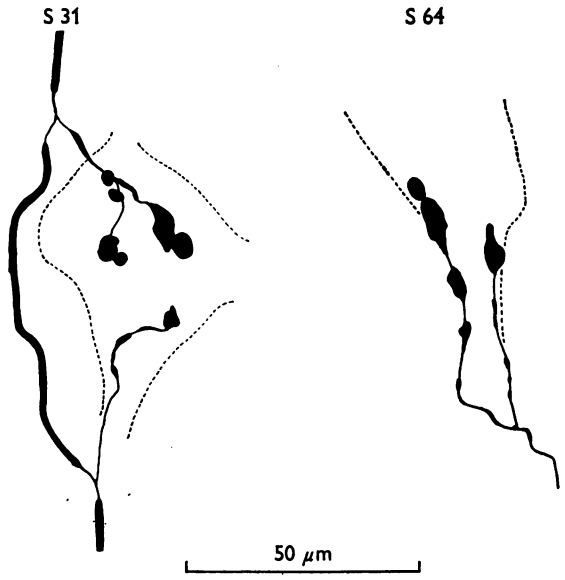
Text-fig. 7. The relative numbers of synaptic structures located at different electrotonic distances from the motoneurone somata.

Synaptic structures on interneurones

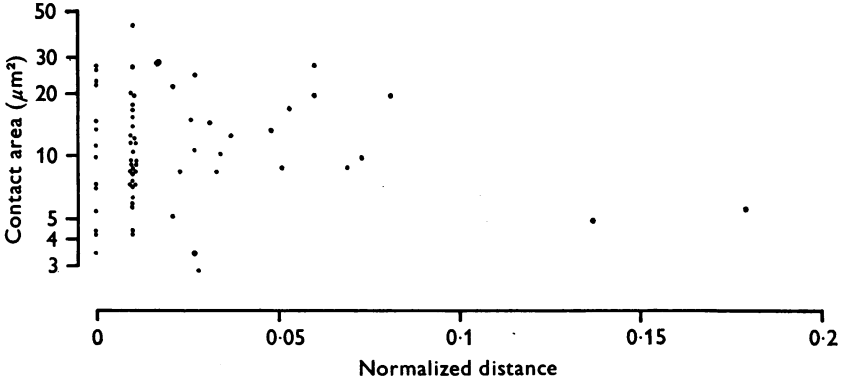
Two small neurones (soma diameters ~ 35 μm) located dorsal to the motoneurones in lamina VII received contact from afferents which continued into the motor nuclei. In one case (Text-fig. 1*E*) the same afferent also made contact with a motoneurone (Text-fig. 4*B*). These small neurones have been tentatively identified as 1a inhibitory interneurones (Jankowska & Lindstrom, 1973). The synaptic structures observed on these cells were somatic and extensive (Text-fig. 8; Pl. 2).

Synaptic contact area

The total area of contact of a synaptic structure was variable as a consequence of variation in both size and number of boutons. There is ultrastructural evidence that in thin sections the width of the region of apposition of boutons formed by spindle afferent fibres is approximately the same as the width of the whole bouton (Ronnevi & Conradi, 1974). Contact areas were thus estimated by treating each bouton as an elliptical contact. The mean area of contact for synaptic structures on motoneurones was 12·3 μm^2 (S.D. = 7·8, n = 63). There was no obvious correlation between contact area and synaptic location (Text-fig. 9).



Text-fig. 8. Drawings of synaptic structures on presumed 1a inhibitory interneurones. Outlines of the interneurones are indicated by interrupted lines. Synaptic structure S31 is also illustrated in Pl. 2 and is formed by the afferent collateral of Text-fig. 1*E*.



Text-fig. 9. Plot of synaptic contact area (μm^2) *vs.* electrotonic distance of the structure from the motoneurone soma. Dendritic synaptic structures located less than 0·02 λ from the soma have all been plotted at 0·01 λ. The ordinate is a logarithmic scale.

The contact areas on the two presumed 1a inhibitory interneurones were 109 and 104 μm^2. This is significantly ($P < 0{\cdot}01$) greater than the contact area on motoneurones. However, there is no ultrastructural evidence that the true area of apposition will be correspondingly larger.

DISCUSSION

Identification of afferents and neurones

It has been assumed that most large cells in the ventral horn are α-motoneurones. Cells of origin of the ventral spinocerebellar tract can be confused with motoneurones. The earliest reports (Cooper & Sherrington, 1940) suggested that such cells do not occur caudal to L6 but a wider distribution has since been described (Ha & Liu, 1968). Some of these neurones receive primary afferent input (Burke, Lundberg & Weight, 1971) and could thus be included in the present sample of afferent–neurone associations although motoneurones will be predominant.

In an earlier study (Iles, 1973) it was assumed that primary afferent collaterals which enter the ventral horn are branches of 1a afferent axons from the primary endings of muscle spindles. It has since been shown that some muscle group II afferents from spindle secondary endings terminate in the ventral horn close to the motor nuclei (Fu & Schomburg, 1974; Fu, Santini & Schomburg, 1974) and furthermore that some make monosynaptic connexion with motoneurones (Kirkwood & Sears, 1974).

