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TRACING OF FROG SENSORY–MOTOR SYNAPSES BY INTRACELLULAR INJECTION OF HORSERADISH PEROXIDASE

BY ROSEMARIE GRANTYN*, THE LATE A. I. SHAPOVALOV AND B. I. SHIRIAEV

From the Laboratory of Physiology of the Nerve Cell, Sechenov Institute, Leningrad, U.S.S.R.

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SUMMARY

1. Monosynaptically connected primary afferent fibres and motoneurones of the isolated spinal cord of the frog were injected with horseradish peroxidase (HRP). Six labelled afferent fibre–motoneurone pairs were reconstructed and subjected to detailed analysis.

2. Frog motoneurones possess eight to twelve dendritic arrays displaying some dorso-ventral asymmetry. Dorsal dendrites exhibit a rostro-caudal extent of 1·7–2·6 mm (average 2·2 mm).

3. Primary afferent fibres bifurcate in the dorsal funiculus. First-order collaterals emanate from the main ascending and descending branches, at an average distance of 407 μm. The average number of boutons per collateral is 670.

4. To reach a contacting bouton the presynaptic spike must pass on average five bifurcations and then zero to twelve boutons *en passant*, attached to a single terminal collateral branch. The structural equivalent of the axon cylinder of the collateral tree roughly preserves cross-sectional area. The branch power ranged between 1·15 and 3·35 (average 2·06).

5. Primary afferent fibres usually form clusters of contacting boutons (contact regions). Connexions between an afferent fibre and a motoneurone comprise from five to twenty-three contact regions (average 12·5). Each contact region contains one to twelve contacting boutons (average 3·3).

6. In two of three experiments contacting boutons were found to be significantly larger than non-contacting boutons. The average diameter of the former was 2·6 μm (range 1·2–4·0).

7. In five out of six cases more than one collateral belonging to the same fibre participated in the connexion with a given motoneurone.

8. The average number of contacting boutons per motoneurone and collateral is 19·1. It was estimated that each collateral could supply not more than thirty-five motoneurones. This would be less than 8·5 % of the motoneurones with their dendrites which cross the termination space of a single collateral. The average number of contacting boutons forming one primary motoneurone connexion was 41·5 (range 21–72).

* Present address: Carl Ludwig Institute of Physiology, 7010 Leipzig, G.D.R.

INTRODUCTION

Intracellular injection of horseradish peroxidase (HRP) has proved to be a powerful tool in studies of connexions established by individual neurones within the central nervous system. This approach was also successfully applied for the analysis of central terminations of Ia afferents in the cat spinal cord (Brown & Fyffe, 1978, 1981; Burke, Walmsley & Hodgson, 1979; Redman & Walmsley, 1981). However, the necessity to apply anaesthetics which may affect HRP transport (Turner, 1977) and the frequent appearance of pathological reactions in response to intracellular impalement and HRP injection (Cullheim & Kellerth, 1978) pose a number of difficulties with the mammalian spinal cord. Furthermore, the attempts to correlate the structural patterns and release characteristics of Ia synaptic connexions are hampered by the peculiarities of amplitude fluctuations of the unitary excitatory post-synaptic potentials (e.p.s.p.s) (Edwards, Redman & Walmsley, 1976; Redman, 1979), the uncertainties concerning the presynaptic invasion by the incoming volley (Edwards *et al.* 1976; Lücher, Ruenzel & Henneman, 1979) and the impairment of quantal release by anaesthetic drugs (Weakly, 1969).

In the amphibian spinal cord the synapses between the individual primary afferent fibres and lumbar motoneurones provide a useful means both for recording of unitary synaptic events (Shapovalov & Shiriaev, 1979) and for tracing the structural pattern with intracellular HRP injection (Motorina, Tamarova, Shapovalov & Shiriaev, 1982*a*). Such features of the amphibian spinal cord as the short distance between the dorsal root entry point and the target motoneurones, the ease of using an isolated preparation in the absence of anaesthetics and the low reactivity to intracellular HRP injection make tracing of interneuronal connexions especially reliable.

The muscle afferents establishing direct connexions with spinal motoneurones in the amphibian derive from muscle spindles, since they are selectively excited by muscle stretch (Shiriaev, 1983) and are homologous to the Ia afferents in mammals (Shapovalov, 1980). This provides a solid basis for comparison of sensory–motor synapses in the mammalian and amphibian cord.

The aim of the present study was the quantitative description of identified sensory–motor connexions in the frog and evaluation of any relationship between their structural pattern and functional properties. It was found that despite the complexity and distributed character of labelled synapses there was a fairly good correlation between the number of contacting boutons and the amplitude of single-fibre e.p.s.p.s. The present paper gives a detailed account of structural features of sensory–motor synapses, and a companion paper (Grantyn, Shapovalov & Shiriaev, 1984) accentuates the association between structural and release parameters of the labelled synapses. A preliminary account has been published elsewhere (Grantyn, Shapovalov & Shiriaev, 1982).

METHODS

The experiments were performed on isolated hemisected spinal cords of frogs *Rana ridibunda*. Full description of the methods used for preparation and simultaneous recording from single primary afferent fibres and motoneurones of the IXth and Xth lumbar segments has been published previously (Shapovalov & Shiriaev, 1980). In the present study preliminary surgery was performed

under ether anaesthesia. Then a 10 min transcardial perfusion with aerated Ringer solution was accomplished prior to laminectomy to wash out the blood from the intraspinal vessels. During this procedure and laminectomy the animals were anaesthetized by cooling on crushed ice. The transcardial perfusion did not reduce viability of the isolated cord, as could be judged by the amplitude of the ventral root response to dorsal root stimulation.

Electrophysiological identification of synaptic connexions. Primary afferent fibres were impaled at the dorsal root entry point, usually at a distance of 2–3 mm from the tip of a second micro-electrode introduced into a motoneurone. Application of brief intracellular current pulses (0·2–0·5 ms, 3–5 nA) evoked an action potential in the impaled afferent fibre and, eventually, a two-component monosynaptic e.p.s.p. in the target motoneurone. For subsequent statistical analysis of amplitude fluctuations 180–430 individual responses were recorded (see Grantyn *et al.* 1984).

Intracellular HRP injection. Micro-electrodes filled with 10–15% HRP in 0·5 M-KCl, 0·05 M-Tris buffer at pH 7·6 were used to inject both interconnected elements. For HRP ionophoresis in the afferent fibre, depolarizing current pulses of 600 ms and 10–15 nA were applied at 1/s for 20–30 min. Motoneurones were injected with lower current intensities ($\leqslant$ 10 nA), for shorter periods of time. The amount of HRP in the motoneurone was sufficient for complete filling of the dendritic tree, while labelling of the recurrent collaterals remained incomplete. Stronger staining of the afferent fibre and relatively weak staining of the motoneurone was advantageous for reconstruction of the connexion; superposition of the recurrent collateral system could be avoided and identification of contacts on more proximal parts of the motoneurones was much easier on the background of comparatively light staining.

Fixation and HRP histochemistry. After HRP injection spinal cords were left for 4–10 h under the same conditions as were adopted for electrophysiological experiments (15–17 °C) and then placed for 30 min into 3% glutaraldehyde in phosphate buffer (pH 7·6) at 5 °C. This was followed by a post-fixation in a mixture of 1% paraformaldehyde and 1·25% glutaraldehyde in phosphate buffer for 4–6 h. Several passages through 30–10% sucrose in phosphate buffer reduced tissue damage during section. The spinal cords were cut on a freezing microtome 24–48 h after HRP injection. Sagittal sections of 60 μm thickness were found to be most convenient both for histological procedures and reconstruction. HRP was demonstrated by the cobalt chloride–diaminobenzidine–glucose oxidase method ($CoCl_2$–DAB–GOD) (Itoh, Konishi, Nomura, Mizuno, Nakamura & Sugimoto, 1979). Sections were kept in the $CoCl_2$–DAB–GOD medium for 2–3 h at 38 °C. The $CoCl_2$–DAB–GOD method appeared to be more sensitive in demonstrating axon terminals than the $CoCl_2$–DAB–H_2O_2 method (Adams, 1977). Pre-incubation in catalase (20 mg/500 ml Tris buffer, pH 7·4, for 15 min) suppressed endogenous activity without much reduction of neuronal stain. Finally, sections were mounted, dried, dehydrated and counter-stained with Carmine Red or Toluidine Blue.

