Exp. Brain Res. 26, 215–234 (1976)

Experimental Brain Research

Spinal Branching of Corticospinal Axons in the Cat

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Summary. Branching patterns of single corticospinal (CS) neurons were studied in the cat by activating these neurons antidromically from various regions of the spinal cord.

1. One hundred and ninety-three neurons were activated antidromically by microstimulation in the gray substance of the cervical cord and the majority of them were found in the forelimb area of the pericruciate cortex.

2. Branches to the lower levels of the spinal cord were found for 30% of the neurons projecting to the cervical gray matter.

3. The remaining 70% sent axons only to the cervical gray matter and some of them sent multiple branches to several segments in the cervical cord.

4. Only a few CS neurons located outside of the forelimb area could be activated from the cervical cord, but all of them also sent branches to the lower levels of the spinal cord. Neurons projecting to both the cervical cord and the lower levels were intermingled in the cortex with those projecting only to the cervical cord.

5. CS neurons activated from a given area of the cervical cord were often clustered together in a small area of the cortex, although some of these CS neurons sent their other branches to other parts of the spinal cord and neurons projecting to other parts were also intermingled among them.

6. The functional significance of multiple axonal branching of CS neurons is discussed in relation to cortical motor functions.

Key words: Corticospinal neuron – Spinal axon branching – Microstimulation

Introduction

Systematic examination of motor effects elicited by stimulation of the motor cortex has revealed the existence of a rather precise topographic organization of the motor cortex (Leyton and Sherrington, 1917; Chang et al., 1947;

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Woolsey, 1958). Asanuma and collaborators (Asanuma et al., 1968; Asanuma and Rosén, 1972; Asanuma and Arnold, 1975) have shown that contraction of individual distal muscles can be elicited at low threshold from discrete areas within the motor cortex, i.e., the cortical efferent zones. They also observed that contraction of another muscle could be recruited with an increase of stimulus intensity (Asanuma et al., 1968). On the other hand, by recording monosynaptic potentials in spinal motoneurons, Jankowska et al. (1975) reported that pyramidal tract cells projecting to motor nuclei innervating different muscles were often located in overlapping areas and, moreover, reported that two or more discontinuous areas in the motor cortex might project to a single motoneuron. In addition to these problems there is still an important question as to whether individual corticospinal neurons terminate in one and only one motoneuron pool or terminate in different motoneuron pools.

It has recently been shown that pyramidal tract cells send branches to various subcortical nuclei (Tsukahara et al., 1968; Endo et al., 1973) and vestibulospinal (Abzug et al., 1974) and reticulospinal (Peterson et al., 1975) neurons branch widely in the spinal cord. The present study was undertaken to investigate whether individual corticospinal neurons also branch and project to separate levels of the spinal cord in the cat. The results obtained by microstimulation of corticospinal axon branches indicate that there exist corticospinal neurons that send axon branches to widely separated levels in the spinal cord, suggesting that single corticospinal neurons may change the excitability of different motoneuron pools.

Methods

A total of 13 cats, weighing between 2.1 and 3.0 kg, was used for the experiments. Operations were performed under sodium pentobarbital (Nembutal 40 mg/kg) anesthesia. The spinal cord was exposed from C3 to Th3 and also at L1, by laminectomy. The dura was opened and the cord was covered with warm mineral oil. The motor cortex was exposed by craniotomy and a closed chamber was installed on the skull over the craniotomy opening. The cisterna magna was opened to prevent swelling of the brain during the experiments. Photographs were taken of the exposed cortex to map the locations of electrode insertions. The animal was then paralyzed by gallamine triethiodide (Flaxedil) and artificial ventilation was initiated. Bilateral pneumothorax was made to minimize movement of the spinal cord. Throughout the experiments, rectal and oil pool temperatures were maintained at 37–38° C. Supplemental doses of Flaxedil and Nembutal (10 mg/kg) were administered at every 2 hrs.

Stimulation and Recording

Activities of single CS neurons were recorded in the pericruciate cortex with microelectrodes made of tungsten wire insulated with a glass micropipette, except for its tip, which was 15–30 μm in length and 10 μm in diameter (Stoney et al., 1968). An array of 5–12 microelectrodes similar to the recording electrodes was inserted into different segments of the cervical gray matter (C4–C8) contralateral to the exposed cortex, to stimulate terminal branches of corticospinal fibers. In addition, two larger bipolar stimulating electrodes separated by 0.5 cm were made of electrolytically etched tungsten wire insulated with lacquer except at the tip. They were implanted in the lateral column at the level of the caudal end of Th3 and at L1 to stimulate the lateral

corticospinal tract contralateral to the cortical recording site. In a few experiments, two pairs of ball electrodes were placed on the surface of the lateral funiculus at the caudal end of Th3 and at L1 levels.

For stimulation of axons of CS neurons, cathodal pulses of 0.3 msec duration and constant intensity were used. They were delivered from the electrodes in the cervical gray matter to an indifferent electrode in the temporal muscle, or bipolarly between electrodes in the lateral funiculus.

Histological Examination

During the experiments, several lesions were made in the motor cortex by passing negative current of 20 μa for 10 sec through the recording electrode. At the end of the experiments, a current of 20 μa was passed for 10–20 sec through each of the cervical stimulating electrodes. The animals were then perfused with saline followed by 10% formalin solution. The motor cortex and cervical cord were removed and 50 μm thick frozen sections were stained by Klüver and Barrera's method (1953). The electrode tracks in the motor cortex and the cervical cord were reconstructed utilizing the lesions made during the experiments.

Results

Criteria for the Antidromic Activation of Corticospinal Neurons

The recording microelectrode was inserted into the pericruciate cortex while stimulating the cervical gray matter through an array of microelectrodes, each delivering current pulses of 50 μa. Whenever negative spikes of a fixed latency were recorded, the electrode eliciting the spikes was determined by sequentially stimulating through each of the electrodes. Threshold current for eliciting the spikes was then measured and using double shocks of 1.5 times the threshold current, the interval at which the second spike was evoked with 50% probability was determined. Cortical cells were considered to be antidromically activated when they (a) responded at a fixed latency to stimuli of 1.5 times threshold and (b) faithfully followed double shocks of that intensity at an interval of 1.4 msec or less. The validity of the above criteria was confirmed in 30 spontaneously active neurons using a collision method. By changing the intervals between the spontaneous discharges and the cervical stimuli, the interval for 50% appearance of the evoked spikes was measured. This interval should be close to the sum of twice the latency of the evoked spike and the refractory period of the stimulated axon if the cervical stimulation in fact activates the cell antidromically. Since the result showed that spikes which followed double shocks with the intervals of 1.4 msec or less and with a fixed latency could be unequivocally classified as antidromic, these two criteria were used throughout the experiments to determine whether cells were activated antidromically.