The present description is concerned with synaptic associations between primary afferents and large neurones in the ventral horn, but the relative proportions of afferents from spindle primary and secondary endings is difficult to assess. Approximately equal numbers of 1a and secondary afferents are found in most hind-limb muscle nerves of the cat (see Boyd & Davey, 1968). The spectrum of diameters of filled axons in the L7 dorsal root (Text-fig. 3) suggests that both types could be visualized by the cobalt iontophoresis technique. If the density of innervation of ankle extensor motoneurones by 1a afferents (Mendell & Henneman, 1971) is compared with equivalent data for secondary afferents (Stauffer, Watt, Taylor, Reinking & Stuart, 1976) then it can be predicted that about 37% of the present sample of synaptic structures could be formed by secondary afferents and 63% by 1a afferents. It should be stressed that these two components of the monosynaptic input to motoneurones from muscle spindles have not been separated in either the ultrastructural or electrophysiological investigations with which the present results are compared below.

General disposition of afferent collaterals

The primary afferent organization observed in the present work (Text-fig. 1) corresponds very closely to that described from Golgi preparations from very young cats (Cajal, 1909; Szentágothai, 1967; Scheibel & Scheibel, 1969) if allowance is made for growth of axon internodes. In cobalt preparations the major collateral in the ventral horn is clearly

myelinated, in agreement with electron microscope observations of degenerating myelinated afferents (1–4 μm diameter) in the ventral horn after dorsal rhizotomy (Conradi, 1969; McLaughlin, 1972*b*). Each major collateral gives off about six very fine branches in the ventral horn which it is proposed form synaptic associations with motoneurones. The fine branches observed in the present material are rather less extensive (relative to the major collateral) than those illustrated from Golgi preparations. This may suggest inability to trace them fully (a few undoubtedly did leave the section which contained the major collateral) or alternatively could be explained by relatively less growth during development.

It is possible to estimate the average number of motoneurones with which each major 1a collateral forms synaptic associations. Mendell & Henneman (1971) have shown that 1a afferents from medial gastrocnemius muscle form monosynaptic connexions with 93 % of homonymous and 65 % heteronymous motoneurones. The number of motoneurones supplying medial gastrocnemius and soleus muscles are 280 and 155 respectively (Boyd & Davey, 1968) and 290 supply lateral gastrocnemius. One 1a afferent thus supplies about 548 motoneurones. The triceps surea motoneurones extend rostrocaudally in a column 9900 μm long in the spinal cord (Romanes, 1951; Van Buren & Frank, 1965). Major collaterals may branch off from a 1a afferent fibre in the dorsal funiculus at intervals of 200 μm (Scheibel & Scheibel, 1969) and further branching can occur within the grey matter. A total of about fifty major collaterals could thus supply the motoneurones, giving eleven motoneurones per major collateral, or about two per fine branch. Afferents from muscle spindle secondary endings in general form monosynaptic connexions with fewer motoneurones (see above).

Morphology of synaptic structures

Very few of the techniques available for staining synaptic boutons for light microscope examination also reveal the preterminal axon. However, unidentified synaptic structures on motoneurones which resemble those described here (Text-figs. 4, 11) are figured by Cajal (1909, Fig. 112; 1935, fig. 22) and Lorente de Nó (1938, fig. 3). Some workers have attempted to identify 1a afferents by degeneration. On this basis Szentágothai (1958) has described 1a afferents forming two *en passant* contacts with motoneurone proximal dendrites followed by a somatic bouton. This description obviously applies very precisely to some of the synaptic structures observed in the present work. Sterling & Kuypers (1967) working on the brachial spinal cord of the cat have suggested that preterminal axons are oriented longitudinally and form several contacts with the longitudinal dendrites of adjacent motoneurones. No evidence of such an arrangement was

obtained in the present material from the lumbar cord (the transverse sections used were thick enough to detect such longitudinal organization).

Electron microscope studies have described primary afferent terminals (M knobs) on motoneurones which are 4–7 μm long (Conradi, 1969; McLaughlin, 1972*a*, *b*). These dimensions are greater than the average diameter ($\sim$ 3·5 μm) of the boutons in cobalt preparations but the difference could well be accounted for by preparative technique. The preterminal axon has rarely been followed far in electron microscope sections but Conradi has described some clustering of M knobs which is consistent with the locally multiple contacts of many of the synaptic structures described here.