Material. A sample of six primary afferent–motoneurone pairs from six different animals was chosen for detailed morphological and statistical analysis. Data from another twelve experiments were used for evaluation of some minor points taken up in the Results or Discussion.

Labelling of neuronal elements by uptake of HRP leaked into the extracellular space as a result of unfortunate micro-electrode positioning or multiple penetrations has been carefully excluded. It should also be noted that the present experiments did not contribute any evidence in favour of transneuronal transport of HRP at sensory–motor synapses, although such transport was noted in the case of certain other classes of primary afferents synapsing on interneurones both in the cat (Hongo, Kudo, Yamashita, Ishizuka & Mannen, 1981) and in the frog (Motorina, Tamarova, Shapovalov & Shiriaev, 1982*b*).

Estimation of morphologic parameters and nomenclature. Reconstruction of motoneurones and afferent fibres was performed at low power magnification ($\times$400), using a camera lucida drawing tube. Size measurements and identification of contacts were accomplished with oil immersion ($\times$2000).

The afferent fibre within the dorsal root will be called the *main axon*. After entering the spinal cord all main axons bifurcate into ascending and descending *main branches*, which in turn split into first, second, third, etc. collaterals. Axonal swellings were considered as *boutons* if the diameter of the swellings exceeded that of the non-enlarged portion of the terminal by at least three times. The criteria adopted for identification of presumed contacts (contacting boutons) follow closely those of Brown & Fyffe (1981): (1) only appositions formed by boutons have been included in the present count of contacts between primary afferents and motoneurones, although recent electron microscopic

studies in the cat demonstrated synaptic specializations also between a motoneuronal dendrite and the non-enlarged part of a terminal collateral branch (Spencer, Evinger & Baker, 1982); (2) all contacting boutons were traced back into the main branch; (3) examination under oil immersion excluded the presence of a gap between the apposed elements. This evaluation procedure tends to underestimate the number of contacts.

Clusters of boutons contacting the motoneurone in close proximity to each other have been classified as separate *contact regions*.

RESULTS

HRP morphology of frog spinal motoneurones. In contrast to the well-known isodendritic geometry of cat lumbar motoneurones (Aitken & Bridger, 1961; Gelfan, Kao & Ruchkin, 1970; Egger, Freeman & Proshansky, 1980; Brown & Fyffe, 1981; Rose, 1981), dendrites of frog spinal motoneurones display a higher degree of individuality (Stensaas & Stensaas, 1971; Szekely, 1976; Bregman & Cruce, 1980). In the sagittal sections used for the present study, dorso-ventral differences of the dendritic tree, especially differences in segment length between bifurcations of dorsal and ventral dendrites, were easily noticeable. Asymmetries of this kind were found to be more pronounced in motoneurones with a more ventral position in the soma layer.

Of special interest was the total rostro-caudal span of dorsal dendrites, which were found to be the main target of primary afferent input to the frog motoneurones (see below). Measurements on six HRP-labelled motoneurones gave an average rostro-caudal span of 2·2 mm (range 1·7–2·6 mm). This is more than estimated in previous Golgi (Stensaas & Stensaas, 1971) and cobalt studies (Szekely, 1976).

It was a common finding that lumbar motoneurones bear spines (see also Motorina *et al.* 1982*a*). Spines occur on both dorsal and ventral dendrites. They are separated by distances of about 1·5–20 μm. As in cat motoneurones (Brown & Fyffe, 1981), spines are carried on short stalks.

In the cat, the existence of motor axon collaterals and their terminations within the motor nuclei is now a well-established fact (Cullheim & Kellerth, 1978; Evinger, Baker & McCrea, 1979). A study with cobalt chloride filling of the ventral roots failed to demonstrate the presence of recurrent axon collaterals in the frog (Szekely, 1976) but they were found after intracellular injection of HRP into motoneurones (Motorina *et al.* 1982*a*). In the present material, which includes a number of motoneurones with relatively light stain (see Methods), recurrent collaterals and their extensive branching in the ventral horn have been observed in four out of six cases suitable for analysis (see Pl. 1 *A*, *C*). The boutons given off by recurrent axon collaterals appeared to be smaller than those of primary afferent fibres (compare *C* and *D*, Pl. 1).

Trajectory and ramification pattern of primary afferent fibres. The general organization of primary afferent fibre arborization is presented in Fig. 1. After entering the dorsal funiculus through the dorsal roots of the IXth and Xth segments the main axon (average diameter 5·1 μm) bifurcates into ascending and descending main branches. The diameter of the ascending main branch (average 3·9 μm, range 3·2–4·5 μm) was only slightly larger than the diameter of the descending one (average 3·4 μm, range 2·6–4·0 μm). In different experiments, diffusion of HRP covered between 4 and 5 mm

of the main branches if the injection site in the dorsal root was sufficiently close (*ca.* 1 mm) to the spinal cord surface. Between nine and thirteen collaterals were stained in these cases. Apparent completeness of HRP labelling was achieved only for three to five collaterals beneath the corresponding dorsal root.

Diameters of first-order collaterals ranged between 2·1 and 3·8 μm (average 2·6 μm, $n = 27$). As in the cat, collaterals issued by the ascending main branch tended to tilt

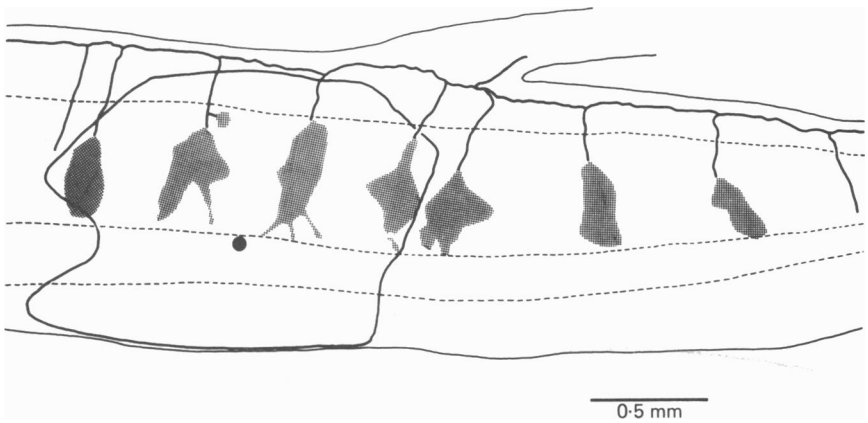

Fig. 1. Sagittal reconstruction of a primary afferent fibre in the frog spinal cord (connexion 5). Profiles of collateral arbors are shown by shading. The location of a motoneurone contacted by this fibre is indicated by filled circle (soma) and continuous line (maximal dendritic extents). The dorsal and ventral limits of grey matter and soma layer are projected with dotted lines

rostrally, especially those just below the dorsal root entry zone. Distances between collateral origins range from 136 to 814 μm (average 407 μm, S.D. = 167 μm, $n = 51$). The sagittal projection of the termination space made up by the collateral arbor usually becomes larger from dorsal to ventral (Figs. 1 and 2). It leaves, however, noticeable gaps even at the most ventral levels.

In contrast to previous studies (Liu & Chambers, 1957; Joseph & Whitlock, 1968) the present material supports the recent evidence (Szekely, 1976; Frank & Westerfield, 1982; Motorina *et al.* 1982*a*) that juxtasomatic dendrites and even the somas of frog motoneurones are within reach of the primary afferent collaterals (Fig. 4). The latter did not usually penetrate the soma layer, but single terminal collateral branches reached far out ventrally to touch the dorsal margin of the motor column (Fig. 2). Such an arrangement suggests that somas of ventrally located motoneurones and ventral dendrites might be free from contacts with primary afferent fibres.

