Functional Specialization Within the Cat Red Nucleus


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Received 29 December 2000; accepted in final form 14 August 2001

Horn, K. M., M. Pong, S. R. Batni, S. M. Levy, and A. R. Gibson. Functional specialization within the cat red nucleus. J Neurophysiol 87: 469–477, 2002; 10.1152/jn.00949.2000. Magnocellular (RNm) and parvicellular (RNp) divisions of the cat red nucleus (RN) project to the cervical spinal cord. RNp projects more heavily to upper cervical levels and RNm projects more heavily to lower levels. The cells in RN are active during reaching and grasping, and the differences in termination suggest that the divisions influence different musculature during this behavior. However, the spinal termination may not reflect function because most rubrospinal terminations are to interneuronal regions, which can influence motor neurons at other spinal levels. To test for functional differences between RNm and RNp, we selectively stimulated RNm and RNp as well as the efferent fibers from each region. Electromyographic activity was recorded from seven muscles of the cat forelimb during reaching. The activity from each muscle was averaged over several thousand stimuli to detect influences of stimulation on muscle activity. Stimulation within the RN produced a characteristic pattern of poststimulus effects. The digit dorsiflexor, extensor digitorum communis (edc), was most likely to show facilitation, and several other muscles showed suppression. The pattern of activation did not differ between RNm and RNp. In contrast, stimulation of RNp fibers favored facilitation of shoulder muscles (spinodeltoideus and supraspinatus), and stimulation of RNm fibers favored facilitation of digit and wrist muscles (edc, palmaris longus, and extensor carpi ulnaris). Fiber stimulation produced few instances of poststimulus suppression. The results from fiber stimulation indicate that the physiological actions of RNm and RNp match their levels of spinal termination. The complex pattern of facilitation and suppression seen with RN stimulation may reflect synaptic actions within the nucleus.

INTRODUCTION

The accompanying report (Pong et al. 2002) demonstrates that projections of the parvicellular red nucleus (RNp) are stronger at upper cervical levels than at lower cervical levels. In contrast, projections of magnocellular red nucleus (RNm) are stronger at lower cervical levels than at upper levels. RNm receives a major input from the cerebellar interpositus nucleus, and RNp receives a major input from the cerebellar dentate nucleus. It is likely that RNm and RNp serve different functions in movement control.

Cells in the red nucleus (RN) discharge strongly during reaching to grasp an object (Gibson et al. 1985, 1994; van Kan and McCurdy 2001). The differential spinal projections indicate that RNp may be more important for control of muscles in the proximal limb, whereas RNm may be more important for control of muscles of the distal limb. However, most terminations of rubrospinal (RST) fibers are to spinal interneurons rather than to motor neurons, and terminations at one spinal level might influence motor neurons at other levels via propriospinal connections (Alstermark et al. 1990; Robinson et al. 1987). The object of the present study was to test the hypothesis that activation of RNp neurons will produce activity in muscles of the proximal limb and activation of RNm neurons will produce activity in muscles of the distal limb.

Fetz and Cheney (1980) developed the technique of spike-triggered averaging of electromyography (EMG) to detect functional relations between cellular activity and muscle activation. By synchronizing the EMG records to the activity of a single neuron it is possible, with sufficient averaging, to detect the contribution that the neuron makes to activation of the target muscle. A variation of spike-triggered averaging is stimulus-triggered averaging, which elicits neural activity with electrical stimulation. Stimulus-triggered averaging has some practical advantages over spike-triggered averaging. One advantage is that the experimental demands are less because single units do not need to be isolated for a prolonged averaging period. A second advantage is that stimulation has a relatively strong effect on muscle activation because more than one neuron is activated by the stimulus pulse. These advantages allow more data to be collected from each subject; this is an important consideration when dealing with behaving animals with EMG electrodes implanted into several muscles. Direct comparisons between spike- and stimulus-triggered averaging in motor cortex (Cheney and Fetz 1985) and red nucleus (Cheney et al. 1991) indicate that patterns of EMG facilitation and suppression are in good agreement between the two methods.

However, stimulus-triggered averaging has a drawback in that activation of fibers passing near the stimulation site may confound the results. This is a major concern with stimulation within the RN because cerebellar efferents course through the nucleus to terminate in more rostral regions of RN as well as thalamus. Additionally, efferents from lateral regions of RN pass through medial regions of the nucleus as they decussate to form the rubrospinal tract (RST). Data from the cases reported in the preceding paper (Pong et al. 2002) show that, for a short distance after decussation, the fibers from RNp and RNm are segregated in the RST. The RNm fibers travel dorsally to fibers from RNp until they reach caudal pontine levels where they intermingle. In this paper, we compare stimulus-triggered averages (SSTAs) of forelimb muscles with stimulation sites in...
RN, RST at caudal mesencephalic levels (segregated fibers), and RST at medullary levels (mixed fibers).