One assumption in the present work is that the synaptic structures observed are typical of the whole population. The method of staining motoneurones which was used does not allow dendrites to be followed for more than about 100 μm from the soma in the transverse plane and the section thickness imposes a 90 μm limit in the longitudinal direction. Thus synaptic structures could only be recognized when they occurred on the soma and proximal 10% of the dendritic tree. The seriousness of this problem can be illustrated by the calculation performed in the previous section. The seven major collaterals shown in Text-fig. 1 would be expected to form synaptic structures on up to eighty motoneurones. In fact four such motoneurones were detected. Presumably the remaining 95% of synaptic structures are located on dendrites which have not been visualized. It is possible that these structures have a different morphology though there was no evidence of any progressive changes with location on the motoneurone in the sample observed (e.g. Text-fig. 9). A further possibility is that a major collateral seen to supply synaptic boutons to the proximal dendritic region of a motoneurone also makes synaptic contact with an undetected distal dendritic region. In such cases the observed synaptic structures would be too small. However, individual excitatory post-synaptic potentials (e.p.s.p.s) generated by an afferent with contacts on both proximal and distal parts of the dendritic tree would be composite in shape. Most individual e.p.s.p.s recorded in the motoneurone have a simple time course and can be ascribed to synaptic action at a restricted region (see appendix).

The majority of the synaptic structures consisted of more than one contact with a motoneurone (mean 1·85, Text-fig. 5). Recently, Edwards, Redman & Walmsley (1976) have observed non-quantal fluctuations in charge transfer at individual 1a synaptic associations with motoneurones in the cat. They have ascribed these fluctuations to multiple synaptic contacts with all or none invasion of each bouton. The average number of boutons per synaptic structure necessary to explain their results is 1·7, which is close to the mean number of boutons observed here (assuming that

the full extent of each synaptic structure has been detected). The preterminal axon which connects the boutons making up a synaptic structure is extremely fine. This fact, combined with the loading effect of a large bouton could produce a lowered safety factor for propagation in the terminal. An interesting consequence of this hypothesis is the possibility that presynaptic inhibition of transmission in the monosynaptic pathway to motoneurones may be mediated by reduced invasion of the synaptic terminals (cf. Wall, 1964). Since the multiple synaptic structures consisted of boutons arranged serially along the preterminal axon a single presynaptic inhibitory neurone could produce a variety of inhibitory effects depending upon which boutons it contacts.

In an earlier study without the benefit of silver intensification (Iles, 1973) the only synaptic structures observed consisted of single contacts by very short branches from a major collateral node. This arrangement is typical of short branches (cf. electron microscope illustrations of Saito, 1972; Skibo, 1972) and the longer fine afferent branches were simply not visible.

Synaptic location

The distribution of synaptic structures on the motoneurone surface observed in the present experiments (Text-fig. 6) is probably distorted due to the difficulty in tracing motoneurone dendrites. However, the observation that each synaptic structure consists on average of 1·85 boutons located fairly close together has facilitated a quantitative comparison of the 1a synapse distributions obtained from electrophysiological experiments (Jack *et al.* 1970, 1971; Iansek & Redman, 1973*b*) with those observed with the electron microscope (Conradi, 1969).

The motoneurones examined by Conradi were situated in the lateral peroneal-tibialis cell group (Balthasar, 1952). Taking a tibialis anterior motoneurone as typical, this would be expected to receive 1a input from tibialis anterior and extensor digitorum longus muscles (Eccles, Eccles & Lundberg, 1957). Using the proportions of homonymous and heteronymous connexions given by Mendell & Henneman (1971) and the spindle counts of sixty-four and thirty-nine respectively for the two muscles (see Boyd & Davy, 1968) one can conclude that on average eighty-five 1a synaptic structures will be formed on a tibialis anterior motoneurone. This corresponds to 159 ($85 \times 1{\cdot}85$) 1a synaptic boutons.

The electrophysiological experiments estimate synaptic distance from the soma in terms of electrotonic distance (i.e. normalized by the dendritic characteristic length λ; see Appendix for a fuller explanation). An average motoneurone will have a soma surface of about 8000 μm^2 and a dendritic area of about 140000 μm^2 (Barrett & Crill, 1974). The average electrotonic length of a motoneurone dendrite is 1·4 λ (see Appendix). The proximal

0·2 λ of the dendritic tree thus includes about 20000 μm^2 of surface (equal increments of electrotonic length correspond to equal increments of membrane area: Rall 1959).

Pooling the electrophysiological data 57 out of 264 individual e.p.s.p.s were allocated to the proximal 0·2 λ of the dendritic tree. The predicted 1a synaptic bouton density is thus $(57 \times 159)/(264 \times 20000)$ $\mu m^{-2} = 0{\cdot}17$ per 100 μm^2. The corresponding M knob density observed by Conradi is 0·1 per 100 μm^2. Considering the assumptions made in this calculation, the small size of the anatomical sample and the unknown contribution of secondary afferents, the agreement is good.

A similar calculation for somatic boutons yields 0·11 per 100 μm^2 from electrophysiological experiments and 0·005 per 100 μm^2 from electron microscopy. This twentyfold discrepancy suggests that electrophysiological methods tend to overestimate the number of somatic e.p.s.p.s. An explanation for this phenomenon is proposed in the Appendix. It is possible that the true discrepancy is less because the anatomical sample of somatic M knobs (Conradi, 1969) is very small and the calculated knob density will be approximate. McLaughlin (1972*b*) did not find any somatic M knobs. A further indication that the true proportion of somatic monosynaptic structures is small can be obtained from the data of Text-fig. 7. Both the ultrastructural and electrophysiological findings agree that there is no marked reduction in the number of 1a structures over the first 0·2 λ of dendrite. The fall off in Text-fig. 7 is thus attributable to sampling. If the number of structures on the first 0·02 λ is then taken as typical of the synaptic density up to 0·2 λ the ratio (number of somatic boutons)/(number of boutons on the first 0·2 λ) can be calculated. The result of 0·05 is close to the value from Conradi (0·02) but distinctly smaller than that obtained from electrophysiology (0·25).