To determine the optimum stimulus duration for exciting the terminal branches of CS neurons, 16 strength-duration curves were constructed. Figure 1 illustrates 7 of these curves chosen randomly from those with a minimum threshold of 5 μa or less. The chronaxies of the 16 fibers ranged from 0.04–0.16 msec (mean and standard deviation being 0.11 ± 0.04 msec).

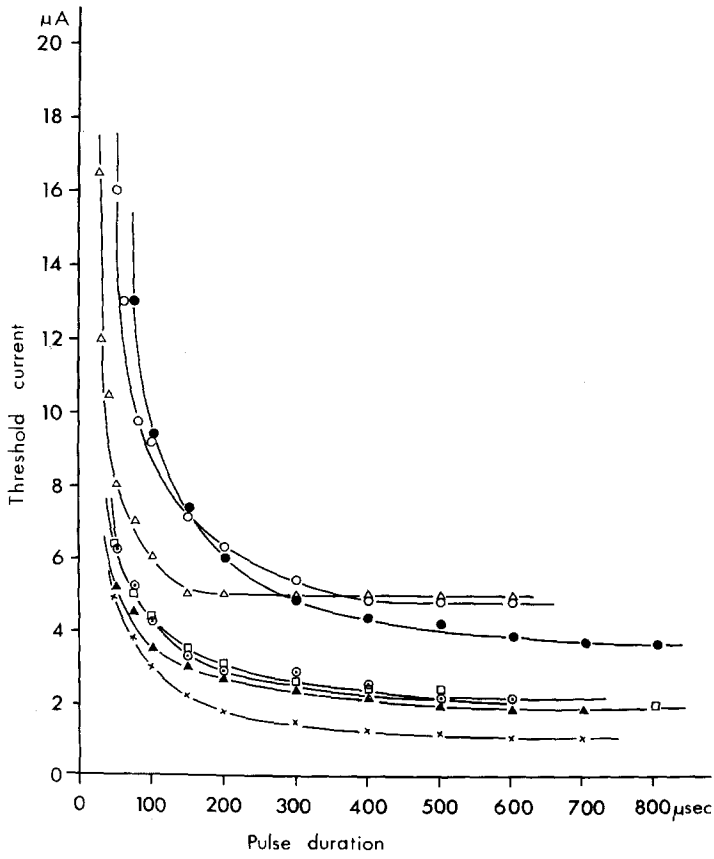

Fig. 1. Strength-duration curves for corticospinal fibers in the cervical gray matter. Six curves for fibers with lowest threshold values of 5 μa or less, are chosen

These values are roughly the same as those obtained for fibers in the spinal cord (BeMent and Ranck, 1969a; Jankowska and Roberts, 1972; Jankowska and Smith, 1973) and axon collaterals of PT cells (Asanuma et al., in press), but shorter than those of PT cells (Stoney et al., 1968). Since the curves approached the rheobase at around 0.3 msec, a pulse duration of 0.3 msec was used throughout the experiments.

In the early stages of the experiments, it was noticed that the antidromic spikes disappeared when stimulus intensity was increased to around 10 times the threshold current, probably due to anodal block at a distant site from the stimulating electrode (Katz and Miledi, 1965; Jankowska and Roberts, 1972). Hence, the stimulus intensity was frequently alternated between 50 μa and 10 μa during the experiments.

Criteria for Stimulating Axon Collaterals of Corticospinal Tract Fibers

Since the purpose of the present experiments was to examine the axonal branching of CS neurons terminating within the cervical gray matter, it was

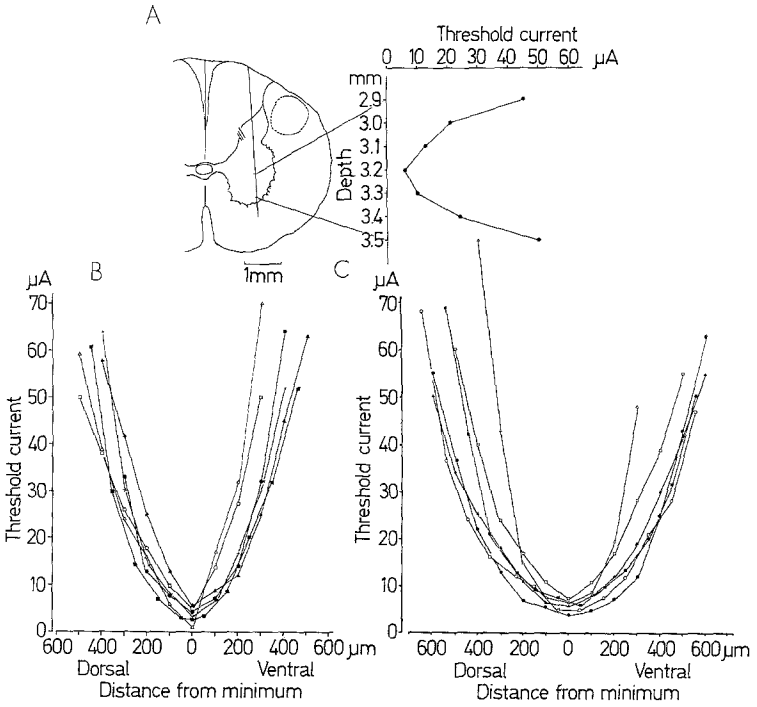

Fig. 2A—C. Depth-threshold for stimulation of axon branches of corticospinal neurons. **A** An example of the electrode track and the curve obtained. Dotted circle represents the lateral corticospinal tract. Threshold currents (abscissa) were plotted against the electrode depth below the cord surface (ordinate). **B** Curves for six axon branches of corticospinal fibers having lowest thresholds of 5 μa or less. The depth is expressed as the distance relative to the point of minimum threshold (zero). **C** Curves for six stem axons in the lateral corticospinal tract with lowest thresholds of 7 μa or less

essential to ascertain that cervical stimulation activated local axon branches in the gray matter and not fibers within the corticospinal tract. To exclude the possibility of current spread to the tract, the following measurements were made. When a stimulating microelectrode was capable of activating a CS neuron with a threshold current of 50 μa or less, the single microelectrode was moved vertically in the same track to find the lowest threshold point. A representative example of the depth-threshold current relationship is shown in Fig. 2A. The lowest threshold point in this track was at a depth of 3.2 mm from the cord surface, indicating that the closest approach of the electrode to the excited axon was achieved at this point. Since the plane perpendicular to the electrode track at this point does not intersect the lateral corticospinal tract (dotted circle in Fig. 2A), the stimulus must have activated a local axon branch and not a fiber in the tract. This method was one of three used to exclude the possibility of current spread to stem axons in the tract and is valid over the entire range of stimulus intensity. In many cases, however, the lowest threshold point was at the same level as the lateral corticospinal tract or the ventral

