Spinal Projections of the Cat Parvicellular Red Nucleus

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Pong, Milton, Kris M. Horn, and Alan R. Gibson. Spinal projections of the cat parvicellular red nucleus. J Neurophysiol 87: 453–468, 2002; 10.1152/jn.00950.2000. Traditionally, the red nucleus of the cat is divided into two parts: a large-celled, magnocellular, division (RNm) and a small-celled, parvicellular, division (RNp). The RNm projects to the spinal cord and receives input from the cerebellar interpositus nucleus. The RNp projects to the inferior olive and receives input from the cerebellar dentate nucleus. In this report, we reexamine the connections of the red nucleus using the bidirectional tracer wheat germ agglutinin-horseradish peroxidase (WGA-HRP). Our findings demonstrate that the cat RNp has a large caudal and lateral region that projects to contralateral spinal cord and not to the inferior olive. The spinally projecting region of RNp receives input from the dentate nucleus and a lateral segment of anterior interpositus. The RNp shows a topography with the lateral region that projects to contralateral spinal cord and not to the cerebellar cortex. For the control of movements such as reaching and grasping, we suggest that RNp and dentate focus on the control of proximal limb musculature, whereas RNm and the anterior interpositus focus on the control of distal limb musculature. We also suggest that other species are likely to have a small-celled area of red nucleus projecting to the spinal cord.

INTRODUCTION

Lesion or inactivation of the lateral cerebellum or its output nucleus, dentate, impairs the ability to make accurate well-coordinated limb movements (Bastian and Thach 1995; Thach et al. 1992). It is generally assumed that the output of lateral cerebellum exerts its influence on limb movements by modifying activity in motor cortex via thalamic connections. However, lesion of the cerebellar receiving area of the thalamus (Bastian and Thach 1995; Fabre-Thorpe and Levesque 1991; Ranish and Soechting 1976) has a relatively minor effect on limb movements in comparison with cerebellar lesion. Therefore it is likely that lateral cerebellum can influence spinal activity via brain stem connections, and physiological studies suggest that this is the case (Hames et al. 1981). One of the major brain stem targets of the dentate nucleus (DN) of the cat is the parvicellular red nucleus (RNp). In this study, we reexamine the connections of RNp to determine whether these cells could provide an anatomical basis for the influence of lateral cerebellum on limb movements.

The mammalian red nucleus (RN) is typically divided into a caudal magnocellular region (RNm) and a rostral parvicellular region (RNp). It is generally assumed that these cytoarchitectonic divisions differ also in connectivity. RNm receives input from the cerebellar interpositus nucleus and projects to the contralateral spinal cord, whereas RNp receives input from the cerebellar dentate nucleus and projects to the ipsilateral inferior olive (Massion 1967). However, in the cat there is no clear separation between large and small cells in the RN, and at least some small cells project to the contralateral spinal cord (Holstege and Tan 1988; Mussen 1927; Pompeianio and Brodal 1957). Could the spinally projecting cells in RNp connect DN output to the spinal cord rather than to the inferior olive? In this study, we address this question by placing injections of wheat germ agglutinin-horseradish peroxidase (WGA-HRP), a bidirectional tracer, into physiologically identified regions of the cerebello-rubro-spinal pathway.

Our results demonstrate that most of the cat RNp projects to the contralateral spinal cord. The spinally projecting cells are largely, if not entirely, separate from cells projecting to the ipsilateral inferior olive. Regions of RNp that project to the contralateral spinal cord receive input from the contralateral cerebellar dentate and a lateral segment of anterior interpositus. For projections to cervical cord, RNp favors upper segments, whereas RNm favors lower segments.

The data do not support the prevalent view of RN connectivity and indicate that the lateral cerebellum can influence limb movements via spinal connections through RNp. The connections of RNp and its physiological actions (see Horn et al. 2002) suggest that the pathway influences upper limb musculature more strongly than distal limb musculature. Dentate may play a role in directing and stabilizing the limb during reaching and grasping.

METHODS

Fourteen male cats (3.5–4.5 kg) were used to trace connections of the red nucleus. Anesthesia consisted of an initial intramuscular injection of ketamine hydrochloride (8 mg/kg) followed by intravenous doses (10 mg) of pentobarbital sodium. Cats were fastened into a stereotaxic frame, and a craniotomy and/or laminectomy was performed to provide access to brain stem and spinal injection sites. After the tracer injections, wounds were sutured, and the cats were placed on a heating pad. Anesthesia was maintained with iv infusion of pentobarbital sodium (2 mg·kg⁻¹·h⁻¹) for the duration of the transport period (approximately 48 h). During the transport period, breathing and temperature were monitored, and the airway was cleared frequently with suction. Prior to perfusion, cats were administered lethal doses of pentobarbital sodium (>250 mg iv as a rapid

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bolus). 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.

Small pressure injections (0.008–0.032 μl) of WGA-HRP (Sigma) were made at physiologically identified sites. Injection sites were initially identified using tungsten microelectrodes. The injection pipettes and the microelectrodes were cross-referenced by using the same optical zero point. Accuracy of final placement was confirmed by recording with the injection pipettes. Table 1 summarizes the injection site or sites for each case.

Perfusion consisted of a saline rinse followed by two liters of freshly made paraformaldehyde (3–4%, depending on the case) and a series of 10, 20, and 30% sucrose/phosphate buffer solutions (0.1 M, pH 7.4). Brains were sectioned at 50 μm in either the parasagittal or frontal planes. Sections were processed with a modified tetramethyl benzidine (TMB) reaction (Gibson et al. 1984; Mesulam 1982) and then stained with thionin.

