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Spinal Branching of Rubrospinal Axons in the Cat

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Summary. The branching patterns of rubrospinal (RS) axons projecting to the cervical spinal cord between C3 and C8 were studied in the cat. RS neurons were identified by their antidromic responses to microstimulation of local axon branches within the cervical gray matter. Twenty-six of 58 RS neurons projecting to the cervical gray matter also sent axon branches to the thoracic spinal cord. Two out of 40 of these RS neurons also sent axon branches to the lumbar spinal cord. Using a collision technique, it was demonstrated that stem axons of rubrospinal neurons commonly sent multiple collaterals to different cervical segments.

Neurons projecting to the cervical spinal cord alone were located in the dorsal quadrants of the red nucleus. Those projecting to cervical, as well as to more caudal segments, were intermingled with the former, and in slightly more ventral portions of the red nucleus. The presence of RS neurons projecting to widely separate levels of the spinal cord suggests that individual RS neurons may be capable of ultimately influencing two or more different motoneuron pools.

Key words: Rubrospinal neurons – Axon branching – Spinal cord – Microstimulation

Introduction

Corticospinal (CS) neurons and rubrospinal (RS) neurons have many common features. Both populations have excitatory monosynaptic connections with interneurons in the intermediate zone of the spinal cord (Lundberg et al., 1972; Shapovalov, 1966; Hongo et al., 1969a; Bayev and Kostyuk, 1973; Illert et al., 1975a; Illert et al., 1975b). In the cat, influences from both CS and RS neurons are transmitted to motoneurons through such interneurons, some of which receive convergent input. Microstimulation within the motor cortex and the red nucleus evokes contraction of single muscles (Asanuma and Ward, 1971; Ghez,

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1975) and the two systems are somatotopically organized. Lesions of either system produces impairments in the control of distal extremity musculature (Kuypers, 1964).

Both CS and RS neurons send axon collaterals to brain stem nuclei (Endo et al., 1973; Anderson, 1971). In addition, it has recently been shown that, in spite of the somatotopic organization of the motor cortex (Asanuma, 1973; Brodal, 1969), many CS neurons terminate in widely separate segments of the spinal cord (Shinoda et al., 1976). Such observations suggest that a subpopulation of cells can influence the activity of several muscles. The present experiments were undertaken to determine if the same principle holds true for the rubrospinal system.

It will be shown that many rubrospinal neurons send axonal branches into the cervical gray matter before terminating at more caudal levels of the spinal cord.

Methods

Twenty adult cats (2.5–3.4 kg) were anesthetized with sodium pentobarbital (Nembutal, 35 mg/kg) and additional doses (10 mg/kg) given when necessary. The spinal cord from C3 to Th3 and from Th12 to L2 was exposed, covered with mineral oil and kept at 36–38° C by radiant heat. The right occipital cortex was exposed and a portion of the cortex was aspirated to allow electrode penetrations in the right red nucleus. The exposed cortex was covered with warm mineral oil. Body temperature was maintained at 36–38° C with a heating pad.

Details concerning the stimulating electrodes and their arrangement, as well as the recording electrodes, have been given elsewhere (Shinoda et al., 1976). Briefly, 12 glass-insulated tungsten microelectrodes were inserted one by one into the cervical gray matter between C4 and C8 as shown in Figure 4A, about 2 mm to the left of the midline and 2.5 mm deep. The electrodes were fixed to a single longitudinal plate which could be moved vertically by a microdrive. Additional electrodes were used to stimulate rubrospinal tract fibers within the lateral funiculus at C3, Th3 and L1. In half of the experiments, these consisted of pairs of silver ball electrodes placed on the surface of the lateral columns, in the other half, single varnished tungsten electrodes (0.5 mm exposed tip) were inserted about 1.5 mm into the lateral funiculus. Cathodal pulses of 0.3 msec were applied through the implanted electrodes relative to a reference electrode in the lower back. Bipolar stimulus pulses of the same duration were used in the case of the pairs of ball electrodes.

Single neurons in the red nucleus were recorded extracellularly with glass insulated tungsten microelectrodes. The region explored went from the stereotaxic planes A 2.5 to 5.5 and L 1.0 to 3.5. In each experiment electrolytic lesions were made in the spinal cord and the stimulating sites were reconstructed from the histological sections. Electrolytic lesions were also made in the red nucleus and the recording sites reconstructed in eight experiments.

Results

Criteria for Antidromic Activation of Rubrospinal Neurons

The recording electrode was positioned in the red nucleus on the basis of the antidromic field potentials evoked by stimulation of the contralateral rubrospinal tract at C3. Characteristic positive-negative antidromic field potentials were recorded when the electrode tip was in the nucleus (Tsukahara et al., 1968; Hongo et al., 1969a). With the recording electrode in the red

