Distribution and Characteristics of Poststimulus Effects in Proximal and Distal Forelimb Muscles From Red Nucleus in the Monkey

ABDERRAOUF BELHAJ-SAIF, JENNIFER HILL KARRER, AND PAUL D. CHENEY

Department of Molecular and Integrative Physiology and Ralph L. Smith Mental Retardation and Human Development Research Center, University of Kansas Medical Center, Kansas City, Kansas 66160

Belhaj-Saïf, Abderraouf, Jennifer Hill Karrer, and Paul D. Cheney. Distribution and characteristics of poststimulus effects in proximal and distal forelimb muscles from red nucleus in the monkey. J. Neurophysiol. 79: 1777–1789, 1998. We used stimulus-triggered averaging (STA) of electromyographic (EMG) activity to investigate two major questions concerning the functional organization of the magnocellular red nucleus (RNm) for reaching movements in the macaque monkey. The first is whether the clear preference toward facilitation of extensor muscles we have reported in previous studies for distal (wrist and digit) forelimb muscles also exists for proximal muscles (shoulder and elbow). The second question is whether distal and proximal muscles may be cofacilitated from RNm suggesting the representation of functional muscle synergies for coordinated reaching movements. Two monkeys were trained to perform a prehension task requiring multijoint coordination of the forelimb. EMG activity was recorded from 24 forelimb muscles including 5 shoulder, 7 elbow, 5 wrist, 5 digit, and 2 intrinsic hand muscles. Microstimulation (20 μA at 20 Hz) was delivered throughout the movement task. From 137 microstimulation sites in the RNm, a total of 977 poststimulus effects was obtained including 733 poststimulus facilitation effects (PSIF) and 244 poststimulus suppression effects (PSIS). Of the PSIF effects, 58% were obtained from distal muscles; 42% from proximal muscles. Digit muscles were more frequently facilitated (35%) than the wrist, elbow, or shoulder muscles (20, 24, and 18%, respectively). The intrinsic hand muscles were infrequently facilitated (3%). At all joints tested, PSIF was more common in extensor muscles than flexor muscles. This extensor preference was very strong for shoulder (85%), wrist (85%), and digit muscles (94%) and weaker for elbow muscles (60%). Of the PSIS effects, 65% were in distal muscles and 35% in proximal muscles. Interestingly, the flexor muscles were more frequently inhibited from RNm than extensor muscles. At 72% of stimulation sites, at least two muscles were facilitated. The majority of these sites (61%) cofacilitated both proximal and distal muscles. At the remaining sites (39%), PSIF was observed in either the proximal (17%) or distal muscles (22%). Facilitation most often involved combinations of shoulder, elbow, and distal muscles (30%) or shoulder and distal muscles (26%). Only rarely were intrinsic hand muscles part of the total muscle synergy. Our results show that the RNm 1) controls both proximal and distal muscles but the strength of influence is biased toward distal muscles, 2) preferentially controls extensor muscles not only at distal forelimb joints but also at proximal joints, and 3) output zones cofacilitate synergies of proximal and distal muscles involved in the control of forelimb reaching movements.

INTRODUCTION

Over the past 30 years, a number of anatomic and electrophysiological studies have investigated the output of the magnocellular red nucleus (RNm) to the spinal cord in both cats and monkeys (e.g., Keifer and Houk 1994; Massion 1967; Padel 1993). From these studies, several suggestions have been made for the role of the RNm in the control of different muscles of the forelimb and the hindlimb. The first important observation concerns the role of the primate RNm in the control of distal versus proximal muscles. Anatomic studies have shown that the projection of rubrospinal fibers is greater to spinal segments controlling distal muscles than proximal muscles in the cat (Holstege 1987; Holstege and Tan 1988; McCurdy et al. 1987) and in the monkey (Holstege et al. 1988; Humphrey et al. 1984; Ralston et al. 1988). Moreover, deficits from lesions of the red nucleus affect movements of the distal joints more severely than the proximal joints (Lawrence and Kuyipers 1968). This anatomic evidence is supported by electrophysiological studies showing that RNm cell activity is highly modulated during movements involving the distal joints of the forelimb and hindlimb (Almaric et al. 1983; Burton and Onoda 1978; Cheney 1980; Dormont et al. 1989; Fromm et al. 1981; Ghez and Kubota 1977; Ghez and Vicaro 1978; Mewes and Cheney 1991; Otero 1976). More recent studies have also shown that many cells in the RNm are more strongly related to movements involving distal joints (wrist and digits) than proximal joints (elbow and shoulder) (Gibson and al. 1985a, b; Kohlerman et al. 1982; Houk et al. 1988; Miller et al. 1993). Intracellular recording has revealed that stimulation of the RNm produces excitatory postsynaptic potentials (EPSPs) preferentially in the motoneurons of distal muscles rather than proximal muscles in the cat (Fujito et al. 1991). However, recent studies have reported that the activity of RNm neurons is more strongly modulated during multijoint reaching movements than during single joint movements (Mewes and Cheney 1994; Miller et al. 1993). This suggests that the output of RNm may be organized for coordinating movements involving not just distal joints but distal and proximal joints together.