Branching pattern of primary afferent collaterals and number of boutons per terminal collateral branch. A representative collateral (Fig. 2, collateral 1; Pl. 2*A*, *B*) from the experiment illustrated also in Fig. 1 was chosen for a schematic presentation of the arborization pattern. Fig. 3 gives information about branch orders, length and diameters of collateral segments and the location of boutons. Unfortunately, it was not possible to determine with certainty which branches were myelinated and which were not.

Although we cannot, at present, judge the complexities introduced by the pattern

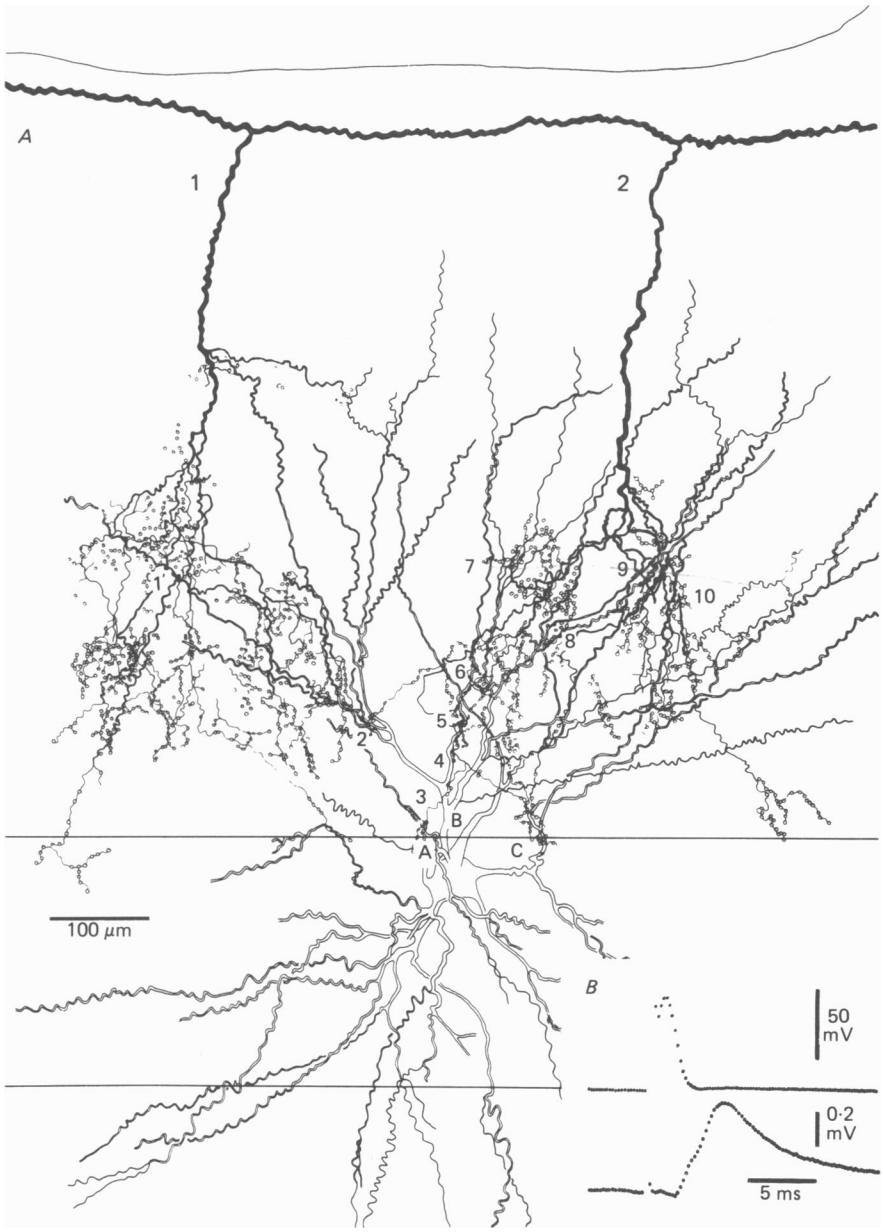

Fig. 2. Sagittal reconstruction (*A*) and averaged records of the presynaptic spike and the e.p.s.p. (*B*) for a primary afferent–motoneurone connexion (connexion 5). Dorsal surface of spinal cord and limits of soma layer are indicated by continuous lines. Small numbers denote contact regions; large numbers, collateral numbers; and letters, dendritic systems.

of nodes of Ranvier and the properties of the myelin sheath, it may be noted that bifurcations of primary afferent collaterals roughly obey a square rule, that is, they conserve cross-sectional area. The branch power (Rall, 1959; Hillman, 1979) ranged from 1·15 to 3·35. The average was 2·06 (S.D. = 0·55, $n = 56$, data from three

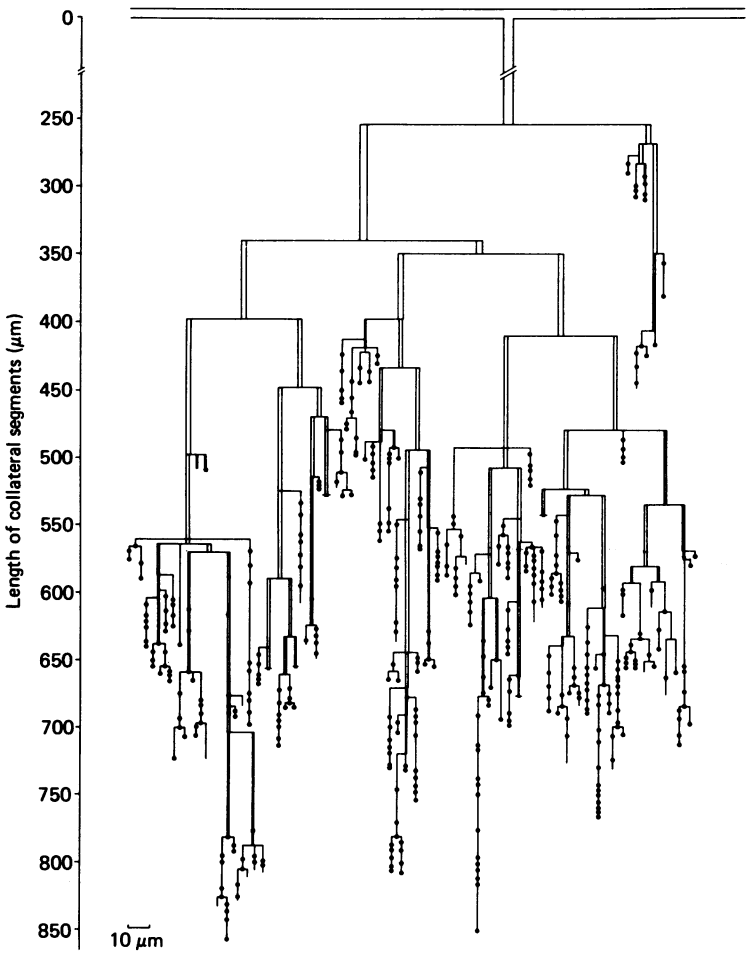

Fig. 3. Schematic presentation of a primary afferent collateral (connexion 5, collateral 1). Segment length and diameters are given according to scales on the left. Location of boutons is shown by dots.

experiments, eight collaterals). Geometrical peculiarities at branch points may come into play under low safety-factor conditions and then influence, in a rather variable fashion, the efficacy of synaptic transmission at contacts established by different branches of the collateral tree (Waxman, 1975).

Plate 2*A* shows a photomicrograph of a terminal branch of collateral 1, Figs. 2 and 3. It can be seen that the majority of boutons were of the *en passant* type. One to thirteen boutons were attached to a single terminal branch. The average number of boutons per terminal collateral branch was 3·2 for collateral 1, Figs 2 and 3.

Number of boutons issued by a single primary afferent collateral. Counting the number of boutons that emerge from a single primary afferent collateral might be useful, since this estimate gives an idea of the fraction of motoneurones that could, potentially, be contacted by a single collateral. In the experiment of Fig. 2, the number of boutons

per collateral ranged from 732 to 781. An average estimate derived from counts of eight collaterals from four experiments is 670.

