J. Physiol. (1982), **324**, *pp.* 479–494
With 4 plates and 8 text-figures
Printed in Great Britain

SYNAPTIC ORGANIZATION OF SENSORY AND MOTOR NEURONES INNERVATING TRICEPS BRACHII MUSCLES IN THE BULLFROG

BY ERIC FRANK AND MONTE WESTERFIELD*

From the Department of Neurobiology, Harvard Medical School, 25 Shattuck St., Boston, MA 02115, U.S.A.

(*Received 30 July 1981*)

SUMMARY

1. The anatomy and physiology of sensory-motor pathways were studied in the brachial spinal cord of adult bullfrogs to characterize the properties and specificity of these connexions.

2. Motoneurones innervating a given forelimb muscle are located in discrete and reproducible regions of the lateral motor column. Yet only a fraction of the motoneurones in a particular region innervates any one muscle.

3. The central projections of sensory afferent axons from the triceps muscles extend throughout the rostro-caudal length of the brachial spinal cord. Within this region these projections terminate in an area containing many motoneuronal dendrites.

4. Within the triceps motor pool sensory neurones from the triceps muscles produce monosynaptic potentials only in triceps motoneurones even though these motoneurones are mingled with motoneurones innervating other muscles.

5. Motoneurones innervating each of the three heads of the triceps muscles, medial, internal and external, receive monosynaptic input from their own, homonymous muscle head. Sensory fibres from the medial head also innervate 98% of the heteronymous motoneurones projecting to the internal or external heads, and nearly 90% of the medial triceps motoneurones are innervated by sensory axons from the other two heads.

6. Similarly, other brachial motoneurones receive monosynaptic input from sensory axons in their own muscle nerves. However, most of the synaptic potentials evoked in triceps motoneurones by stimulation of muscle nerves other than triceps are of longer latency and probably involve polysynaptic pathways.

7. Thus, the pattern of synaptic connexions between muscle sensory afferents and motoneurones in the frog's spinal cord is specific. Furthermore, comparison with homologous pathways in the cat's spinal cord suggests that the strength and pattern of these connexions are similar.

INTRODUCTION

The adult nervous system is characterized by highly ordered synaptic connexions among neurones. One example is the sensory innervation of spinal motoneurones

* Present address: The Institute of Neuroscience, University of Oregon, Eugene, OR 97403, U.S.A.

(Eccles, 1957). Motoneurones receive strong monosynaptic excitatory input from muscle spindle afferents in their own muscles, somewhat weaker input from spindles in synergistic muscles, and virtually no direct excitatory input from antagonists (Eccles, Eccles & Lundberg, 1957).

Most of our knowledge about the cellular basis of this reflex comes from intracellular studies of hind limb motoneurones in the cat. In amphibians, stretch reflexes can be elicited (Chambers & Simcock, 1960), but comparatively little is known about their specificity. In fact, earlier studies of synaptic potentials in hind limb motoneurones of the frog suggested that muscle sensory afferents from many different, often antagonistic, muscles could project to a single motoneurone (Simpson, 1976). These observations were interpreted as consistent with the fact that the hind limbs do not support the frog against gravity and that during a jump, antagonistic muscles are activated simultaneously.

Because stretch reflexes had been demonstrated in the triceps brachii muscles of amphibians (Chambers & Simcock, 1960) we used this muscle system to study the specificity of sensory-motor synapses in the bullfrog. The triceps muscles are powerful elbow extensors of the forearm, which normally support the rostral half of the frog and catch its entire weight at the completion of a leap. In the results presented here, we demonstrate that these connexions are remarkably analogous to those of the triceps brachii system in the cat, both in terms of specificity and amplitudes of synaptic potentials.

In addition to their usefulness in studies of synaptic mechanisms, sensory-motor synapses in the frog are well suited for studying synaptic development and possible plasticity in the central nervous system. The synapses develop relatively late during larval life, and intracellular recordings can be made from motoneurones before and during the period of synaptogenesis (Westerfield & Frank, 1980). Moreover, this development occurs in a large, free swimming tadpole, where surgical manipulations can be made with relative ease before sensory-motor synapses form. In the following paper (Frank & Westerfield, 1982), we exploit this feature to show that foreign sensory neurones can establish novel but functionally appropriate monosynaptic connexions with motoneurones.

METHODS

Animals. Bullfrogs (*Rana catesbiana*) of both sexes were used in all experiments. They were kept at room temperature (20–24 °C) and fed trout chow (Purina) three times weekly. Most animals were juveniles, 3–5 cm in length (rump to snout). Surgical procedures were performed on frogs anaesthetized by immersion in tricaine methane sulfonate (Eastman Chemicals).

Anatomy. Central projections of sensory fibres and locations of motoneurones were determined by labelling peripheral nerves with horseradish peroxidase (HRP). Neurones were labelled by cutting the appropriate muscle nerve or spinal nerve and placing the cut end in contact with a small piece of gelfoam impregnated with HRP, or by placing the nerve inside a small cuff made from polyethylene tubing and filled with 60 % HRP, 1 % lysolecithin, in water (Frank, Harris & Kennedy, 1980). Survival times ranged from 2–4 days for labelling spinal nerves to 6 days to 2 weeks for labelling the central projections of muscle sensory afferents. Tissue fixation and processing have been described in detail (Frank *et al.* 1980).

Physiology. Intracellular recordings from motoneurones were made using a semi-isolated preparation of the spinal cord. The frog was chilled in ice water, decapitated, skinned and eviscerated. The rest of the dissection was performed in 4 °C oxygenated saline of the following composition (mM): Na^+, 116; K^+, 2; Ca^{2+}, 1·8; Cl^-, 122; glucose, 15; HEPES buffer, 5 at pH 7·2. The cord was

quickly exposed by a complete dorsal laminectomy, and the choroid plexus, arachnoid and dura were removed. In later experiments, the spinal cord was hemisected longitudinally near the dorsal–ventral midline. Individual peripheral nerves in the arm were dissected and placed in suction electrodes. Once the brachial nerve was freed of all its peripheral connexions, the vertebral column, together with the spinal cord and brachial nerve, was separated from ribs, limbs and body wall, and placed in the experimental chamber for recording. The preparation was perfused with oxygenated saline at 14 °C. Resting, action and synaptic potentials had stable amplitudes for many hours. A diagram of the preparation is shown in Fig. 1.

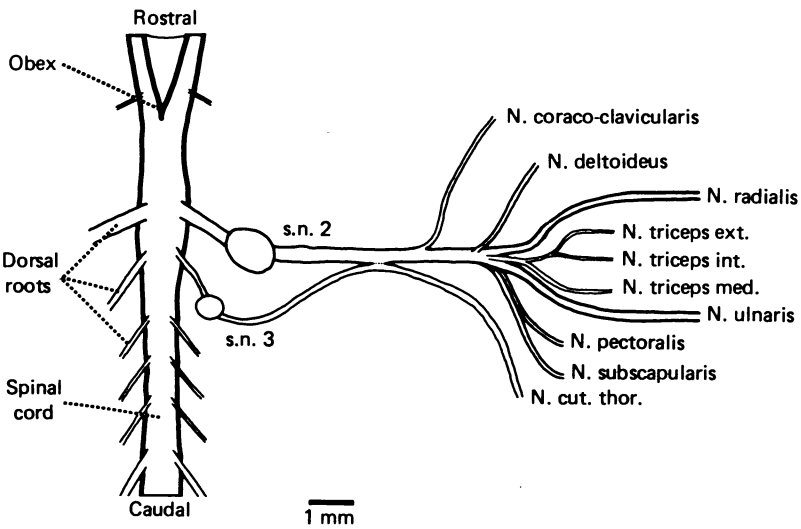


Fig. 1. Experimental preparation. The second spinal nerve (s.n. 2) normally provides the entire innervation of the arm. All forearm, hand and finger muscles are innervated by the ulnar or radial nerves. The third spinal nerve (s.n. 3) normally innervates cutaneous targets (muscle and skin) in the ventral thorax. Not all nerve branches are shown.