corticospinal tract as indicated on the figures of Chambers and Liu (1957), Petras (1967) and the atlas of Verhaardt (1964). In such cases it was necessary to estimate the extent of current spread to exclude the possibility of direct activation of the fibers in the corticospinal tract. For this purpose, the changes in threshold with depth were established for 36 axon branches in the gray matter and 12 stem axons in the lateral corticospinal tract. We compared distance-threshold curves of axon branches and stem axons since the larger stem fibers should have a lower threshold (BeMent and Ranck, 1969a) and should be activated over a greater distance (Jankowska and Roberts, 1972). Distance-threshold curves for six axon branches, having lowest thresholds of 5 μa or less, are shown in Fig. 2B. The curves were normalized by plotting the depth of the stimulating electrode relative to the point of minimum threshold for each axon. The lowest threshold intensity of 293 fibers activated by stimuli in the gray matter was 1.0 μa. Judging from these curves, stimulation with less than 50 μa will activate an axon branch at a distance no greater than 500 μm (Fig. 2B). On the assumption that threshold current for excitation of an axon is approximately proportional to the distance from the site of excitation to the tip of the stimulating electrode (BeMent and Ranck, 1969b; Bean in Appendix of Abzug et al., 1974), the square of the threshold current as a function of the squared diplacement from the minimum threshold point was plotted using 22 neurons (not illustrated). The result indicated that the relationship was approximately linear except for the very low threshold range. The proportionality constant rangend from 4.7–12.0 μm/μa (mean and standard deviation, 8.8±1.2 μm/μa). Figure 2C represents the distance-threshold curves of six stem axons with lowest thresholds of 7 μa or less. The curves are broader than those for axon branches. The relationship between the threshold and the distance, calculated in the same way, was not linear. Therefore, the proportionality constant (μm/μa) is different for different parts of the curves. From the curves represented in Fig. 2C, it is concluded that the maximum effective spread of stimulus current is 500 μm at 20 μa, 600 μm at 35 μa and 700 μm at 50 μa. To exclude the possibility that the stimulating current activated the fibers in the corticospinal tract directly, the distance between each stimulating site and the nearest region of the lateral corticospinal tract was measured in each experiment by histological examination. The nearest region of the tract was determined from the figures of Chambers and Liu (1957), Petras (1967) and the atlas of Verhaardt (1964). This distance ranged from 580–1700 μm. Taking into account the above estimated values of current spread and the distances between the corticospinal tract and the stimulated sites in respective cases, it was possible to exclude the possibility of direct activation of the stem fibers. The measured distances represent a highly conservative estimate because most stem axons were likely to be located further away from the electrodes and the shortest possible distance was used in the above assessment. From these considerations it can be safely concluded that the CS neurons sampled in the present experiment were activated antidromically by stimulation of local branches in the cervical gray matter, and not by stimulation of the main axons in the lateral corticospinal tract. So far, the current spread to the lateral corticospinal tract has been discussed but the

same criterion was applied to analysis of current spread to the ventral corticospinal tract and we could also exclude the possibility that the stimulus current spread to the ventral corticospinal tract. This conclusion was strengthened further by collision experiments which will be described in the following section.

Branching of Individual Corticospinal Neurons to Multiple Cord Levels

Whenever a CS neuron was activated antidromically by stimulation of the cervical gray matter, the corticospinal tract was stimulated at the thoracic and lumbar level to determine whether the same neuron sent axons to the lower levels of the spinal cord. When a neuron was activated from two or more of the electrodes, collision of the impulses initiated from two different sites was studied by delivering a pair of stimuli in a conditioning-testing fashion. This procedure allowed us to ascertain that both stimuli activated the same neuron antidromically, and to calculate the conduction time between the branching point of the axon and the site of excitation. If both responses are antidromic and the same neuron is activated, the maximal interval between two stimuli for the blockade of the second impulse should correspond to the sum of the conduction time between the two stimulating sites and the refractory period of the axon at the second site. Only such neurons that satisfied the above condition were accepted in this study. On the other hand, if one of the responses is synaptically activated, it should be blocked by a preceding evoked spike only during the refractory period of the soma or the initial segment of the axon (Darian-Smith et al., 1963). An example of such a collision experiment is illustrated in Fig. 3. This neuron responded to stimulation of the gray matter at the level of C6 and also of the thoracic corticospinal tract. The latency of the spikes evoked from the cervical cord (L_c) was 2.22 msec (Fig. 3A) and from the thoracic cord (L_t) was 2.37 msec (Fig. 3B). The refractory periods for the axons in the cervical gray (R_c) and in the thoracic cord (R_t) were 0.58 msec (A) and 0.81 msec (B) respectively. The collision was tested by delivering a stimulus to the thoracic corticospinal tract followed by a stimulus to the cervical gray matter. As shown in Fig. 3C–E, the second spike appeared with 100%, 50% or 0% probability depending on the interval between the two successive stimuli. The inter-stimulus interval which produced disappearance of the second spike in about 50% of the trials (I_{tc}) was 1.38 msec (Fig. 3D). When the order of the stimuli was reversed (Fig. 3F–H), the interval for 50% collision (I_{ct}) was 1.47 msec (Fig. 3G). Assuming that branches of the same neuron have been activated from different sites, the conduction time (X_c) between the branching point of a local axon at the corticospinal tract and the site of excitation in the cervical gray matter may be calculated as follows:

$$X_c = {}^1/_2 \, (I_{tc} + L_c - L_t - R_c) \qquad (1)$$

where I_{tc}, L_c, L_t, and R_c are as already defined. In the example of Fig. 3, the

calculated X_c was 0.32 msec. The latent periods for spike generation are absent from this equation for reasons given in the Appendix[3].