The locations of anterogradely labeled terminals and fibers and retrogradely labeled cell bodies were plotted onto high-resolution digital images using a computerized plotting system (Image Tracer, Translational Technology). The plotting system registered microscope stage position (±1-μm resolution) to the digital image for accurate marking of label location. All plotting was performed under high-powered (×100) observation using polarized light illumination.

Overlap between the label from two different cases was assessed by first creating computer images of the sections with the plotted label. Corresponding sections from the two cases were scaled to the same size and manually overplotted with the aid of a computer drawing program (Canvas, Deneba). When the location of label from one case fell within 100 μm of label from the other case, the area was marked as a site of overlap. For the red nucleus, the location of the caudal and ventral large cells provided the most reliable alignment between cases. Stereotaxic coordinates were assigned based on comparison of the sections with the cat atlas of Berman (1968).

The parafomaldehyde fixation produced severe and variable tissue shrinkage, so our estimates of cell sizes within RN are not comparable to those made by previous investigators. However, comparisons between RN areas within a subject are still meaningful. To measure the size of spinally projecting cells, high-power bright field microscopic images were made of selected regions within the nucleus. Using Image Tracer, we then marked the retrogradely labeled cells in the image under darkfield polarized light illumination. The width and length of marked cell somas were then measured from the brightfield image. Measurements were orthogonal to each other and made at the points of maximal cell dimensions. When the section appeared to include the apical dendrite, an estimate of soma size was extrapolated from cell-wall curvature. Attempts were made to measure every labeled cell, but in some instances, cell membranes could not be visualized. The product of each pair of measurements was used as an estimate of soma area.

**RESULTS**

**Projections to the cervical cord**

RED NUCLEUS LABELING FROM SPINAL INJECTIONS. Our first objective was to identify regions of the red nucleus that project to the spinal cord. A single rubrospinal fiber can terminate along several segments of the cord (Shinoda et al. 1977), so we maximized the number of labeled rubrospinal cells by making closely spaced injections across several segments. To simplify the study, we examined projections only to cervical cord. A series of WGA-HRP injections were made into the intermediate spinal gray every 2–3 mm from C3 to C6 on the right side of the cord (case CN10, Table 1). Recordings through the injection pipette helped distinguish cells in the intermediate laminae from the large motoneurons in the ventral horn.

The frontal sections through the left RN in Fig. 1 were photographed with a combination of semi-polarized darkfield and transmitted light to visualize both labeled and unlabeled cells. At the caudal RN pole (Fig. 1, A3.8), the labeled cells are confined to approximately the dorsal half of the nucleus. Spinal projections of the RN have a well-recognized topography (Holstege and Tan 1988; Pompeiano and Brodal 1957; Robinson et al. 1987), and the unlabeled cells in the ventral half of the nucleus project to lower levels of the cord. Although the labeled cells vary in size, the high percentage of large cells identifies this level as the RNm.

Rostrally (Fig. 1, A4.6), two groups of labeled cells are apparent. Lying medially and consisting mainly of large cells, one group appears to be a rostral continuation of RNm. A different group of labeled cells can be seen lateral to RNm. Although this group contains cells of various sizes, the cells appear to be smaller than those of the mediod group.

Further rostrally (Fig. 1, A5.2) there is no clear RNm, but the smaller celled lateral group is prominent. In this report, we consider the rostral lateral group of cells as part of RNp, which is consistent with their designation in the Berman (1968) cat atlas.

Measurements of cell areas within the groups (see Method) supported our visual impressions. We compared the cells in Fig. 1. Cells in RNp (lateral group) at A5.2 and A4.6 had median soma areas of 275 and 273 μm², respectively. Cells in RNm at A4.6 (medial group) and A3.8 (all cells) had median soma areas of 420 and 418 μm², respectively. A one-way ANOVA between groups (Kruskal-Wallis ANOVA on ranks) indicated significant differences (P < 0.05), so we ran pairwise comparisons (Dunn’s method) between the groups. Comparisons between measurements of RNm and RNp cells were significantly different (P < 0.05), whereas comparisons within either RNm or RNp were not significantly different (P > 0.05).

A complete representation of the retrograde labeling from A3.1 to A6.9 for case CN10 is shown in Fig. 2, left. The smaller-celled lateral group becomes prominent at about A4.5 and continues to A5.9. The medial RNm group extends from the caudal end of the nucleus (A3.1) to approximately A5.4. The spinal projection from both groups of cells is almost entirely contralateral. An outline of the labeled RN cells on the

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**TABLE 1. Injection sites and volumes**

<table>
<thead>
<tr>
<th>Cat</th>
<th>Injection Site(s)</th>
<th>WGA-HRP (1%) Injected, μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRN1</td>
<td>RNp (left) and RNm (right)</td>
<td>0.008 (RNp) 0.008 (RNm)</td>
</tr>
<tr>
<td>BRN3</td>
<td>RNp (left)</td>
<td>0.004 (2%)</td>
</tr>
<tr>
<td>C4-1</td>
<td>C₄ spinal segment (right)</td>
<td>0.016 × 4</td>
</tr>
<tr>
<td>C4-2</td>
<td>C₄ spinal segment (right)</td>
<td>0.008 × 2</td>
</tr>
<tr>
<td>C8-1</td>
<td>C₈ spinal segment (right)</td>
<td>0.008</td>
</tr>
<tr>
<td>CN9</td>
<td>Dentate (right)</td>
<td>0.012</td>
</tr>
<tr>
<td>CN10</td>
<td>C₅–C₆ spinal segments (right)</td>
<td>0.016 × 10, 0.020, 0.032</td>
</tr>
<tr>
<td>CN11</td>
<td>Dentate (left) and IO (left)</td>
<td>0.014 (DN) 0.0080.012 (IO)</td>
</tr>
<tr>
<td>CN12</td>
<td>Dentate (bilateral)</td>
<td>0.008/0.008</td>
</tr>
<tr>
<td>PS1</td>
<td>C₇ spinal segment (right)</td>
<td>0.016 × 2, 0.008 × 3, 0.012</td>
</tr>
<tr>
<td>SC9</td>
<td>C₆ spinal segment (left)</td>
<td>0.012</td>
</tr>
<tr>
<td>SC12</td>
<td>C₅–C₆ spinal segments (right)</td>
<td>0.016 × 20</td>
</tr>
<tr>
<td>ZII</td>
<td>Zona incerta (left)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