The number of motoneurones with dendrites which cross the terminal space of a collateral was determined based on the average rostro-caudal span of the dorsal dendritic arbor (2·2 mm, see above), and the average number of motoneurones in the soma column of Nissl-stained sagittal sections from six spinal cords. It was found that

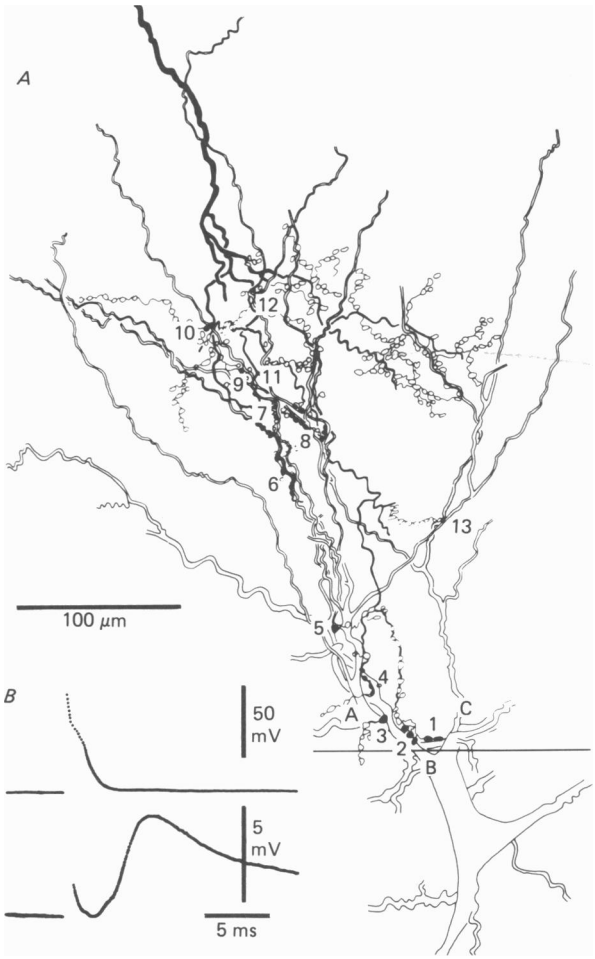

Fig. 4. Sagittal reconstruction (*A*) and averaged records (*B*) for a primary afferent–motoneurone connexion (connexion 4). The horizontal continuous line indicates the dorsal limit of the soma layer.

a column of 2·2 mm length contained on average 412 motoneurones. Ignoring the rostro-caudal spread of the collateral arbor, it may be conjectured that a single collateral with its 670 boutons can contact at maximum 412 motoneurones. Provided that all boutons were destined for motoneurones only, the average number of boutons per collateral and motoneurone would be 1·65.

However, it will be shown below that a single stained collateral contacts a single stained motoneurone on average with 19·1 boutons. That is, a single collateral

contacts as in cats (see Brown, 1981) less than thirty-five motoneurones. If some of the primary afferent terminal branches have other targets apart from motoneurones, it can be concluded that only a relatively small fraction (less than 8·5%) of the motoneurones crossing the termination space of a collateral will actually be contacted.

Contacts between primary afferent collaterals and motoneurones. Whenever direct stimulation of a primary afferent fibre evoked a monosynaptic e.p.s.p. in a motoneurone, subsequent adequate staining revealed contacts between both elements. It was found that one motoneurone established connexions with one, two or three collaterals (Fig. 2, also see Table 1, Grantyn *et al.* 1984).

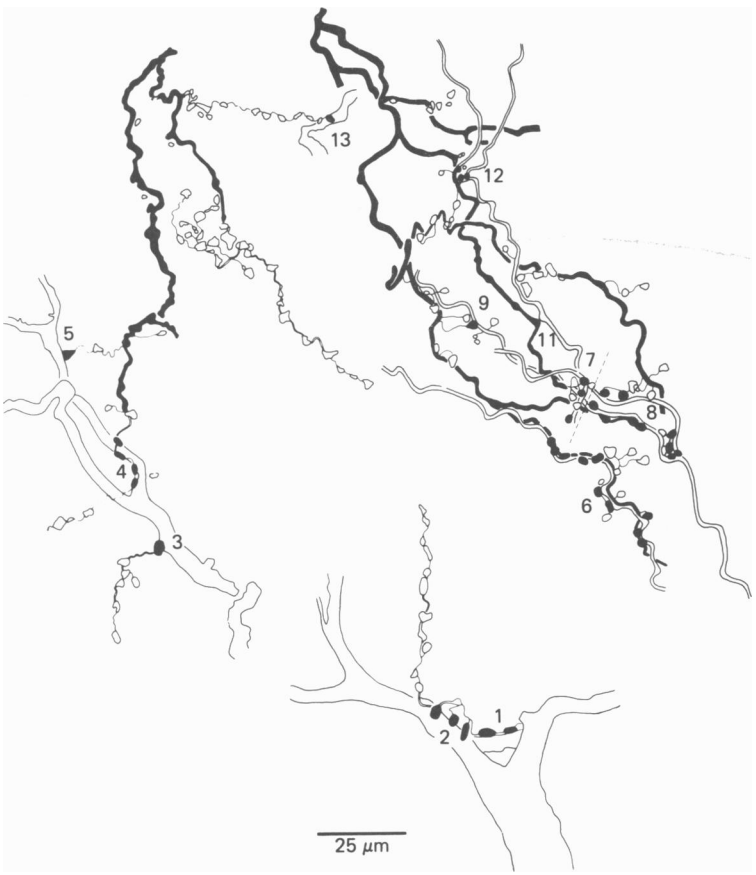

Fig. 5. Arrangement of contacts between a primary afferent collateral and a motoneurone (connexion 4). Numbers refer to contact regions as denoted in Fig. 4.

Plate 3 shows boutons in contact with proximal (*A*, *B*) and distal (*C*, *D*) portions of motoneuronal dendrites. All contacts were also drawn under oil immersion as illustrated by Figs. 5 and 6. Numbers refer to contact regions of the reconstructed dendritic trees (Figs. 4, 9 and 10).

The most salient feature in the arrangement of primary afferent–motoneuronal connexions is the pronounced clustering of contacting boutons. Chains of four to

twelve contacting boutons attached to a single terminal collateral branch have been quite common (see Fig. 5, site 6; Fig. 6, sites 8 and 9). This is in keeping with the relatively high number of boutons given off by a single terminal branch (see Fig. 3). Such a terminal collateral branch bearing a large number of boutons *en passant* is often restricted to a single dendritic segment. Apart from this 'climbing type' contact region there also exists a relatively large number of contact regions of the 'point type' (see Fig. 6, sites 2, 3 and 20). The latter type was usually formed by terminal boutons. The average number of contacting boutons per contact region was 3·3 for all six connexions.

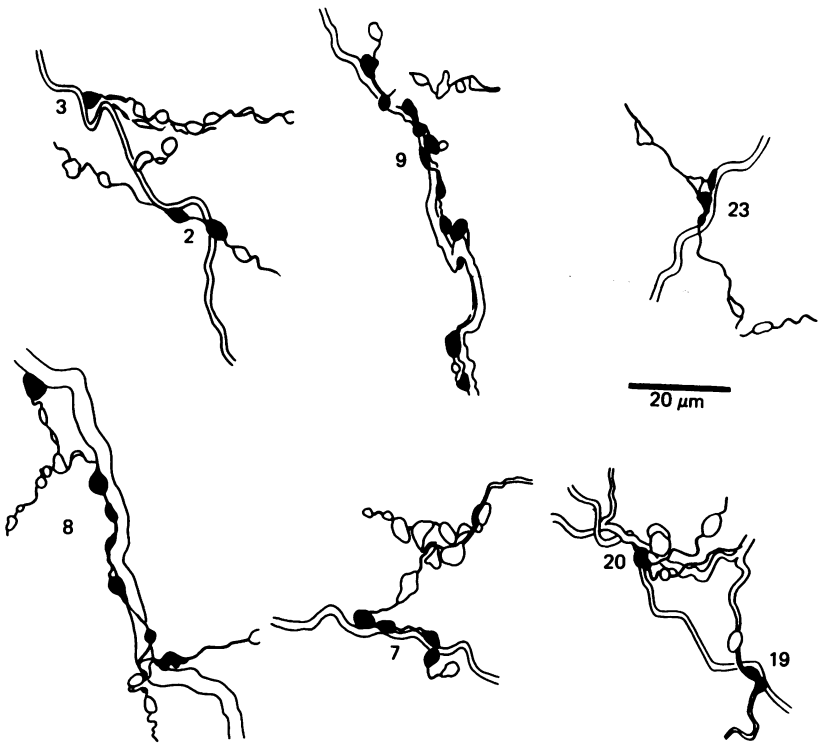

Fig. 6. Arrangement of contacts between a primary afferent collateral and motoneuronal dendrites (connexion 3). Numbers refer to contact regions as denoted in Figs. 9 and 10.