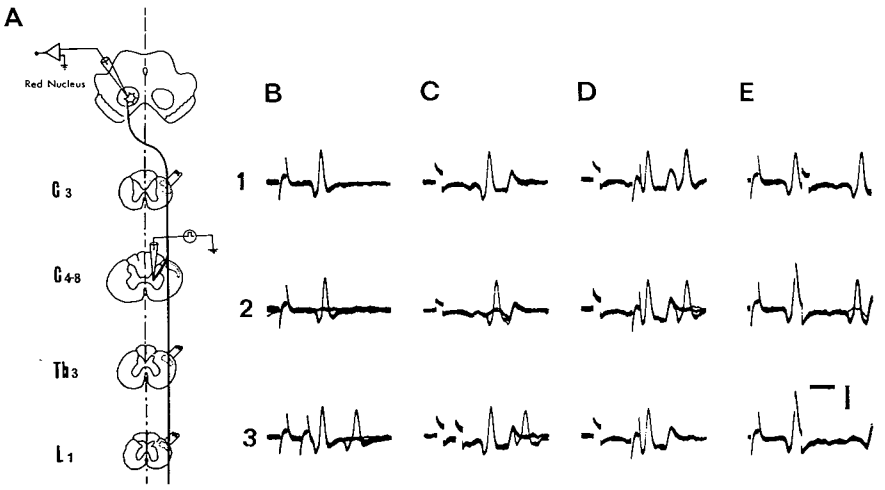

Fig. 1. Schematic diagram of the experimental arrangement **(A)**. Twelve microelectrodes were implanted in the cervical gray matter between C4 and C8. To stimulate the rubrospinal tract, electrodes were implanted at C3, Th3 and L1. **B–C** Antidromic activation of a rubrospinal neuron from the cervical gray matter at C6 **(B)** and the thoracic cord **(C)**. B2 and C2 at threshold, B1 and C1 at 1.5 times threshold, and B3 and C3, double shock stimuli at 1.5 times threshold. Threshold currents for C6 and Th3 stimulation were 8 μA and 50 μA. **D–E** Collision tests. Stimulation of Th3 preceded that of C6 by 1.59 msec. (D1); 1.58 msec (D2) and 1.53 msec (D3). C6 stimulation was followed by Th3 stimulation (E) and interstimulus intervals were 2.06 msec (E1), 1.77 msec (E2) and 1.70 msec (E3). This neuron could not be activated from L1. Calibrations: 1 msec, 200 μV

nucleus in place, stimulating currents were applied in parallel through all 12 electrodes in the cervical gray matter (50 μA for each electrode). Whenever spikes were evoked from the cervical gray matter, the effective stimulating electrodes were determined by passing current through each electrode in sequence. Spike potentials were considered to be evoked antidromically if they (1) appeared in an all or none manner at threshold and with fluctuations in latency of less than 0.1 msec (Fig. 1, B2 and C2), (2) responded with fixed latency to stimulus currents of 1.5 times threshold (Fig. 1, B1 and C1), and (3) faithfully followed double shocks at an interval of 2.0 msec or less at 1.5 times threshold (Fig. 1, B3 and C3). These antidromic spikes sometimes showed IS-SD block in response to double shock stimuli (Coombs et al., 1957; Eccles et al., 1975). In such cases, IS spikes responded to double shocks with shorter intervals (0.74 ± 16 msec; mean and S.D., N = 7) than SD spikes (1.29 ± 0.32 msec). Of the neurons that satisfied the first two criteria, only about one-fourth met the third criterion. A large proportion of the remaining neurons seemed to be activated antidromically because double shocks at just below threshold with various inter-stimulus intervals revealed no facilitatory effects. It is possible that inhibitory effects produced by co-stimulation of axon collaterals of pyramidal tract cells (Tsukahara et al., 1968) might have prevented the antidromic invasion of the second spikes. We nevertheless, confined our observations to RS neurons which satisfied all three of the above criteria.

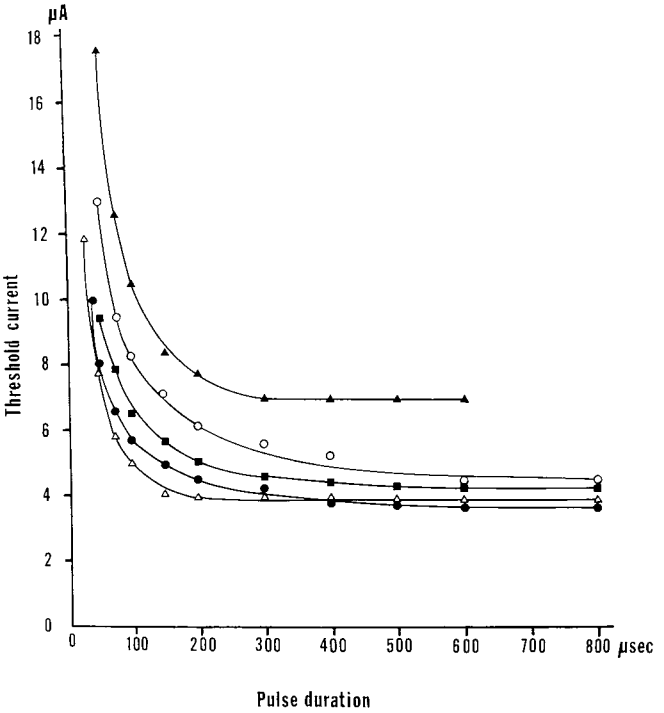

Fig. 2. Strength-duration curves. Five examples for rubrospinal fibers activated in the cervical gray matter are represented. The ordinate indicates the stimulus current required to activate an axon with a firing index of about 50%

The relation between threshold intensity and duration of stimulus current was examined in eight axon branches of RS neurons activated within the cervical gray with lowest thresholds of 10 μA or less. Figure 2 illustrates the strength-duration curves for five axon branches whose lowest thresholds were 7 μA or less. The curves approach rheobase values at around 0.3 msec. Hence, to simplify theoretical considerations, we used stimulus pulses of 0.3 msec duration throughout the experiments. The chronaxies for the examined eight axons ranged from 0.04 to 0.09 msec (0.07 ± 0.02 msec, mean and S.D., $N = 8$). These values are similar to those obtained by Jankowska and Roberts (1972) for axons of spinal interneurons, and by Jankowska and Smith (1973) for Renshaw cell fibers, but smaller than the values for axons of corticospinal neurons (Shinoda et al., 1976).