Motoneurones were impaled with glass micropipettes (Omega dot tubing, Frederick Haer) filled with 3 M-KCl or 0·5 M-K citrate. 0·5% Fast Green was added to the filling solution to make the electrode tip visible. The tips of the filled pipettes were often boiled in 0·5 M-K citrate for 3 min to lower their resistances by 25–50 MΩ (about one third). Electrodes were advanced through the dorsal mid line, or through the cut medial surface of the hemisected cords, with a stepping motor manipulator (California Institute of Technology Engineering Services). Impaled cells were identified as motoneurones by antidromic activation from one of the peripheral nerves (Frank & Fuortes, 1955). We recorded only from cells with resting potentials greater than −40 mV; the average resting potential was $-53{\cdot}4 \pm 10$ mV (mean ± S.D.). The results described here are based on recordings from 516 motoneurones in forty-three adult bullfrogs.

Synaptic potentials were recorded in response to stimulation of peripheral nerves at frequencies of 0·5–2 Hz. There was no obvious facilitation or depression of monosynaptic potentials at these frequencies. Measurements of amplitude and latency were made from two to twenty individual responses using a signal averager (Dagan) to reduce the effects of electrode noise and spontaneous synaptic activity. After withdrawing the pipette from the cell we subtracted the extracellular field potential from the intracellular record electronically.

In about one third of the frogs we found one or more nerve twigs that branched off from the external triceps nerve to innervate the internal triceps muscle. Axon counts of the internal-external triceps nerve revealed extensive axon branching (Leon Nawrocki, personal communication). We also found that about one third of internal (or external) triceps motoneurones also projected through the external (or internal) branch, as determined by antidromic stimulation. A similar situation has been described for lumbrical muscles in the cat's hind foot (Emonet-Denand, Laporte

& Proske, 1971). For these reasons, we tentatively regarded the internal and external triceps muscles as functionally equivalent, and usually stimulated both nerve branches together.

Electrical coupling among motoneurones. Motoneurones within the frog's spinal cord are electrically coupled to each other (Grinnell, 1966; Erulkar & Soller, 1980). Thus it was important to determine how this coupling interfered with the measurement of sensory-motor synaptic potentials. In experiments described elsewhere (Westerfield & Frank, 1982) we demonstrated that homonymous motoneurones (innervating the same muscle) are often electrically coupled to each other, but in general not to motoneurones innervating other muscles including the functionally related heteronymous motoneurones. For triceps motoneurones, only homonymous sensory synaptic potentials are contaminated by electrical coupling among motoneurones.

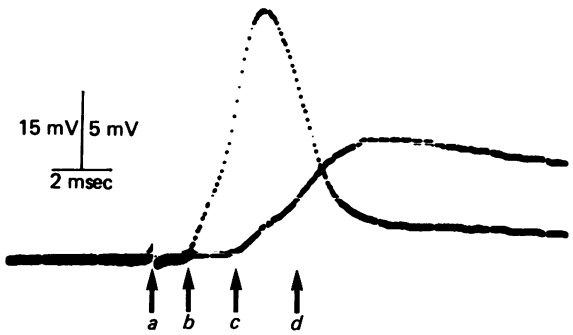


Fig. 2. Antidromic action potential and homonymous synaptic potential recorded intracellularly from a medial triceps motoneurone (resting potential, −65 mV). Stimulation of the nerve to the medial head of triceps (*a*) elicited an antidromic action potential (*b*). At a slightly lower stimulus strength (also at *a*), only a synaptic potential was evoked. Early (*c*) and late (*d*) components of the synaptic potential can be clearly distinguished. The trace of the synaptic potential is the average of three responses.

Mode of synaptic transmission and measurement of latencies. The latencies of the various synaptic potential components were measured with respect to the time of peripheral nerve stimulation. Fig. 2 illustrates two superimposed traces obtained upon stimulation of the medial triceps nerve suprathreshold (upper trace) and subthreshold (lower trace, recorded at higher gain) for activation of this motoneurone's axon. The upper trace shows the antidromic action potential whose latency was 1·2 msec (*b–a*). The synaptic potential (lower trace), elicited at a slightly lower stimulus strength, shows the two components characteristic of amphibian sensory-motor synaptic potentials. The latency of the early component (*c–a* = 2·8 msec) corresponded to the time of arrival of action potentials in the terminals of sensory afferents (Alvarez-Leefmans, de Santis & Miledi, 1979) and the time of active invasion of motoneuronal somata by the antidromic impulses (Shapovalov & Shiriaev, 1978). This component is produced both by electrical coupling between sensory afferents and this motoneurone and by electrical coupling between this neurone and some of its antidromically activated neighbours (Grinnell, 1966). The later, presumably chemical, component of the synaptic potential has an additional latency (*d–c*) of 2·0 msec, giving a total latency of 4·8 msec (*d–a*).

Synaptic transmission between muscle sensory afferents and motoneurones in this preparation is mediated both electrically and chemically. If the ventral roots are cut to abolish antidromic action potentials and hence motoneuronal coupling potentials, both early (electrical) and late (chemical) components of the purely sensory triceps e.p.s.p.s are visible (Westerfield & Frank, 1982, also see Fig. 3 of Frank & Westerfield, 1982). Heteronymous triceps e.p.s.p.s, which are not contaminated by motoneuronal coupling, also show both components (upper trace of Fig. 8). Finally, only the early component persists in calcium-deficient solutions (Westerfield & Frank, 1982), suggesting it is mediated electrically, as previously shown for dorsal root e.p.s.p.s (Shapovalow & Shirlaev, 1980; Alvarez-Leefmans *et al.* 1979).

Only synaptic potentials with latencies less than or equal to 5·5 msec were classified as monosynaptic. This classification was based upon two observations: (1) virtually all triceps e.p.s.p.s

in triceps motoneurones had latencies shorter than this, and (2) the fastest i.p.s.p.s, which are thought to be disynaptic in this preparation, had latencies only slightly greater than 5·5 msec. Thus, any synaptic potential with a latency greater than this may be polysynaptic.

RESULTS

Anatomy of sensory and motor neurones

Motor nuclei. The locations of motoneurones innervating several different forelimb muscles were determined using retrograde labelling with horseradish peroxidase