RNm and RNp, magnocellular and parvocellular divisions, respectively, of the red nucleus; IO, inferior olive.

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TOPOGRAPHY OF RN PROJECTIONS TO THE SPINAL CORD. The and ICA, and, more rostrally, the Fields of Forel (FF). in the interstitial nucleus of Cajal (ICA), the area between RN of section). Most cells projecting to the ipsilateral cord are located right to compare locations of cells projecting to the ipsilateral left (contralateral to the injection) has been transposed to the right to compare locations of cells projecting to the ipsilateral and contralateral cord. Only a few ipsilateral cells overlap with the area of contralateral projection (Fig. 2, column 1, right side of section). Most cells projecting to the ipsilateral cord are located in the interstitial nucleus of Cajal (ICA), the area between RN and ICA, and, more rostrally, the Fields of Forel (FF). TOPOGRAPHY OF RN PROJECTIONS TO THE SPINAL CORD. The results from the spinal injection case (CN10) indicated that dorsolateral RN contains smaller cells that project to the contralateral cord. Do the cells in dorsolateral RN terminate at the same spinal levels as cells in medial RN? We compared the locations of retrogradely labeled neurons in the RN between cases with injections restricted to one segment of the cervical cord (Fig. 3). Although the injections sometimes included fibers in the ventral funiculus (i.e., Fig. 3, C1), the dorsolateral funiculus, where fibers from RN travel (Fig. 6), was not included by the injection sites. Therefore retrogradely labeled cells within the RN were due to pickup by terminals in the spinal gray and not by fibers of passage.

The C1 injection (Fig. 4, left) labeled cells across the medial to lateral extent of RN, although the largest number of labeled cells are in the most lateral section (L3.2). The C4 injection produced a relatively even distribution of labeled cells throughout the nucleus. (A replication of the C4 injection, case C4-2, resulted in the same distribution.) The C6 injection labeled a large number of cells in the most medial section (L1.4), but few cells were labeled in the lateral sections. The C8 injection produced a pattern similar to the C6 injection.

We compared the location of labeled RN cells from the C1 and C4 injections in greater detail (Fig. 5). At caudal levels (A3.4), there is total overlap between C1 and C8 projecting cells with more cells labeled by the C6 injection. At more rostral levels, the distribution of labeled cells shifts laterally with proportionately more resulting from the C1 injection. The largest amount of overlap occurs at mid-levels of the nucleus. The data indicate a modest topography in the cervical spinal projection of RN. Although all segments seem to receive some input from the entire RN, more cells in caudal and medial RN (RNm) project to C6 and C8, whereas more cells in rostral and lateral RN (RNp) project to C1. The intermediate segments show more even distributions.

SPINAL LABELING FROM RED NUCLEUS INJECTIONS. To confirm the pattern of RN projections to cervical cord, we made small injections of WGA-HRP into selected regions of the RN and traced anterograde labeling in the cord. With anterograde tracing, both the laminar and segmental distribution of terminations can be determined.

To accurately place the injection sites, we first located the caudal pole of RN by recording with a tungsten microelectrode and used those coordinates to calculate the location of the desired injection sites. In one case (BRN3, Table 1), we placed a single injection in the rostral RN on the left side 2 mm rostral and 0.5 mm lateral to the caudal pole. Cells at the injection site fired during forelimb withdrawal in response to hard pinches of the paw.

The center of the BRN3 injection (Fig. 2, middle) was located in the lateral RN at A5.0 (reaction product above the injection resulted from bleeding along the track). The injection site covered slightly more than the rostral half of the RN. There was little encroachment into the region dorsal and medial to RN that contains cells with ipsilateral spinal projections.

Contralateral to the injection, labeled fibers can be seen in the dorsolateral funiculus (Fig. 6, left). Only a few labeled fibers could be found in the dorsolateral and ventral funiculi on the ipsilateral (left) side, and only a small amount of terminal label was present on this side. Most of the ipsilateral label was located in Rexed’s lamina VII (Rexed 1954) near the central canal and appeared to result from fibers crossing at segmental levels.

On the contralateral side (right), many labeled fibers could be seen streaming from the dorsolateral funiculus into the spinal gray, and terminal label was present in laminae V, VI,
and VII. At upper cervical levels (C₁–C₄), the labeled terminals formed a focus in the lateral regions of lamina V and VI (no label was present in the lateral cervical nucleus). At C₄, the labeled terminals extended deeper into the dorsal part of lamina VII, and the projection to lamina VII was pronounced at C₇ and C₈.