During the experiments, it was sometimes noted that the stimuli became ineffective when the stimulus intensity was increased to 7–14 times the threshold intensity. This was most likely due to anodal surround (Katz and Miledi, 1965). Thus, when determining the electrode which was effective in evoking a spike, the stimulus current was varied in each case between 50 μA and lower values.

Criteria for Activating Terminal Branches of Rubrospinal Neurons in the Gray Matter

Since the purpose of the present study was to examine the branching patterns of RS neurons terminating within the cervical gray matter, it was essential to activate axon branches in the gray matter and not nearby fibers in the rubrospinal tract.

Current spread to stem axons in the RS tract was excluded by estimating the extent of effective current spread. For this purpose we measured the threshold current required to activate individual RS axons while moving the stimulating electrode vertically. Representative curves of threshold current versus electrode depth (aligned relative to the lowest threshold points) are shown for five RS axon branches activated from the gray matter in Figure 3B and for six stem axons in the rubrospinal tract in Figure 3C. These curves were selected for their monophasic and symmetric profiles around the lowest threshold points and the constant latencies of spikes evoked at each depth. Other curves, as shown in Figure 3A, had more than one low threshold point. Since different regions of the same axon were presumably activated in the latter case, only the curves of the former type were used to establish estimates of current spread. The curves for stem axons were much wider than those for axon branches of RS neurons. Using a triangulation procedure (BeMent and Ranck, 1969; Bean, Appendix in Abzug et al., 1974; Peterson et al., 1975), distance-threshold curves were established (not illustrated) to assess the extent of effective stimulus spread. The curves were not linear and the slopes increased slightly with distance from the focus. The slopes were lower and nonlinearity was greater for stem axons than for terminal branches in the gray matter. Judging from 13 distance-threshold curves for stem axons in the lateral funiculus, the estimated upper limits of the extent of stimulus action were 450 μm at 20 μA, 570 μm at 30 μA, 680 μm at 40 μA and 760 μm at 50 μA. These values are similar to those found by Roberts and Smith (1973) for dorsal spinocerebellar tract axons and Shinoda et al. (1976) for stem axons of corticospinal neurons.

For each neuron activated from the cervical gray matter, the distance from the electrode tip to the rubrospinal tract was measured. The location and outlines of the rubrospinal tract were determined from the outermost fibers in the illustrations of Petras (1967) and Verhaart (1964). These correspond to the more recent autoradiographic findings of Edwards (1972; also personal communication). The distance from the stimulated sites to the closest border of the rubrospinal tract ranged from 880 to 2440 μm and was greater than 1000 μm in 80% of the cases. The shortest distance (880 μm) was thus longer than the maximum estimate of the range of stimulus action at 50 μA (760 μm). When the observed thresholds were taken into account, the distance between the upper limit of current spread (from distance threshold curves of stem axons) and the closest border of the rubrospinal tract was greater than 500 μ for 87% of stimulus sites. Of the remaining rubrospinal axons whose thresholds implied a limit of current spread 200 to 500 μ from the tract border, only 25% could be activated from the thoracic electrode in the lateral funiculus as well as from the cervical gray matter. The corresponding proportions for sites where the upper