Another important finding concerns the differential control of extensor versus flexor muscles by the RNm. Early studies reported that macrostimulation of the RNm produces contraction of flexor muscles of the hindlimb and forelimb (Massion 1967; Pompeiano 1957; Sasaki et al. 1960; Thulin 1963). While recording the activity of RNm cells during locomotion in the thalamic cat, Orlovsky (1972) found that the peak discharge of most rubrospinal neurons occurred during the swing phase when flexors were most active. Other studies have shown that stimulation of the RNm in cats may produce contractions of either extensor or flexor muscles (Ghez 1975). EPSPs in interneurons controlling motoneurons of some extensor hindlimb muscles in the cat have also

0022-3077/98 $5.00 Copyright © 1998 The American Physiological Society
been demonstrated (Hongo et al. 1969). Recently, several studies have demonstrated a strong preference of rubrospinal cells for control of extensor muscles both in the cat (Holstege 1987; McCurdy et al. 1987) and in the monkey (Cheney et al. 1991; Gibson et al. 1985a; Houk et al. 1988; Mewes and Cheney 1991; Miller et al. 1993). However, this extensor muscle preference has only been systematically tested at distal joints (wrist and digits).

From previous work, it seems clear that RNm preferentially controls distal muscles and, in the monkey, has its strongest excitatory effects on extensor muscles. Nevertheless, the actions of the rubrospinal system may vary from one joint to another and from forelimb to hindlimb. Therefore the goals of this study were to (1) examine the action of RNm neurons on different muscle groups of the forelimb, including proximal muscles, during a coordinated multit joint movement task that engaged all the recorded muscles, (2) to test the extensor preference at proximal forelimb joints using stimulus-triggered averaging of electromyographic (EMG) activity, and (3) to assess the extent to which sites in RNm influence combinations of distal and proximal muscles as potential synergies for coordinated reaching movements.

METHODS

Animals and training procedures

Data were collected from the left red nuclei of two adult rhesus monkeys (Macaca mulatta). The animals were placed in a primate chair with a padded restraint for the left forelimb, and with freedom of movement of the right arm. The monkeys were trained on two different tasks (prehension and push-pull) involving the activity of shoulder, elbow, wrist, digit, and intrinsic hand muscles.

The prehension task consisted of four different phases. The task begins with the monkeys right hand resting on a home plate device at wrist height and his elbow flexed at approximately 90°. Pushing on the home plate activated a microswitch, and, after a 1-s delay, a small food pellet was delivered automatically into a target cylinder as a reward. In the second phase of the task, the monkey reached into the target cylinder to grasp the food pellet with its fingers. During this phase, the arm was fully extended. In the third phase, the monkey flexed its elbow and wrist to bring the pellet to its mouth. Finally, in the last phase of the task, the monkey returned its hand to the starting position (home plate). In general, each trial lasted approximately 4 s. Task performance was controlled and monitored using custom-written software for an IBM-compatible computer. The size of the target cylinder could be decreased in six steps by insertion of progressively smaller concentric cylinders. In this way, the task could be made more difficult by requiring greater skilled use of the digits. The cylinder diameters used to collect data in this study ranged from 16 to 47 mm.

The push-pull task was used on some occasions to provide a greater background of proximal muscle EMG activity for the purpose of confirming poststimulus effects obtained during the prehension task. The push-pull task required the monkey to grip a manipulandum with his right hand in a pronated position and its elbow at approximately 90° at the midpoint in the movement trajectory. The monkey was required to move the manipulandum between two target position zones: one in flexion and the other in extension. Movement away from the zero position was opposed by springs connected to the manipulandum. Successful performance of the task required holding in each zone for at least 0.8 s, after which the monkey received an applesauce reward. Behavioral program control was implemented with an IBM-compatible computer. Rectangular target zones were displayed on a color monitor that the monkey viewed. Arm position was represented as a moving vertical line cursor on the screen. Tone bursts of different pitch signaled entry into the correct target zone and successful completion of the hold phase of the task. The push-pull task was used in some cases to enhance proximal muscle activity.

The monkeys were trained daily on these two tasks for several months before a cranial chamber was implanted for microelectrode recording. Once trained, monkeys worked steadily on the task for 3–5 h daily, completing 3,000–10,000 responses for both tasks.

Chamber and EMG implants

After training was complete, a recording chamber and EMG electrodes were implanted in each monkey. For all implant surgeries, the monkeys were tranquilized with ketamine (10 mg/kg) and anesthetized with isoflurane gas. Surgeries were performed in an AAALAC-accredited facility using full sterile procedures. Postoperatively, monkeys received prophylactic antibiotic and analgesic medication. All work involving these monkeys conformed with the procedures outlined in the Guide for the Care and Use of Laboratory Animals published by the U.S. Department of Health and Human Services, National Institutes of Health.

