Chapter 3
Caudal medullary pathways to lumbosacral motoneuronal cell groups in the cat; evidence for direct projections possibly representing the final common pathway for lordosis

ABSTRACT
The nucleus retroambiguus (NRA) projects to distinct brainstem, and cervical and thoracic cord motoneuronal cell groups. The present paper describes NRA projections to distinct motoneuronal cell groups in the lumbar enlargement. Lumbosacral injections of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) were made to localize and quantify the retrogradely labeled neurons in the caudal medullary lateral tegmentum. These injections were combined with spinal hemisections in order to distinguish between neurons having ipsi- or contralaterally descending axons. The NRA-lumbosacral fibers desend almost exclusively contralaterally, but neurons in areas surrounding the NRA project mainly ipsilaterally. In an anterograde tracing study, injections of WGA-HRP or tritiated leucine were made in the region of the NRA to determine the NRA targets in the lumbosacral cord. Hemisections in C2 made it possible to distinguish between NRA projections and projections from neurons in the adjoining lateral tegmentum. The results show delicate NRA projections to distinct lumbosacral motoneuronal cell groups innervating specific hindlimb muscles (iliopsoas, adductors, and hamstrings), as well as axial muscles (medial longissimus and proximal tail muscles). The projection is bilateral with a contralateral predominance. Ipsilaterally terminating fibers are derived from NRA neurons which axons cross the midline at the level of the obex, descend through the contralateral spinal white matter, and recross at the level of termination. A concept is presented in which the periaqueductal gray-NRA-lumbosacral projections form the final common pathway for lordosis in the cat.

INTRODUCTION
The nucleus retroambiguus (NRA), first described in humans by Olszewski and Baxter in 1954, is a rostrocaudally organized column of interneurons in the ventrolateral part of the medulla oblongata. In the cat, it extends from 1 to 8 mm caudal to the obex and corresponds with the caudal part of the ventral respiratory group (for review see Feldman, 1986). NRA interneurons are known to project to specific brainstem and spinal cord motor nuclei. In the brainstem, the NRA projects to somatic motoneurons located in the nucleus ambiguus, innervating the larynx, pharynx and soft palate (Holstege, 1989; Zhang et al., 1992). NRA neurons project to the spinal cord as well. In the cervical cord, NRA fibers terminate in motoneuronal cell groups innervating the diaphragm, pectoral muscles, and cutaneous trunci muscles (Holstege and Kuypers, 1982; Feldman et al., 1985; Holstege and Blok, 1989). In the thoracic and upper lumbar cord (T1-L3), the NRA projects to motoneurons innervating intercostal and abdominal muscles (Holstege and Kuypers, 1982). In the sacral cord (S1-S2) the NRA has been shown to project to the nucleus of Onuf innervating the pelvic floor musculature including the external urethral and anal sphincters (Holstege and Tan, 1987). Electrophysiologically, it has been shown that the NRA contains expiration related neurons, although its rostral part contains inspiration related cells as well (Merrill, 1970; 1974). NRA interneurons are known to be involved in expiration, as well as in vomiting, defecation and vocalization (Fukuda and Fukai, 1986a,b; Miller et al., 1987; Holstege, 1989; Zhang et al., 1992). The NRA receives, in addition to projections from other respiration related neurons in the brainstem (for review see Feldman, 1986), specific projections from the midbrain periaqueductal gray (PAG) (Holstege, 1989; Davis and Zhang, 1991). The PAG is the final integrator for various components of emotional behavior, such as vocalization, jumping, blood pressure control, micturition and lordosis (Sakuma and Pfaff, 1979b; Holstege, 1989; Bandler et al., 1991; Blok and Holstege, 1994). The intense PAG-NRA projection raises the question whether the NRA is involved in behavior other than expiration or expiration related activities. Therefore an attempt was made to carefully study its descending pathways to the spinal cord. In this paper the NRA projections to the lumbosacral cord are presented. The results strongly suggest a role for the NRA in lordosis.
Chapter 3