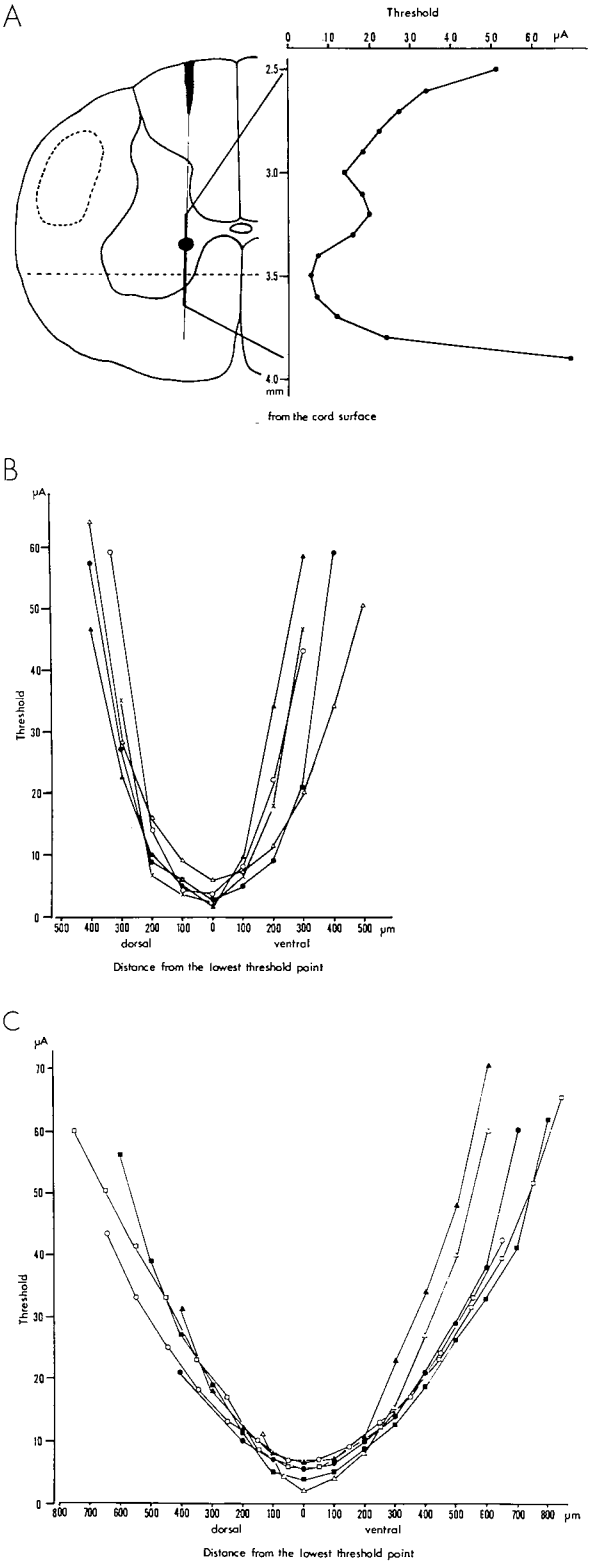
A
Threshold
0 10 20 30 40 50 60 μA
2.5
3.0
3.5
4.0
mm
from the cord surface
B
μA
60
50
40
30
20
10
0
Threshold
500 400 300 200 100 0 100 200 300 400 500 μm
dorsal
ventral
Distance from the lowest threshold point
C
μA
70
60
50
40
30
20
10
0
Threshold
800 700 600 500 400 300 200 100 0 100 200 300 400 500 600 700 800 μm
dorsal
ventral
Distance from the lowest threshold point

Fig. 3A–C

limit was 500 to 1000 μ and 1000 to 2000 μ from the rubrospinal tract were 29% and 48% respectively. This indicates that significant numbers of stem axons descending outside the main body of the rubrospinal tract (possibly closer to the gray matter) were not activated at the stimulus currents used.

Moreover in several cases, such as that illustrated in Figure 3A, the transverse plane (perpendicular to the stimulating track) passing through the lowest threshold point was situated ventral to the rubrospinal tract. In such cases current spread to the tract may be excluded since the stimulated portion of the axon must lie in this plane.

We therefore concluded that the RS neurons sampled in the present study were activated antidromically by stimulation of terminal fibers in the cervical gray, not by stem axons in the tract. This conclusion was in fact supported by the evidence described below.

Branching of Individual Rubrospinal Axons

Whenever a RS neuron was activated antidromically from one or more electrode in the cervical gray matter, it was tested to determine whether it also projected to more caudal levels of the spinal cord. When a neuron was activated by stimuli applied both to the cervical gray and the thoracic or lumbar lateral columns, we applied a collision test as described in a previous study (Shinoda et al., 1976). This procedure allowed us, (1) to ascertain that both stimuli activate the same neuron, (2) to provide additional support that both responses were antidromic, and in addition, (3) to estimate the conduction time between the branching point of axon collaterals and their stimulated portion.

An example of such a collision experiment is illustrated in Figure 1. This neuron was activated from an electrode in the cervical gray at C6 and an electrode in the thoracic lateral funiculus. The latency of the spike potentials evoked from the cervical gray (Lc) and from the thoracic cord (Lt) were 1.30 and 1.76 msec (Fig. 1, B1 and C1). The refractory periods determined as the interval between two stimuli in the cervical gray (Rc) and the thoracic cord (Rt) were 0.80 msec (B3) and 0.77 msec (C3). To test for collision, stimulation of the cervical gray preceded that of the thoracic cord (Fig. 1E). At the conditioning-test interval of 2.06 msec (E1), a response to the thoracic stimulus appeared in all trials, but when the interval between two successive stimuli

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Fig. 3. Threshold current for antidromic activation as a function of stimulating electrode depth. **A** An example of a stimulating electrode track at C6. A dashed line encloses the rubrospinal tract. Threshold for evoking discharges (abscissa) was plotted against the electrode depth below the cord surface (ordinate). Dark areas indicate the electrode track and the electrolytic lesion. The horizontal dashed line which passes through the track at the point of minimal threshold does not intersect the rubrospinal tract, thus excluding current spread to the stem axon. **B** Depth-threshold curves for five rubrospinal axon branches activated within the cervical gray matter with minimum thresholds of 7 μA or less. Electrode depth is expressed as distance dorsal or ventral to the point of minimum threshold (zero). **C** Depth-threshold curves for six rubrospinal stem axons with the lowest thresholds of 7 μA or less