Stimulation within RN produced both facilitation and suppression of limb muscle activity and showed a strong bias in favor of facilitating a digit muscle, extensor digitorum communis (edc). However, the overall pattern of muscle activation was similar for stimulation sites in RNm and RNp. At the mesencephalic stimulation site, stimulation of dorsal regions of the RST (RNm fibers) produced strong facilitation of edc but weak facilitation of the shoulder muscles spinodeltoideus (SD) and supraspinatus (ss). Stimulation in the ventral regions of the RST (RNp fibers) produced strong facilitation of the shoulder muscles but weak facilitation of edc. Stimulation of the RST at medullary levels produced facilitation in all limb muscles with few instances of suppression. The results indicate that anatomical differences in spinal terminations between RNp and RNm are reflected by physiological action and suggest that the complex effects seen with RN stimulation may be due to activation of local neural elements.

**METHODS**

**Behavioral paradigm**

Five cats were trained to reach, grasp, and retrieve a handle on presentation of a tone. The cats received a small quantity of pureed chicken and cod liver oil extruded from the end of the handle. Cats typically performed 200–500 trials during daily training sessions of 1–2 h. The cats were provided supplemental food in their home cages to maintain their body weights between 80 and 100% of free-feeding weight.

**Implant surgery**

Surgery was performed in an American Association of Laboratory Animal Care-approved surgical suite using aseptic techniques. All procedures were approved by the St. Joseph’s Hospital Institutional Animal Care and Use Committee and were in accordance with National Institutes of Health guidelines. Each cat was initially anesthetized with an intramuscular injection of ketamine hydrochloride (10–15 mg/kg), and anesthesia was maintained with intravenous administration of pentobarbital sodium. A recording chamber and a head restraint device were fastened to the skull with stainless steel screws and dental acrylic. For three cats, the chamber was positioned to provide access to the RN (A4.0, Berman 1968). In one cat, the chamber was positioned (P2.0) to provide access to the RST at mesencephalic levels, and, in another, the chamber was positioned to provide access to the RST at medullary levels (P10.0).

For each cat, seven pairs of insulated multi-stranded stainless steel wire were implanted into forelimb muscles. Each electrode had a tip exposure of 4–6 mm, and the tips of each pair were separated by 5–10 mm. Placement was confirmed by electrical stimulation through the electrodes. Table 1 lists the implanted muscles and their physiological actions.

**RST tracing**

The RST tracing presented in this paper is based on case BRN1 of the previous paper (Pong et al. 2002), and the anatomical methods are fully described in that paper. For case BRN1, injections of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) were placed in RNm on the right side and in RNp on the left side (Fig. 3, see also Fig. 2 of Pong et al. 2002). By plotting the location of anterogradely labeled fibers, the trajectories of the descending fibers from these two regions could be compared across sides of the same frontal sections.

**Neural and EMG recordings**

Neural activity within the RNm or RST was recorded with tungsten microelectrodes. Cells in RNm and RNp discharged during movements of the contralateral limbs. Spike amplitudes from cells in RNp tended to be smaller than amplitudes of cells in RNm, and fiber recordings in RST were characterized by waveforms with initial positive deflections (van Kan et al. 1993).

EMG activity was recorded with a band-pass of 10–10,000 Hz, full-wave rectified, integrated with a 1-ms time constant and sampled at 4 kHz. Prior to each recording session, the amplifier gain for each muscle was set to provide peak amplitudes of 5 V during the behavioral task. This procedure was meant to help compensate for changes in electrodes over time as well as for variations between muscles and cats. Examples of the rectified and integrated EMG signals from four muscles during a reach trial are illustrated in Fig. 1A.

**StTA and data analysis**

StTAs of rectified EMG activity were calculated for the different RN and RST sites. The techniques used in the present study are similar to those of Cheney et al. (1991). Stimuli were applied at 500- or 1,000-μm increments along each electrode track. During stimulation, monophasic negative pulses (0.2-ms duration, 10 Hz, 5–30 μA) were delivered through the tungsten recording electrode while the cat performed the reaching task. The patterns of muscular activation near threshold (5–10 μA) resulted in similar StTA patterns as generated with the suprathereshold current of 20 μA. Data presented in this report were collected using stimuli of 20 μA with EMG averaged for 2,000 pulses.