A circular stainless steel recording chamber allowing exploration of a 22-mm-diam area was attached to the skull at an angle of 30° to the midsagittal plane. The center of the chamber was positioned at stereotaxic coordinate A8, based on the atlas of Snider and Lee (1961). The recording electrodes were positioned within the chamber using an X-Y coordinate manipulator. A beveled and sharpened guide cannula containing the microelectrode was used to penetrate the dura and brain to within 8–10 mm of the red nucleus. The electrode was then advanced into the nucleus using a manual hydraulic microdrive. According to atlas coordinates, the RNm is located from A5 to A7 and L1.2 to L2.5. However, these coordinates were slightly different in our monkeys. The weights of the monkeys in this study were three times that of the ones used in the Snider and Lee atlas. Our coordinates showed that the red nucleus was located more anteriorly and ventrally. Humphrey et al. (1984) reported the same disparity. The RNm is located 1.5–2 mm lateral to the oculomotor nucleus and extends over approximately the same anteroposterior coordinates. Localization of the red nucleus was aided by using the oculomotor nucleus as a landmark. Oculomotor neurons were identified on the basis of their distinctive discharge properties, including (1) firing rates clearly related to eye position, (2) little variability in interspike interval at a constant eye position, and (3) a large range of repetitive firing rates extending from 0 to 700 Hz (Fuchs and Luschi 1970). Oculomotor neurons are easily recognizable on the basis of these characteristics and therefore represent ideal landmarks for confirming brain stem stereotaxic coordinates (Cheney 1980).

EMG activity was recorded from 24 muscles representing shoulder, elbow, wrist, digit, and intrinsic hand muscles. Five muscles were recorded from the shoulder: pectoralis major (PEC), anterior deltoid (ADE), posterior deltoid (PDE), teres major (TMAJ), and latissimus dorsi (LAT); seven muscles from the elbow with three extensors: triceps long head (TLON), triceps lateral head (TLAT), and biceps long head (BIL); seven muscles from the wrist with three extensors: extensor carpi radialis (ECR), extensor carpi ulnaris (ECU), and flexor carpi radialis (FCR); five muscles from the wrist including, two extensors: extensor carpi radialis (ECR), extensor carpi ulnaris (ECU), and three flexors: flexor carpi radialis (FCR), flexor carpi ulnaris (FCU), and palmaris longus (PLF); five forearm digit muscles including three extensors: extensor digitorum communis (EDC), extensor digitorum 2.3 (ED2.3), and extensor digitorum 4.5 (ED4.5), and two flexors: flexor digitorum superficialis (FDS) and flexor digitorum profundus (FDP), and two intrinsic hand muscles, abductor pollicis brevis (APB) and first dorsal intersosseus (FDI). Recordings were made using pairs of multistranded stainless steel
wires (AS-632 Bioflex insulated wire, Cooner Sales, Chatsworth, CA). With the monkey anesthetized, each wire of a pair was back fed into a 22-gauge hypodermic needle and inserted transcutaneously into the muscle belly. Separation at the point of insertion was 5 mm, and tip exposure was 1–2 mm (Loeb and Gans 1986). Electrode locations were confirmed by stimulating through the electrode pair and observing appropriate evoked movements. Electrode wires and connector terminals were anchored in position using medical adhesive tape (Johnson and Johnson Medical). The monkey was returned to the cage wearing a vest and sleeve to protect the implant. The monkeys adapted readily to this procedure, and implants typically remained functional for 5–8 wk.

Stimulus-triggered averaging procedures

The stimulus-triggered averaging technique used in this study was developed by Cheney and Fetz (1985) and is described fully in the work of Cheney et al. (1991). In contrast to spike-triggered averaging of rectified EMG activity, which reveals the synaptic effects of a single cell on motoneuron firing, stimulus-triggered averaging reveals the effects of the neuronal aggregate activated by the stimulus. Most of the stimulated neurons are probably in the vicinity of the electrode tip, but activation of distant neurons through axon collaterals is also possible. EMGs were digitized at a rate of 5 kHz, and averages were generally compiled over a 60-ms epoch including 20 ms before the trigger to 40 ms after it. Assessment of effects was based on stimulus-triggered averages of at least 500 trigger events. Microstimuli were applied during all phases of arm movement. However, to avoid averaging segments of EMG when muscle activity was minimal or absent, the averaging program checked the segment of EMG activity associated with each stimulus before accepting it. The average of all EMG data points over the entire 60-ms epoch had to equal or exceed 5% of full-scale input to be accepted. Stimuli were applied at rates from 15 to 20 Hz and generally at a current of 20 μA. Individual stimuli were symmetrical biphasic pulses (negative-positive) with a total duration of 0.4 ms.

Quantitation and measurement of poststimulus effects

Poststimulus facilitation and suppression were computer measured as described in detail by Mewes and Cheney (1991). The onset latency of poststimulus facilitation effects (PSIF) and poststimulus suppression effects (PSIS) was generally measured as the point where the envelope of the effect intersected the line representing 2 SDs from the baseline. SDs were typically calculated from the 1st 20 ms of the average. The magnitude of PSIF and PSIS was expressed as the percent increase or decrease in EMG activity above (facilitation) or below (suppression) baseline. Peak values were measured as the highest point in the peak of facilitation or lowest point in the trough of suppression.

Expressions for these methods of quantifying poststimulus effects are documented extensively in previous papers (Cheney and Fetz 1985; Cheney et al. 1991; Kasser and Cheney 1985). Results

Data were collected from the left red nucleus in two rhesus monkeys (M. mulatta) at or near sites of cells related to forelimb movements. From 137 microstimulation sites in the RNm, a total of 977 poststimulus effects was obtained, with 733 (75%) PSIF effects and 244 (25%) PSIS effects.