was shortened, the test response failed to appear (E3). The maximal conditioning-test interval for blockage of the test response, i.e., the maximal blocking interval between the successive stimuli (Ict) was 1.58 msec (E2). When the stimulus order was reversed with the stimulus to the thoracic cord leading (Fig. 1D), the maximal blocking interval (Itc) was 1.77 msec (Fig. 1, D2). When separate branches of the same neuron are activated, the approximate conduction time (Xc) between the branching point of one branch from the main axon in the tract to the stimulated site of the branch in the gray matter has been given by the following equation (Shinoda et al., 1976):

$$Xc = \frac{1}{2}(Itc + Lc - Lt - Rc) \tag{1}$$

Itc, Lc, Lt and Rc are as defined above. As pointed out by Shinoda et al. (1976, appendix), the true value of the refractory period of the axon may differ slightly from Rc (minimal interstimulus interval evoking two spikes), therefore the true conduction time along the branch is given by:

$$Xc^* = \frac{1}{2}(Itc + Lc - Lt - Rc + \varepsilon) \tag{2}$$

where ε represents an error factor.

The possible origins of the error ε will be discussed below (see Discussion), however, the net ε was estimated experimentally. Two separate stimulating electrodes were placed in the lateral funiculus at cervical and thoracic levels to excite stem axons in the rubrospinal tract. The cervical electrode thus corresponded to one placed on a branch having a distance of zero from the stem axon. Under these conditions, the true conduction time Xc* should be zero and the difference between this and Xc provides an estimate of ε since $Xc = Xc^* - \frac{\varepsilon}{2}$. For nine neurons activated from the separate tract electrodes and examined using the conditioning-test technique described above, the value of Xc calculated using equation (1) was 0.02 ± 0.03 msec (mean and standard deviation) and ranged from +0.5 to –0.3 msec. These values were almost the same as those obtained for corticospinal neurons (0.0 ± 0.03 msec) (Shinoda et al., 1976). The error term was small enough that equation (1) could be used to obtain an approximation of the conduction time along individual local branches (see Fig. 4C).

In the present study a total of 75 RS neurons could be considered to be activated antidromically by stimulation of terminal axons in the cervical gray matter on the basis of estimates of current spread. In 17 of these, however, the estimated conduction times (Xc) along the axon branches had values which were close to zero or even negative. It may be argued that these 17 RS fibers stimulated in the cervical gray were stem axons, some of which coursed in an aberrant location close to or within the gray matter. It is, however, also possible that the negative values calculated for the conduction time along axon branches arose from underestimation of Xc*, since the error factor described above was not determined for each RS neuron and the error could be larger in some cases

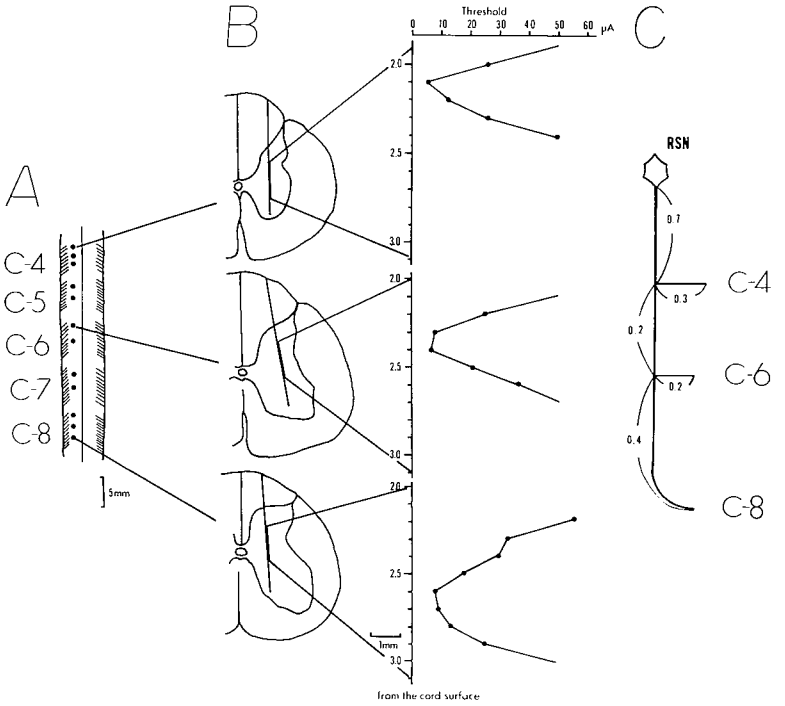

Fig. 4. Multiple axon branching of a rubrospinal neuron in the cervical gray matter. Stimulating electrodes were implanted as shown in **A**. While recording from the same neuron, an array of the electrodes were moved vertically and the effects of stimulation were examined at each depth. In **B** threshold currents (abscissa) were plotted against depths (ordinate). Thin and thick lines represent electrode tracks reconstructed from the histological sections and the surveyed areas in each track. **C** Branching pattern of this neuron. Numbers in the diagram indicate conduction times (msec) calculated from the values obtrained from collision tests. Utilization time of about 0.2 msec war subtracted from the latencies in **C**. The neuron was activated antidromically from Electrodes Nos. 1, 3, 6, 8 and 12 from top to bottom, but collision of evoked spikes from Electrodes 3 and 8 with those from the other electrodes should not be completed before the neuron died

especially when soma-dendritic invasion of antidromic spikes tended to be blocked (see Discussion).