MATERIALS AND METHODS

Retrograde tracing study

In order to localize NRA neurons projecting to the lumbosacral cord, in six adult female cats (3.2-6.7 kg) injections of 2.5% wheatgerm agglutinin-horseradish peroxidase (WGA-HRP) were made in the L6 (cases 2249, 2278, and 2280), L7 (case 2295), S1 (case 2261) and S2 (case 2265) segments. See Chapter 1 for the general surgical and histological procedures. After laminectomy of the lumbar vertebrae, multiple injections of WGA-HRP were made with a glass pipette using a pressure pump. The injections occupied the entire rostrocaudal extent of the respective segments, but were kept unilaterally, except for case 2295 in which a bilateral injection was made. Unilateral injections were made to distinguish between neurons projecting to the ipsi- and contralateral spinal gray matter, respectively. In order to differentiate between ipsilateral or contralateral descending pathways, prior to the L6 injection an ipsilateral L3 hemisection was made in case 2278, and a contralateral hemisection was made in case 2280. In case 2295 the bilateral L7 injection was preceded by a left sided L3 hemisection. All hemisections were made by aspiration with a glass pipette. After 3 days survival time, the animals were perfused and spinal cords were cut and incubated in TMB in order to verify if the hemisections were complete. Finally, the material (only 1:10 non serial sections were coated with Ilford G5 emulsion by dipping, and stored in the dark at 5 °C for 3 months. Subsequently, the material was developed with Kodak D19 at 16 °C, fixed, and counterstained with cresyl violet. The sections were studied with a Wild darkfield M7S microscope, and photomicrographs were taken of representative spinal cord sections. In each experiment, the injection area was defined as that area in which the silver grains over the cell bodies were either as numerous as, or more numerous than those over the surrounding neuropil (Holstege et al., 1977).

WGA-HRP cases

Although the autoradiographical tracing results demonstrated NRA-lumbar motoneuronal projections, the material (only 1:10 non serial sections were taken. The collected sections (1:4) were grouped into nine levels between the obex (level 0 in our study) and 8 mm caudal to the obex (level 8). Each level represented six sections. For each level, the retrogradely labeled cells were counted and plotted into one drawing by means of a drawing tube.

Anterograde tracing study

Tritiated leucine cases

In seven cats (all females except for cases 1183 and 1472), weight 2.0-3.5 kg, single injections with L-(4,5-3H)-leucine (specific activity > 100 Ci/mmol) were placed in the region of the NRA. In two of these cases (1019 and 1183) the injections were made at a more rostral level than in the other five cases (1471, 1472, 1571, 1683, and 1684). After dorsal approach and exposure of the caudal medulla, the injection in each cat was placed stereotactically with a Hamilton microsyringe fitted with a 22-gauge needle. In all cases 0.5 µl tritiated leucine was injected over a period of 5 minutes except for case 1571, in which 0.25 µl tritiated leucine was injected. The needle was left in place for an additional 30 minutes to minimize the spread along the needle track. After a survival period of 6 weeks (Holstege et al., 1979), the animals were deeply anesthetized and perfused with saline followed by 10% formalin. After postfixation in 10% formalin for at least one week, brain and spinal cords were cut into transverse 25 µm frozen sections. One series of every tenth section was mounted, coated with Ilford G5 emulsion by dipping, and stored in the dark at 5 °C for 3 months. Subsequently, the material was developed with Kodak D19 at 16 °C, fixed, and counterstained with cresyl violet. The sections were studied with a Wild darkfield M7S microscope, and photomicrographs were taken of representative spinal cord sections. In each experiment, the injection area was defined as that area in which the silver grains over the cell bodies were either as numerous as, or more numerous than those over the surrounding neuropil (Holstege et al., 1977).

Table 1. Number of counted neurons in the NRA and adjoining lateral tegmental field after spinal injections of WGA-HRP

<table>
<thead>
<tr>
<th>Case</th>
<th>Injection site</th>
<th>WGA-HRP</th>
<th>Hemisection</th>
<th>labeled neurons in NRA</th>
<th>group of labeled neurons ventromedial to NRA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>absolute number %</td>
<td>absolute number %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ipsi contra total</td>
<td>ipsi contra total</td>
</tr>
<tr>
<td>2249</td>
<td>caudal L5-rostral L7</td>
<td>4 µl 2.0%</td>
<td>ipsi L3</td>
<td>115 326 441 26 74</td>
<td>16 24</td>
</tr>
<tr>
<td>2278</td>
<td>caudal L5-rostral L7</td>
<td>3 µl 2.5%</td>
<td>contra L3</td>
<td>119 128 93 7 15 15</td>
<td>100 100</td>
</tr>
<tr>
<td>2280</td>
<td>caudal L5-rostral L7</td>
<td>3 µl 2.5%</td>
<td>left L3</td>
<td>24 334 358 7 93 349</td>
<td>389 90 10</td>
</tr>
<tr>
<td>2295</td>
<td>caudal L6-L7</td>
<td>6 µl 2.5%</td>
<td></td>
<td>384 19 403 95 5 18</td>
<td>311 5 95</td>
</tr>
<tr>
<td>2261</td>
<td>caudal L7-S1</td>
<td>3 µl 2.5%</td>
<td></td>
<td>101 259 360 28 72</td>
<td>350 112 462 76</td>
</tr>
<tr>
<td>2265</td>
<td>caudal S1-S2</td>
<td>2 µl 5.0%</td>
<td></td>
<td>109 248 357 30 70</td>
<td>330 97 427 78</td>
</tr>
</tbody>
</table>
cord and extended into the contralateral lamina X, except unilateral injections involved the entire left side of the hemisections as well as the amounts of tracer. All cord. Table 1 shows the level of the injections and In 6 cases WGA-HRP was injected in the lumbosacral Location of injections and hemisections Retrograde tracing study