Of the facilitation effects, 42% were biphasic. This type of facilitation was obtained in shoulder, elbow, wrist, and digit muscles. Biphasic effects consisted of facilitation followed by suppression (Fig. 9, PDE and ED45). In a few cases, an initial suppression effect was followed by facilitation. Cheney et al. (1991) also noted biphasic poststimulus effects in the forearm muscles from RNm. The second phase of biphasic effects most likely results from activation of spinal inhibitory interneurons, although postexcitatory depression of motoneuron excitability cannot be ruled out.

Because the SD of variability in baseline points should decrease as the square root of the number of trigger events, we normalized all SDs to 1,000 trigger events (Kasser and Cheney 1985). Three classes of PSIF were defined based on peak magnitude: weak, defined as PSIF with peak magnitudes two to three times the SD of the baseline; moderate, defined as PSIF with peak magnitudes three to six times the SD; and strong, defined as PSIF with peak magnitudes greater than six times the SD. Of 733 PSIF effects obtained, 33% were weak, 38% moderate, and 29% strong according to these criteria.

Latency and magnitude

Because of the greater certainty with which onset could be identified, latency data are based on moderate and strong PSIF only. The average PSIF onset and peak latencies were 7.9 and 10.0 ms, respectively, compared with 12.3 and 14.4 ms for PSIS. PSIS onset latency and peak were both 4.4 ms longer than PSIF onset and peak latencies confirming earlier studies (Cheney et al. 1991). Table 1 shows the average latency and magnitude for moderate and strong PSIF at different joints. The following PSIF mean latency comparisons showed statistically significant differences (P < 0.05): (1) shoulder, elbow, wrist, and digit latencies < intrinsic hand muscles, (2) shoulder < elbow, wrist, and digit, and (3) digit < elbow and wrist. Many of these differences can be attributed to differences in conduction distance. At 60% of stimulation sites that produced PSIF in at least one shoulder and one distal muscle, the latency of the shoulder PSIF was shorter (0.5–2 ms) than PSIF in the distal muscles.

The magnitude of the PSIF was not significantly different among shoulder, wrist, digit, and intrinsic hand muscles (Table 1). However, the magnitude of elbow PSIF was significantly weaker than the magnitude of shoulder, wrist, or digit PSIF (P < 0.01).

Figure 1 shows the distribution of PSIF onset latency for muscles acting at different joints of the arm. Some differences were noted between the distributions for proximal and distal muscles. First, 70% of the onset latencies of digit muscle PSIFs were distributed within an interval of 1 ms, between 7.5 and 8.5 ms. For the shoulder and elbow, the onset latencies showed a much broader peak with 86% of shoulder effects falling between 6.5 and 9.5 ms and 95% of elbow effects falling between 7 and 10.5 ms. Variability in onset latency evident from the distributions in Fig. 1 could result from activation of rubrospinal cells conducting at different velocities, the type and strength of synaptic linkage to motoneuron pools, and speed of conduction along the motoneurons and muscle fibers mediating the effects.

Figure 2 shows that there was tendency for stronger PSIF to be associated with shorter latencies (<7.5 ms). This tendency was most pronounced for digit PSIF (P < 0.001) but also significant for shoulder PSIF (P < 0.05). The slopes for elbow and wrist did not achieve statistical significance.
TABLE 1. Latency and magnitude of PStF effects

<table>
<thead>
<tr>
<th>Joint</th>
<th>Onset Latency, ms</th>
<th>Peak Onset Latency, ms</th>
<th>Magnitude, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoulder</td>
<td>7.3 ± 0.9</td>
<td>9.4 ± 0.8</td>
<td>37.4 ± 25.8</td>
</tr>
<tr>
<td>Elbow</td>
<td>8.2 ± 1.3</td>
<td>10.0 ± 1.3</td>
<td>27.2 ± 11.3</td>
</tr>
<tr>
<td>Wrist</td>
<td>8.1 ± 1.2</td>
<td>11.0 ± 1.2</td>
<td>36.0 ± 18.2</td>
</tr>
<tr>
<td>Digit</td>
<td>7.7 ± 0.8</td>
<td>9.7 ± 1.2</td>
<td>38.5 ± 22.1</td>
</tr>
<tr>
<td>Intrinsic</td>
<td>11.1 ± 0.8</td>
<td>12.7 ± 1.7</td>
<td>33.0 ± 29.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. Data based on moderate and strong PStF effects. PStF, poststimulus facilitation effects.

The inverse relationship between magnitude and latency suggests that stronger PStF effects are mediated by faster conducting RNm cells and/or motoneurons or that the synaptic linkage is direct.

Distribution of poststimulus effects

Figure 3, A and B, shows the distributions of the poststimulus effects across shoulder, elbow, wrist, digit, and intrinsic hand muscles. Of 733 PStF effects, more than one-half (58%) were from distal muscles including 20% from wrist, 35% from digit, and only 3% from intrinsic hand muscles. Forty-two percent of PStF effects were from proximal muscles including 18% from shoulder and 24% from elbow muscles. Considering only moderate and strong PStF, 67% of PStF effects were from distal muscles (23% from the wrist, 41% from digit, and 3% from intrinsic hand muscles), and 33% were from proximal muscles (17% from shoulder and 16% from elbow).