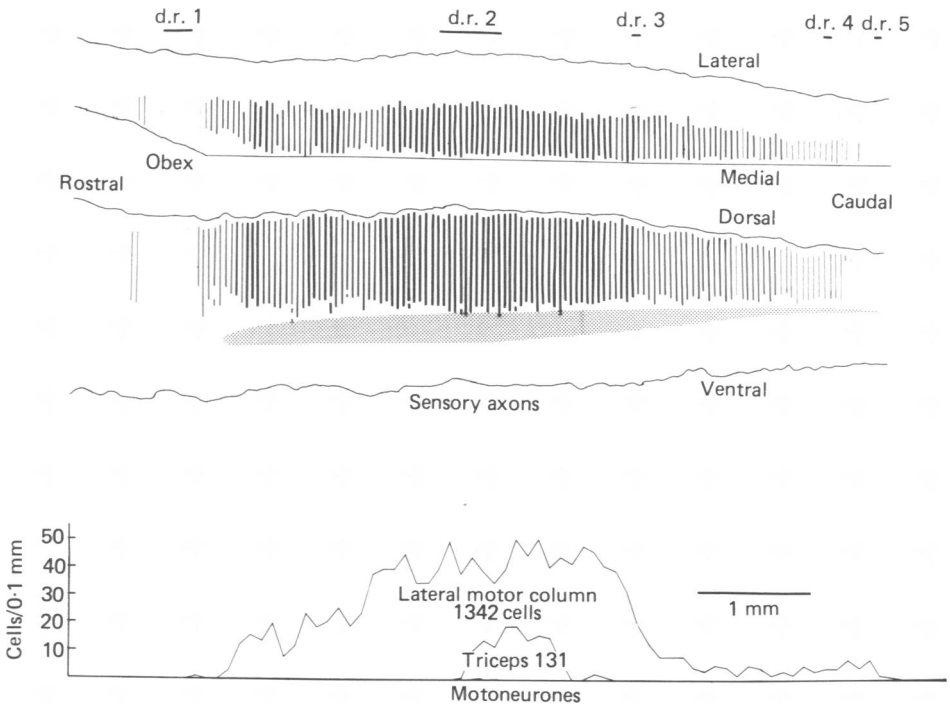


Fig. 3. Projection of triceps muscle sensory afferents (above) and location of the triceps motor pool in the brachial spinal cord. Triceps sensory afferents were labelled with HRP and their projection pattern was determined from serial 50 μm transverse sections such as the one shown in Pl. 3. The lateral and ventral extent of the triceps projection in each section is indicated by a line in the horizontal (upper diagram) and sagittal (middle diagram) views. The thickness of the line corresponds to the density of the projection. The shaded area corresponds to the location of the brachial motor column. The triceps motor pool occupies only the central one fourth of the lateral motor column (lower graph), but the triceps sensory axons project throughout the brachial spinal cord. d.r., dorsal root.

(HRP). Pl. 1 illustrates the position of the triceps motor pool in the brachial spinal cord. The triceps motoneurones were labelled by placing HRP on the cut ends of all the branches of the triceps nerves bilaterally. The labelled neurones were grouped together within a discrete region of the lateral motor column, and the distribution was the same on the two sides. To determine the anatomical location of the triceps motor pool, we made serial reconstructions of the spinal cord from transverse sections and counted the number of labelled and unlabelled motoneurones in the lateral motor

columns. One of these reconstructions is shown in the lower part of Fig. 3. Each triceps motor nucleus contained 115–135 ($n = 4$) neurones, occupied approximately one quarter of the total motor column, and was consistently ($n = 20$) located just caudal to the rostro-caudal mid-point. This location has been confirmed in many electrophysiological experiments.

Within the region of the triceps motor pool, fewer than half of the motoneurones innervated triceps muscles (see Pl. 1 and the lower part of Fig. 3). This was confirmed by recording intracellularly within the triceps motor pool where only one third of the

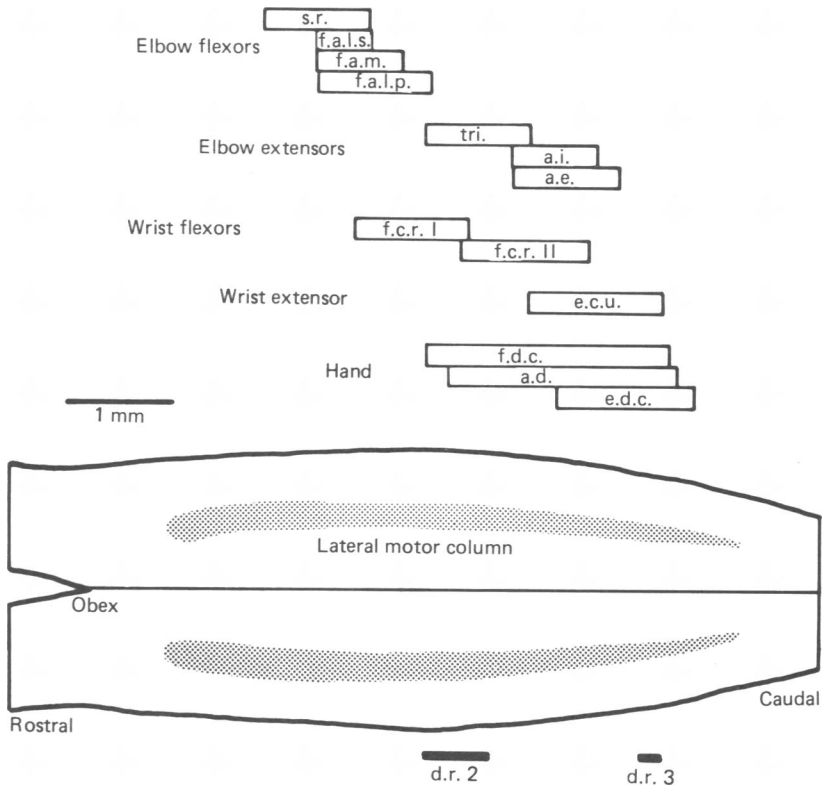


Fig. 4. Location of forelimb motor pools. A diagram of the spinal cord, showing the location of the second and third dorsal roots (d.r.) and the entire extent of the brachial motor column, is shown below. The positions of individual motor pools, as determined by labelling motor nerves with HRP, are drawn above to the same scale. The muscles are grouped, along the rostral-caudal axis only, according to their primary functions. a.d., abductor digiti II; a.i., internal anconeus; a.e., external anconeus; e.c.u., extensor carpi ulnaris; e.d.c., extensor digitorum communis; f.a.l.p., flexor antibrachii lateralus profundus; f.a.l.s., flexor antibrachii lateralus superficialis; f.a.m., flexor antibrachii medialis; f.c.r. I and II, flexor carpi radialis; f.d.c., flexor digitorum communis and palmaris brevis; s.r., sternoradialis; tri., triceps.

motoneurones could be activated by antidromic stimulation of the triceps nerves (see below). The subscapular, pectoral and deltoid muscles were innervated by other motoneurones within this region.

A summary diagram of the positions of the main forelimb motoneuronal pools is

shown in Fig. 4. The cell bodies of motoneurones innervating the sternoradialis and flexor antibrachii muscles which flex the elbow and are thus antagonists of triceps, were rostral and adjacent to the triceps motor pool, showing no overlap in territory. On the other hand, the anconei muscles which extend the elbow, as do triceps muscles, but are located in the lower arm, had motor nuclei that were nearly co-extensive with the triceps pool. Motor pools innervating some of the muscles that move the wrist were also co-extensive with triceps while motoneurones innervating the fingers were further caudal.

Sensory projections. The central projections of sensory fibres from the arm were determined by placing a pellet of HRP on the cut end of the second spinal nerve, which usually provides the entire innervation of the arm. An example is shown in Pl. 2. Sensory fibres enter the spinal cord through the dorsal roots, branch to run longitudinally within the dorsal columns, and send axon collaterals ventrally through the grey matter. The axons terminate principally in two areas within the cross-section of the spinal cord, one in the dorsal grey matter and the other more ventrally in a region occupied by the dendrites of motoneurones. This region of overlap presumably contains the monosynaptic connexions between sensory and motor neurones.

The central projections of sensory afferents innervating individual muscles in the arm were studied by labelling single muscle nerves with HRP (Frank *et al.* 1980). Pl. 3 shows the central projections of triceps sensory axons visualized with this procedure. The dorsal neuropile, seen in Pl. 2, is missing; instead the muscle sensory afferents project directly down into the more ventral neuropile, in juxtaposition with the dendrites of motoneurones. Finer details of this projection can be seen in Pl. 4. Sensory axons terminate in chains of varicosities, most of them approximately 100–200 μm from the somata of motoneurones. A few varicosities, however, are located only 20–30 μm from the cell bodies. These projections are very similar to those of *Ia* fibres in the cat, which have been shown, by direct injection of HRP into functionally identified sensory axons (Brown & Fyffe, 1978; Burke, Walmsley & Hodson, 1979), to contact both proximal and distal dendrites of individual motoneurones.