If the stimulus current spreads directly to the fibers in the corticospinal tract, the conduction time (X_c) is expected to be zero. This was tested

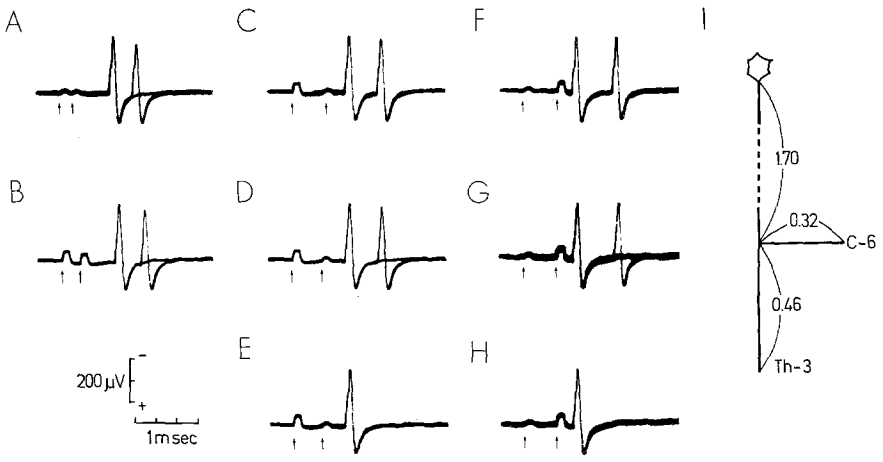

Fig. 3A–I. Measurement of the conduction time along the branches of a corticospinal fiber. **A and B** Measurement of refractory periods by double shock stimuli at cervical gray matter and thoracic cord. **C–E** Measurement of collision time between thoracic and cervical branches. With the interval shown in D the second spike appeared in 50% of the trials. **F–H** Same as C–E, but the sequence was reversed. Six sweeps are superimposed in each case. **I** Schematic drawing of branches and the conduction times along the branches obtained from the measurements shown in A–H. The latent periods for spike generation (about 0.2 msec) were subtracted from the measured conduction times. The same consideration was applied to Fig. 4 and Fig. 5. Further details are in the text

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[3]*Appendix.* Equation (1) is derived as follows.

Let: X_t = the conduction time from the point of stimulation in the thoracic cord to the point (z) at which the fiber branches to the cervical gray matter.

X_c = the conduction time between the point of activation in the cervical gray and point z.

X_z = the conduction time between point z and the PT cell.

u_c, u_t = latent periods for spike generation after the start of stimulating pulse at cervical and thoracic points of stimulation, respectively.

R_c, R_t = refractory periods measured by double shock stimuli, at cervical and thoracic points of stimulation, respectively.

I_{tc} = maximal conditioning-test interval when the spikes evoked from the cervical cord are blocked by the spikes from the thoracic cord.

I_{ct} = maximal conditioning-test interval when the stimulus order is reversed.

Then: $L_c = u_c + X_c + X_z$

$L_t = u_t + X_t + X_z$

$I_{tc} = u_t + X_t + X_c + R_c - u_c$

In the last equation, $(R_c - u_c)$ was used instead of R_c, because in this case the latent period for initiation of the first spike should not be included. Solving these equations for X_c, equation (1) results.

$$X_c = {}^1/_2 \, (I_{tc} + L_c - L_t - R_c) \qquad (1)$$

experimentally by implanting separate electrodes in the lateral corticospinal tract at cervical and thoracic levels. Using the same collision method, the value (X_c) was calculated and it was 0.0±0.03 msec (mean and standard deviation, N = 12), the maximum value being 0.13 msec. Taking this experimental error into account, we considered that the axon collaterals from the corticospinal tract were activated in the gray matter when the conduction time (X_c) along the branches was 0.15 msec or more. The conduction time (X_c) from the branching point to the stimulated site in the cervical gray matter ranged from 0.15–0.80 msec. All the neurons that satisfied the above criterion also satisfied the previous criterion derived from estimation of current spread. The results conclusively demonstrated that local axon branches were activated antidromically within the cervical gray matter and the possibility of current spread to both lateral and ventral corticospinal tract was excluded.

Branching Pattern in the Cervical Cord

An attempt was made to assess the pattern of terminal arborization of individual CS neurons in the cervical cord by defining axon branches from which they were activated antidromically. When a neuron was activated from at least one of the cervical electrodes, 8–12 electrodes implanted in the cervical gray matter were moved vertically over a distance of 1.5–2.5 mm and the effects of microstimulation from each electrode were examined at various depths. In addition, when several electrodes activated the same neuron antidromically, the collision test was performed between each possible pair of them and the conduction time along each axon branch was estimated using equation (1). Figure 4 illustrates an example of the longitudinal distribution of axon collaterals of a CS neuron in such an experiment. This neuron was activated antidromically from four electrodes arranged as shown in A and the dimensions of the axonal arborization of this neuron were determined as shown in C. It was found that this neuron sent at least four independent axon branches into the spinal gray matter over a distance of four segments (Fig. 4B and C).

Stimulating microelectrodes were moved to find the lowest threshold areas in about one-fourth of the experiments and fixed in the other experiments. Table 1 summarizes the results obtained from both types of experiments. Altogether 193 corticospinal neurons of the cat pericruciate cortex were identified as antidromically activated by stimulation of the cervical gray matter with the currents of 50 μa or less. Of these, 57 neurons (30%) were also activated by stimulation of the thoracic cord. Eleven of the latter group (6% of

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In the equation (1), R_c indicates the refractory period of the axon branch at the cervical point of stimulation. But in the motoneurons, the refractory period of the soma or the initial segment of an axon is longer than that of the axon (Coombs et al., 1957). Therefore, the refractory period of the soma of Cs neuron may have been measured by double shock stimuli rather than the refractory period of the axon in the present study. Hence, we may have overestimated R_c, which would lead to an underestimate of X_c. In spite of this, the calculated values of X_c were invariably greater than 0.15 msec, indicating that axon branches were activated.

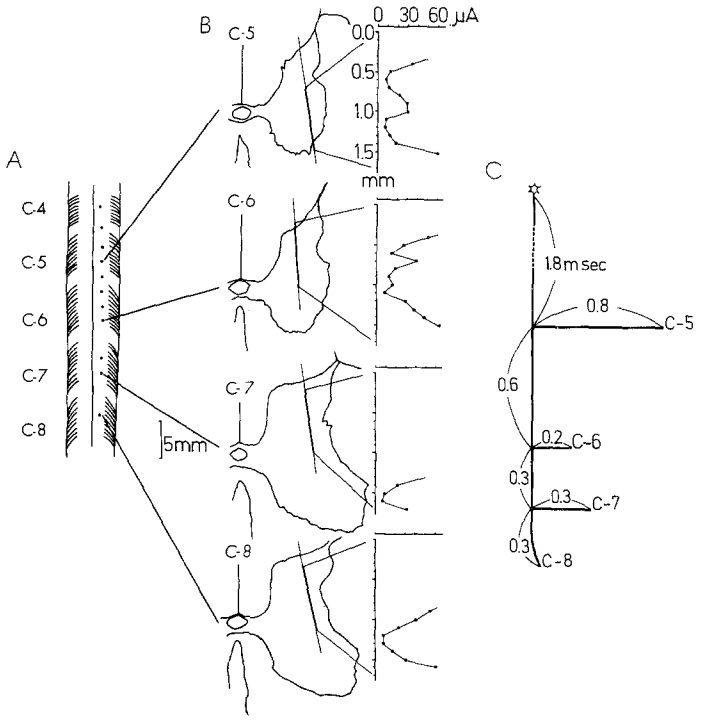

Fig. 4A–C. Multiple branching of a corticospinal fiber in the cervical gray matter. The neuron was activated antidromically from C5, C6, C7 and C8. Stimulating electrodes were placed as shown in **A**. While recording from the same neuron, stimulating electrodes were moved vertically and threshold currents at each position were determined. In **B**, threshold currents (abscissa) were plotted against depths (ordinate). Electrode tracks were reconstructed from the histological section. Thin and thick lines represent an electrode track and the surveyed area in that track respectively. The branching pattern of this neuron is shown in **C**. Numbers in the diagram indicate conduction time (msec) calculated from collision tests