Synaptic contact area

There is some evidence that large synapses release more transmitter and produce a greater post-synaptic response than smaller ones (Kuno, Turkanis & Weakly, 1971; Kuno *et al.* 1973). Iansek & Redman (1973*b*) have shown that the charge transferred at 1a synapses on motoneurones increases with distance from the soma (synapses located 0·59 λ from the soma transferred ten times the charge of somatic synapses, the relationship up to 0·6 λ was approximately logarithmic). Evidence for an increase in contact area with distance was sought in the present material but not observed. However, contact area estimated by light microscopy may be a poor index of the amount of active synaptic area. Alternative explanations for the physiological result can also be envisaged.

Significance of the observed synaptic organization for electrophysiological estimation of synaptic location

The majority of synaptic structures observed in the present material consisted of more than one contact with a motoneurone at different distances from the soma. The consequences of multiple location on the *distal* dendritic tree have already been described (Rall, 1967; Jack & Redman, 1971; Iansek & Redman, 1973*b*). E.p.s.p.s produced by such multiple synapses would be allocated to a single distance from the soma somewhat greater than the true mean distance of the individual contacts. In the appendix it is shown that the limited spread of the multiple contacts in the present material would in most cases not lead to distance allocations significantly different from the actual mean distance. However, some multiple structures on *proximal* dendrites would tend to be incorrectly classified as somatic. Only in the most extreme cases observed would e.p.s.p.s with a composite shape be predicted.

I am grateful to Drs J. J. B. Jack and S. J. Redman for discussion of this work. The study was supported by M.R.C. Project Grant G 974/359/B to Dr Jack.

APPENDIX

Electrophysiological methods of estimating synaptic location on the soma-dendritic surface utilize a model of the motoneurone and assume a point synaptic location. Provided that the electrical constants of a motoneurone are measured then the shape of an e.p.s.p. recorded from it can be used to predict the synaptic location and synaptic current time course. It is the function of this appendix to examine the responses of such a model to multiple synaptic inputs of the kind observed in the anatomical study, to compare these with point inputs, and to estimate the error in synaptic distance allocation that will be introduced.

Computational methods

The motoneurone model used was the lumped soma short dendritic cable model of Jack & Redman (1971). In their notation X and L refer respectively to synaptic distance and dendrite length (both normalized by the characteristic length λ). Time is denoted by T (normalized by the membrane time constant, τ). The dendrite to soma conductance ratio ρ is replaced by the parameter ρ_∞ (Rall, 1959) where $\rho_\infty = \rho/\tanh L$. Dendrites were assumed to have open-circuit terminations.

The voltage response at the soma to an impulse of current applied at distance X on the dendritic tree $V(\rho_\infty, L, X, T)$ was calculated using an ALGOL programme on an ICL 1906*A* computer (Oxford University Computing Laboratory).

$$V(\rho_\infty, L, X, T) = C_0 \rho_\infty \, e^{-T} 2^{\frac{1}{2}} \sum_{n=0}^{\infty} (-1)^n \times \{F(X+2nL, n, \rho_\infty, T) + F(-X+2(n+1)L, n, \rho_\infty, T)\}, \quad (1)$$

where C_0 = constant,

$$F(y, n, \rho_\infty, T) = e^{-y^2/4T} \sum_{r=0}^{n} \frac{(-n)_r}{r!} (2\rho_\infty)^r (2T)^{\frac{1}{2}r} \psi(-(r+1), w),$$

$$w = 2^{\frac{1}{2}} \left(\rho_\infty T^{\frac{1}{2}} + \frac{y}{2T^{\frac{1}{2}}} \right),$$

$$(-n)_r = (-n)(-n+1)(-n+2) \ldots (-n+r-1).$$

The parabolic cylinder function was calculated from

$$\psi(0, x) = 1$$

$$\psi(-1, x) = (\tfrac{1}{2}\pi)^{\frac{1}{2}} e^{\frac{1}{2}x^2} \operatorname{erfc}(x/\sqrt{2})$$

and the recurrence relationship

$$\psi(-\nu-1, x) = -\frac{1}{\nu} \{x\psi(-\nu, x) - \psi(-\nu+1, x)\}.$$

Terms in the summation in eqn. (1) were ignored when they contributed less than 1% to the total. The results were checked against those of Rall (1969).

The response to a smooth time course of current injection simulating the synaptic current given by

$$I(T) = \alpha^2 T e^{-\alpha T}$$

was obtained by numerical convolution for α = 10, 20, 40, 80. The response transients were characterized by 10–90% rise time and half-width and fully checked against the results of Jack & Redman (1971).