The distribution of triceps sensory afferents within the spinal cord was determined with this method by serial reconstruction of the spinal cord from transverse sections in two animals, whose ventral roots had been cut one week before the labelling experiments so that only sensory elements were labelled. The same distribution was observed in both cases and the results from one animal are shown in the upper half of Fig. 3. The location of the triceps motor pool, as determined in another animal, is shown below for comparison. Triceps sensory afferents projected quite evenly throughout the entire brachial region of the spinal cord, well beyond the triceps motor pool, even though they do not extensively innervate motoneurones outside this pool (see below).

To see if the HRP labelled all the triceps sensory and motor cells, we compared the number of labelled cells to the total number of myelinated axons in the unlabelled triceps nerve (141–163, $n = 3$). There were approximately thirty ($n = 3$) labelled sensory axons in the dorsal root, and 130 labelled motoneurones in the spinal cord. These observations demonstrate that essentially all sensory and motor cells are labelled with this technique.

Physiology of the sensory-motor pathway

Functional projections of triceps sensory neurones. Synaptic interactions between sensory and motor neurones innervating the three heads of the triceps muscle were strong; stimulation of the triceps nerve evoked synaptic potentials in almost all triceps motoneurones. These synaptic potentials were seen when the stimulus was just subthreshold for antidromic activation of the cell. An example of this potential is illustrated in Fig. 2. This trace shows the synaptic potential evoked at a slightly lower stimulus strength than was required for activation of the antidromic action potential. The latencies of these synaptic potentials in triceps motoneurones ranged from 2·0 to 5·5 msec, which is the range we considered to be monosynaptic (see Methods). Triceps synaptic potentials with longer latencies were seldom seen. In 87% of 143 triceps motoneurones studied, we evoked triceps synaptic potentials below threshold for antidromic activation. The remaining nineteen neurones may also have received triceps inputs, but at a threshold higher than the antidromic threshold.

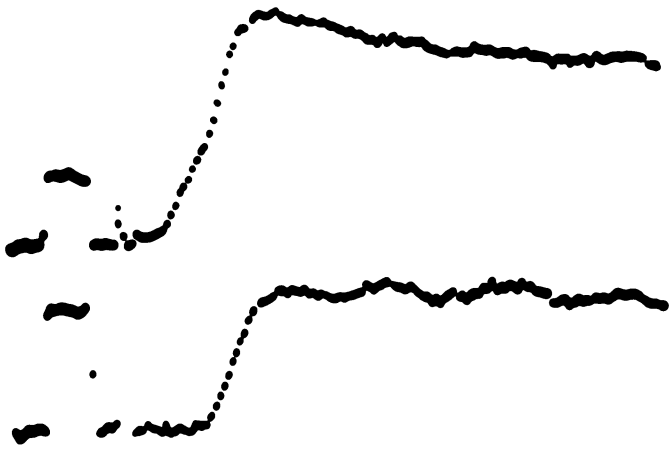


Fig. 5. Homonymous and heteronymous synaptic potentials in a medial triceps motoneurone (resting potential −68 mV). The homonymous input, at a stimulus intensity just subthreshold for antidromic activation of the cell, is shown in the upper trace. The heteronymous input from the external triceps nerve is shown in the lower trace. Only a late component is present in this particular heteronymous e.p.s.p. Each trace is the average of five to ten responses; extracellular fields have been subtracted. Calibration pulses are 0·5 mV and 2 msec.

Homonymous synaptic potentials. Homonymous sensory inputs are readily apparent despite the problems associated with their measurement (see small print section below). In preparations with cut ventral roots, over 90% of all sixty-three cells receiving any input from triceps afferents were innervated by medial triceps axons. Since few motoneurones other than triceps receive triceps input (see below) and about one half of all triceps motoneurones innervate the medial head of the triceps muscle, most medial triceps motoneurones must receive homonymous input. Moreover, it was often (fifty out of sixty-six motoneurones) possible to see a component of the homonymous synaptic potential that was larger and had a longer latency ($4{\cdot}77 \pm 0{\cdot}59$ msec, $n = 32$, see examples in Fig. 2 and upper trace of Fig. 5) than the

motoneuronal coupling potential. These late components must have been the result of homonymous sensory input.

Measurements of homonymous inputs to triceps motoneurones are subject to two difficulties. First, stimulus strengths sufficient to activate all the sensory fibres also elicited the antidromic impulse which obscured the synaptic potential. If we cut the ventral root to eliminate the antidromic impulse, we could no longer identify the motoneurone. Thus, it was never possible to measure the maximum amplitude of the homonymous synaptic potential in identified motoneurones. Second, these potentials were contaminated by coupling potentials from the antidromic impulses in neighbouring motoneurones (see Methods). Thus, an unknown part of the e.p.s.p. elicited by stimulation from a motoneurone's own muscle nerve was not from sensory axons but from motoneuronal coupling potentials. For these reasons we did not include homonymous triceps synaptic potentials in our analysis of amplitudes (Figs. 6 and 7).

Amplitudes of homonymous e.p.s.p.s were estimated by indirect methods. The contribution of motoneuronal coupling was estimated in experiments where the dorsal roots were cut to abolish sensory input. In this manner we measured the amplitude of the homonymous coupling potential at a stimulus strength just subthreshold for the antidromic impulse. The average amplitude of coupling was subtracted from the average amplitude of the homonymous synaptic potential measured in preparations with intact dorsal and ventral roots. With this correction, the average medial triceps homonymous e.p.s.p. was $1{\cdot}94 \pm 1{\cdot}55$ mV ($n = 33$) in amplitude. The corresponding figure for the internal-external triceps e.p.s.p.s was $0{\cdot}92 \pm 0{\cdot}58$ mV ($n = 24$). Since for the homonymous e.p.s.p.s, only a fraction of the sensory afferents could be stimulated below the threshold for antidromic activation of the motoneurone, our figures represent lower estimates of their maximum amplitudes. Because muscle sensory and motor axons in the frog have similar conduction velocities and hence thresholds (Tamarova, 1977), on the average we activated approximately half of the homonymous sensory fibres. Therefore our estimates of homonymous e.p.s.p. amplitudes may be low by a factor of two. The average amplitudes of homonymous synaptic potentials in triceps and other brachial motoneurones are presented in Table 1.

Heteronymous synaptic potentials. Synaptic projections from sensory fibres innervating heteronymous muscles were also apparent. For example, sensory afferents in the medial triceps muscle nerve evoked an e.p.s.p. of 0·4 mV or larger in 98 % (45/46) of the motoneurones innervating the internal or external heads of triceps. Similarly, stimulation of sensory afferents in the internal or external triceps nerve evoked an e.p.s.p. of at least 0·4 mV in 89 % (32/36) of the medial motoneurones tested (Fig. 5, lower trace). These synaptic potentials are not contaminated by motoneuronal coupling, nor are they obscured by antidromic action potentials. The average medial triceps heteronymous e.p.s.p. was $1{\cdot}60 \pm 1{\cdot}16$ mV ($n = 46$) while the corresponding figure for the internal-external triceps e.p.s.p. was $0{\cdot}86 \pm 0{\cdot}54$ mV ($n = 28$). Thus the heteronymous sensory input from the medial triceps muscle is approximately twice as large as the heteronymous input from the combined internal-external triceps muscle. Amplitude histograms of triceps heteronymous synaptic potentials are presented in Fig. 6 (see also Table 1).

Triceps input to other motoneurones. In contrast, relatively few motoneurones innervating muscles other than triceps receive significant monosynaptic input from triceps sensory afferents. In deltoideus, subscapular and pectoralis motoneurones, only 5 % of 179 examples studied received synaptic input greater than 0·4 mV from

either the medial or internal-external triceps nerves. Amplitude histograms of triceps e.p.s.p.s in these motoneurones are presented in Fig. 6 (see also Table 1).

For motoneurones projecting through the ulnar or radial nerves, the input from triceps was somewhat stronger; 17 % of these cells (21/124 examples) received triceps input greater than 0·4 mV. These nerves innervate the entire forearm, wrist, and hand, so it is possible that ulnar and radial motoneurones innervating specific muscles receive specific projections from triceps sensory axons.