Fig. 7 gives the distribution of bouton size (mean of maximal bouton diameter and the diameter perpendicular to it). Data were derived from two different experiments (see legend to Fig. 7). It can be seen that contacting boutons (crosses) tend to belong to a class of large size boutons within the sample of non-contacting boutons of the same collateral (filled circles). In the two cases of Fig. 7 diameters of contacting and non-contacting boutons differed significantly ($t = 5{\cdot}067$, $P < 0{\cdot}001$ for case A and $t = 6{\cdot}52$, $P < 0{\cdot}001$ for case B) by Student's t test. The corresponding average diameters and standard deviations for contacting and non-contacting boutons were 2·65 μm (S.D. = 0·5) and 2·2 μm (S.D. = 0·7 μm) for the experiment of Fig. 7*A*, and 2·7 μm (S.D. = 0·55 μm) and 2·0 μm (S.D. = 0·65 μm) for the experiment of Fig. 7*B*. In a third case (Fig. 5, size distribution not shown) the corresponding average values

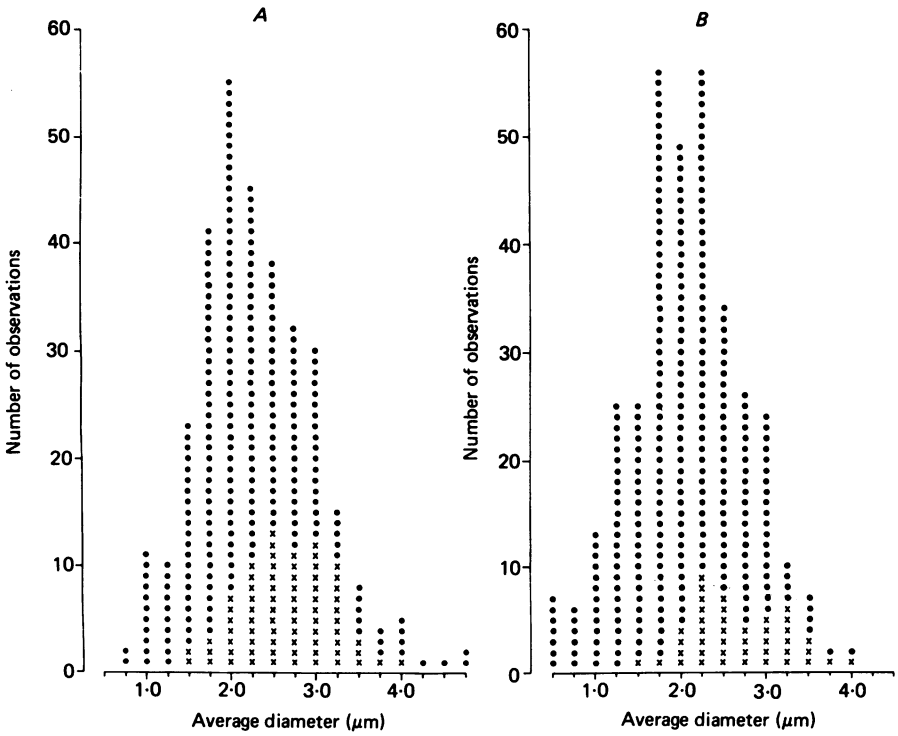

Fig. 7. Histograms of the average diameters of contacting (crosses) and non-contacting (filled circles) boutons of a primary afferent fibre. *A* connexion 3, *B* connexion 5.

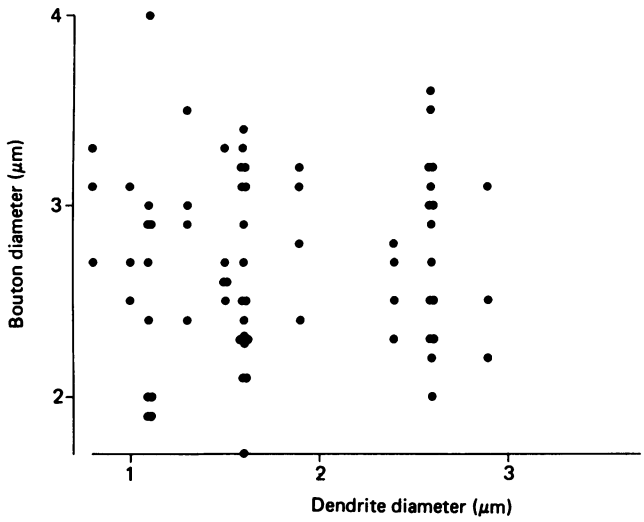

Fig. 8. Relation between average diameters of contacting boutons (ordinate) and diameter of the underlying dendritic segment (abscissa). Connexion 3.

and standard deviations were 2·45 μm (S.D. = 0·4 μm) and 2·25 μm (S.D. = 0·75 μm). This difference was statistically not significant.

Fig. 8 shows that there is no correlation between the size of contacting boutons and the diameter of underlying dendrites.

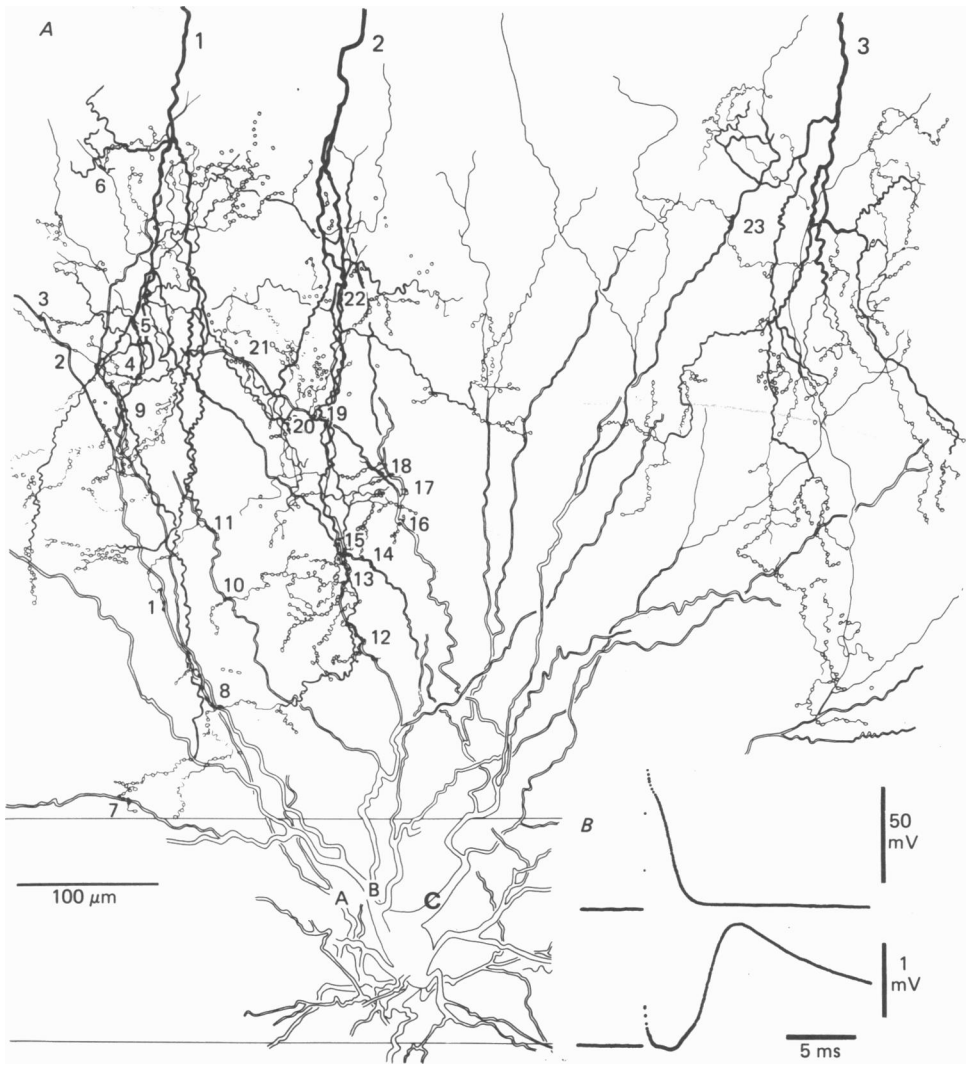

Fig. 9. Sagittal reconstruction (*A*) and records (*B*) for a primary afferent–motoneurone connexion (connexion 3). See legend to Fig. 2 for further explanations.