total) could also be driven from the first lumbar segment. The remaining 136 neurons (70%) were activated only from the cervical area. Of these 136 neurons, 99 neurons were activated from only one or two electrodes located in one segment and 37 neurons were driven from two or more segments (Table 1–I). Twenty of these 37 were activated from two adjacent segments, and the rests were activated from more widely separated areas. Of the 57 neurons activated from both the cervical gray matter and the thoracic cord including the ones driven from the lumbar cord as well (Table 1–II, 1–III), 41 were activated from only one cervical segment. The remaining 16 neurons sent two or more branches to different cervical segments before terminating in the thoracic or lumbar cord. Since all axons were tested for thoracic and lumbar projection using the current of 2 ma it is unlikely that there were fibers in these regions of the tract which escaped from stimulation. This conclusion is supported by the observation that all neurons activated from the lumbar cord could also be driven from the thoracic cord.

Table 1. Patterns of branching of corticospinal neurons (No. = 193)

I. Neurons activated only from the cervical gray matter (No. = 136).

A. Activated from one cervical segment

	C4	*C5*	*C6*	*C7*	*C8*	*Total*
No.	28	13	24	27	7	99

B. Activated from two cervical segments

	C4+C5	C5+C6	C6+C7	C7+C8	C4+C6	C5+C7	C4+C7	C5+C8	C4+C8	Total
No.	2	7	9	2	2	2	1	1	3	29

C. Activated from three or more cervical segments

	C4+C5+C7	C5+C6+C7	C5+C7+C8	C4+C5+C8	C4+C6+C7+C8	C5+C6+C7+C8	Total
No.	1	2	1	2	1	1	8

II. Neurons projecting to both cervical and thoracic cord (No. = 46).

A. Activated from one cervical segment

	C4	*C5*	*C6*	*C7*	*C8*	*Total*
No.	1	6	7	13	4	31

B. Same as A, but two or more branches in the cervical cord were activated.

	C4+C5	C5+C6	C6+C7	C7+C8	C6+C8	C5+C8	C5+C6+C7	Total
No.	1	4	6	1	1	1	1	15

III. Neurons activated from both cervical and first lumbar cord (No. = 11).

A. Sites of branching in the cervical cord.

	C4	*C5*	*C6*	*C7*	*C8*	*C6*+C7	Total
No.	1	2	2	5	0	1	11

Among the CS neurons activated from three or more different sites in the spinal cord, there were three patterns of branching from the main fiber. In one pattern, two local axons arose from different sites of the tract as shown in Fig. 5A. In the second pattern, represented in Fig. 5B, only one local axon originated from the tract and then split into two branches. In the third pattern, shown in Fig. 5C, one local axon stemmed from the tract which was activated from two different sites. In cases when spikes were evoked from multiple electrodes in one segment, the latter two types were common, although differentiation between these two was sometimes difficult. Therefore, neurons belonging to the latter two patterns were classified in the Table 1 as the group of neurons projecting to only one cervical segment. The first type was observed when spikes were elicited from areas more than two segments apart. All neurons in the groups classified as IC, IIB and III belonged to the first type.

When neurons were activated from only one electrode, we could not exclude the possibility that these neurons sent other undetected axon collaterals to the cervical cord. The shortest distance between stimulating electrodes was 2.0 mm and the maximum current used was 50 μa. The current is likely to have activated only those axon collaterals located within a radius of about 500 μm, and hence, it is expected that the number of neurons sending multiple branches to the cervical cord was underestimated.

The locations of effective stimuli in the cervical gray matter with currents of 10 μa or less are summarized in Fig. 6. Most of these spots were located in laminae IV, V, VI and VII of Rexed (Rexed, 1954), but some were found in laminae VIII and IX. Triangles indicate the sites where CS neurons sending

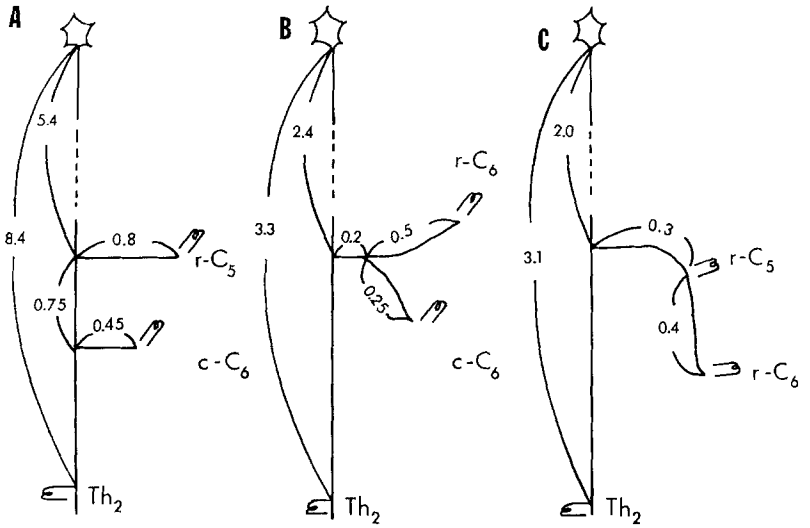

Fig. 5A–C. Three different examples of branching patterns in the cervical cord from corticospinal fibers sending axons to the thoracic cord. **A** axon collaterals are given off at different levels of the tract. **B** one collateral is given off and it then bifurcates into multiple axon branches. **C** one axon collateral travels a long distance within the cervical gray matter. Numbers in the diagram represent conduction times along the distances indicated