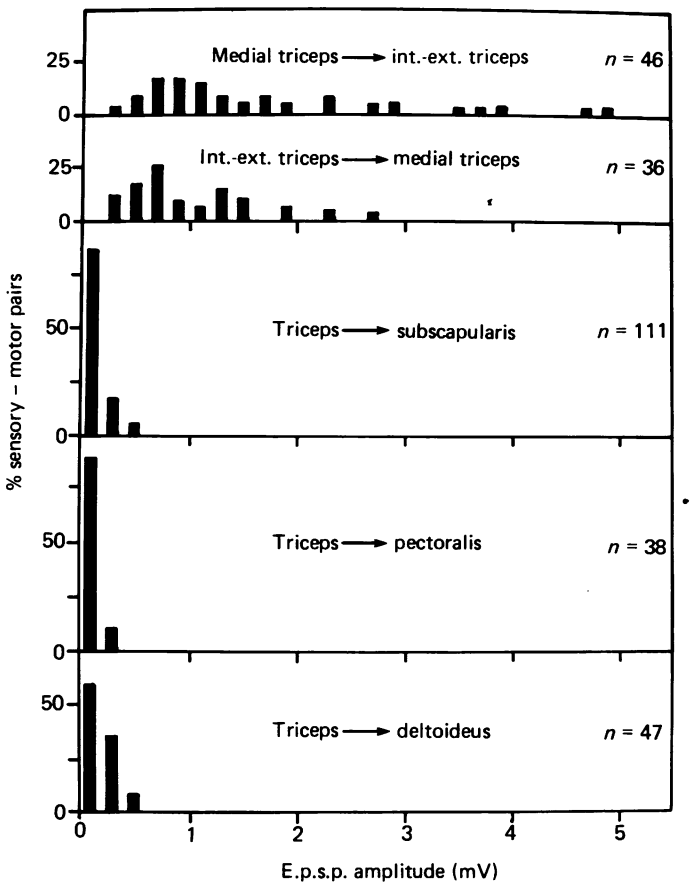


Fig. 6. Amplitude histogram of triceps e.p.s.p.s in five classes of motoneurones. Only those potentials with latencies less than 5·5 msec, and therefore probably mediated monosynaptically, are included (see text for details). E.p.s.p.s elicited from triceps sensory afferents are larger in heteronymous triceps motoneurones than in subscapular, pectoral or deltoid motoneurones. Results are from 175 motoneurones in thirteen frogs.

Thus the specificity of triceps sensory projections onto brachial motoneurones is high. Virtually every triceps motoneurone received monosynaptic input from the triceps nerves while only a small percentage of non-triceps motoneurones did. This specificity is seen within the triceps motor nucleus, where only about one third of the cells are triceps motoneurones (see Fig. 3). Most of the non-triceps cells we tested were located within this region, yet few of them recieved monosynaptic input from triceps sensory axons even when immediately adjacent to a triceps motoneurone that

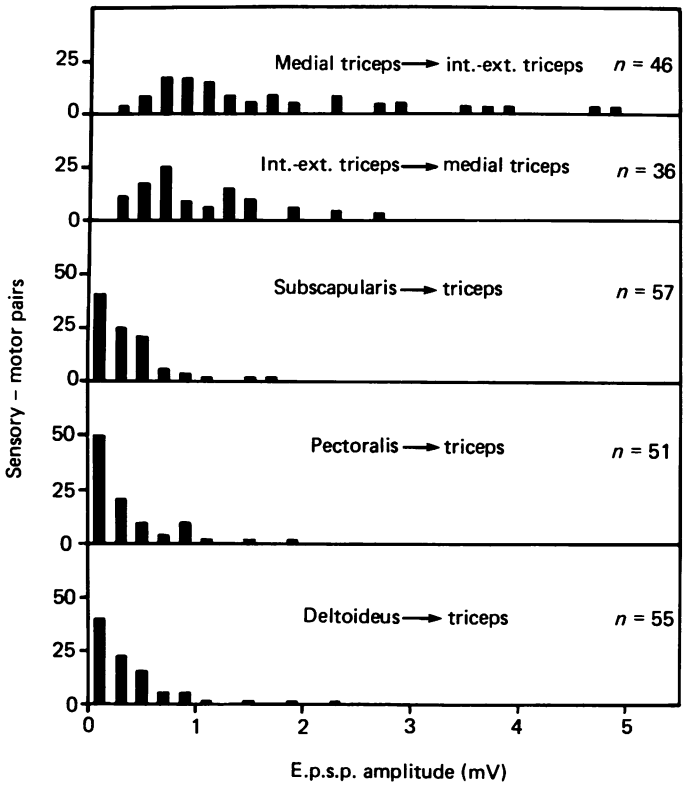


Fig. 7. Amplitude histograms of monosynaptic (as defined by latencies less than 5·5 msec) muscle sensory input to triceps motoneurones. The upper two panels show the heteronymous input to triceps motoneurones and the lower three panels show the input these same motoneurones receive from three different muscle nerves (subscapular, pectoral and deltoid). In general, monosynaptic e.p.s.p.s from other muscle nerves were smaller, although large inputs were seen occasionally. Data are from the same experiments as in Fig. 6.

TABLE 1. Amplitudes of sensory-motor e.p.s.p.s. All synaptic potentials with latencies less than 5·5 msec were used to calculate the averages for each class of sensory-motor pairs. Amplitudes are expressed in millivolts. The homonymous synaptic potentials (along the diagonal) are lower estimates (see text). Number of sensory-motor pairs in parentheses, and errors are ±S.E.M.

	Sensory				
Motor	Medial triceps	Int. and ext. triceps	Subscapularis	Pectoralis	Deltoideus
Medial triceps	⩾1·94±0·27 (33)	0·86±0·10 (28)	0·24±0·07 (24)	0·22±0·06 (21)	0·58±0·16 (24)
Int. and ext. triceps	1·60±0·17 (46)	⩾0·92±0·12 (24)	0·52±0·06 (29)	0·45±0·09 (30)	0·34±0·07 (30)
Subscapularis	0·15±0·05 (47)	0·18±0·05 (44)	⩾2·18±0·37 (23)	0·67±0·11 (26)	0·09±0·03 (40)
Pectoralis	0·08±0·03 (20)	0·08±0·03 (20)	0·71±0·29 (18)	⩾1·17±0·24 (9)	0·08±0·03 (18)
Deltoideus	0·23±0·03 (24)	0·08±0·03 (22)	0·17±0·09 (20)	0·05±0·02 (13)	⩾1·83±0·25 (19)
Radialis	0·22±0·07 (35)	0·17±0·04 (34)	0·01±0·01 (26)	0·05±0·02 (16)	0·54±0·21 (22)
Ulnaris	0·20±0·07 (23)	0·43±0·17 (22)	0·47±0·25 (13)	0·05±0·04 (14)	0·23±0·15 (16)

was innervated. Apparently triceps axons discriminate among motoneurones within the same region of the spinal cord.

Other sensory input to triceps motoneurones. In contrast to the strong input that triceps motoneurones receive from their own muscle afferents, the monosynaptic input from other nerves was usually weaker. The amplitudes of probable monosynaptic, non-triceps e.p.s.p.s in triceps motoneurones are shown in Fig. 7 as an amplitude histogram. The amplitudes of heteronymous triceps e.p.s.p.s are shown for comparison. Just as triceps sensory axons provide very little input to most non-triceps motoneurones (see Fig. 6), so many non-triceps sensory axons provide little or no monosynaptic input to triceps motoneurones (see also Table 1).