A gap in labeling can be seen in medial regions of laminae VI and VII; this gap was most prominent at C₇. The gap was not apparent at C₈ (Fig. 6, BRN3). Below the cervical enlargement (T₂ was the lowest segment examined), the pattern of terminal labeling resembled that seen at high cervical segments with most of the labeling confined to a lateral focus in laminae V and VI. While the pattern of labeling was similar, the density of labeling in the lower cervical and upper thoracic was significantly lower than at the higher cervical levels. No terminal label was present in lamina IX (motoneuronal cell groups) at any level.

COMPARISON OF SPINAL TERMINATIONS OF RNP TO RNM. The RNP case (BRN3) confirmed a projection to the contralateral spinal cord. To compare projections of RNM and RNP, we placed injections in different RN regions on opposite sides in case BRN1. The bilateral injection allowed us to compare across sides at spinal levels so the relative density of labeling could be judged in tissue processed under identical conditions.

Ipsilateral spinal projections from RN could confound bilateral injections. However, the results from CN10 and BRN3 indicated that ipsilateral projections from rostral RN are insignificant. Furthermore, CN10 (Fig. 2) as well as the other spinal injection cases (6 cases, Table 1) indicated that caudal RN and immediately surrounding regions do not have a significant projection to the ipsilateral cervical cord. The lack of ipsilateral spinal projections from RNm agrees well with previous studies (McCurdy et al. 1987; Pompeiano and Brodal 1957; Robinson et al. 1987). One case (1383 in Holstege 1987) has suggested ipsilateral spinal projections from RN; however, the injection site for this case included the region dorsal and medial to rostral RN. Our data (i.e., CN10) also indicate an ipsilateral projection from this region, but it was not included by our RN injections (Fig. 2).

The injection sites were identified as in BRN3 except that the
RNp injection was placed 1.0 mm further rostral to the estimated caudal RN border. Recordings at the injection site revealed responses to tapping or squeezing of the right (contralateral) forelimb, and cells ventral to the injection site responded to taps and squeezes of the right hind limb. On the opposite side, we placed an injection at the caudal pole of the RN. The RNm injection (Fig. 2, BRN1, right side) was largely limited to the caudal 0.5 mm of the RN and did not extend far beyond A4.5. Although the injection was well confined to the borders of the RN, the entire nucleus was covered at caudal levels. The RNp injection (Fig. 2, BRN1, left side) was centered in the dorsolateral RN at A5.4. There was essentially no overlap between the corresponding RN areas covered by the two injections. The unilateral RN injection (BRN3) and the RNp injection of BRN1 have extensive overlap, but the BRN1 injection did not extend as far caudally as the BRN3 case.

Anterograde labeling of terminals in the spinal cord demonstrated that RNp and caudal RNm, for the most part, terminate at different levels of the spinal gray (Fig. 6, right). Due to the decussation of the rubrospinal tract, label from the RNp is on the right and RNm on the left. The RNp label was clear at high cervical segments (levels above C4 were densely labeled but were not photographed prior to fading) and focused in the lateral parts of laminae V and VI. As in the BRN3 case, a gap in labeling is seen in the medial regions of laminae V and VI. Unlike the BRN3 case, the density of label decreased at levels caudal to C4 and was essentially absent at C7 and C8. We believe this difference can be attributed to the more rostral position of the BRN1 RNp injection.

The RNm injection labeled many fibers in the dorsolateral funiculus (Fig. 6, BRN1, left side of sections) but produced only a small amount of label in the spinal gray at upper cervical levels. The label that did exist was focused medial to the location of label from RNp in central regions of laminae VI and VII (Fig. 6, BRN1, C3 and C4, left side). Below C4 the amount of label present in the spinal gray increased rapidly and showed a peak in density at C7. At C7 the label was evenly distributed across lamina VI and the dorsal regions of lamina VII. The C7 label from caudal RN included the area left bare in the BRN3 case. The label at the higher cervical segments also appeared to fill the gap in the RNp projections. RNm and RNp appear to project to different areas of the spinal gray in the same cervical segment. At C8 the density of the labeling dropped, but a substantial amount was still present in the central portions of laminae VI and VII.

The RNm injection also produced a small amount of label in the dorsolateral motoneuronal pools (lamina IX) at C8 (barely visible in Fig. 6). Neither of the RNp injections led to label in motoneuronal pools. Below the cervical enlargement (segments down to T3 were processed), a moderate density of labeling was present in the central portions of laminae V, VI, and VII.

To gain a more representative comparison of the label between side for BRN1, digital photographs were analyzed using Adobe Photoshop. A pixel intensity and color range was set to select label in polarized light darkfield images. The number of selected pixels within the spinal gray served as an estimate of the area of anterograde label. The same pixel selection template was used for all sections, and counts from three to six sections at each segment were averaged. Reaction strength varied between sections, so the comparison was made as a ratio of the pixel count between sides as well as by the absolute count.

Between C1 and C3 the pixel count was higher on the side corresponding to the RNp injection (Fig. 7, top). The ratio was highest at C1 with approximately three to four times as many selected pixels on the RNp side. At C4, the pixel count was approximately equal between sides. Below C4, the count was greater on the side corresponding to the RNm injection, and the ratio peaked at C7–C8 with approximately 10 times as much label resulting from the RNm injection as from the RNp injection.

The RNp injection produced a relatively steady decrease in count with spinal level (Fig. 7, bottom). The highest level was found at C1 and the lowest at T3. The RNm injection produced a low count at C7 that rapidly increased to a peak at C7 and then declined to a low number by T3.
The anterograde results are in good agreement with the retrograde results. Large cells at the caudal pole of the nucleus have a projection to laminae VI and VII at all cervical levels, but the projection is denser at lower segments. Smaller cells in the rostral pole of RN project mainly to laminae V, VI, and VII at upper cervical segments.