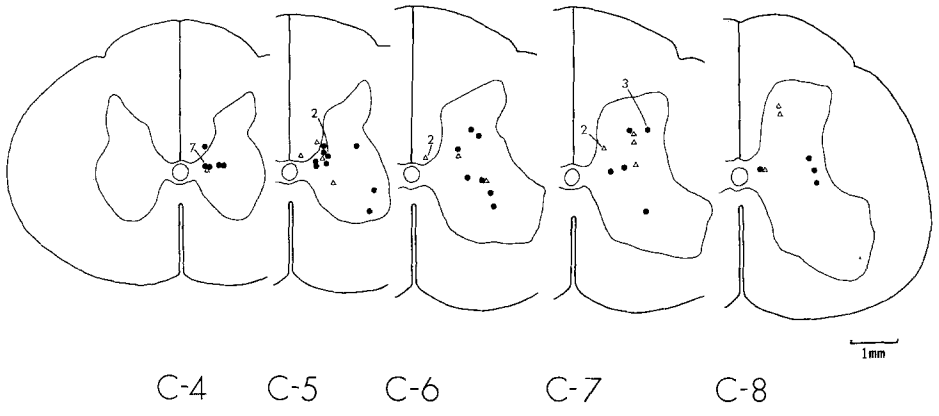

Fig. 6. Distribution of the sites of stimulation where corticospinal neurons were activated antidromically with currents of 10 μa or less. Dots represent sites where neurons sending their axon only to the cervical cord were recorded and open triangles represent sites where neurons sending their axon to the thoracic cord as well as to the cervical cord were recorded. The numbers in the figure show the number of neurons activated from the same spots

axons to the cervical gray and also to the thoracic cord were activated. These sites were interspersed among others and there was no obvious topographical difference in their distribution. When moving the stimulating electrodes up and down in search of the lowest threshold point to activate an axon

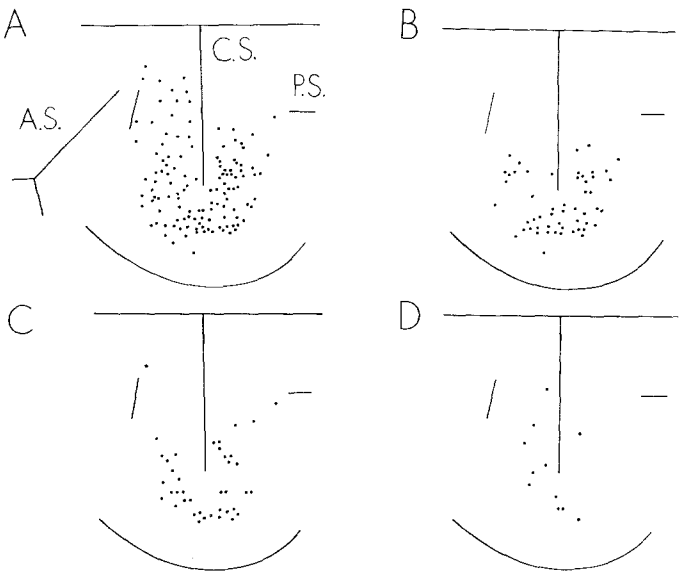

Fig. 7A–D. Distribution of corticospinal neurons projecting to various levels in the spinal cord. Locations of neurons were represented by the sites of electrode insertions. **A** total sites of electrode insertions made throughout the experiments. **B** distribution of neurons projecting only to the cervical cord. **C** neurons projecting to the cervical and thoracic cord. **D** neurons projecting to the cervical and lumbar cord. Abbreviations: *A.S.* ansate sulcus, *C.S.* cruciate sulcus, and *P.S.* presylvian sulcus

antidromically, it was not unusual to find more than one low threshold site in the gray matter in one track. Such an example is shown in Fig. 4B, in which two low threshold sites were separated by about 500 μm. This observation suggests that individual corticospinal neurons may send axons to more than one lamina in the gray matter.

Cortical Distribution of Neurons Activated from Different Levels in the Cervical Cord

Recording electrodes were inserted not only into the pericruciate but also into the postcruciate gyrus including forelimb and hindlimb areas (Fig. 7A). These areas included area 4γ and a portion of 3a of Hassler and Muhs-Clement (1964). CS neurons activated only from the cervical gray matter were located in the area surrounding the lateral end of the cruciate gyrus (Fig. 7B). These neurons were found exclusively in the forelimb area of the pericruciate cortex, or nearby 3a, but not in the hindlimb area which is medial to the level of the presylvian sulcus (Armand et al, 1974; Woolsey, 1958; Thompson and Fernandez, 1975). The distribution of neurons driven from the cervical gray matter as well as the thoracic cord, but not from the lumbar cord, is shown in

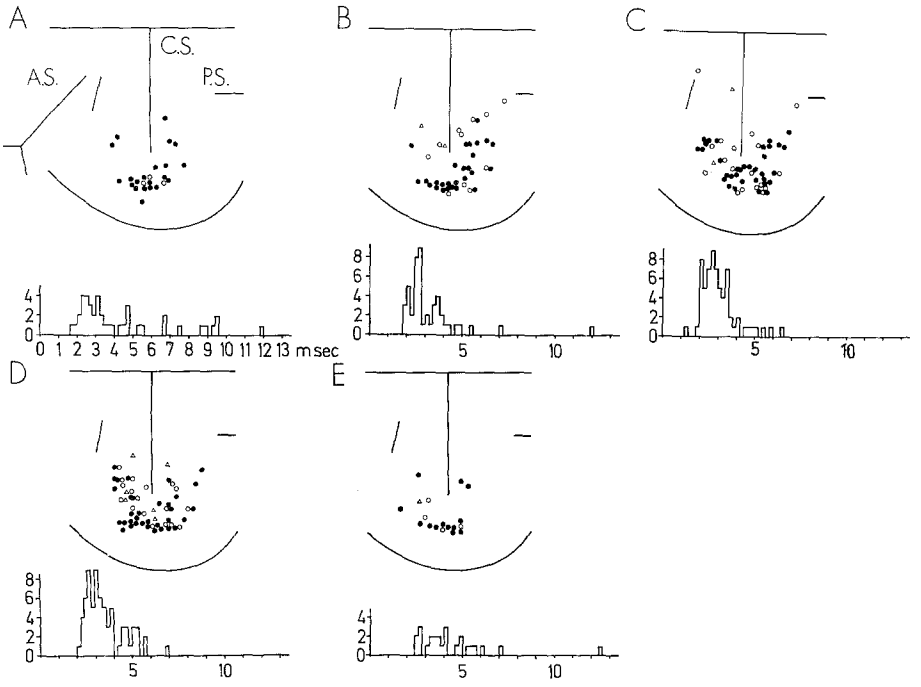

Fig. 8A–E. Distribution of corticospinal neurons activated antidromically from different cervical segments. Each symbol represents the site of electrode insertion in which one to 11 neurons were recorded. **A–E** neurons activated from C4 (A), C5 (B), C6 (C), C7 (D) and C8 (E) respectively. Latencies of evoked spikes from each segment are shown in each histogram attached to the diagrams. When neurons were activated from more than one segment, they were plotted in all appropriate diagrams. Filled circles show neurons projecting only to the cervical cord; open circles, neurons projecting to both the cervical and the thoracic cord; triangles, neurons projecting to both the cervical and the lumbar spinal cord

Fig. 7C. These neurons were scattered in the area where corticospinal neurons projecting only to cervical spinal cord were located. This result was in good agreement with the finding that in many cases, neurons recorded in one penetration were a mixture of neurons projecting only to cervical segments and projecting to both cervical and thoracic cord. There were very few corticospinal neurons which were driven from both the cervical and the lumbar cord and their location is illustrated in Fig. 7D.