StTAs were calculated by computing an average baseline activity and standard deviation (SD) from 10-ms periods preceding the stimulus pulses. Data were standardized relative to the SD of the baseline activity. Figure 1B illustrates examples of significant StTAs for the four muscles shown in A. Several criteria were required for a StTA to be considered significant. First, the amplitude of the waveform needed to exceed ±3 SDs of baseline activity. Second, the duration of the significant elevation needed to exceed 2 ms. Third, only StTAs with waveforms beginning between 3 and 15 ms following the stimulus pulse were considered as occurring within a physiologically relevant time frame.

**Verification of stimulation sites**

Marking lesions (−10 μA for 10 s) were placed at the end of the experiment. Prior to perfusion, cats received an intramuscular injection of ketamine (20 mg/kg) followed by a lethal dose of sodium pentobarbital (approximately 25 mg/kg) delivered in a single rapid iv bolus. Cats were perfused with 10% formalin. The brains were frozen and sectioned at 50 μm. Every section through areas of interest was collected and stained with either cresyl violet or neutral red/luxol blue.
Locations of unmarked sites were reconstructed relative to lesion locations.

RESULTS

Stimulation in RN

We first attempted to determine functional relations of RNm and RNp by stimulating at 51 sites within the RN of three cats. Sites located in the caudal 2 mm of the RN (n = 35) are referred to as RNm, and sites in the rostral 2 mm of the nucleus (n = 16) are referred to as RNp. Figure 2 illustrates averages from a proximal (ss) and a distal (edc) forelimb muscle for one stimulating track that passed through RNm.

For the track illustrated by Fig. 2, two sites (black circles) within RNm produced significant facilitation. Cells at the dorsal site were active during movements of the contralateral forelimb, and cells at the ventral site were active during movement of the contralateral hind limb. Presumably, the current spread of the 20-μA pulses either included forelimb regions of RN or activated passing fibers related to forelimb musculature. The StTAs from the illustrated track (Fig. 2) mirror the results obtained from all of the RN stimulation tracks. No significant StTAs were produced by stimulation outside of the borders of the RN (27 sites dorsal to RN and 28 ventral), and the pattern of poststimulus facilitation and suppression was consistent between cats. Figure 6A plots the percentage of significant StTAs for three cats with stimulation sites in RNm. For each cat, the most frequently facilitated muscle was edc (91% of sites), while several other muscles, such as palmaris longus (pl), displayed a high incidence of poststimulus suppression. For both RNm and RNp, the distribution of poststimulus effects between muscles differed significantly from chance (RNm, χ² = 48.2, 6 df, P < 0.01; RNp, χ² = 22.8, 6 df, P < 0.01).

Our hypothesis predicted that proximal limb muscles would more likely be activated by RNp stimulation and distal muscles by RNm stimulation. Although the shoulder muscles, sd and ss, were more likely to be facilitated by RNp stimulation (49 vs. 21%), the overall pattern of muscle activation between RNm and RNp was not significantly different (χ² = 2.9, 6 df, P > 0.50). Therefore stimulation within RN did not support a functional difference between RNm and RNp.

Anatomical separation of RNm and RNp fibers in the RST

Stimulation within RN is likely to be confounded by activation of passing fibers. The double injection case (BRN1) of the previous paper (Pong et al. 2002) indicated that stimulation of the RST at the appropriate level might avoid this problem. Figure 3, A and B, illustrates injections (0.008 μl) of WGA-HRP (1%) made in RNm (A) and RNp (B) on opposite sides of the brain. Figure 3, C and D, illustrates the locations of RST fibers as they descend to the spinal cord. Position of the labeled fibers was plotted onto images of the sections with the use of a computer-aided plotting system (Image TracerTM). Because the fibers exiting from the RN cross immediately to the opposite side, labeled fibers from the RNp injection (vertical striping) are on the right and those from RNm (horizontal striping) are on the left. The section shown in Fig. 3C is at the level of the caudal mesencephalon and rostral pontine nuclei (A1.6). At this level, fibers from RNm (left) travel immediately below fibers of the superior cerebellar peduncle (BC). Fibers from RNp (right) lie ventral to those from RNm and are bounded on their ventral border by the medial lemniscus (ML). Therefore a stimulating track through the RST at this level would pass first through RNm efferent fibers and then through RNp efferent fibers. Figure 3D illustrates the location of labeled RST fibers at the level of the rostral inferior olive (P12.7). At this level, fibers from RNm and RNp are intermingled and occupy corresponding locations on either side of the brain.