**Spinal Versus Olivary Projections.** Traditionally, RNp has been thought to transmit information from the dentate nucleus of the cerebellum to the principle subnucleus of the ipsilateral inferior olive. Because both our retrograde and anterograde studies indicated that a substantial portion of the cat RNp projects to the contralateral spinal cord, could the same cells provide input to the inferior olive (IO)? The BRN3 injection produced some anterograde label in the ipsilateral IO (mostly in the dorsal lamella of the principal olive), but the BRN1 injections produced no visible label in the IO. Therefore it is likely that spinally projecting and olivary projecting cells in RNp are largely from different populations of neurons.

To determine the location of RN cells projecting to the IO, we made injections centered on the dorsal lamella of the principle subnucleus of the inferior olive (PO). Several studies have indicated that a substantial portion of the cat RNp projects to the contralateral spinal cord, could the same cells provide input to the inferior olive (IO)? The BRN3 injection produced some anterograde label in the ipsilateral IO (mostly in the dorsal lamella of the principal olive), but the BRN1 injections produced no visible label in the IO. Therefore it is likely that spinally projecting and olivary projecting cells in RNp are largely from different populations of neurons.

Source of input to the red nucleus

**Labeled Cells in the Cerebellar Nuclei.** The retrograde labeling from the RN injections allowed us to compare inputs to RNp and RNm. The heaviest source of input to the RN arises from the deep cerebellar nuclei. Both dentate and interpositus provide input to RN. The injection into the caudal pole of RNm labeled a large number of cells in posterior interpositus (NIP) and the medial two-thirds of anterior interpositus (NIA) but few cells in DN or lateral NIA (Fig. 10, right). In contrast, the RNp injections labeled cells in lateral NIA and DN (Fig. 10, left and middle). The mutually exclusive areas of label dem-
PROJECTIONS FROM DENTATE TO RN. We confirmed the retrograde results from RN injections with anterograde labeling from three cerebellar nuclear injections. One case (CN9) was sectioned in the parasagittal plane, so that the large cells in caudal RN could be used for accurate rostral-caudal alignment between cases. The DN injection (Fig. 11A) was centered in medial ventral DN, although dorsal spread may have included a small ventral region of dorsolateral NIA. The injection did not encroach on NIP.

Because the IO has a precise topographic relationship with the cerebellar nuclei (Groenewegen et al. 1979; Tolbert et al. 1976), labeling in IO provides a method for estimating effective pickup at cerebellar nuclear injection sites. The DN has reciprocal connections with the principle division of the contralateral IO (PO), whereas NIA has reciprocal connections with the contralateral rostral dorsal accessory division (rDAO).

The olivary label in case CN9 indicated that the effective injection site did not include NIA because no label was present in rDAO. The presence of label in the lamellae of the PO indicated that the DN was included by the injection. The parasagittal section in Fig. 11B illustrates anterograde label in the ventral lamella and vlo (but not in rDAO) from the CN9 injection.

For comparison with the anterograde label in RN, an additional spinal injection case (SC12) was sectioned in the parasagittal plane. We placed injections of 1% WGA-HRP every 2 mm from C1 to mid C6 (Table 1). In rostral and lateral regions of the RN, cells labeled from the spinal injections overlapped with anterograde label from the DN injection. The cells projecting to the cervical cord lie in the dorsal rostral part of the RN (Fig. 12A), which matches the anterograde label from the DN (Fig. 12B).

The pattern of overlap through the RN between the dentate projection and the spinally projecting cells (Fig. 13C) indicates that most of the rostral-lateral region of RN that projects to the cervical cord receives input from medial DN. Only medial RN (L1.1–1.9) contains a large number of cells projecting to cervical cord that are not included in the terminal area of the CN9 injection.

Two additional injections of the cerebellar nuclei confirmed the retrograde results. The two injections (CN12 bilateral) were slightly different from the CN9 injection in that one was centered more laterally in dentate whereas the other injection was centered more medially in dentate and included some of lateral NIA. The amount of rDAO label in each case corresponded with the amount of NIA included in the injection site. The lateral dentate injection led to label in the lateral junction of the lamellae of the PO but none in rDAO. The medial dentate/lateral NIA injection labeled the ventral lamella of the PO as well as rostral and medial rDAO. The anterograde label from the lateral dentate injection overlapped with the labeled cells from the spinal injection case (CN10, Fig. 2) in the lateral areas of the RN cells in sections A4.5 to A5.9. The overlap from the medial dentate/NIA injection extended further caudally and covered lateral RN from A3.6 to A5.9.

BRAIN STEM INPUTS. Connections of the RN have been described in detail (Edwards 1972; Robinson et al. 1987), but the current injections into RN revealed some differences between RNm and RNP connections that have not been well described. Although the cerebellar nuclei provide the largest input to both

FIG. 5. Distribution of RN cells projecting to C1 and C8. Area graphs show the number of labeled cells at different lateralities and rostral-caudal levels in the RN. G, the region projecting to both C1 and C8. The C1 injection (●) labeled cells throughout the RN but labeled more cells in the rostral-lateral region than the C8 injection (□). Conversely, the C8 injection labeled more cells in the caudal-medial RN.

onstrate the topographic nature of the cerebellar projection and indicate that passing fiber pickup was minimal at the injection sites. Fibers terminating in RNp pass through RNm (Figs. 11B and 13B), and fibers terminating in RNm continue through rostral RN to terminate in the thalamus. Pick-up by these fibers would have produced overlap of labeled areas in the cerebellar nuclei.