Figs. 9 and 10 illustrate the location of contracting boutons on the motoneuronal dendritic tree in connexion 3 and indicate their relationship to the collateral arbor (Fig. 10). In this experiment contacts were found at geometrical distances between 200 and 625 μm from the soma. In cases where the motoneuronal soma was more dorsal, contacts were found also at juxtasomatic sites (Fig. 4, Pl. 3*A*, *B*). The most

peripheral portions of the dorsal dendritic arbor remained free of contacting boutons from primary afferents. No contact was found on dendritic spines.

The schematic presentation of the connexion shown in Fig. 9 also gives an impression of the position of contacting boutons on the collateral arbor. It may be seen that the propagating spike must pass on average five bifurcations of the collateral tree to reach the contacting bouton (range: two or nine bifurcations).

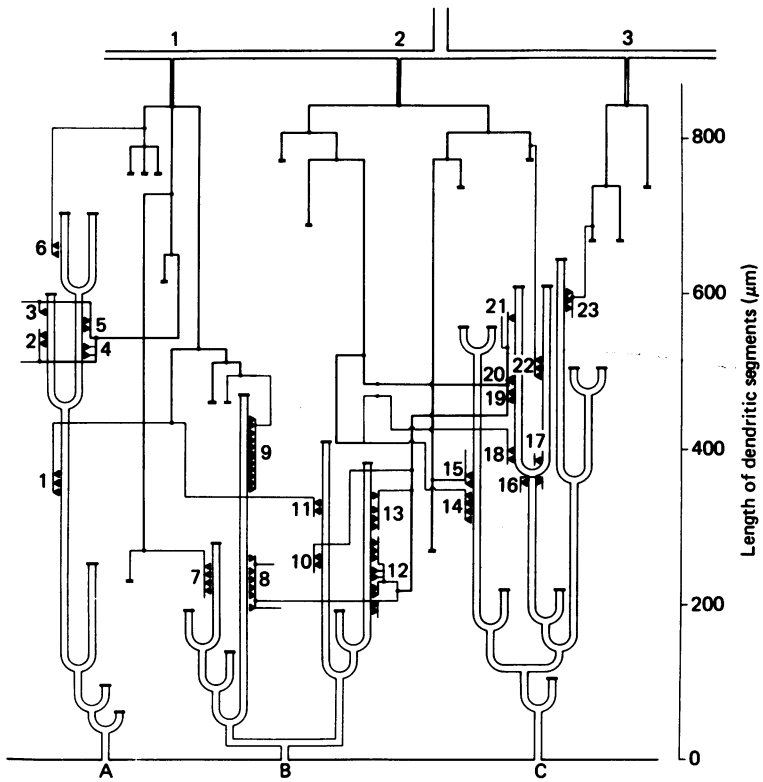

Fig. 10. 'Wiring diagram' of a primary afferent–motoneurone connexion (connexion 3). Contacting boutons are indicated by triangles. Scale on the right gives the length of dendritic segments. Zero corresponds to the dendritic origin at soma. Segmental length of the afferent fibre and diameters of collateral and dendritic branches are not drawn to scale.

Correspondingly, contacts were formed by fourth- to tenth-order collateral branches. After having reached the terminal collateral branch the presynaptic spike must pass on average two boutons *en passant* to get to the most distal contacting bouton. Again, there is a large range: for different contact regions this estimate could vary between zero and ten to twelve boutons *en passant*.

Number of contact regions and number of contacting boutons. It was mentioned above that the afferent terminals tended to form clusters of boutons, most frequently in a climbing type of arrangement. Since the number of contact regions turned out to be a useful parameter (see companion paper), care was taken to establish appropriate criteria for defining contact regions as separate entities (see Methods). The number

of contact regions per motoneurone ranged between five and twenty-three in the six cases analysed so far. The average value is 12·5.

The number of contacting boutons per motoneurone was in all cases considerably higher than in the Ia motoneuronal connexion of the cat (Burke *et al.* 1979; Redman & Walmsley, 1981; Brown & Fyffe, 1981) although the criteria for identification of contacting boutons have been equally strict (see Methods). The number of contacts could even be higher if synaptic specializations exist also between the non-enlarged part of the axon and a motoneuronal dendrite (Spencer *et al.* 1982). Plate 2*D* shows a relatively thick collateral branch that seems to fuse over a certain distance with a dendritic segment.

With the limitation that only contacts established by boutons have been considered in the present count, we found between twenty-one and seventy-two separate contacts forming the connexion between a single afferent fibre and one motoneurone. The average was 41·5. The average number of contacting boutons per collateral and motoneurone was 19·1.

An obvious correlation was found between the size of the e.p.s.p. and the number of contacting boutons (see Grantyn *et al.* 1984).

DISCUSSION

In addition to their usefulness in studies of transmissional processes (Shapovalov & Shiriaev, 1979, 1980), frog's sensory–motor connexions turned out to be well suited for studying synaptic morphology and the relationship between structural and functional characteristics of a central excitatory synapse. In contrast to a widely accepted notion that axons in the central nervous system usually make only one or two synaptic contacts on a given neurone (cf. Stevens, 1979) it was found that sensory–motor connexions involve, as a rule, several tens of synaptic contacts. These contacts were widely distributed along the post-synaptic cell and there is clear evidence that presynaptic collaterals may produce an effect at many electrotonic distances from the soma. Thus, even with the single connexion it appears very difficult to calculate accurately the synaptic location from classical analysis using the time course of an averaged e.p.s.p.

HRP labelling of presynaptic terminals in the frog spinal cord has revealed a structural pattern consisting of numerous boutons and varicosities connected by very thin fibres which might suggest a low safety factor for spike propagation, especially at branch points. However, the existence of the stable (non-fluctuating) electrotonic component of the e.p.s.p. suggests that an action potential propagates reliably along the presynaptic terminals (Shapovalov & Shiriaev, 1980). Ultrastructural observations suggest that boutons in the frog spinal cord, which probably correspond to terminals of the monosynaptic reflex pathway, contain structural specialization characteristic of mixed synapses (Sotelo & Grofova, 1976). A recent electron-microscopic study with HRP injection into dorsal roots (Adanina & Shapovalov, 1983) has shown that in fact synapses combining both an active zone and a gap junction derive from primary afferents.