FIG. 1. Records generated during a reaching trial. A: leg position was monitored with a lever attached at the wrist. Examples of the rectified and integrated (1 ms electromyogram (EMG) for 4 four-limb muscles [extensor digitorum communis (edc), palmaris longus (pl), brachialis (br), and supraspinatus (ss)] are illustrated below the position trace. B: the stimulus-triggered averages (StTAs) from the muscles after averaging of 2,000 stimulus pulses. Red nucleus (RN) stimulation produced significant StTAs for all of the illustrated muscles, although pl responded with poststimulus suppression rather than facilitation. The long horizontal dashed lines represent ±3 SDs of pretrigger EMG activity.
Stimulation of RST

MESENCEPHALIC LEVEL. A fourth cat received RST stimulation in the caudal mesencephalon. The RST was localized by recording fiber discharge during movement of the ipsilateral limb, and its ventral border was identified by the sensory responses of the underlying ML.

Figure 4 illustrates StTAs produced on one track through the mesencephalic RST. An outline of the corresponding frontal section from BRN1 is included to demonstrate the relative location of fibers from RNm and RNp (labeled fibers from the RNm injection have been transposed to the right side). The plane of section was not identical between the cases so sections were matched by comparing ventral structures.

StTAs were collected at 0.5-mm steps in depth along the track. Stimulation sites above the BC or below the ML did not elicit significant StTAs (Fig. 4, white circles). During stimu-
lation, significant StTAs ceased abruptly as the electrode entered the ML. However, the anatomical reconstruction of this track placed the two most ventral stimulation points within ML fibers. It is likely that the anatomical reconstruction has some inaccuracy because marking lesions were made during electrode withdrawal.

Stimulation sites within the RST produced significant post-stimulus facilitation (black circles, Fig. 4). Dorsal stimulation sites facilitated edc but not ss, whereas ventral stimulation sites facilitated ss but not edc. Similar muscle activation patterns were seen on the other stimulation tracks. The histograms in Fig. 6, C and D, illustrate the probability of significant StTAs for the various muscles at stimulation sites within either the dorsal (C) or ventral (D) halves of the RST. The probability of obtaining significant StTAs for edc, pl, and extensor carpi ulnaris (ecu) is higher at dorsal sites, whereas the probability for obtaining significant StTAs for triceps (long head; tr), sd, and ss is higher at ventral sites. Brachialis (br) was activated with approximately equal frequency from dorsal and ventral sites. Poststimulus facilitation was much more commonly produced by mesencephalic stimulation than was poststimulus suppression (88% of the stimulation sites produced facilitation).

The largest differences in activation were seen for edc, sd, and ss. At dorsal stimulation sites edc was activated 77% of the time but only 15% of the time at ventral sites. In contrast, sd and ss were activated 7 and 3% at dorsal sites but 35 and 65% at ventral sites. The patterns of activation between dorsal and ventral stimulation sites were significantly different ($\chi^2 = 41.2, 6$ df, $P < 0.01$).

MEDULLARY LEVEL. At the level of the caudal pons, the fibers of the RST form a relatively compact bundle that travels along the ventrolateral edge of the brain stem. At this level, fibers from RNm and RNp intermingle and disperse evenly throughout the RST. Therefore stimulation should provide an overall picture of relations between RN output and forelimb muscles regardless of the site within the RST.

Figure 5 illustrates results from one track passing through
the RST at medullary levels. Stimulation dorsal and ventral to the RST (white circles) failed to produce significant StTAs, but stimulation within the RST (black circles) did. StTAs for ss and edc are illustrated in Fig. 5, right. Significant StTAs commence at the same depth for both muscles and the largest StTAs are elicited at the same stimulation site.

To test for a potential topography within the RST at medullary levels, we grouped the stimulation sites into dorsal and ventral halves based on recording depth (as was done for the RST at mesencephalic levels). Figure 6, E and F, illustrate the results. The patterns of activation for the dorsal and ventral sites were not significantly different ($\chi^2 = 0.93, 6$ df, $P > 0.50$). As with stimulation of the mesencephalic RST, few instances of poststimulus suppression were observed (95% of the sites produced facilitation).

DISCUSSION

The primary objective of this study was to determine if spinal terminations of RNm activate different forelimb musculature than those of RNp. Stimulation of fibers from RNm activated muscles of the distal limb (edc, pl, and ecu) more strongly than muscles of the proximal limb and shoulder (tr and ss). Stimulation of fibers from RNp activated muscles of the proximal limb and shoulder more strongly than those of the distal limb. Therefore the physiological actions of RNm and RNp are consistent with the anatomical observation that RNm fibers terminate more heavily at lower cervical segments and RNp fibers at upper segments (Pong et al. 2002).