Was this distribution significantly influenced by the number of muscles recorded at each joint? Five muscles were sampled for the shoulder, wrist, and digit categories, but seven were sampled at the elbow and only two intrinsic hand muscles. Normalizing did not significantly change the distribution of facilitation except that the frequency of intrinsic hand muscle suppression became much more prominent. Overall, suppression effects were observed more frequently in distal muscles (65%) than in proximal muscles (35%).

Extensor preference of RNm output

Of 137 stimulation sites, 136 (99%) showed excitatory effects in at least one extensor muscle. Eighty-two percent of all PStF effects were in extensor muscles; only 18% in flexor muscles. If only strong and moderate PStF effects are considered, the disparity becomes larger (86% for extensor muscles and 14% for the flexor muscles). Figure 4 shows that this marked preference for facilitation of extensors exists not only for wrist and digit muscles, as previously reported by Mewes and Cheney (1991) and Cheney et al. (1991), but also for shoulder and elbow muscles. However, the extensor preference appeared to be stronger for distal muscles where 90% of PStF effects were in extensors compared with proximal muscles, where 71% of PStF effects were in extensors. Figure 4A shows the distribution of PStF in the shoulder, elbow, wrist, and digit muscles for both extensors and flexors. PStF occurred much more frequently in extensor muscles at all forelimb joints. Preferential facilitation of extensors was greater for shoulder (85%), wrist (85%), and digit muscles (94%) than for elbow muscles (60%). If we consider only the moderate and strong PStF, the percentages increase to 90 and 96% for wrist and digit muscles, respectively; facilitation of shoulder and elbow extensors remained at 85 and 60%.

FIG. 1. Distribution of poststimulus facilitation effects (PStF) onset latency for muscles at shoulder, elbow, wrist, and digit joints. Intrinsic hand muscles not included because of limited sample size. Values given in parentheses for each graph represent means ± SD of the onset latency of the PStF. Data based on moderate, strong, and weak effects.
Interestingly, just the opposite pattern was found for suppression effects. At all joints, there was a clear flexor preference in the number of PStS effects observed (Fig. 4B). This preference was strongest for wrist muscles (80%). Figure 5 shows the number of the PStF and PStS effects observed in each of 24 sampled forelimb muscles. The muscles most frequently facilitated by stimulation of the RNm were PDE for the shoulder, ECU and ECR for the wrist, and EDC, ED2,3, and ED4,5 for the digits. The most frequently suppressed muscles were the wrist flexor muscles (PL and FCU). It is also of interest that the intrinsic hand muscles were more frequently suppressed than facilitated. All muscles were facilitated and inhibited from some site in RNm except PEC, which showed frequent suppression but no facilitation. However, it is possible that facilitation would have been found with more extensive sampling.

These data show that the output of the RNm is not uniformly distributed to flexor and extensor muscles. Rather, at both distal and proximal joints, there is a strong preference favoring facilitation of extensor muscles and suppression of flexor muscles. This preference is most prominent for distal muscles.

**PStF muscle field**

The term “muscle field” has been defined as the set of muscles with significant facilitation from single cells in spike-triggered averages of EMG activity (Cheney et al. 1991; Fetz and Cheney 1978, 1979). Muscle fields can also be characterized for sites within motor cortex or red nucleus activated by microstimuli. Such muscle fields will reflect the output effects of a collection of neurons activated by the stimulus.

For the purposes of this study, muscle field was defined as the number of muscles showing PStF and PStS from sites of stimulation within RNm. The mean muscle field was 6.7 for all sites of stimulation, with 5.4 muscles showing PStF and 2.3 showing PStS (Fig. 6). However, at 38 sites (28%),

![Fig. 2. Relationship between onset latency and magnitude of PStFs (moderate and strong effects) for shoulder, elbow, wrist, and digit muscles. Linear regression lines are plotted, and correlation coefficients (r) and P values are given. Intrinsic hand muscles not included because of limited sample size.](http://jn.physiology.org/)

![Fig. 3. Distribution of PStF (A) and poststimulus suppression effects (PStS; B) in shoulder, elbow, wrist, digit, and intrinsic hand muscles.](http://jn.physiology.org/)
sites facilitated at least one muscle at all joints (shoulder, elbow, wrist, digit, and intrinsic hand). Twenty-six percent of the sites yielded facilitation of at least one shoulder muscle and one distal muscle (Fig. 8) and 11% facilitated an elbow muscle together with a distal muscle.

Cofacilitation of muscles at proximal and distal joints remained prominent even when the analysis was limited to moderate and strong PStF. Fifty-three percent of sites produced effects in at least one proximal and one distal muscle. Thirty percent of sites cofacilitated a shoulder, elbow, wrist, and digit muscle; two of these sites also cofacilitated an intrinsic hand muscle. Nineteen percent of sites cofacilitated shoulder and distal muscles. Table 2 summarizes the frequency with which different combinations of facilitated muscles were observed. At 10 stimulation sites we examined the extent to which muscle field varied with stimulus intensity. Figure 9 shows a typical result. Overall, lower stimulus intensities yielded slightly smaller muscle fields. Although

PStF appeared in just 1 muscle, although only 15 of these PStFs were moderate or strong. Eleven of the 15 were in extensor muscles (3 in PDE, 1 in TLAT, 5 in EDC, 2 in ED45); 3 were in intrinsic hand muscles (1 in APB, 2 in FDI); and only 1 was in a flexor muscle (FDP). At 99 sites (72%), stimulation facilitated two or more muscles of the same joint or different joints. The largest number of facilitated muscles at 1 RNm site was 19 (15 if weak PStF is excluded).