Figure 8 shows the cortical distribution of CS neurons activated from different cervical segments. Neurons projecting to thoracic (open circles) and lumbar levels (triangles) as well as to the cervical gray matter were intermixed with neurons activated only from cervical segments (filled circles). No systematic distribution of neurons projecting to different cervical segments could be found.

The latencies of antidromic spikes evoked by stimulation of the cervical gray matter at different segments are plotted in the inset histograms in Fig. 8A–E. These indicate that neurons with shorter latencies predominated in our sample.

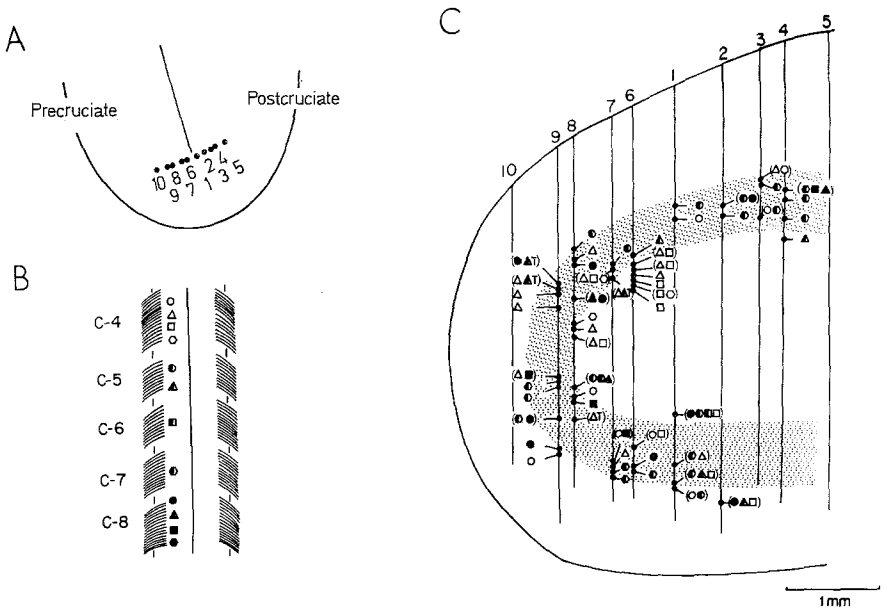

Fig. 9A–C. Distribution, within the forelimb area of the pericruciate cortex, of corticospinal neurons activated from different cervical segments. **A** sites of electrode penetration into the cortex. **B** locations of cervical electrodes. Each electrode is marked by a different symbol. **C** reconstruction of electrode tracks from the histological examination. Antidromically activated neurons are marked with different symbols depending on the effective stimulating electrode. The letter *T* indicates the electrode in the corticospinal tract at Th3. The stimulating electrodes were fixed at the same positions throughout the experiment. When a neuron was activated from two or more different sites, those stimulating sites were indicated in parentheses. The lower portion of Fig. 9C belongs to area 3a according to Hassler and Muhs-Clement

During the course of the experiments, it was noted that neurons located close together in the cortex were often activated from the same electrode in the cervical cord. In the entire series of experiments, two or three corticospinal neurons were recorded at the same recording site in 15 cases. In seven out of these 15 cases, two corticospinal neurons were activated from the same electrode at a fixed position in the cervical gray matter with thresholds of 42 μa or less. This finding suggested that CS neurons projecting to a narrow area in the spinal cord may be clustered together in the cortex. To examine this possibility, we recorded as many corticospinal neurons as possible in one plane of the pericruciate cortex and examined the sites of the spinal cord from which these neurons could be activated (Fig. 9). An array of 12 electrodes was implanted in the cervical gray matter as shown in Fig. 9B, and fixed in the same positions throughout the experiment. Altogether 10 cortical penetrations were made in one plane (A) and recording sites were reconstructed (C) in reference to seven electrolytic lesions made during the experiment. Six out of nine cells, in penetrations of No. 2, 3, and 4, were activated from No. 8 electrode at C–7 (half-filled hexagon), although neurons projecting to other parts were intermingled among them (neurons marked by open triangle and circle, and by half-filled triangle). Among these neurons, the neurons

projecting to the widely separated cervical levels (C4 and C7, C5 and C8) were intermixed. In the upper portion of penetrations No. 6 and 7, seven cells were driven from either No. 2 electrode at C–4 (open triangle) or No. 3 electrode at C–4 (open square), or both, which were 2.0 mm apart. In addition, a neuron projecting to both cervical and thoracic cord was intermingled with the neurons projecting only to the cervical level.

Discussion

I. Technical Considerations

In the present study, corticospinal neurons activated by stimulation of the axons in the cervical gray matter were sampled. Three methods were used to ascertain that intraspinal microstimulation activated axon branches and not stem axons in the corticospinal tract: estimation of current spread, measurement of lowest threshold point along the track and calculation of conduction time along axon collaterals. Our calculations indicated the extent of effective stimulus for axon collaterals in the gray matter was 8.8 ± 1.2 μm/μa (mean and S.D.). The maximum value obtained was 12 μm/μa. These values are in good agreement with those obtained by BeMent and Ranck (1969a) and Abzug et al. (1974). On the other hand, the slopes of distance-threshold curves were less for stem axons in the tract and the nonlinearity of the threshold-distance relations were more pronounced. These characteristics were closer to those obtained from studies of Ia interneurons by Jankowska and Roberts (1972) and dorsospinocerebellar tract by Roberts and Smith (1973). Therefore, we estimated the extent of effective stimulus from distance-threshold curves of stem axons (Fig. 2C) to exclude the possibility of direct activation of the stem axons in the corticospinal tracts.