Fig. 8. Differences in synaptic latencies of e.p.s.p.s from internal-external triceps afferents (upper trace) and pectoral afferents (lower trace) in a medial triceps motoneurone. The earliest input from the pectoralis nerve was at 7·2 msec, later than either the early (3·0 msec) or late (5·4 msec) component from the heteronymous sensory input. Resting potential, −53 mV; averages of five to ten responses. Calibration pulses are 0·5 mV and 2 msec. Extracellular fields have been subtracted.

The synaptic input from the ulnar and radial nerves to triceps motoneurones was often of short latency (less than 5·5 msec) and sufficiently powerful to activate the motoneurones orthodromically which triceps afferents rarely did. Triceps sensory axons also innervated 17% of ulnar and radial motoneurones (see above), suggesting the possibility of reciprocal innervation. We did not include these ulnar and radial inputs in our analysis, however, because these nerves are very large, innervating the entire forearm, wrist and hand, and they contain both cutaneous and muscle sensory afferents. Stimulation of these nerves produced large extracellular field potentials which complicated the measurements of amplitudes and latencies. In contrast, the synaptic input from the subscapular, pectoral and deltoid nerves provided a meaningful comparison with triceps inputs because these nerves are similar in size to the triceps nerves and they innervate only muscles.

These non-triceps muscle nerves often produced longer latency, presumably polysynaptic, potentials in triceps motoneurones. An example is shown in Fig. 8, where the synaptic input from the internal and external triceps nerves (upper trace) was compared to that from the pectoral nerve (lower trace) in a medial triceps motoneurone. The triceps e.p.s.p. had early and late components of 3·0 and 5·4 msec, while the earliest potential after stimulation of the pectoral nerve began at 7·2 msec. Other components had even longer latencies. These longer latency potentials were probably polysynaptic and not the result of longer peripheral conduction times since pectoralis motoneurones receive short latency input from their own sensory axons, analogous to the input that triceps motoneurones receive from triceps sensory afferents.

DISCUSSION

Anatomy of the sensory-motor system. Motoneurones innervating forelimb muscles are localized to discrete regions of the lateral motor column within the brachial region of the spinal cord. The general pattern is similar to that reported by Cruce (1974*a*) for lumbar motoneurones in the bullfrog. As in higher vertebrates, more distally located limb muscles are usually innervated by more caudally located motoneurones. This is not a strict rule, however, as occasionally we encountered motoneurones innervating finger muscles within the triceps region. When triceps motoneurones were labelled with HRP it was apparent that only a fraction of the motoneurones within the triceps region were triceps motoneurones. Often, triceps and non-triceps somata were immediately adjacent (see Pls. 1 and 4).

Labelling of triceps sensory fibres with HRP also demonstrates the large degree of overlap between dendritic arbors of motoneurones and terminal fields of muscle sensory afferent axons. Few, if any, synaptic varicosities are present in the more dorsal regions of the spinal cord (Pl. 4). On the basis of degeneration studies, Joseph & Whitlock (1968) concluded that primary sensory afferents innervated only the most distal tips of motoneuronal dendrites. This anatomical finding was correlated with the physiological results of Brookhardt & Fadiga (1960) who found that dorsal root e.p.s.p.s tended to have long, slow rising phases. Our findings, however, confirm more recent results in the frog (Szekeley, 1976) and the cat (Brown & Fyffe, 1978; Burke *et al.* 1979). Some sensory varicosities actually extend to within 20–30 μm of the motoneuronal somata. This is less close than in the cat, but much closer than the earlier anatomical and physiological results had suggested for the frog.

Specificity of sensory-motor connexions. The characterization of projections from triceps brachii muscle sensory afferents onto brachial motoneurones extends the relatively few intracellular studies that have been made on reflex specificity in the frog's spinal cord. In the lumbar spinal cord many motoneurones receive synaptic input from several different muscle nerves and stretch reflexes are difficult or impossible to evoke (Cruce, 1974*b*; Simpson, 1976). The absence of an anti-gravity posture and the strong co-activation of hind limb extensors and flexors during jumping may both contribute to the lack of any obvious synaptic specificity of muscle sensory afferents onto motoneurones (Simpson, 1976). In the forelimb muscle system of the toad, however, reflex specificity has been demonstrated, perhaps because the forelimbs, especially in toads, are used to support the body against gravity. After ablation of the cerebrum and cerebellum, overt stretch reflexes can be elicited in the triceps brachii muscles (Chambers & Simcock, 1960). Fukami (1961) reported that individual brachial motoneurones in the toad were synaptically excited only by one or the other of the biceps or triceps muscle nerves; an individual neurone never responded to both inputs. Although the identity of the motoneurones was not established because the ventral roots were cut, the result suggested that muscle sensory inputs to motoneurones were specific.

Our results show that triceps muscle sensory afferents specifically innervate certain classes of brachial motoneurones. The system shares many of the characteristic features of the sensory-motor pathway in the cat. There, muscle sensory afferents tend to produce the largest e.p.s.p. in homonymous motoneurones, next largest in

heteronymous motoneurones, and least in antagonistic or functionally unrelated motoneurones (Eccles *et al.* 1957). Similarly, in the frog (Table 1), medial or internal-external triceps sensory afferents form strong connexions with homonymous and heteronymous triceps motoneurones, but provide much weaker inputs to the pectoral, subscapular or deltoid motoneurones which act on the shoulder joint rather than the elbow. Many of these pectoral and subscapular motoneurones are co-extensive with triceps suggesting that sensory afferents discriminate among adjacent motoneurones.

In both the cat and the frog, some synaptic interconnexions exist between muscles that are not strictly heteronymous. Thus in the cat, soleus motoneurones (ankle extensors) are innervated by muscle sensory afferents from the vastus and crureus muscles, which are knee extensors. Triceps sensory afferents in the frog also innervate some motoneurones whose axons run in the ulnar and radial nerves. In terms of the functional role of these connexions, co-activation of these muscles may be required for the maintenance of normal posture in both the frog and the cat.

Although it is generally thought that the stretch reflex is much stronger in mammals than in lower vertebrates, the amplitudes of the e.p.s.p.s in motoneurones evoked by stimulation of heteronymous muscle sensory afferents were comparable in the frog and the cat. Eccles *et al.* (1957) reported that the average amplitudes of input from caput longus (equivalent to the medial triceps brachii muscle in frogs) onto caput lateral and caput medial (external and internal triceps muscles) were 1·03 and 1·26 mV; we found an average amplitude of 1·60 mV. The reciprocal inputs of caput lateral and medial onto longus motoneurones were 0·13 and 0·58 mV in the cat; we found the input from the combined internal and external triceps branches onto medial triceps motoneurones to be 0·86 mV. Thus 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 sensory afferent axons.

It is a pleasure to acknowledge useful discussions with Drs J. Dodd, W. Harris, J. Horn, D. Hubel, J. Jansen and J. Lichtman and the expert technical help of M. Nastuk and L. Nawrocki. Supported by NIH grants NS 00212 and NS 14451 to E.F. and an M.D.A. fellowship to M.W.

REFERENCES

ALVAREZ-LEEFMANS, F. J., DE SANTIS, A. & MILEDI, R. (1979). Effects of some divalent cations on synaptic transmission in frog spinal neurones. *J. Physiol.* **294**, 387–406.

BROOKHART, J. M. & FADIGA, E. (1960). Potential fields initiated during monosynaptic activation of frog motoneurones. *J. Physiol.* **150**, 633–655.

BROWN, A. G. & FYFFE, R. E. W. (1978). The morphology of group *Ia* afferent fibre collaterals in the spinal cord of the cat. *J. Physiol.* **274**, 111–127.

BURKE, R. E., WALMSLEY, B. & HODSON, J. A. (1979). HRP anatomy of group *Ia* afferent contacts on motoneurones. *Brain Res.* **160**, 347–352.

CHAMBERS, W. W. & SIMCOCK, J. P. (1960). Postural reflexes in the forelimb of the toad, *Bufo marinus*. *J. Neurophysiol.* **23**, 54–61.