If we discard the seventeen neurons in which the collision and current spread criteria gave discrepant results, 26 of 58 RS neurons (45%) which sent axon branches to the cervical gray matter, also projected to the third thoracic level. If we include the 17 neurons in our sample, the percentage of neurons projecting to both the cervical gray and thoracic cord becomes 57% (43/75). The real proportion of neurons sending axon branches to both the cervical gray and the thoracic cord among neurons activated from the cervical gray matter is likely to lie between these two values. In addition, forty of the 58 RS neurons were tested to determine whether they could also be activated from the lateral funiculus at L1. Only two of these neurons (5%) sent axon branches to the lumbar cord as

well as to the cervical gray matter. We confined more detailed analysis to the 58 RS neurons in which collision and current spread criteria gave concordant results.

Branching of Rubrospinal Neurons in the Cervical Gray

In half of the experiments the pattern of axon branches in the cervical gray matter was examined by moving the microelectrode array. When a neuron was activated from one of the electrodes, the array of 12 electrodes was moved vertically over 2–3 mm and stimuli were delivered through the other electrodes to determine if the neuron could be activated from other sites. An example of such an experiment is shown in Figure 4. Electrodes were positioned as shown in Figure 4A and the neuron was driven from 5 electrodes in the cervical gray. The branching pattern of the neuron was assessed using the collision method described above (Fig. 4C). This neuron sent different axon branches to C4, C6 and C8. The data obtained from experiments in which the cervical electrodes were moved an those in which they remained fixed are summarized together in Table 1. Of 32 neurons projecting only to the cervical cord, five neurons sent separate axon branches to two different cervical segments and five neurons sent axon branches to three or more segments. Of the 58 RS neurons projecting to the cervical cord, 26 sent axon branches to the thoracic cord and five of these gave off two or more axon branches into the cervical gray matter. The locations of stimulus sites from which 39 axons were activated with thresholds of 15 μA or less are illustrated in Figure 5. These sites were in Rexed's Laminae IV, V, VI and VII and correspond well with the area of termination of rubrospinal neurons determined by anatomical (Nyberg-Hansen and Brodal, 1964; Petras, 1967) and other physiological methods (Bayev and Kostyuk, 1973).

Table 1. Patterns of spinal branching of rubrospinal neurons (N = 58)

I. Neurons activated only from the cervical cord (N = 32)

A. Activated from one segment.

	C4	C5	C6	C7	C8	Total
No.	11	3	4	1	3	22

B. Activated from two or more segments

	C4+C5	C6+C7	C4+C6	C6+C8	C4+C5+C7	C4+C5+C6	C5+C6+C8	C6+C7+C8	Total
No.	1	1	2	1	2	1	1	1	10

II. Neurons projecting both to the cervical gray and the thoracic cord (N = 26)

A. Activated from one cervical segment

	C4	C5	C6	C7	C8	Total
No.	4	5	4	4	4	21

B. Activated from two or more cervical segments

	C7+C8	C6+C8	C4+C8	C4+C6+C7	C5+C6+C7	Total
No.	1	1	1	1	1	5

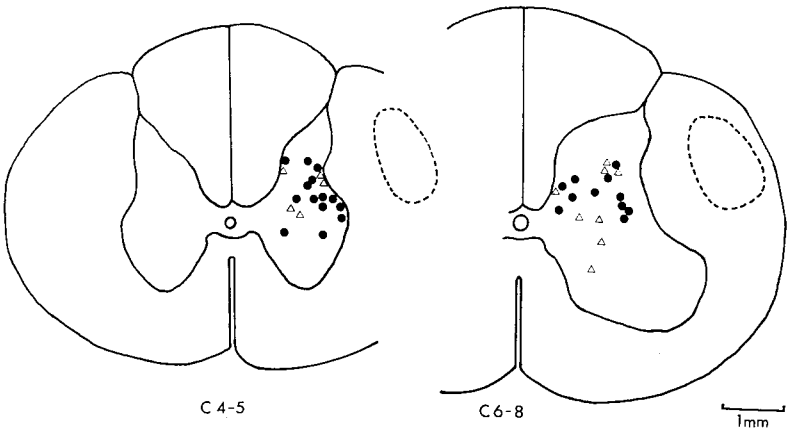

Fig. 5. The localization of the electrode tips from which rubrospinal fibers were antidromically activated with 15 μA or less. Dots represent activation points for neurons which send their axon only to the cervical gray matter and triangles indicate points for neurons which project to the thoracic cord as well as to the cervical cord. Dotted circles show the rubrospinal tract as determined from the figures of Petras (1967) and Verhaardt (1964)