The RNp injections (BRN3 and BRN1, Fig. 2, left) produced similar patterns of retrogradely labeled cells in the cerebellar nuclei (Fig. 10, left and middle). However, the BRN3 injection, which included a larger portion of RN (Fig. 2) and used 2% rather than 1% WGA-HRP, labeled a higher number of cells. At rostral levels, labeled cells were mainly located in the dentate nucleus (DN, Fig. 10, P7.9, left and middle). More caudally, labeled cells were numerous in the lateral parts of anterior interpositus (NIA) and medial DN. The RNp injections labeled only a few cells in NIP, and caudal lateral regions of DN were sparsely labeled.
RNm and RNp, our retrograde results indicate that RNp receives significant input from additional brain stem areas. The largest input arises from the ZI and FF. The RNp also has extensive reciprocal connections with the ventral half of the anterior pretectal nucleus and with a region of the central tegmental field dorsal to RNp. The connections with the central tegmental field are probably analogous to those that have been described for the rat (Cadusseau and Roger 1992; Roger and Cadusseau 1987). A rather diffuse connection may exist with the mesencephalic tegmentum contralateral to the injection site. Although all of these inputs require verification with anterograde tracing, we were particularly interested in the large ZI input because this nucleus might provide a pathway allowing basal gangliar activity access to brain stem and spinal levels.

The BRN3 injection into RNp produced a dense focus of labeled cells in the medial part of ZI where it fuses with FF (Fig. 14A). A less dense layer of labeled cells trails laterally in the dorsal half of ZI. In all areas, the appearance of the label also suggests the presence of anterograde label, indicating a bidirectional connection with RN.

Both RNp injections (BRN3, BRN1) labeled cells in ZI and FF (Fig. 14B). In case BRN3, RN projections from ZI/FF were mainly ipsilateral, with a small contralateral component. The ZI/FF labeling from the dual RN injection (BRN1) was essentially the same as from the unilateral RNp injection (BRN3), suggesting that RNm is not likely to be a major component of the connection with ZI/FF.

To verify the ZI input to RN, we placed a WGA-HRP injection into ZI (case ZII). The injection site (Fig. 15C) spread dorsally into the thalamus and ventrally into the subthalamic nucleus (not visible in the photograph, Fig. 14C).
However, neither of these areas contained retrogradely labeled cells after RN injections, so it is unlikely that label in RN was confounded by injection site spread. Anterograde label from the ZI injection (Fig. 12C) overlaps with terminal label from DN as well as cells retrogradely labeled from cervical cord. RNm was unlabeled except for a strip along its ventral border. In more medial sections, the strip of label included the caudal as well as ventral borders of the nucleus but most of RNm was unlabeled. ZI apparently has a widespread input to RNp but a restricted input to the ventral and caudal borders of RNm. A significant number of retrogradely labeled cells were present in lateral RNp (and surrounding regions), thereby supporting the bidirectional nature of this connection.

The ZI injection retrogradely labeled cells in the entopeduncular nucleus (the feline equivalent of GPi), so it is possible that ZI provides an interface between basal gangliar output and RNp. However, this connection needs verification because the entopeduncular label may have resulted from fibers of passage. In addition to this potential indirect entopeduncular input to RNp, a small number of entopeduncular cells were retrogradely labeled following the BRN3 injection. Therefore RN probably has a minor direct input from the entopeduncular nucleus, and a larger indirect input via ZI (and adjoining FF). Using autoradiography, Nauta (1979) also found evidence for a small, direct entopeduncular projection to RN and acknowledged the possibility that ZI and FF receive entopeduncular input.

BRAIN STEM TARGETS. Anterograde labeling from the RN injections revealed no new brain stem projections, but it did provide additional detail about the topography of known projections. The sensory trigeminal nucleus, facial nucleus and spinal trigeminal nucleus were labeled by the RNp injection of case BRN1 but not by the RNm injection. Label in the lateral reticular nucleus and main cuneate was present after injections into either the RNm or RNp.

DISCUSSION

Although many of the details that we report in this paper have been demonstrated or suggested by previous studies, our results are the first to identify and describe the connections of a spinally projecting region of the cat RNp. We demonstrate that the region of RNp projecting to the cord is large and has different connectivity than either RNm or the olivary-projecting region of RNp. For the cervical cord, projections of RNp favor upper segments, whereas those from RNm favor lower segments. Although both RNp and RNm receive major inputs from the cerebellar nuclei, the input to RNp originates from lateral NIA and medial DN, whereas input to RNm originates from medial NIA and NIP. Additionally, the spinally projecting region of RNp receives a large input from ZI and FF, whereas RNm receives a highly restricted input from these nuclei. The anatomical differences are likely to reflect functional differences between subregions of the RN.

Other studies have reported small cells in rostral RN projecting to the contralateral spinal cord (Holstege and Tan 1988;
Mussen 1927; Pompeiano and Brodal 1957). However, our data indicate that the cells do not mix with cells projecting to IO but rather form a large homogeneous region. The spinally projecting region of RNp appears to be more extensive in our study, but this is probably because we made a number of closely spaced WGA-HRP injections in upper cervical segments. The closely spaced injections would result in more tracer pickup by the extensively branched RN axons (Shinoda et al. 1977, 1988), and RNp terminates most heavily at upper segments, especially C1. Previous studies of RN spinal projections would not have identified cells projecting selectively to upper cervical segments because they either sectioned the tract or injected tracers at spinal levels caudal to C1.