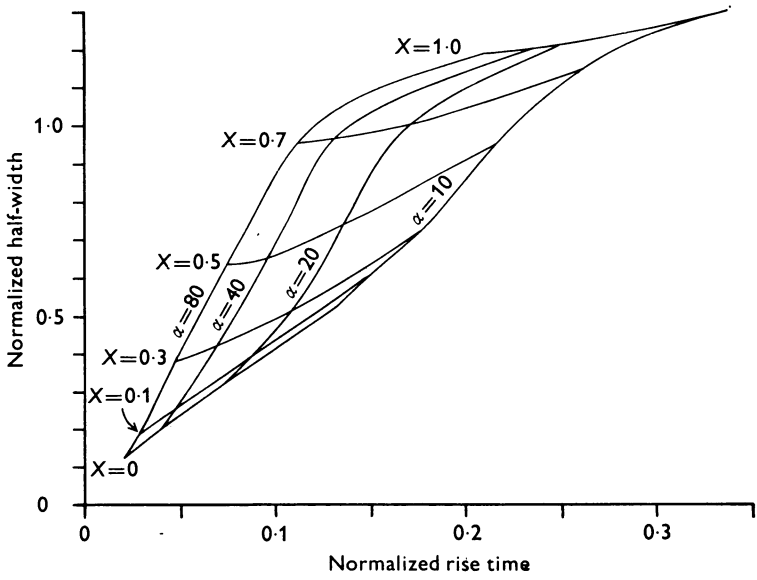
E.p.s.p.s generated at a single point

Since there were no electrophysiological measurements available for the motoneurones studied histologically, average values for the electrical parameters were chosen (τ = 5 msec, L = 1·4, ρ_∞ = 10). These values were obtained from the published results of several electrophysiological and combined anatomical studies (Nelson & Lux, 1970; Lux *et al.* 1970; Burke & Ten Bruggencate, 1970; Jack *et al.* 1971; Iansek & Redman, 1973*a*; Barrett & Crill, 1974).

Normalized half-widths and rise times for e.p.s.p.s generated by a single synaptic contact distance X from the model soma and with various synaptic current time course (α) are plotted in Text-fig. 10.

E.p.s.p.s generated by multiple synaptic contacts

The two synaptic structures chosen for modelling are illustrated in Text-fig. 11 (see also Pl. 1). These examples had boutons widely distributed on the motoneurone surface and were thus the most likely to differ in their behaviour from a single point location. The parameters of these structures which were incorporated in the model are given in Table 1. An additional variable included in the calculations was the conduction delay between current injection at each bouton. Conduction velocities from 0·1 to 0·5 m/sec as well as simultaneous current injection were studied.



Text-fig. 10. Normalized half-widths and normalized rise times of e.p.s.p.s generated by a single synaptic contact and recorded at the model motoneurone soma. Average motoneurone parameters are assumed ($\rho_\infty = 10$, $L = 1{\cdot}4$). Synaptic locations from somatic to 1·0 λ from the soma (X) and four different time courses of synaptic current (α) are illustrated. The e.p.s.p. shapes for intermediate values of X and α can be obtained by interpolation.

TABLE 1. Dimensions of two multiple synaptic structures incorporated in the motoneurone model

Synaptic structure 12

Bouton number	1	2	3
Distance from soma (X)	0·043	0	0
Distance between boutons (μm)	56·8	6·6	

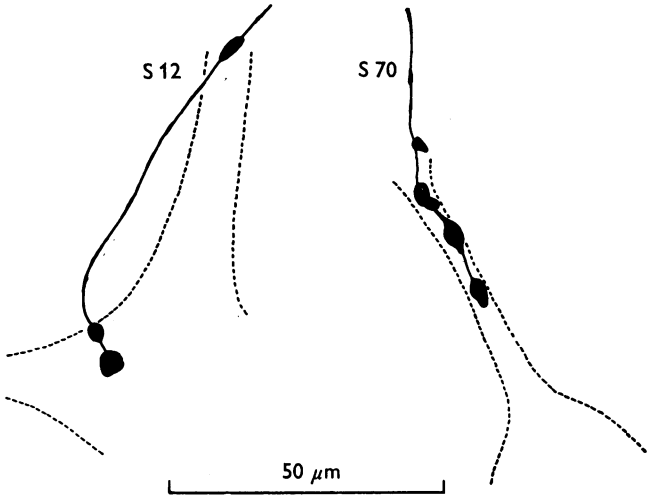
Synaptic structure 70

Bouton number	1	2	3	4
Distance from soma (X)	0·081	0·069	0·055	0·039
Distance between boutons (μm)	8·0	9·5	10·9	

Conduction delays were normalized by the average membrane time constant. It was assumed that the same amount of charge would be injected at each bouton. In all the synaptic structures examined the morphology of the afferent terminal indicated that conduction was towards the motoneurone soma. This arrangement would be expected to maximize e.p.s.p. amplitude at the soma (Rall, 1964).