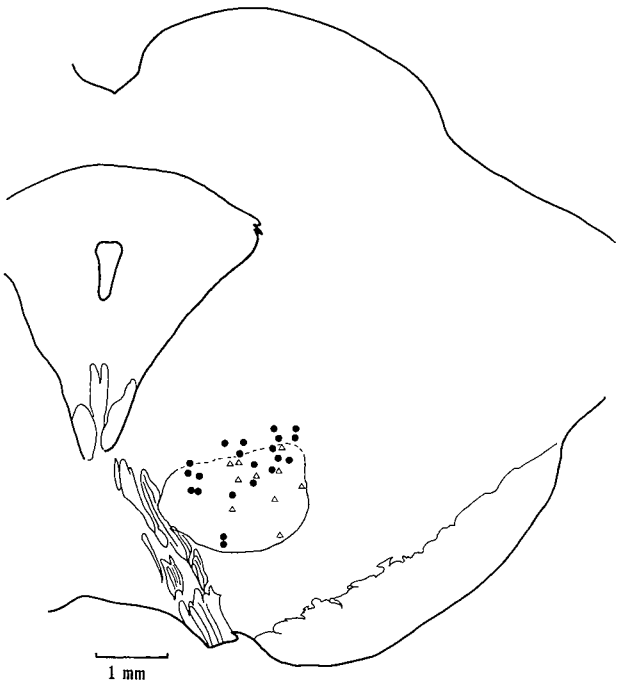

Fig. 6. Distribution of rubrospinal neurons in the red nucleus projecting to the cervical cord and lower levels. Filled circles represent neurons sending axons only to the cervical cord and triangles indicate neurons projecting to the thoracic cord as well as to the cervical gray matter. Data were reconstructed from Klüver Barrera stained sections. The dorsal border of the red nucleus is shown with an interrupted line because of the difficulty in ascertaining the limits of the nucleus in this area (see text)

Distribution of Rubrospinal Neurons Projecting to Different Spinal Levels

When the recording electrode tip was located in the dorsal portion of the red nucleus, spike activity superimposed upon clear antidromic field potentials was evoked by stimulation of the cervical gray matter through all 12 electrodes. Stimulation of the thoracic and sometimes the lumbar lateral funiculus produced only antidromic field potentials in this portion. On the other hand, when the recording electrode was advanced to the ventral portion of the nucleus, antidromic field potentials and unit activity evoked from thoracic and lumbar electrodes were present. From these areas, unit activity evoked from the electrodes in cervical gray was rare.

In Figure 6, the locations of neurons recorded in the red nucleus are represented on a drawing of a transverse section of the midbrain at the level of the oculomotor nucleus where most of the cells were found. At this level the dorsal regions of the red nucleus consist of irregular extensions separated by fibers of the brachium conjunctivum. Thus, the dorsal border is less well defined than the ventral one and its position varies more from section to section. Neurons projecting only to the cervical gray matter (filled circles) were most abundant in the dorsal portions of the nucleus. Neurons projecting to both cervical and thoracic cord (open triangles) were intermingled with neurons sending axons only to the cervical levels although their proportion was greater in the more ventral part of the nucleus.

Discussion

The anatomical studies of Brodal and Pompeiano (1957) and Nyberg-Hansen and Brodal (1964) have shown that RS neurons in the dorsomedial and ventrolateral quadrants of the red nucleus send their axons to the cervical and lumbar spinal cord, respectively. The locations of neurons antidromically identified from spinal segments C2 and L2 confirmed this somatotopic arrangement (Tsukahara et al., 1967; Eccles et al., 1975). In the present study, neurons projecting only to the cervical gray matter were similarly located in the dorsal portions of the red nucleus. In addition, however, a substantial proportion of neurons were found to project to both the cervical, as well as to mere caudal levels of the spinal cord. While most of these neurons were interspersed with the former, a few were in a somewhat more ventral region of the red nucleus (Fig. 6).

Collision experiments made it possible to ascertain that many RS neurons sent independent axon branches to separate segments of the cervical spinal cord and to obtain an estimate (Xc) of the conduction time along individual branches (Fig. 4, Table 1). It must, however, be recognized that equation (1) which was used to calculate Xc neglects an error term ε. This error resulted primarily from the necessity of estimating the refractory period of axons from recordings of spikes generated at the cell body. Nevertheless, the magnitude of the error was assessed experimentally and found to be negligible.

The factors contributing to the error term ε figuring in equation (2), where Xc* gives the true conduction time along axon branches, must be further considered.

(1) Rc is expressed in terms of the interval between two successive stimuli in the cervical gray. The utilization time for spike generation in response to the second stimulus may be longer than that to the first stimulus, since the second stimulus may occur during the relative refractory period of the axon. Therefore, the refractory period of the axon expressed by the stimulus interval may be shorter than the least interval of two successive spikes. This possible error, however, also holds for the collision test and is thus cancelled out when the value of Rc determined by double shocks is applied to the collision experiment, because Itc is also expressed in terms of the interval between the conditoning and test stimuli (Shinoda et al., 1976).

(2) Rc is in fact determined by monitoring SD or IS spikes instead of axon spikes. In spinal motoneurons the refractory period of the soma or the initial segment is known to be longer than that of the axon (Coombs et al., 1957). We also found that, when IS-SD block was clearly seen in RS neurons, Rc measured with SD spikes tended to be longer than that with IS spikes (approximately 0.5 msec difference on the average) (see also Eccles et al., 1975), and the axon may have a shorter refractory period than the initial segment. This would cause an error ε having a positive sign (overestimation of the refractory period of the axon) and lead to an underestimation of Xc*.