CRUCE, W. L. R. (1974*a*). The anatomical location of hindlimb motoneurones in the lumbar spinal cord of the frog, *Rana catesbiana*. *J. comp. Neurol.* **153**, 59–76.

CRUCE, W. L. R. (1974*b*). Supraspinal monosynaptic input to hindlimb motoneurones in the lumbar spinal cord of the frog. *Rana catesbiana*. *J. Neurophysiol.* **37**, 691–704.

ECCLES, J. C. (1957). *The Physiology of Nerve Cells*. Baltimore: Johns Hopkins Press.

ECCLES, J. C., ECCLES, R. M. & LUNDBERG, A. (1957). The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones. *J. Physiol.* **137**, 22–50.

MONET-DENAND, F., LAPORTE, Y. & PROSKE, U. (1971). Contraction of muscle fibers in two adjacent muscles innervated by branches of the same motor axon. *J. Neurophysiol.* **34**, 171–187.

RULKAR, S. D. & SOLLER, R. W. (1980). Interactions among lumbar motoneurones on opposite sides of the frog spinal cord: morphological and electrophysiological studies. *J. comp. Neurol.* **192**, 473–488.

FRANK, E., HARRIS, W. A. & KENNEDY, M. B. (1980). Lysophophatidyl choline facilitates labelling of CNS projections with horseradish peroxidase. *J. Neurosci. Meth.* **2**, 183–189.

FRANK, E. & WESTERFIELD, M. (1982). The formation of appropriate central and peripheral connexions by foreign sensory neurones of the bullfrog. *J. Physiol.* **324**, 495–505.

FRANK, K. & FUORTES, M. G. F. (1955). Potentials recorded from the spinal cord with microelectrodes. *J. Physiol.* **130**, 625–654.

FUKAMI, Y. (1961). Postsynaptic potentials in toad's spinal motoneurones due to muscle afferent volleys. *Jap. J. Physiol.* **11**, 596–604.

GRINNELL, A. D. (1966). A study of the interaction between motoneurones in the frog spinal cord. *J. Physiol.* **182**, 612–648.

JOSEPH, B. S. & WHITLOCK, D. G. (1968). Central projections of selected spinal dorsal roots in anuran amphibians. *Anat. Rec.* **160**, 279–288.

SHAPOVALOV, A. I. & SHIRIAEV, B. I. (1978). Two types of electrotonic e.p.s.p. evoked in amphibian motoneurones by ventral root stimulation. *Expl Brain Res.* **33**, 313–323.

SHAPOVALOV, A. I. & SHIRIAEV, B. I. (1980). Dual mode of junctional transmission at synapses between single primary afferent fibres and motoneurones in the amphibian. *J. Physiol.* **306**, 1–15.

SIMPSON, J. J. (1976). Functional synaptology of the spinal cord. *Frog Neurobiology*, ed. LLINAS, R. & PRECHT, W. Berlin: Springer-Verlag.

SZEKELEY, G. (1976). The morphology of motoneurones and dorsal root fibres in the frog's spinal cord. *Brain Res.* **103**, 275–290.

TAMAROVA, Z. A. (1977). Excitatory postsynaptic potentials induced in the frog lumbar motoneurones by muscle and cutaneous nerve stimulation. *Sechenov J. Physiol. U.S.S.R.* **63**, 806–813.

WESTERFIELD, M. & FRANK, E. (1980). Development of the monosynaptic reflex in the frog's spinal cord. *Soc. Neurosci. Abs.* **6**, 846.

WESTERFIELD, M. & FRANK, E. (1982). Specificity of electrical coupling among neurones innervating forelimb muscles of the adult bullfrog. *J. Neurophysiol.* (in the Press).

EXPLANATION OF PLATES

PLATE 1

Location of triceps motoneuronal pools. Horizontal section through the brachial spinal cord, rostral is to the left. Triceps nerves on both sides were labelled with HRP for 6 days. The labelled triceps neurones appear black (TMB reaction) and form a distinct cluster on each side, near the centre of the lateral motor column. The remainder of the motoneurones in the lateral motor column, counterstained with Neutral red, appear grey. Scale, 500 μm.

PLATE 2

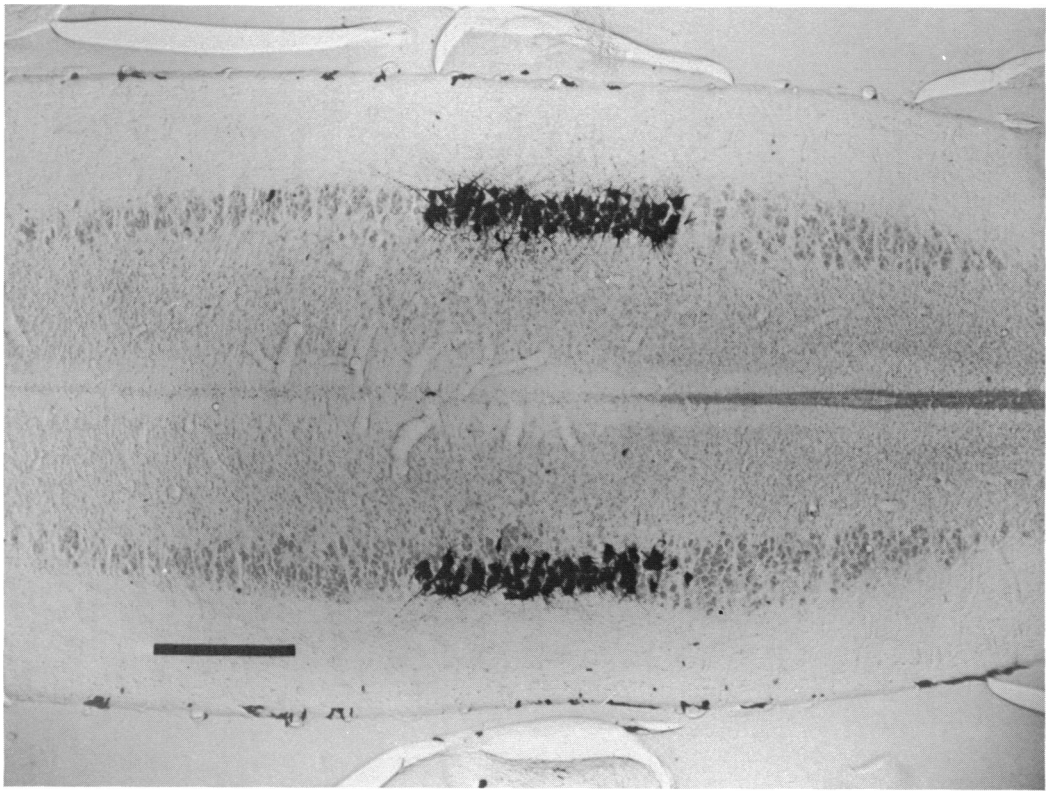
Sensory axons and motoneurones revealed by labelling the entire second spinal nerve (s.n. 2) with HRP. Transverse 50 μm section reacted with DAB after a 4 day exposure. Sensory fibres enter through the dorsal root (upper right), course longitudinally in the dorsal columns of the spinal cord and project ventrally into the two neuropile regions. Brachial motoneurones, located in a discrete column (arrow) in the ventral horn, have dendrites that arborize over a wide region, including the more ventral neuropile area of the sensory axons. Scale, 200 μm.