The rise time and half-width of computed transients generated by these

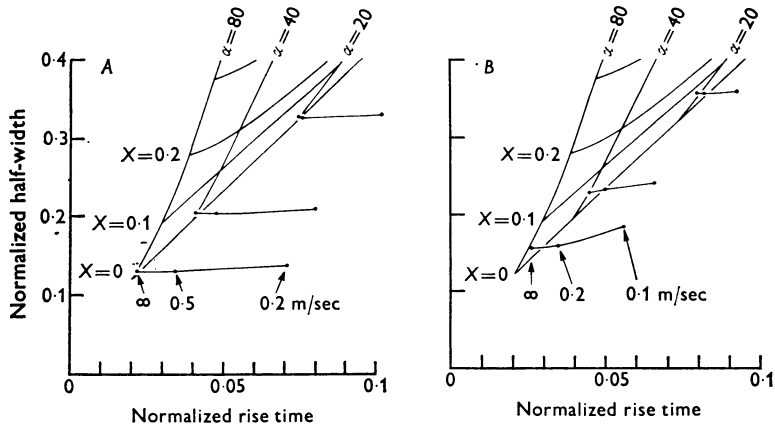
multiple structures with α = 20, 40, 80 were plotted on a diagram of the same form as Text-fig. 10. When current injection at each site was simultaneous the rise time and half-width combinations were indistinguishable (within computational error) from those expected for point current injection at the mean synaptic distance and with the same value of α (Text-



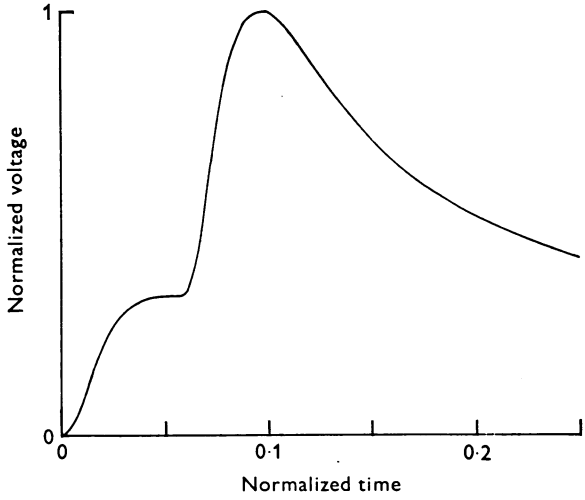
Text-fig. 11. Drawings of two multiple synaptic structures on proximal dendrites of motoneurones. Outlines of the motoneurones are indicated by interrupted lines. Synaptic structure S70 is also illustrated in Pl. 1.

fig. 12*A*, *B*). Introduction of a conduction delay, however, increased the rise time and produced rise time and half-width combinations which could not be accounted for by any single location (except by postulating a new function to describe the time course of synaptic current). The effect was most marked for the structure consisting of boutons on both the proximal dendrite and soma (S12). In the extreme case of brief synaptic current (α = 80) and slow afferent conduction velocity (0·2 m/sec) the modelled e.p.s.p. was clearly composite (Text-fig. 13). A few individual e.p.s.p.s with inflexions on the rising phase were found by Mendell & Henneman (1971), thus providing some evidence in favour of this analysis. The precise shape of the e.p.s.p. will depend upon bouton location and the conduction velocity of the afferent relative to electrotonic conduction in the motoneurone dendrite. Since the behaviour of synaptic stuctures with multiple contacts is so critically dependent upon the conduction velocity in the afferent terminal it is necessary to consider this point further.

The fine afferent terminals could not be accurately measured under the light microscope but were estimated to be considerably less than 1 μm in



Text-fig. 12. Normalized half-widths and normalized rise times of e.p.s.p.s generated in the motoneurone model by multiple synaptic structures based on S12 (*A*) and S70 (*B*). The half-width and rise time combinations for a single point location on the proximal dendrite are reproduced (enlarged) from Text-fig. 10. Half-widths and rise times for the e.p.s.p.s of the multiple structures are plotted for three different time courses of current injection (α = 20, 40, 80) and three afferent conduction velocities (normalized by a 5 msec time constant). Note that slow afferent conduction has a disproportionate effect on the e.p.s.p. rise time.



Text-fig. 13. Time course of the e.p.s.p. generated in the motoneurone model by a synaptic structure based on S12 with a brief time course of synaptic current injection (α = 80) and slow afferent conduction (0·2 m/sec).

diameter. Unfortunately the relationships between diameter and conduction velocity for both myelinated and unmyelinated small fibres are unclear (Jack, 1975). If the afferent terminals are unmyelinated then they have diameters comparable to the smallest C fibres in the vagus nerve (Keynes & Ritchie, 1965). The slowest conducting fibres recorded from the vagus nerve have conduction velocities of 0·5 m/sec (Iggo, 1958). Parallel fibres in the cerebellar cortex have diameters of about 0·2 μm (Palkovits, Magyar & Szentágothai, 1971) and conduct at 0·3 m/sec (Eccles, Llinás & Sasaki, 1966). If the loading effect of synaptic boutons (see *Morphology of synaptic structures*) is also considered then afferent conduction velocities of less than 1 m/sec in the terminal are plausible.