Divergence of output effects to muscles at multiple joints

RNm output effects associated with stimulation show a clear pattern of cofacilitation of muscles at proximal and distal joints. Of 137 sites investigated, 84 (61%) showed facilitation of both proximal (shoulder and elbow) and distal muscles (wrist, digit, and intrinsic hand; Fig. 7A). Of the remaining 39% of the sites, PStF was limited to proximal muscles (17%) or distal muscles (22%). Including both PStF and PStS, 88 (64%) of sites influenced both proximal and distal muscles.

Of the 61% of stimulation sites that cofacilitated proximal and distal muscles, over one-half (63%) facilitated at least one shoulder, elbow, and distal muscle (Fig. 7B). A few

![Graph A](image1)

**FIG. 4.** Distribution of PStF (A) and PStS (B) in extensor and flexor muscles of the shoulder, elbow, wrist, and digits. The frequency of PStF is higher in extensor muscles at all joints, and the frequency of PStS is higher in flexor muscles at all joints.

![Graph B](image2)

**FIG. 5.** Distribution of PStF (right) and PStS (left) obtained from 24 distal joints. Of 137 sites investigated, 84 (61%) showed facilitation of both proximal (shoulder and elbow) and distal muscles (wrist, digit, and intrinsic hand; Fig. 7A). Of the remaining 39% of the sites, PStF was limited to proximal muscles (17%) or distal muscles (22%). Including both PStF and PStS, 88 (64%) of sites influenced both proximal and distal muscles.

![List of Muscles](image3)

**FIG. 6.** Number of sites (cumulative) out of 100 that positively influenced each muscle. Each muscle is represented with a hash symbol, and the number of sites is indicated on the right. The number of sites increased as the arm was extended, with the greatest number of sites in the shoulder muscles. The number of sites also increased as the hand was extended, with the greatest number of sites in the wrist and digit muscles.
some clear discrepancies exist between the muscle fields at 20 and 10 μA (e.g., ECR, ED2.3, PL), cofacilitation of proximal and distal muscles was prominent at both stimulus intensities. At 5 μA all effects were lost.

**DISCUSSION**

In this study we used stimulus-triggered averaging of EMG activity from 24 muscles of the forelimb to investigate aspects of the output organization of the RNm in the macaque monkey. The results show that 1) the RNm controls both proximal and distal muscles of the forelimb, although influence over distal muscles is generally more prominent, 2) RNm output preferentially excites extensor muscles not only at distal joints but also at the shoulder and elbow, 3) RNm output preferentially inhibits flexor muscles at proximal and distal joints, and 4) the majority of sites within the RNm cofacilitate muscles at proximal and distal joints.

**Origin and detection of poststimulus effects**

Two factors may influence the interpretation of the poststimulus effects. The first is related to the origin of poststimulus effects and the second to their detection at the level of muscle EMG activity.

We interpret the poststimulus effects in this paper as originating primarily from the activation of rubrospinal elements. Because axons and cell somas both have comparable thresholds (i.e., Jankowska and Roberts 1972; Jankowska et al. 1975), the possibility arises that poststimulus effects might be due to activation of collaterals of corticospinal or cerebellar nuclear neurons (Humphrey and Reitz 1976; Humphrey et al. 1984; Ralston 1994). These two possibilities have been discussed extensively in previous work (Cheney and Fetz 1985; Cheney et al. 1991; Ghez 1975; Kasser and Cheney 1985; Larsen and Yumiya 1980). All of these authors have concluded that effects obtained by microstimulation within the RNm are primarily the result of rubrospinal neuron activation, not activation of axon collaterals from cerebral cortex or cerebellum.

The ability to detect postspike and poststimulus effects in a particular muscle will depend on 1) the strength of synaptic coupling between the premotor cells and motoneurons, 2) the distribution of synaptic influence to individual motoneurons (motor units) within the motoneuron pool, 3) the number of motor unit action potentials recorded by the intramuscular EMG electrodes, and 4) the presence of activity in motor units. Without EMG activity, no effects can be detected. Electrodes that minimize the number of motor unit action potentials recorded in a muscle could potentially reduce the chances of detecting effects, particularly in a muscle where the synaptic linkage from premotor cells to motoneurons is distributed narrowly to a small number of motoneurons. We estimate that our EMG electrodes “pick up” the spikes of possibly 10–15 motor units, and this does not appear to be highly muscle specific. The muscle fibers belonging to different motor units are generally distributed broadly throughout the muscle so regardless of the specific electrode placement or factors such as innervation ratio, our EMG electrode technique should have provided the opportunity to record a similar size population from each muscle (Roy et al. 1984; Streuli Messmer et al. 1990). Moreover, Palmer and Fetz (1985) showed that on average, 95% of recorded single motor units within a muscle are influenced by microstimulation at individual cortical sites. Similarly, Lemon et al. (1990) showed that single cortical cells tested with spike-triggered averaging may facilitate many, if not all, motor units within a muscle. These findings suggest that the terminations from single cortical cells are distributed.