(3) With double stimuli, the least interval between two successive spikes of the stimulated axon may be longer than the true refractory period of the axon expressed by stimulus interval as indicated earlier in (1). Moreover, the conduction velocity of the second spike may be slower than that of the first (Tasaki, 1959) thus prolonging further the interval between two successive spikes arriving at the soma. As a consequence, the second spike may invade the soma even if the interval of double stimuli is close to the true refractory period of the axon. This prolongation of the interval of two spikes along the axon should therefore cause a reduction of the error factor described in (2) and tends to compensate for the overestimation of the refractory period of the axon. In fact, the net value of ε was small enough (see Results) that the true refractory period of the axon can be estimated fairly precisely even by recording spikes from the cell soma.

In the collosion experiment, stimulation of the thoracic cord preceded that of the cervical gray in order to estimate the conduction time between the site of excitation in the cervical gray and the branching point of the local axon (0.3 msec at C4 and 0.2 msec at C6 in Fig. 4C). When the sequence of the conditioning and test stimuli was reversed, we could estimate the conduction time (Xt) from the point of stimulation in the thoracic cord to the point at which the local axon branches to the cervical gray matter according to the following equation:

$$Xt = \frac{1}{2}(Ict + Lt - Lc - Rt) \tag{3}$$

where Ict = maximum conditioning-test interval when the spikes evoked from the thoracic cord are blocked by the spikes from the cervical cord Rt = refractory period at the thoracic point of stimulation. Here again, Xt must include some error as in the case of Xc. However, the present technique allows us to obtain a true value of conduction time between the two branching points in the cervical cord (0.2 msec between C4 and C6 in Fig. 4C) regardless of the value of error. Although small, the error which is included in Rt will be cancelled out when the conduction times from the point of stimulation in the thoracic cord and two different points of local branching in the cervical cord are compared. This indicates that the two axon branches at C4 and C6 in Figure 4C originate from separate points along the stem axon and do not derive from a common branch originating at a single point.

Separate axon branches must certainly occur in a larger proportion of cells than observed here since only a small proportion of the cervical gray matter was examined. Indeed, we used stimuli of 50 μA to search for antidromically activated neurons and the extent of stimulus spread of this current intensity is less than 800 μm from the electrode tip. Since only 12 electrodes separated by 3 to 7 mm (and spanning 3–5 segments) were inserted, large areas were left unstimulated. Nonetheless, about one fifth of the neurons sent independent axon branches to cervical levels three or more segments apart. A still larger proportion also projected caudal to Th3.

These observations raise the possibility that individual RS neurons may influence more than one motoneuron pool which, for the cat forelimb, extend only 1 to 3 segments (Thomas and Wilson, 1967; Sterling and Kuypers, 1967). Moreover, while monosynaptic connections from rubrospinal neurons to motoneurons are scarce (Shapovalov, 1966) or absent (Hongo et al., 1969a) in the cat, rubral volleys typically evoke di- or poly-synaptic postsynaptic potentials in motoneurons (Hongo et al., 1969a; Bayev and Kostyuk, 1973). These effects are principally mediated by interneurons (Hongo et al., 1972; Baldissera et al., 1972) and propriospinal (Illert et al., 1975b; Illert et al., 1975a) neurons intercalated in reflex pathways (Hongo et al., 1969b) and have convergent peripheral and central inputs. Some of these interneurons should project to several motoneuron pools (Hultborn et al., 1976). By contrast, some interneurons activated by rubrospinal volleys may not be activated by peripheral nerve stimulation (Kostyuk and Pilyavsky, 1969; Bayer and Kostyuk, 1973; cf. however, Hongo et al., 1972). These interneurons have been postulated to constitute a private pathway mediating rubral effects on motoneurons. Regardless of which interneuronal pathway is involved, the occurence of widely spaced rubrospinal axon branches strongly suggests that some RS neurons will ultimately influence the activity of several muscles.

It now appears that axons of all major descending systems send collaterals to multiple levels of the spinal cord. This feature was first described by Abzug et al. (1974) who found that 50% of vestibulospinal neurons with axon collaterals in C6–Th1 segments were also antidromically driven by stimulation of the lumbar vestibulospinal tract. A similar percentage (67%) of reticulospinal neurons sent branches to the cervical gray matter as well as to the first lumbar segment (Peterson et al., 1975). In contrast, Shinoda et al. (1976) found that 12 out of 193 (6%) corticospinal neurons activated from the cervical gray matter (C4–C8) also projected to the first lumbar segment. Similarly, in the present study only 2 out of 40 RS neurons (5%) projecting to the cervical gray matter (C4–C8) sent axon branches to the first lumbar level. Neurons in the former two phylogenetically older descending systems thus appear to give off axon collaterals to a wider territory in the spinal cord which may enable the simultaneous control of several muscles during postural adjustment. Most neurons of the latter two descending systems seem to project to more restricted areas of the spinal cord and are therefore likely to control the activity of smaller groups, or single, contralateral limb muscles more specifically. Nevertheless, recent evidence suggests that fibers of these two systems also converge on a propriospinal system with widespread actions (Illert et al., 1975a, 1975b).

It remains to be determined how these systems with divergent projections are still capable of mediating the localized motor effects elicited by microstimulation. It is possible that neurons in a given sector may have predominant effects in a narrow spinal territory and weaker effects over an extensive area. The latter could serve to coordinate the actions of multiple muscles which are required in the performance of skilled movements.

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