The general organization of primary afferent fibres found in the frog spinal cord

TABLE 1. Parameter estimates for the sensory–motor connexions in the cat and frog

Parameter	Cat	Frog
Spacing of collaterals (μm)	1062 (100–300)[2, 4]	407 (135–815)
Diameter of collaterals emerging from the main ascending branch	2·4 (1·5–3·0)[4]	2·6 (2·1–3·8)
Number of boutons per collateral	M. soleus: 184[4] M. gastrocn. med.: 264[4]	670 (515–757)
Number of boutons per terminal collateral branch	1·73 (1–7)[4]	3·2 (1·13)
Average bouton diameter (μm)	3·4 (2·5–5·3)[2]	2·13 (0·8–4·6)
Number of collaterals participating in one connexion	1[3, 6]	2·17 (1–3)
Number of contacting boutons per collateral and motoneurone	3·6 (2–6)[3, 6]	19·1 (10·5–43)
Total number of contacting boutons per motoneurone	3·6 (2–6)[3, 6]	41·5 (21–72)
Number of clusters (contact regions) per motoneurone	2·1 (1–4)[3, 6]	12·7 (5–23)
Number of contacting boutons per cluster (contact region)	1·7 (1–3)[3, 6]	3·3 (1–12)
Average diameter of contacting boutons (μm)	—	2·6 (1·2–4·0)
Amplitude of single-fibre e.p.s.p. (chemical component, μV)	137 (30–450)[1]	222 (20–1700)[5]

[1] Mendell & Weiner, 1976; [2] Brown & Fyffe, 1978; [3] Burke *et al.* 1979; [4] Ishizuka *et al.* 1979; [5] Shapovalov & Shiriaev, 1980; [6] Brown & Fyffe, 1981.

is in many respects similar to the intraspinal projections of homologous Ia fibres in the cat lumbar cord (Iles, 1976; Brown & Fyffe, 1978, 1981; Burke *et al.* 1979; Ishizuka, Mannen, Hongo & Sasaki, 1979). However, certain quantitative differences may be noted.

Contrasting the average estimates for collateral spacing and the number of boutons per collateral in cat and frog (Table 1), it becomes clear that the projection of a single primary afferent in the frog spinal cord is much denser, in spite of the fact that the main axons and the main ascending and descending branches were of smaller calibre than in the cat (Ishizuka *et al.* 1979). This high density of primary afferent input is matched by an equally high density of motoneuronal packing. The number of motoneurones contacted by a single collateral may be therefore the same as in the cat (Brown, 1981). Our calculations indicated a value of about thirty-five motoneurones per collateral. It was shown electrophysiologically that a single fibre excites several different motoneurones (Shapovalov & Shiriaev, 1980) and on the other hand every

motoneurone may be innervated by at least ten to twenty individual dorsal root fibres.

In the frog, usually two to three collaterals synapse with the same motoneurone. In contrast, in the cat each motoneurone is reported to establish contacts with only one primary collateral, even though its dendritic tree crosses the termination space of several collaterals of the same Ia afferent (Brown, 1981). As might be expected such different structural organization is correlated with differences in function. For example, the stretch reflex is thought to be poorly developed in amphibians (Bremer & Moldaver, 1934; Mashima, 1955; Cruce, 1974). However, recently Frank & Westerfield (1982) have shown that the monosynaptic projection from triceps muscle afferents to brachial motoneurones shares many of the characteristic features of the sensory–motor pathway in the cat. The absence of overt stretch reflexes in the frog may be a function of the state of excitability of the motoneurones rather than an absence of significant and specific input from muscle spindles.

Not only the sensory–motor connexion as a whole but also the organization of individual contacts appear to be more complex in the frog than in the cat (Table 1). On average, many more boutons tend to form clusters or contact regions which may be interpreted as functional subunits (see Grantyn *et al.* 1984). This coincides with the fact that in the frog more boutons are found to be attached to a single terminal branch. This difference may be a specific difference in structural patterns of sensory–motor synapse in mammals and amphibians, but it may also reflect the better conditions for complete HRP labelling of contacts in the isolated frog spinal cord.

In spite of the striking complexity which characterizes the primary afferent fibre–motoneurone connexion in the frog it is remarkable that a correlation may be found between the number of contacts and the amplitude of e.p.s.p. generated at that connexion. This justifies further analysis of the structural–functional relationship at sensory–motor synapses presented in the following paper (Grantyn *et al.* 1984).

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EXPLANATION OF PLATES

PLATE 1

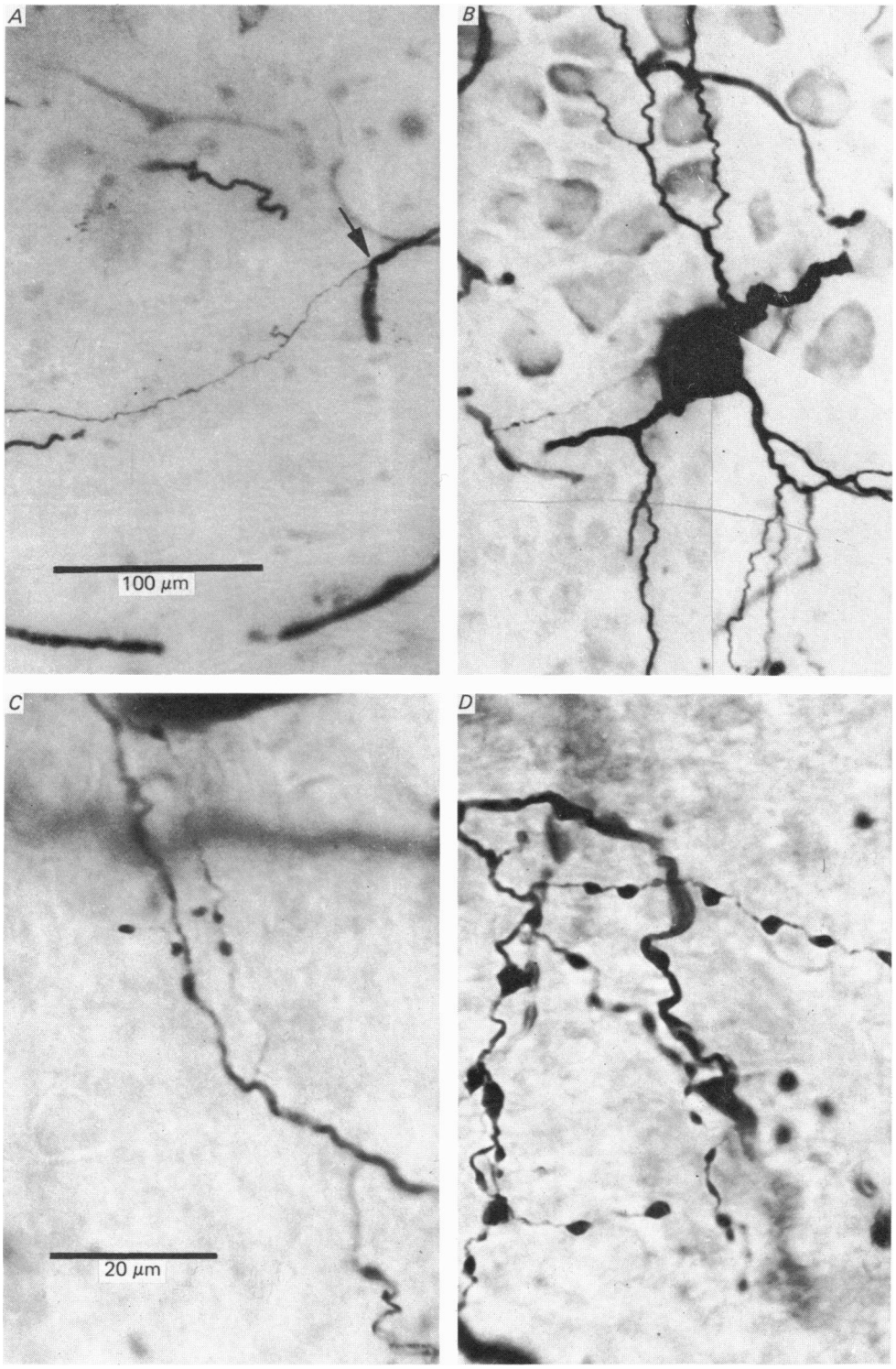
A, recurrent collateral emerging from the main axon of the motoneurone shown in *B*. *C*, collateral branches and boutons given off by the recurrent collateral shown in *A*. Note the smaller size of boutons as compared to the boutons carried by a primary afferent collateral of the same preparation (*D*). Scales in *A* and *C* apply also to *B* and *D*, respectively. All photomicrographs were taken from connexion 2.