In each of 8 female cats (cases 2237, 2251, 2256, 2258, 2267, 2271, 2286, and 2290) 2-5 injections of 20-30 nl 2.5% WGA-HRP were rostrocaudally placed in the region of the NRA. In all cases the injections were placed after dorsal approach and exposure of the caudal medulla and in cases 2286 and 2290 of the first two cervical segments. The injections not only involved the NRA, but also the adjacent lateral tegmentum which implies that the labeled fibers in the spinal cord could be derived from NRA neurons or from neurons in the adjacent tegmentum. However, the retrograde tracing experiments showed that more than 93% of the NRA fibers crossed the midline and descended in the contralateral funiculi, while neurons in the tegmentum medial to the NRA sent their fibers through the ipsilateral spinal cord. Therefore, interruption of the ipsi- or contralateral pathways at upper cervical levels would allow to distinguish between the termination sites of the contralateral NRA-spinal and ipsilateral reticulospinal pathways. An ipsilateral C2 hemisection was made in case 2286, and a contralateral C2 hemisection was made in case 2290.

In all anterograde tracing cases the same histological procedures were applied as described for the retrograde WGA-HRP experiments. After perfusion, brain and spinal cord were removed and the injection site was determined using the DAB procedure. Segments L1 to S3 were cut on a freezing microtome into 40 µm thick, transverse sections and a 1 out of 4 series of consecutive sections was processed using the TMF procedure and microscopically studied using the same technique as described for the retrograde tracing study. Photomicrographs were taken from representative sections. In case 2286 with the ipsilateral C2 hemisection, the labeled fibers of every 6 consecutive collected sections were plotted into one drawing. In case 2290, showing the ipsilateral pathway, the distribution pattern of anterogradely labeled fibers was similar throughout the length of the lumbosacral cord. For each segment, one drawing was made showing the labeling of 3 consecutive collected sections.

RESULTS

Retrograde tracing study

Location of injections and hemisections

In 6 cases WGA-HRP was injected in the lumbosacral cord. Table 1 shows the level of the injections and hemisections as well as the amounts of tracer. All unilateral injections involved the entire left side of the cord and extended into the contralateral lamina X, except for case 2278 in which the dorsal funiculus and contralateral lamina X were not involved. In case 2265 (S2 injection) the injection site extended into larger portions of the contralateral gray. The L3 hemisections (cases 2278, 2280, and 2295) were complete and did not extend across the midline.

Cases without hemisections (2249, 2261, and 2265)

Ipsilateral side At the level caudal to the obex two groups of labeled neurons were found; one group within the confines of the NRA and another group in the tegmentum just medial to it. In the NRA the bulk of the labeled neurons was found at levels 2 to 6 (2-6 mm caudal to the obex; Figs. 1 and 2 left). The neurons in the medially adjacent tegmentum formed a compact cell group between levels 3 and 8. Further rostrally this cell group became more dispersed and contained more neurons. The tegmental group was found exclusively ipsilaterally. Some other dispersed neurons were found in the tegmentum between NRA and central canal. Other labeled neurons were present in the ventral part of the ipsilateral gracile nucleus and a few in lamina 1 at the transition between the caudal spinal trigeminal nucleus and the C1 spinal cord.

Contralateral side In all 3 cases a large number of labeled neurons were present in the NRA, some in the lateral tegmental field between NRA and central canal, and some in the ventrolateral solitary nucleus. At levels just caudal to the obex, some neurons were located just medial to the lateral reticular nucleus.