In the study of Iansek & Redman (1973*b*) three out of four e.p.s.p.s allocated to somatic synapses had excessively long rise times and could not be fitted to the motoneurone model. This is the type of deviation predicted by the present calculations. An alternative explanation would be a deficiency in the model itself (Iansek & Redman, 1973*a*, J. J. B. Jack and J. F. Iles unpublished). Either or both explanations could be correct. In some earlier experiments (Jack *et al.* 1971) motoneurone parameters were not separately estimated. By choosing a large value of ρ_∞ e.p.s.p.s with long rise times can be fitted to the model for a single synaptic location. However, these authors noted that some of the e.p.s.p.s which they classified as somatic could not be modelled by a single point of current injection even if ρ_∞ was increased to infinity. One possibility is that some of the e.p.s.p.s classified as somatic in these experiments were in fact generated by synaptic structures having more than one bouton on the proximal dendrite and possibly also the soma. If the proportion of aberrant 'somatic' e.p.s.p.s (0·75) found by Iansek & Redman (1973*b*) is taken as typical and the whole electrophysiological sample adjusted accordingly then a new ratio (number of somatic boutons)/(number of boutons on the first 0·2 λ of dendrite) can be calculated. The new value of 0·05 is very much closer to that obtained from ultrastructural studies (see *Synaptic location*).

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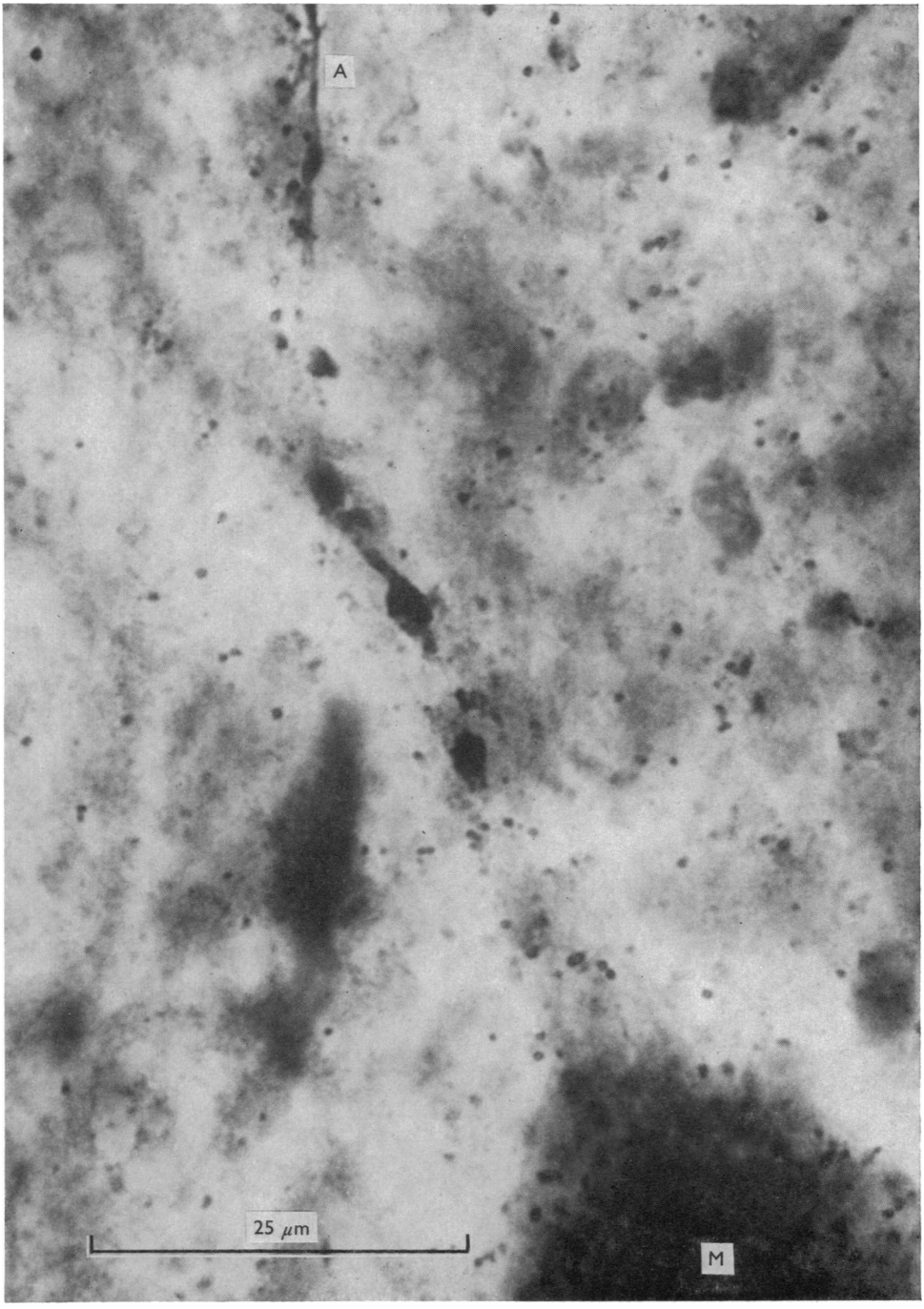