Patterns of activation

Stimulation within the RN produces a highly characteristic pattern of StTAs. Digit (edc) and wrist (ecu) extensor muscles are strongly facilitated, whereas other limb muscles often show instances of suppression as well as facilitation. There are no other reports of StTAs resulting from stimulation of the cat RN, but stimulation in the monkey produces similar effects (Belhaj-Saif et al. 1998; Mewes and Cheney 1991). Digit and wrist extensor muscles are strongly facilitated, and several limb muscles show a high incidence of suppression (especially pl).
Surprisingly, neither a favoring of edc nor a significant number of poststimulus suppressions occurred with stimulation of the RST. At medullary levels, RST stimulation produced a high probability of facilitation for all seven muscles (range 55–90% facilitation), and poststimulus suppression was limited to one muscle at one site.

The lack of preferential facilitation of edc from the RST stimulation is especially surprising, since motor pools at C8 receive a selective input from the RN for the cat and monkey (Fujito et al. 1991; Holstege and Tan 1988; McCurdy et al. 1987; Ralston et al. 1988). However, most RN projections to the cord terminate in interneuronal regions rather than in motoneuronal pools, and it may be that the motoneuronal projections account for a relatively small portion of the poststimulus effects. If this is the case, why does stimulation within RN favor edc?

One possibility is that input to edc (and other digit muscles) might arise from RN neurons with larger dendritic fields. Input to C7–C8 motoneuronal pools arises from the caudal RNm (Pong et al. 2002), which contains the largest cells in the nucleus. The dendritic fields of the large cells extend over a substantial portion of the nucleus in both cat and monkey (Burman et al. 2000; Conde and Conde 1973; Wilson et al. 1987), and stimulation in the nucleus could favor these cells. Afferents to the nucleus might also favor neurons projecting to edc, and stimulation anywhere within the nucleus could activate these afferents. The large dendritic fields of RNm neurons and fiber activation might contribute also to the failure to find...
a significant difference in the pattern of muscle activation when stimulating within RNm and RNP.

Poststimulus suppression

Activation of synaptic mechanisms could account for the high incidence of poststimulus suppression seen with RN stimulation. Poststimulus suppression requires that some inhibitory mechanism be activated by the stimulation. Inhibitory mechanisms might be located within the RN, spinal cord, or at both locations. Intracellular recording from spinal motor neurons during RN stimulation indicates that only 6% of motor neurons at C6–C8 respond with inhibitory postsynaptic potentials (IPSPs) (Fujito et al. 1991). The percentage of IPSPs is too low to account for the 21% incidence of poststimulus suppression observed with RN stimulation. [Behaj-Saif et al. (1998) reported a 25% incidence of poststimulus suppression from RN stimulation in the monkey.]

It is possible that poststimulus suppression results from activation of inhibitory synapses within the RN, which would not be activated by RST stimulation. There is evidence that the RN contains inhibitory interneurons as well as inhibitory afferents from a variety of sources (Katsumaru et al. 1984; Padel and Gorska 1980; Whishaw and Gorny 1996). It is likely that the RST most strongly affect these regions (Lawrence and Kuypers 1968; Schrimsher and Reier 1993; Sybirska and Gorskya 1980; Whishaw and Gorny, 1996), and temporary inactivation of RNm (Gibson et al. 1994) or interpositus (Mason et al. 1998; Milak et al. 1997) impairs the ability to properly position the digits for a variety of tasks such as grasping, walking, and standing.

If the RN is specialized for control of distal musculature, why does stimulation of the RST produce postspike effects in proximal as well as distal muscles? Lesions or inactivation of the RNm (Gibson et al. 1994) or interpositus (Mason et al. 1998; Whishaw and Gorny 1996) are especially well during digit extension (Gibson et al. 1985, 1994; Jarratt and Hyland 1999; van Kan and McCurdy 2001). Cells in interpositus, the major input to RNm, discharge only if hand movements are included in the behavioral task (van Kan et al. 1994). Lesions of the RST most strongly affect this use (Lawrence and Kuypers 1968; Schrimsher and Reier 1993; Sybirska and Gorskya 1980; Whishaw and Gorny 1996), and temporary inactivation of RNm (Gibson et al. 1994) or interpositus (Mason et al. 1998; Milak et al. 1997) impairs the ability to properly position the digits for a variety of tasks such as grasping, walking, and standing.

This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-36820 (A. R. Gibson) and National Research Service Award NS-10726 (M. Pong).

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