In the L6-injected case (2249), the total number of retrogradely labeled NRA neurons was slightly larger than in the S1- and S2-injected cases (2261 and 2265; see table 1). In each of the 3 cases, 70-74% of the NRA neurons was found on the contralateral and 26-30% on the ipsilateral side.

Cases with hemisections (2278, 2280, and 2295)

Although the 3 unilaterally injected cases revealed the side of termination of the lumbosacral cord projecting NRA neurons, the question remains whether the NRA-lumbosacral pathways descend ipsi- or contralaterally through the spinal cord. Figure 3 depicts all six theoretically possible trajectories (A to F). Combining the unilateral L6-injections with ipsi- and contralateral L3 hemisections reveals the trajectories of these pathways.

Contralateral hemisection (case 2280) In this case, the distribution pattern of labeled neurons in the NRA was similar to the cases without hemisection, except that on the ipsilateral side labeled neurons in the NRA were sparse and amounted to only 7% of the total number of labeled NRA neurons, while in non hemisected cases the ipsilaterally located NRA neurons amounted 26-30%
Chapter 3

Average number of retrogradely labeled neurons in the NRA per section for levels 0 to 8

(Table 1; Figs. 1 and 2 middle). Apparently, these 7% descended to the lumbosacral cord via trajectory A or E of Figure 3, and more than 93% of all NRA neurons project to the lumbosacral segments via the contralateral cord (trajectories C or F). It also means that from all NRA neurons projecting to the ipsilateral lumbosacral segments (26-30% of the total population) the great majority send their fibers by way of the contralateral cord. These fibers cross the midline twice (trajectories D or F), first at the level of the NRA itself ventral to the central canal and for the second time, since the hemisection is in L3, at the level caudal to L3, probably at the level of termination (see also the anterograde experiments).

Ipsilateral hemisection (case 2278). After a hemisection ipsilateral to the side of the injection only those NRA neurons will be labeled, which send their fibers via the contralateral spinal cord, but which axons terminate ipsilaterally, i.e. cross the midline twice (trajectories D or F in Fig. 3). Furthermore, NRA neurons will be labeled, which send their fibers through the ipsilateral spinal cord, but which terminate contralaterally, i.e. cross once at the level of termination (trajectories B or E). These latter neurons will be found in the NRA contralateral to the side of the injection. In case 2278, with an ipsilateral hemisection 119 labeled NRA neurons were found on the ipsilateral side and 9 on the contralateral side (see Table 1). Apparently, nearly one third of the total number of NRA lumbosacral neurons have fibers, which cross twice, while approximately 3% of the total population has fibers which descend ipsilaterally, and cross at the level of termination.

In contrast to cases 2249 and 2280, no retrogradely labeled neurons were found ventromedial to the ipsilateral NRA (see Figs. 1 and 2 right), indicating that this group of neurons projects to the ipsilateral lumbosacral cord by means of ipsilaterally descending axons (however, see case 2295 in table I).

Are recrossing fibers collaterals or not?
The question arises whether or not the recrossing fibers are collaterals of fibers crossing at the level of the NRA and terminating contralaterally in the lumbosacral cord (trajectory D or F in Fig. 3). The same question applies to the ipsilaterally descending NRA-lumbosacral pathways (trajectory B or E). Theoretically, a double sided injection combined with a hemisection would solve this problem, provided that 1) in all cases the same number of NRA lumbosacral neurons exists, and 2) WGA-HRP does not label fibers of passage. In that case, assuming that no collateralization exists, a bilateral injection would result in a number of labeled NRA neurons ipsilateral to the hemisection which is 1 1/3 times higher than the number of ipsilateral NRA neurons after a unilateral hemisection (trajectories C plus D). Suppose all recrossing NRA-lumbosacral fibers are collaterals, no difference would be found (trajectory F). In case 2295 such a bilateral injection was made. The result was that not 1 1/3, but 1 1/6 times the number of contralaterally projecting neurons was present in the ipsilateral NRA (see Table 1). This would mean that 50% of the contralaterally descending NRA-lumbosacral fibers descend via trajectory F, while the other 50% descend via trajectories C or D. However, it must be emphasized that such a conclusion should be taken with.
In conclusion, the retrograde tracing results indicate that: 1) the bulk (70-74%) of the NRA-lumbosacral neurons have axons descending in the contralateral cord to terminate in the contralateral lumbosacral gray matter; 2) 26-30% of the NRA-lumbosacral neurons project to the ipsilateral spinal gray, most of which descend by way of the contralateral cord. The fibers of these neurons cross the midline twice; and 3) the impression is gained that part of the recrossing fibers are collaterals from contralaterally terminating axons and part of them are not.