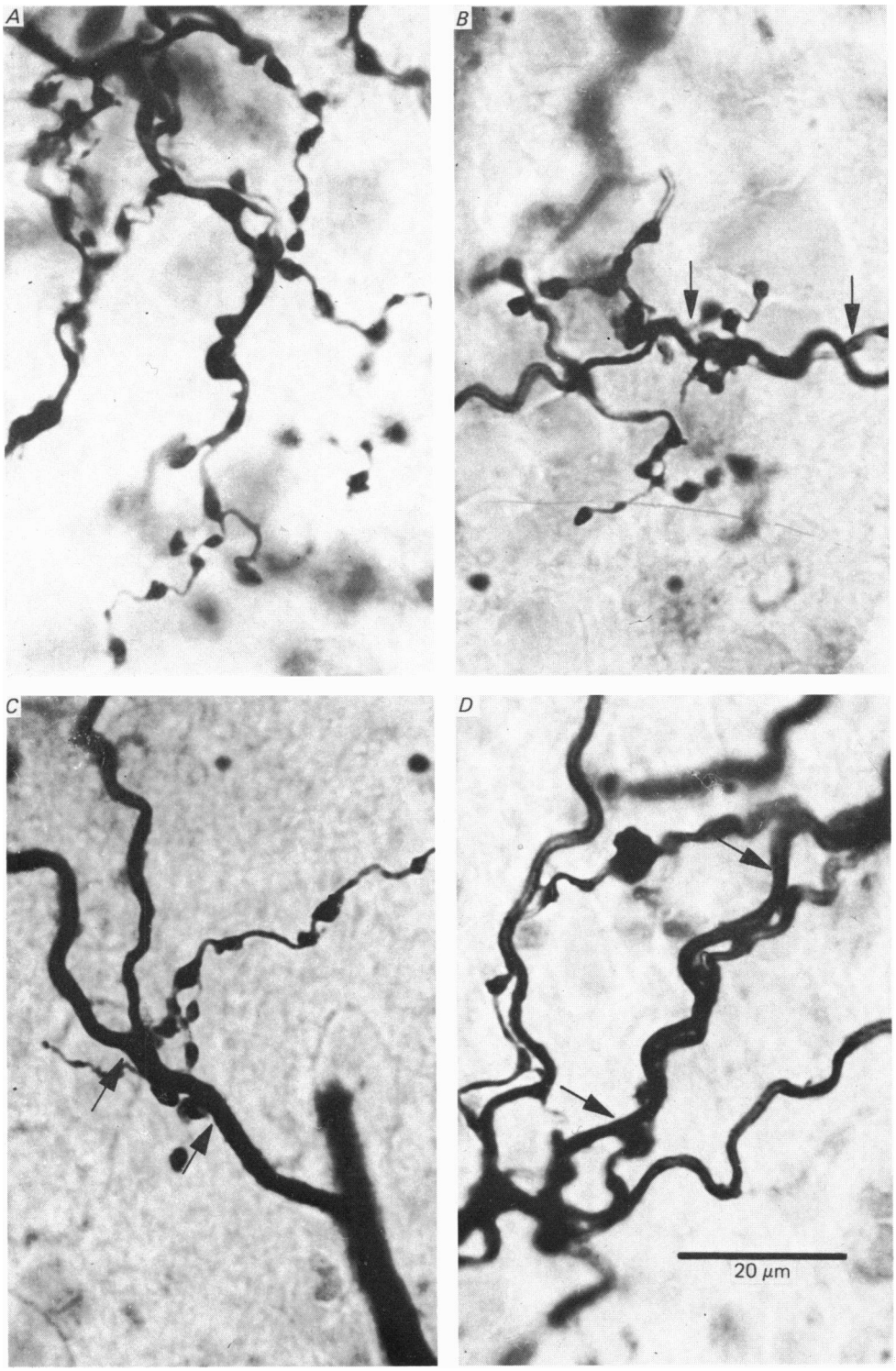
PLATE 2

A, *B*, terminal collateral branches and boutons of collateral connexion 5. *B*, between arrows, contact region number 1 (see Fig. 2). *C*, *D*, same connexion, contacts established by collateral 2. *C*, contact region 2. *D*, contact region 8. Scale refers to *A*–*D*.

PLATE 3

Synaptic arrangements at low (*A*, *C*) and high (*B*, *D*) magnification. *A*, *B*, connexion 4, contact regions on proximal dendrites. Contact regions 3, 4, 5 (see Figs. 4 and 5). *C*, *D*, connexion 3, contact region on a more distal dendrite. Contact region 7 (see Fig. 9).


A
100 μm
B
C
20 μm
D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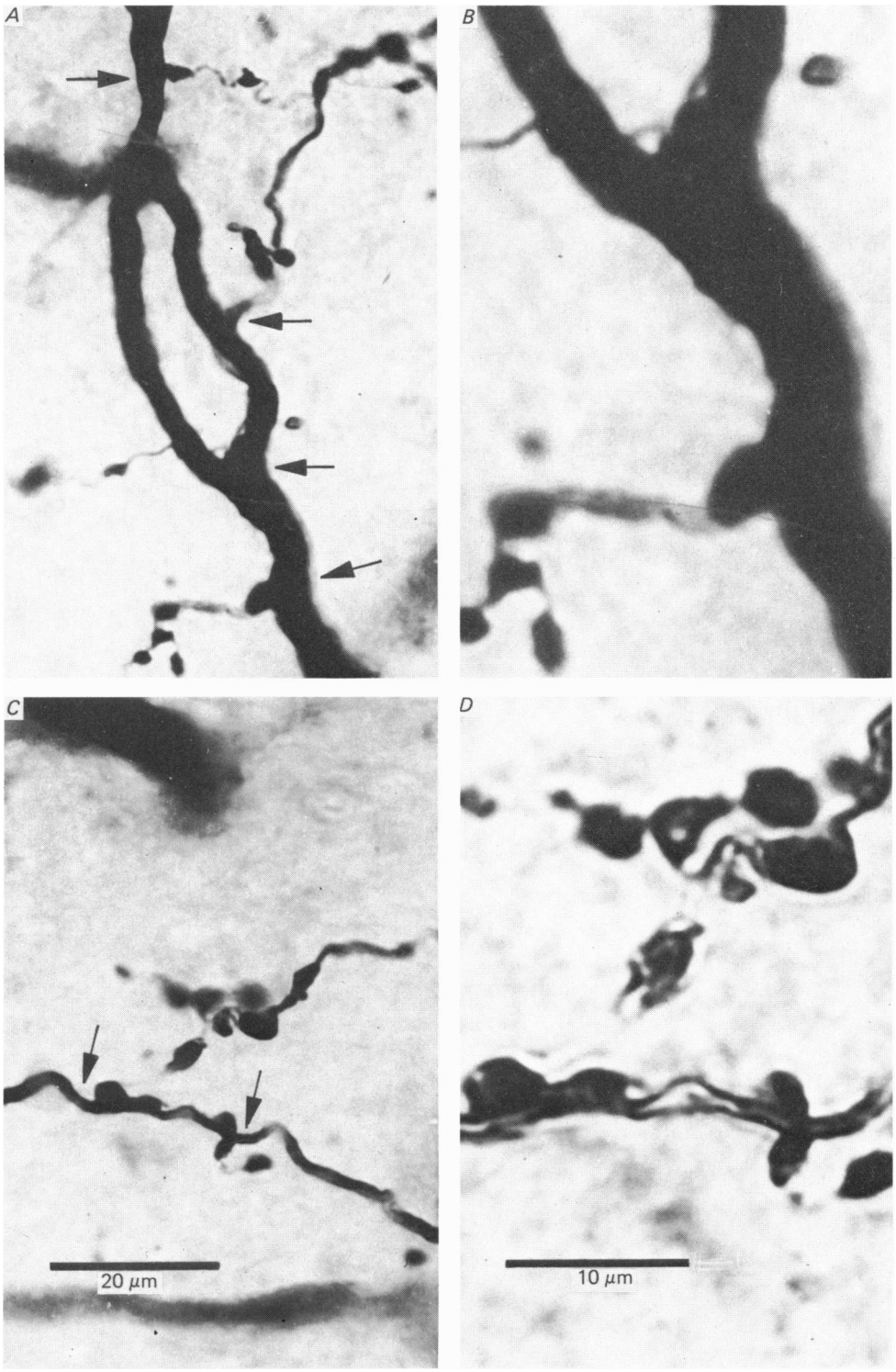
A
B
C
D
20 μm
10 μm