Our results indicate that the RNp populations projecting to the spinal cord and the IO are almost entirely separate, which is in good agreement with previous studies (Huisman et al. 1982; Spence and Saint-Cyr 1988). The mesencephalic input to the IO in the cat arises from a complex of nuclei that includes only a restricted rostral and medial portion of RNp (Onodera 1984; Saint-Cyr and Courville 1981; Walberg and Nordby 1981). The separation of olivary and spinally projecting neurons supports the suggestion by Holstege and Tan (1988) that RNp be divided into subregions. However, projection targets are just one possible basis for subdividing RN. Equally important considerations are the source of inputs to the subregions.

Using degeneration techniques, Voogd (1964) identified a dorsolateral region about midway along the rostral caudal extent of the RN that receives input from the cerebellar dentate nucleus. Our data support his observation and also identify this as a spinally projecting region of RN. These neurons may mediate the short latency activation of spinal neurons following stimulation of the dentate nucleus in the decerebrate cat (Hames et al. 1981).

Based on spinal connections, the smaller cells in lateral RN could be grouped with RNm. However, based on cerebellar

FIG. 9. Limited overlap of olivary and spinally projecting cells. Left: cells (●) labeled after a C3–C6 spinal injection (case CN10, Fig. 2). Middle: cells labeled after the IO injection (Fig. 5). Right: areas of potential overlap of olivary and spinally projecting cells. An outline of the RN projection to the contralateral cord has been included in the enlarged series. The largest potential for overlap occurs at A5.4 but is modest even at this level. FF, fields of Forel; FR, fasciculus retroflexus; ICA, interstitial nucleus of Cajal; ND, nucleus Darkshewitsch; PTA, anterior pretectal nucleus; PTP, posterior pretectal nucleus.
Figure 15 summarizes the major input-output relations of spinally projecting regions of the RN. The figure shows two different pathways from the cerebellum. The first pathway (light gray) starts from a strip that includes lateral NIA and medial DN. The rostral-lateral RN receives this projection as well as one from ZI and FF and then projects to the lateral areas of the upper cervical cord. The second pathway (black) originates in medial NIA and then projects to the caudal-medial RN. Cells in this area of RN project to the lower cervical segments including motoneurons at C₈. The topography consists of two pathways with an intermediate overlapping region (dark gray). Alternatively, the cerebellar nuclei from lateral to medial could map to the cervical cord from rostral to caudal in a continuum.

Comparisons between species. Parvicellular RN projects to the spinal cord in species other than cat. The most intensively studied species has been the rat (see Ruigrok and Cella 1995 for review). As in the cat, the rat RN is not sharply divided into magnocellular and parvicellular regions (Reid et al. 1975a,b), and at least some of the parvicellular neurons project to the spinal cord (Huisman et al. 1981, 1983; Shieh et al. 1983). Additionally, the rat RNp does not supply input to the inferior olivary (Ruigrok et al. 1993; Rutherford et al. 1984). Dorsolateral regions of the rat RNp receive input from caudal regions of the cerebellar lateral (dentate) nucleus (Angaut and Cicirata 1988; Ruigrok et al. 1993; Teune et al. 2000). Therefore a large portion of the rat RNp receives input from the cerebellar lateral nucleus, has little or no projection to the IO, and has, at least, some projection to the spinal cord. It appears that the connections are similar, perhaps identical, to the connections of the spinally projecting region of the cat RNp.

The RN of the North American opossum is organized much like the cat and rat. There is no distinct parvicellular division of the opossum red nucleus (King et al. 1971; Martin et al. 1974), and the cells projecting to the IO lie rostral and medial in the RN (Martin et al. 1983). As in the cat, there is only a small region of spatial overlap between the olivary and spinally projecting cells. Even in this overlap region, the populations are distinct because very few cells project to both the spinal cord and IO (Martin et al. 1983).

Studies in monkey indicate spinal terminations similar to the cat, including direct connections to motoneuronal pools that innervate muscles that move the digits (Holstege et al. 1988; Ralston et al. 1988). Early studies using degeneration tracing techniques identified small- and medium-sized cells in or near lateral RN projecting to the contralateral spinal cord (Kuyper and Lawrence 1967; Poirier and Bouvier 1966); however, HRP tracing studies failed to demonstrate this population (Castiglioni et al. 1978; Kneisley et al. 1978). More recently, Burman et al. (2000) used WGA-HRP to confirm the existence of...
smaller cells dorsolateral to the RN that project to the contralateral cord. It seems likely that these smaller cells are analogous to spinally projecting RNp cells of the cat.

In the human RN, there are few large cells, so most of the nucleus is presumed to be parvicellular and therefore olivary projecting (Massion 1967). However, as Pompeiano and Brodal (1957) pointed out, if many spinally projecting cells in the human are small, the spinal projection may be greatly underestimated. Degeneration studies following brain stem lesions in human infants support this suggestion (Papez and Stottler 1940). The authors conclude that most of the rubrospinal fibers in the human arise from small cells caudal and lateral in the nucleus. Such a projection fits well with the data from the degeneration studies in the monkey as well as with the data from our study.

It is likely that species differences in RN connections are not as large as previously supposed. All of the aforementioned species have large cells projecting to the contralateral spinal cord that receive input from interpositus. All have small cells in the rostral medial part of the nucleus that project to the ipsilateral IO. Finally, we would like to suggest that all have a lateral area consisting of smaller cells that project to the contralateral cord and receive input from DN.