**Only part of the entire NRA population projects to the lumbosacral cord**

In case 2295 with a bilateral L7 injection, according to Table 1, 384 retrogradely labeled neurons were counted in the NRA on the side ipsilateral to the hemisection. Carefull analysis of the ipsilateral NRA itself clearly shows that not all neurons within the confines of the nucleus are labeled (Fig. 4). This finding suggests that the NRA contains many neurons not projecting to the lumbosacral cord, and which therefore are involved in other activities than the labeled neurons (see also Holstege 1989, p. 248).

**Reticulospinal pathway**

The retrograde tracing results indicate that the tegmental neurons medial to the NRA project mainly ipsilaterally to the lumbosacral gray matter. However, according to case 2295, a few (5%) of these neurons project via the contralateral spinal cord. In respect to the cases without hemisections, in which considerable numbers of labeled tegmental neurons were found at the contralateral side, it should be recalled that in these cases the injection site extended into the contralateral lamina X. This might have

---

**Figure 2** Schematic representation of the WGA-HRP labeled neurons in the caudal medulla in cases 2249, 2280, and 2278. Each drawing represents six sections around the levels 0, -2, -4, -6, and -8 mm caudal to the obex.
resulted in retrograde labeling of the reticulospinal neurons because the anterograde tracing observations (see further) indicate that the reticulospinal fibers terminate in this lamina.

**Anterograde tracing study**

**Injections involving the rostral NRA**

*Location of injections*  In two cases 1183 and 1019 the injection sites involved the rostral NRA and adjoining lateral reticular formation (see Fig. 5). The injection in case 1019 extended much further medially in the tegmentum than in case 1183. Both injections were present at the level of the obex, but in case 1183 the injection site extended further rostrally and in case 1019 further caudally.

*Contralateral projection*  A specific contingent of labeled fibers, after crossing the midline at the level of the obex, descended in the contralateral ventral funiculus throughout the length of the spinal cord. In the lumbosacral cord from these fibers many were distributed to mainly the lateral part of the L1-L3 motoneuronal cell groups, containing abdominal muscle motoneurons (Miller, 1987; Holstege et al., 1987). These projections were bilateral, with a slight contralateral preponderance. In case 1183, but not in case 1019, at the levels L6 and L7 a very small number of labeled fibers were distributed to mainly the ventral part of the contralateral ventral horn. Virtually no labeled fibers were found in the Onuf’s nucleus in both cases. In conclusion the rostral NRA does not, or to only a very limited extent, project to the L4-S3 ventral horn.

*Ipsilateral projection*  In both cases many labeled fibers descended in the ipsilateral dorsolateral funiculus, but in case 1183 they were very sparsely present at lumbosacral levels. In case 1019 the ipsilaterally descending fibers traveled throughout the length of the spinal cord and terminated in laminae V to VIII and in lamina X of the ipsilateral gray matter. However, in case 1183, with the more lateral injection, in the lumbosacral gray matter labeled fibers were sparse.

**Injections involving the caudal NRA**

*Location of injections*  In 4 cases (1471, 1571, 1683 and 1684; see Fig. 5) injections of 3H-leucine were made in caudal portions of the NRA and adjacent tegmentum. In a fifth case (1472) the injection site avoided the NRA, but occupied the tegmentum between NRA and solitary nucleus. The injection site extended laterally into the caudal spinal trigeminal nucleus and dorsally into the dorsal column nuclei. The two most caudally located injections were made in cases 1471 and 1571. In case 1471 the injection was relatively large and involved the lateral tegmental field, including the caudal NRA and the most medial part of the caudal spinal trigeminal nucleus. The smallest injection was made in case 1571 and was present in the white matter of the lateral funiculus, but extended medially into the lateral half of the caudal NRA. Similar injection sites were present in cases 1683 and 1684, but these injections extended further medially and further rostrally in the tegmentum than the injection in case 1571.