**FIG. 6.** Number of facilitated and/or suppressed muscles at each magnocellular red nucleus (RNm) site tested. A: all PSTFs. B: moderate and strong PSTFs. C: PSTF and PSTS combined (total muscle field). D: all PSTS. Values given in parentheses for each graph represent means ± SD of the muscle field size.
movements at distal joints of the forelimb and hindlimb (77 and 83%, respectively) and only a much smaller number of cells was involved with movements at proximal joints (23 and 17%, respectively). Similar differences were reported by Gibson et al. (1985a). Our results lead to a somewhat different conclusion. In fact, the number of PStF effects was only 5% less for proximal muscles than distal muscles. The difference increased to 20% if only moderate and strong PStF were considered. Nevertheless, it seems clear that RNm effects on proximal muscles are common and can be as powerful as its effects on distal muscles.

This brings us to another question concerning the functional role of RNm actions on proximal muscles. RNm not only has affects on proximal muscles, but it is important to note that, at the majority of sites, these effects were linked to one or more effects on distal muscles. Seventeen percent of sites facilitated only proximal muscles, and 22% of sites facilitated only distal muscles, whereas 61% of sites facilitated a combination of proximal and distal muscles. However, cofacilitation of proximal and distal muscles in stimulus-triggered averages must be interpreted cautiously. Some possible interpretations are illustrated in Fig. 10. One possibility is that the cofacilitation of proximal and distal muscles is not due to activation of individual rubrospinal neurons having terminations in both proximal and distal motoneuron pools but rather to activation of two populations of neurons that individually facilitate either distal or proximal motoneurons but not both (Fig. 10A). Additional variations of this theme are illustrated in Fig. 10, C and D. In one case, excitatory collaterals between proximal and distal muscle rubrospinal cells might yield cofacilitation of both sets of muscles when, in fact, individual neurons have their terminations confined to motoneurons of only one muscle group. However, it should be noted that there is no evidence that such collaterals exist within the RNm. Moreover, effects mediated indirectly involve an additional synapse and would be expected to be weaker and somewhat longer latency than the direct effects. Cofacilitation of proximal and distal muscles might also occur by stimulation of afferent input fibers that

Distribution of the PStF in proximal versus distal muscles

One of the significant findings of the present study is that proximal muscles are common targets of RNm action. In several previous studies, the role of RNm in movements at distal joints has been emphasized (Cheney et al. 1991; Gibson et al. 1985a,b; Holstege et al. 1988; Houk et al. 1988; Humphrey et al. 1984; Kennedy 1987; Kohlerman et al. 1982; Lawrence and Kuyers 1968; McCurdy et al. 1987; Mewes and Cheney 1991, 1994; Miller et al. 1993; Ralston et al. 1988; Sinkjaer et al. 1995; Sybirska and Gorska 1980). For example, Kohlerman et al. (1982) reported that many RNm cells were strongly modulated in association with

<table>
<thead>
<tr>
<th>Shoulder</th>
<th>Elbow</th>
<th>Wrist</th>
<th>Digit</th>
<th>Intrinsic</th>
<th>Number of Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>2</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Number of stimulation sites showing PStF in muscles at the joints indicated

Data based on moderate and strong PStFs. “X” indicates the joints showing at least one facilitated muscle. PStF, poststimulus facilitation effects.
select separate populations of proximal and distal muscle rubrospinal neurons (Fig. 10D). Although this possibility cannot be completely ruled out, accepting it would simply shift the attributes of functional organization that had been ascribed to individual rubrospinal neurons one level back to cortical or cerebellar afferent input fibers.

An alternative possibility, which we favor, is that many individual rubrospinal neurons actually do terminate within the motoneuron pools of both proximal and distal muscles (Fig. 10B). This conclusion is supported by the previous findings showing that for both motor cortex and red nucleus cells, the profile of PStF across forearm muscles (and the muscle field) closely matches that of postspike facilitation (PSpF) from individual neurons recorded at the site of stimulation (Cheney and Fetz 1985; Cheney et al. 1991). The implication of this finding is that neighboring output neurons in motor cortex and red nucleus must have similar muscle fields. In fact, Cheney et al. (1991) showed that at 83% of sites, the muscle with the strongest PStF at 10 μA was also the muscle with the strongest PSpF from a rubromotoneuronal (RM) cell at that site; at 20 μA, 68% of sites matched. Therefore it seems reasonable to conclude that pattern of proximal and distal muscle cofacilitation we obtained in this study using stimulus-triggered averaging would also be expected for individual neurons using spike-triggered averaging. In fact, our preliminary spike-triggered averaging studies have revealed individual RM cells that produce postspike facilitation in both proximal and distal muscles. The fact that PStF in distal and proximal muscles is often lost together when stimulus intensity is decreased also suggests that the same neurons are mediating effects in both muscle groups. Nevertheless, it will be important to examine the issue of RM cell branching to both proximal and distal motoneuron pools using the spike-triggered averaging method (Fetz and Cheney 1980). In other studies from our laboratory, cells facilitating both proximal and distal muscles have also been found in the forelimb representation of primate motor cortex (McKiernan et al. 1994). Moreover, using the stimulus-triggered averaging method for mapping the output of the motor cortex, Karrer et al. (1995) demonstrated the existence of regions in primate motor cortex yielding cofacilitation of proximal and distal muscles of the forelimb.