The greatest confusion about RN connections may be the result of a lack of correspondence between similarly named regions. In the monkey, the area identified as RNp is probably confined to the olivary projecting region of RNp. In the rat and cat, the area identified as RNp includes both olivary and spinally projecting regions.

FUNCTIONAL IMPLICATIONS. Most RN output projects to interneurons, but, in some instances, the RST terminates among motoneurons. Probably the strongest motoneuronal projection is to the facial nucleus (Courville 1966; Holstege et al. 1984; Robinson et al. 1987). Both of our RNp injections but not the RNm injection produced anterograde label in the facial nucleus. Inactivation of RN blocks the expression of a classically conditioned eye-blink response (Chapman et al. 1990) as does inactivation of interpositus (Yeo et al. 1985), so it appears that RNp provides lateral interpositus with a pathway to the facial nucleus.

The involvement of lateral NIA with facial movements agrees well with its olivary connections. Medial regions of the rDAO project to lateral NIA, which, in turn, projects to dorsolateral RN (Daniel et al. 1987; Gibson et al. 1987; Robinson et al. 1987). Cells in medial rDAO (and the adjoining medial portion of the ventral lamella of the PO) respond to stimulation of the face and head (Gellman et al. 1983; Weiss et al. 1993) and receive input from the trigeminal nucleus (Berkley and Hand 1978). The trigeminal nucleus also connects to a caudal region of dorsolateral RN (Robinson et al. 1987). Therefore RNp is likely to be involved in facial movements.

The only clear RN projection to spinal motor pools is to lateral pools at C8 (Fujito et al. 1991; Holstege 1987; McCurdy et al. 1987; Robinson et al. 1987). Our data indicate that RNm is the source of C8 motoneuronal terminations. Although the projection to motor pools is small in comparison to projections to spinal interneurons, many studies have indicated that the RNm and interpositus are especially important for the control

Despite the focus of RNm on digit control, inactivation and lesion studies (Bracha et al. 1999; Gibson et al. 1994; Lawrence and Kuypers 1968; Martin et al. 1993; Schrimsher and Reier 1993; Sybirska and Gorska 1980) indicate that the RN influences proximal as well as distal limb musculature [although Levesque and Fabre-Thorpe (1990) report minimal deficits in reaching with chemical lesions of the RN]. The RN projection to upper cervical levels suggests that this region may be important for proximal limb control. Recordings at the RNp injection sites indicated that the cells discharge during withdrawal of the forelimb, and the retrograde labeling indicated that they receive input from the cerebellar nuclei which interconnect with the lamellae of the PO. Cells in the PO respond to stimulation of the limbs (Gellman et al. 1983), and it is likely that RNp and DN form a second cerebello-spinal circuit involved in limb movement control. The findings reported in the accompanying paper (Horn et al. 2002) indicate that the output from RNp does have a strong influence on musculature of the shoulder.

RNp terminations at C1–C2 could be involved in the control of head movements because motor neurons at these levels innervate neck muscles (Gordon and Richmond 1991; Kitamura and Richmond 1994). However, RNp terminations favor lateral interneuronal regions, which project to motoneurons at other spinal levels. Interneuronal regions of C1–C2 receive input from many descending motor systems (Gibson et al. 1998; Holstege and Kuypers 1982), including the tecto-spinal pathway (Cowie and Holstege 1992; Huerta and Harting 1982), which has been implicated in limb movement control (Werner et al. 1997). This region of interneurons may integrate a number of inputs important for orienting a variety of body parts.
FIG. 15. Major elements of the cerebello-rubro-spinal pathways to cervical cord. Two pathways from the cerebellar nuclei are shown. In one pathway, lateral cerebellum (dentate/lateral NIA, light gray) projects to rostral-lateral RNp, which then projects to the upper cervical cord. This pathway converges with input from ZI and FF. In the other pathway, output from medial NIA and NIP (black) projects to the medial-caudal RNm, which then projects to intermediate spinal regions and motoneurons at C8. The spinal projection is heaviest at lower cervical. The darker gray indicates areas of potential overlap in RN and spinal cord.

such as the head, limbs, and trunk. Propriospinal interneurons at C3–C4 are likely to be involved in limb control (Illert et al. 1977) and interrupting propriospinal connections from these segments does produce deficits in reaching (Alstermark et al. 1981).

Inactivation of the monkey cerebellar nuclei can also affect limb guidance, although there is some debate about which regions of the nuclei are critical. Both dentate (Thach et al. 1992) and NIP inactivation (Mason et al. 1998) have been reported to disrupt reach accuracy. If the topography of cerebellar projections to RN is similar between cat and monkey, injections into NIP might also affect DN cells projecting to RNp. Alternatively, both NIP and DN might be important for reach accuracy. Posterior interpositus projects to the medial border of the cat RN (Robinson et al. 1987), and our data demonstrate that cells in medial regions of RN project to all cervical levels. Therefore NIP may play an integrative role influencing activity in both proximal and distal musculature during limb movements. Dentate may focus more on proximal musculature, but deficits in either system could produce inaccuracy during reaching.

Humans with lesions of the lateral cerebellum reach inaccurately. In fact, lesions of the lateral cerebellum disrupt reach accuracy more than lesions of the thalamus, which suggests that dentate output projects to areas important for reaching without passing through thalamus (Bastian and Thach 1995). It is tempting to speculate that the small-celled lateral region of human RN (Papez and Stottler 1940) allows dentate output to influence activity of the upper cervical cord.

Testing of the previous speculation will require a great deal of additional research. At least for the cat, it is likely that the spinal projections of RNp and RNm serve different roles in the control of limb movements—it would be surprising if this were not also the case for other mammalian species.

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