J. F. ILES

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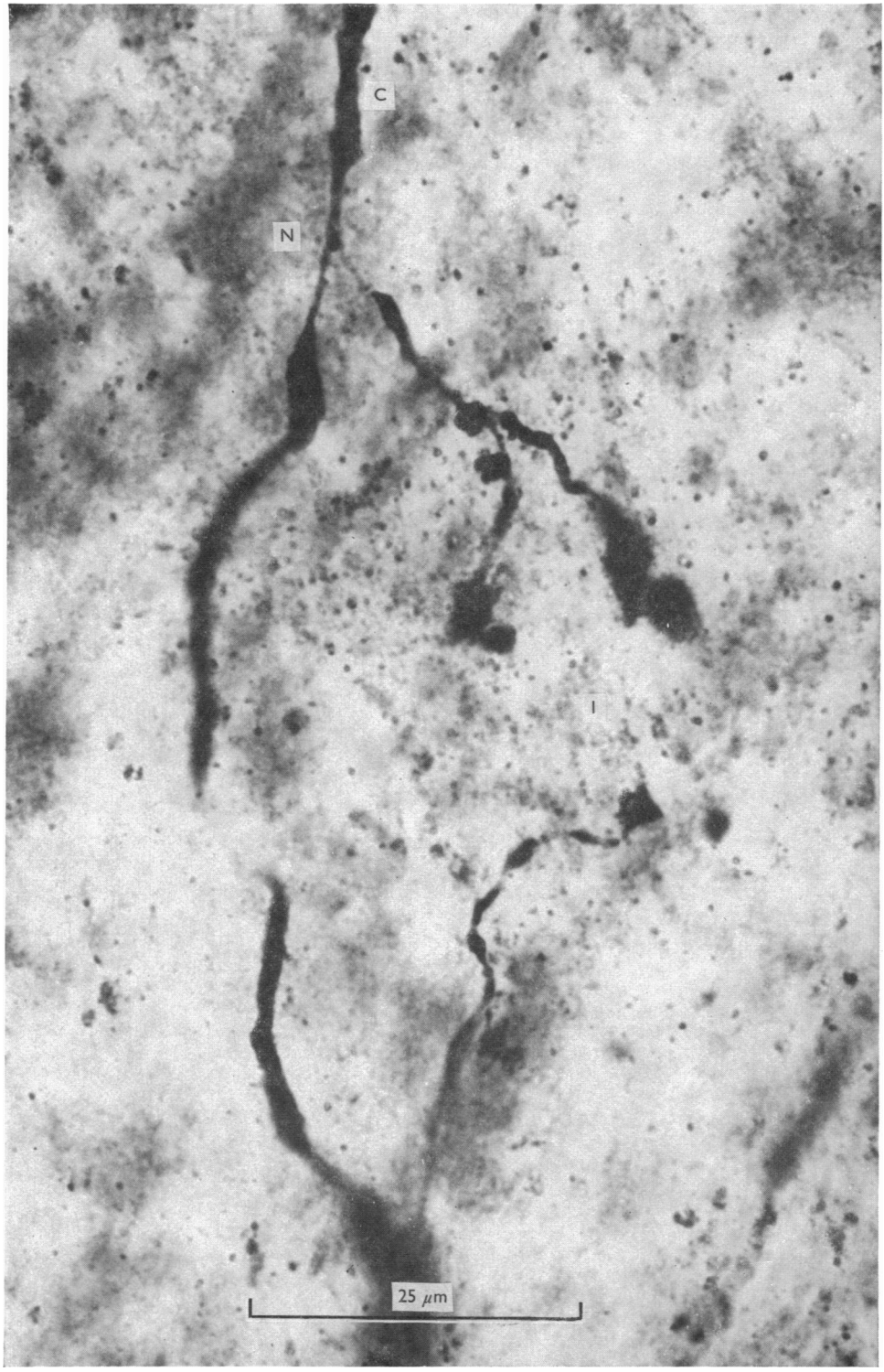

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EXPLANATION OF PLATES

PLATE 1

Cobalt-filled and intensified afferent axon fine branch (A) and synaptic structure consisting of four boutons making *en passant* contacts with a proximal dendrite of a motoneurone (M). Viewed with an oil-immersion objective (× 100) after counter-staining with thionine. This synaptic structure (S70) is also illustrated in Text-fig. 10.

PLATE 2

Extensive synaptic structure on a presumed 1a inhibitory interneurone (I). Two fine branches from the same major afferent collateral (C) form contacts on the neurone. The branch at the proximal node (N) is in focus. Viewed with an oil-immersion objective (× 100) without counter-staining. This synaptic structure (S31) is also illustrated in Text-fig. 7.