II. Functional Significance of Branching Fibers

CS neurons were sampled in the pericruciate cortex corresponding to area 4γ of Hassler and Muhs-Clement (1964). As shown in Fig. 7, all the neurons activated only from the cervical cord were located in the forelimb area and lateral border of the trunk area (Armand et al., 1974; Asanuma and Sakata, 1967; Woolsey, 1958). Most of the neurons projecting to both the cervical gray matter and the lower level of the spinal cord were located within the same area and a small fraction of them were also found at about the level of the ansate sulcus which is the lateral border of the hindlimb area. The existence of neurons projecting from the forelimb area of the motor cortex to the thoracic and lumbar cord was first described by Sherrington (1889) based on the Marchi method in the monkey. Recently, this problem was reinvestigated in cats (Chambers and Liu, 1957; Nyberg-Hansen and Brodal, 1963) and monkeys (Kuypers and Brinkman, 1970; Liu and Chambers, 1964) using the Nauta method. These investigators found terminal degenerations

predominantly in the cervical gray matter after making discrete lesions in the forelimb area of the cat and monkey cortex. In addition, they observed that a considerable fraction of fibers from the forelimb area projects to the upper thoracic cord and that a small fraction projects to the lumbar cord. Our present results are in good agreement with these anatomical results. These anatomical studies, however, could not distinguish neurons projecting only to the thoracic or the lumbar cord from neurons projecting to both the cervical gray matter and the lower levels. The present study resolved this important question. It is demonstrated that 30% of 193 CS neurons projecting from the forelimb area to the cervical cord also send branches to the lower levels. These neurons are intermingled with those projecting only to the cervical cord. Whether or not there exist neurons in the forelimb area of the cortex which terminate only at the thoracic or lumbar cord remains unsolved because of the methods employed. However, it may be relevant to state that practically no neurons in the hindlimb area projected only to the cervical cord (Fig. 7B).

Among the 136 neurons terminating only in the cervical cord 80% were activated only from one cervical segment. For these neurons, we still cannot conclude that they actually sent axons only to one site of the cord. However, as shown in Fig. 9, CS neurons in a small area of the cortex often send at least one of their branches to a common area of the cervical cord. This finding might explain why weak intracortical microstimulation elicits contraction in a single muscle (Asanuma et al., 1968) whereas stronger microstimulation often produces contraction of multiple muscles (Asanuma et al., 1968; Andersen et al., 1975). In most of the cases when neurons were activated by 2 or more consecutive electrodes 2.0 mm apart, our calculation showed that the stimuli activated different parts of the same terminal bush (Fig. 5B and C). The results suggested that the size of the terminal arborization of single branches is about 2–3 mm longitudinally in the spinal gray matter. Of the 136 neurons, 13% (17) sent branches to wide areas extending for 3 or more segments (Table 1). It should be reiterated that this proportion of neurons with multiple cervical branches represents an underestimation because of wide interelectrode distance relative to the effective extent of the stimuli and the limited number of electrodes.

Our results are in good agreement with the anatomical data reported by Scheibel and Scheibel (1966). They showed that most of the individual corticospinal fibers leave the body of the tract and enter the spinal gray at right angles and ramify over 1–2 segments. In the present study, the maximum extent of distribution of all the axon branches in single corticospinal neurons that we observed was as long as 5 segments in the cervical cord and in addition 30% of the total population which sent axons to the cervical cord also sent branches to the lower level of the spinal cord. Recent studies by Abzug et al. (1974), Peterson et al. (1975) and Shinoda, Ghez and Arnold (in preparation) also demonstrated the existence of neurons projecting to both the cervical gray matter and the lumbar cord in other long descending fiber systems.

The presence of corticospinal neurons projecting to widely separated levels of the cord raises the possibility that single corticospinal neurons are capable of influencing two or more motor neuron pools. It is accepted that corticospinal

neurons in the cat do not have monosynaptic connections with motoneurons but cause excitatory postsynaptic potentials in them through interneurons (Lloyd, 1941; Brooks and Stoney, 1971). Illert et al. (1974) recently described propriospinal neurons at C3 conveying corticospinal impulses to the motoneurons innervating forelimb muscles of the cat. There are, however, no available data that show how segmental interneurons or other propriospinal neurons innervating a particular motor nucleus are arranged spatially in the cervical cord. Therefore, it must be considered that there are two possible types of cortico-motoneuronal connections made by corticospinal neurons with multiple axon branches in the cat. In one type a single corticospinal neuron has connections with more than one motoneuron nucleus through different interneurons. In the other, individual corticospinal neurons may synapse on more than one interneuron terminating in one motor nucleus. In the first instance, individual corticospinal neurons are related to two or more different motoneuron pools and in the second, they are related to individual motoneuron pools. Both anatomical and physiological evidence show that motoneurons supplying one muscle are aligned longitudinally in the spinal cord (Reed, 1940; Romanes, 1951; Thomas and Wilson, 1967; Sterling and Kuypers, 1967). The distribution of one motor nucleus spreads over one to three segments (Romanes, 1951; Thomas and Wilson, 1967). The present results indicate that the projection area of some corticospinal neurons having multiple branches is much wider than the distribution area of any single motor nucleus. This finding strongly suggests that single corticospinal neurons may influence different motor nuclei through interneurons, especially those neurons projecting both to the cervical and to the thoracic or lumbar level. It cannot, however, be excluded that widely separate corticospinal axon branches terminate on interneurons which in turn project to the same motoneuron pool. A further consideration is that some branches may well impinge upon interneurons controlling transmission of afferent or ascending pathways (Lundberg et al., 1963).

If single corticospinal neurons influence more than one motor nucleus, the next important question will be what are the functional groups of motor nuclei influenced by each group of corticospinal neurons. Various interpretations may exist. One of the interpretations relates to the observation that stimulation on the surface of the cortex always produces a co-ordinated contraction of a group of muscles (Sherrington, 1906). It has been shown that the motoneurons receive muscle spindle afferents from functionally related groups of muscles (Eccles et al., 1957; Eccles and Lundberg, 1958). Also shown is that some interneurons terminate onto different motoneurons of close synergists (Hultborn et al., 1971). By the same token, it is possible that corticospinal neurons projecting to serveral cervical segments influence a group of motor nuclei which are functionally related to each other. On the other hand, it has been known that antagonists contract simultaneously during voluntary movements in man (Dodge and Bott, 1927) and microstimulation of the motor cortex can exert excitatory effects on antagonists simultaneously (Asanuma and Ward, 1971). In addition, the existence of corticospinal neurons projecting to both the cervical gray and lower levels of the cord may indicate that single

CS neurons are related to yet another type of functional group of motor nuclei. Analysis of the functional groups of muscles influenced by each set of corticospinal neurons may provide a new clue to the understanding of the function of the motor cortex.

Acknowledgements. This research was supported by Grant NS-10705 from the National Institute of Health.

The authors would like to express their gratitude to Ms K. Alexieva and Mrs. A. Jean-Mary for their technical assistance and Ms Ada Watson for her assistance in preparing the manuscript.

Dr. A. Arnold was the recipient of the NIH Fellowship MH 00559.

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Received April 2, 1976