Anatomic and physiological studies from other laboratories have provided additional supporting evidence. Shi-noda et al. (1977, 1982) investigated the branching of individual rubrospinal fibers in the spinal cord of the cat using intra-axonal injections of tracer (horseradish peroxidase). They showed that some axons projected widely to two or three segments of the spinal cord. Injections of neuroanatomic tracers confined to the forelimb region of RNm have shown a widespread pattern of input to all levels of cervical cord, although clearly this method does not reveal single fiber branching patterns (Robinson et al. 1987). However, few rubrospinal fibers (2–3%) project to both cervical and lumbar cord (Huisman et al. 1982). Additional evidence comes from electrophysiological studies showing that the

![Image](Fig. 8. Stimulus-triggered averages from a RNm site that cofacilitated muscles at shoulder, wrist, and digit joints. Significant PStF (bold) was obtained at the shoulder (PDE and TMAJ), the wrist (ECR and ECU), and the digits (EDC, ED4,5, and ED2,3). PStS (*) was obtained at the wrist (FCU, FCR, and PL) and elbow (BR and DE). The number of trigger events is given in parentheses.)
FIG. 9. Stimulus-triggered averages from the same RNm site of 16 muscles at 20, 10, and 5 μA. Muscle fields at 20 and 10 μA were similar, and all effects were lost at 5 μA. The number of trigger events is given in parentheses.

43F4
activity of the RNm cells is strongly modulated during movements involving coordinated multijoint reaching movements (Gibson et al. 1985a,b; Kohlerman et al. 1982; Mewes and Cheney 1994; Miller et al. 1993). Based on these results and the findings of this paper, we suggest that some red nucleus output zones are organized to produce a basic pattern of functional synergy in proximal and distal muscles needed for reaching movements involving extension of the arm for the purpose of acquiring and grasping an object. Variations in the details of the movement could be achieved by activation of neurons with more restricted muscle fields that would sculpt the basic template of synergy among muscles at different joints for the purpose of producing the specific intended movement.

**Extensor preference**

In this study we have shown that the strong extensor muscle preference in RNm output demonstrated previously for distal muscles also applies to shoulder and elbow muscles. Ninety-nine percent of sites tested at 20 μA either facilitated extensors exclusively or produced stronger facilitation of extensors than flexors. Seventy-one percent of proximal muscle PSTF effects were in extensors; 90% of distal muscle PSTF were in extensors. The extensor muscle preference was also clear at each joint; 85% at the shoulder and wrist, 60% at the elbow, and 94% at the digits.

This result is contrary to some early work that suggested that the RNm predominantly facilitates contralateral flexor muscles (Hongo et al. 1969; Massion 1967; Orlovsky 1972; Pompeiano 1957; Sasaki et al. 1960; Thulin 1963). However, it should be pointed out that this early work was based on effects in hindlimb muscles of the cat following stimulation of the red nucleus with large tip electrodes. Using intracellular recordings from hindlimb motoneurons in the cat, Hongo et al. (1969) confirmed the presence of EPSPs in flexor motoneurons and inhibitory postsynaptic potentials (IPSPs) in extensor motoneurons but also noted that the predominant effect on some extensor motoneuron pools (toe extensor muscles) was excitatory.

More recently, Cheney et al. (1991) used StTA of EMG activity from RNm in the awake monkey performing a wrist movement task to test output effects on motoneurons of distal extensor and flexor muscles. They reported that 94% of RNm sites tested at 20 μA either exclusively or preferentially facilitated extensor muscles. Similar results were obtained with lower intensities (5 and 10 μA) of stimulation. Stimulation was applied at the sites of RM cells in the study by Cheney et al. (1991), but it was also reported that stimulation at many non-RM cell sites yielded preferential facilitation of extensor muscles. Mewes and Cheney (1991) also reported that a large majority of single RM cells (69%) tested with spike-triggered averaging of EMG preferentially facilitated forelimb extensor muscles. The present study extends this finding to include muscles at proximal forelimb joints.

**Conclusions**

In conclusion, RNm output sites most frequently cofacilitate both proximal and distal muscles as a synergy suggesting that RNm may be preferentially involved in the control of movements requiring coordination of proximal and distal joints. At the same time, the potential versatility of RNm output appears to be somewhat restricted by the strong preference favoring facilitation of extensor muscles and inhibition of flexor muscles, which is prominent at all forelimb joints. The action of RNm also seems rather selective in some cases, for example, PDE at the shoulder. This pattern of output would suggest that RNm cells might be preferentially involved in reaching movements that involve multijoint coactivation of extensor muscles. This output pattern is consistent with the coactivation of shoulder (both ADE and PDE), elbow, wrist, and forearm digit muscles that characterizes the ‘‘reaching phase’’ and the ‘‘in target cylinder phase’’ of the prehension task. These issues will be tested further by correlating the activation patterns of individual RM cells during the prehension task with the muscle fields of the same cells.

We thank T. Novak, T. Gleason, J. Kenton, and J. Rengel for expert technical assistance.

This work was supported by grant 1657 from the Paralyzed Veterans of American- Spinal Cord Research Foundation, grant NS-25646 from the National Institute of Neurological Disease and Stroke and grant HD-02528 from the National Institute of Child Health and Human Development.

Address for reprint requests: P. D. Cheney, Smith Mental Retardation and Human Development Research Center, University of Kansas Medical Center, Kansas City, KS 66160.