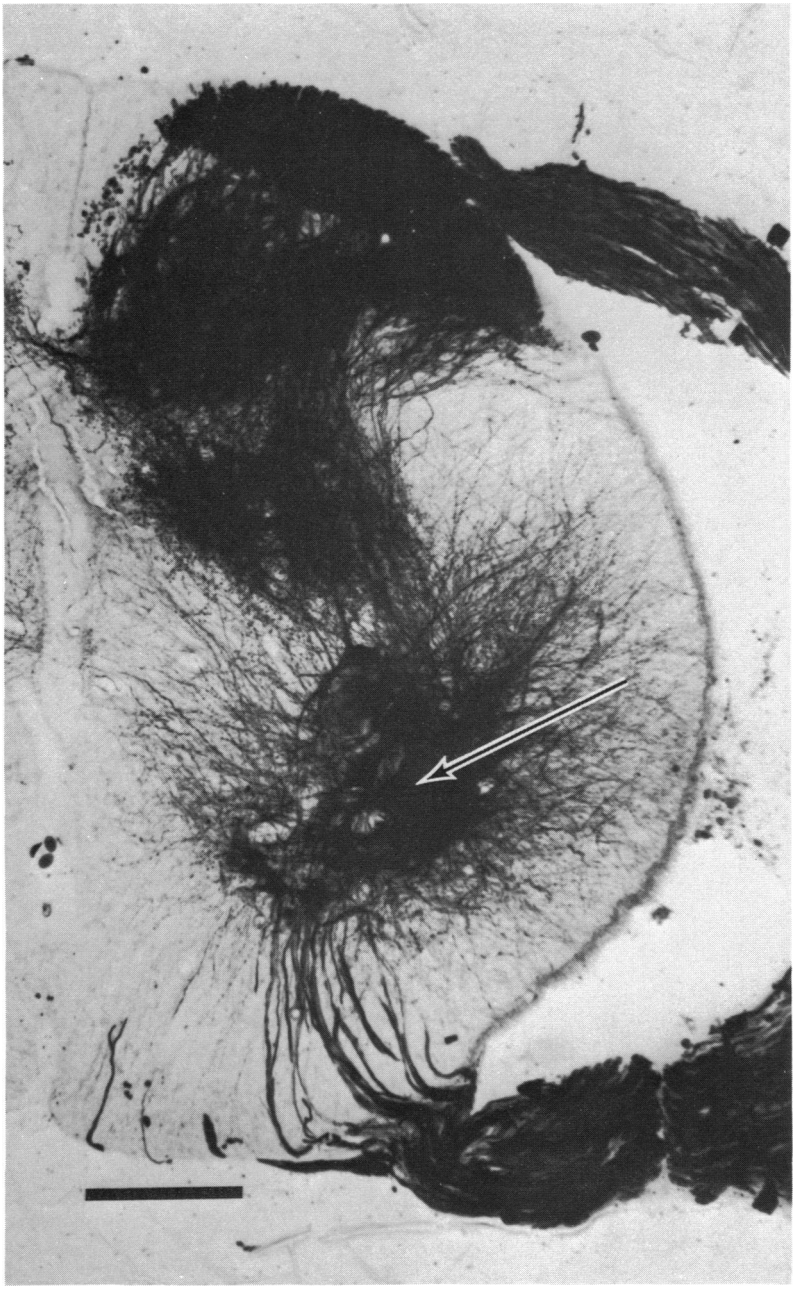
PLATE 3

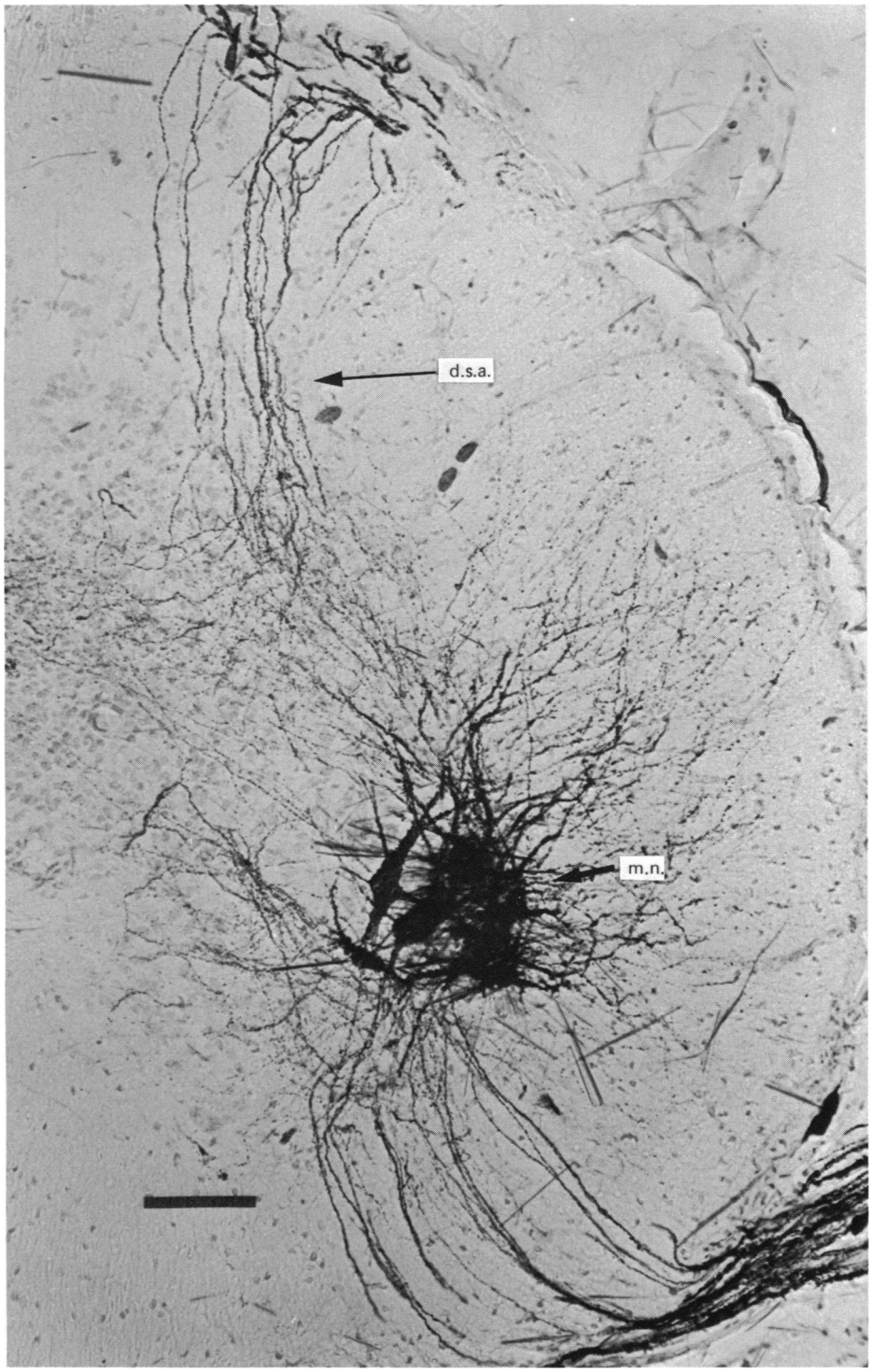
Triceps muscle sensory axons and motoneurones labelled with HRP. The triceps nerve was labelled with HRP and lysolecithin in a cuff placed in the arm 6 days previously, and the fixed tissue sections were reacted with TMB. This transverse section shows that the descending axon collaterals of the sensory fibres (d.s.a.) do not arborize in the more dorsal neuropile area shown in Pl. 2, but only in the ventral neuropile, where they overlap with dendrites of triceps motoneurones (m.n.). Scale, 100 μm.

PLATE 4

Contacts between triceps sensory and motor cells labelled with HRP. The triceps nerve was exposed for 13 days to lysolecithin and HRP, and tissue sections were reacted with DAB. Sensory fibres terminate in long chains of varicosities; one such chain (arrow) can be seen running near the dendrite of a labelled triceps motoneurone. Triceps motoneuronal somata are seen in the lower right; some sensory varicosities extend to within 20–30 μm of the cell bodies. Scale, 10 μm.







E. FRANK AND M. WESTERFIELD