*Contralateral projection*  The contralateral pathway consisted of fibers that crossed the midline at the level of the injection or just rostral to it and descended in the contralateral ventral funiculus and more caudally in the ventrolateral funiculus as well. The fibers of the contralateral fiber bundle descended throughout the length of the spinal cord, except in case 1472, in which they were not found beyond upper lumbar levels. At upper lumbar levels, similar to the rostral NRA injected cases, many labeled fibers were distributed to abdominal muscle motoneurons bilaterally (see Fig. 6).
At these levels, many labeled fibers were observed to cross in the ventral commissure, indicating that the projection to the ipsilateral abdominal muscle motoneuronal nuclei is at least partly derived from the contralaterally descending pathway. Yet, in cases 1471 and 1472, but not in case 1571, a few labeled fibers descended in the ipsilateral ventral funiculus and may have contributed some fibers to the ipsilateral abdominal motoneuronal cell groups. Further caudally, in all cases, except 1472, a clear distribution was observed to distinct parts of the motoneuronal cell groups at the levels L5 to S1 and to the nucleus of Onuf at the level S1-S2 (see Fig. 6). These motoneuronal projections were bilateral, but, in contrast to the projections to the abdominal wall motor nuclei, they had a very strong contralateral preponderance.

Ipsilateral projection. In all 5 cases an ipsilateral bundle descended in the dorsolateral funiculus. In cases 1571 and 1683, with injections not extending into the lateral tegmental field, this bundle gradually disappeared at low cervical and upper lumbar levels, respectively. In cases 1471, 1472 and 1684 the ipsilateral bundle continued throughout the length of the spinal cord and labeled fibers were distributed to all parts of the intermediate zone including lamina X (see Fig. 6). In the upper lumbar cord a few labeled fibers were distributed to the intermediolateral cell column, and in the sacral cord a very few labeled fibers were found in the sacral parasympathetic cell group.

In cases 1471, 1472 and 1684 labeled fibers were distributed to lamina I throughout the lumbosacral cord (see also Fig. 4 of Holstege, 1988). These projections were found on both sides, but showed an ipsilateral predominance. Lamina I projections do not seem to originate in the NRA, because the NRA is not injected in case 1472 and lamina I projections were absent or sparse in the other 3 cases with injections involving the NRA.

Summarizing: in all 5 cases with injections in the caudal medulla approximately the same projection pattern was observed i.e. two different descending pathways, an ipsilateral one in the dorsolateral funiculus, distributing fibers to the intermediate zone and lamina I, and a contralateral one in the ventral and ventrolateral funiculus, distributing fibers to motoneurons innervating abdominal musculature. In all cases in which the caudal NRA was injected, strong projections to motoneuronal cell groups at levels L5 to S1 and to the nucleus of Onuf were found. All motoneuronal projections were bilateral, but in respect of the L5-S1 motoneuronal projections they showed a strong contralateral preponderance.

WGA-HRP experiments

Location of injections and hemisections. In 8 cases (cases 2237, 2251, 2256, 2258, 2267, 2271, 2286, and 2290) multiple injections of WGA-HRP were made in the caudal medulla oblongata. All injections involved the NRA as well as the adjoining lateral tegmental field and the lateral funiculus (see Fig. 7). In two of the 8 cases hemisections were made in the second cervical segment, ipsilaterally in case 2286 and contralaterally in case 2290. The hemisections were complete and did not extend across the midline.

As has been shown in the retrograde tracing study more than 90% of the NRA neurons sent their fibers through the contralateral spinal cord, while a group of neurons located in the tegmentum medial to the NRA projected almost exclusively ipsilaterally. In order to distinguish between these two projection systems, the descending projections will be described on the basis of the findings of cases 2286 for the contralateral pathway, and 2290 for the ipsilateral pathway. It should be emphasized that, depending on the site of the injection, in the other 6 WGA-HRP cases (all without hemisections) both the ipsi- and contralateral pathways were found.

Contralateral pathway in the segments L1 to S3

Location of descending fibers. In case 2286, with the ipsilateral C2 hemisection, descending labeled fibers were found in the dorsolateral, ventrolateral, and ventral funiculi. From L1 to S3 their number gradually decreased. From rostral L4 to caudal L5, the labeled
fibers in the ventrolateral funiculus gradually shifted into a more peripheral position. Only a very limited number of retrogradely labeled neurons was found in the lumbosacral cord (see Fig. 8), which indicates that the bulk of the labeled fibers in the white matter represent descending fibers.

**Non-motoneuronal termination sites** Throughout the length of the L3-S3 spinal cord, labeled fibers were distributed to laminae V to VII and to lamina X bilaterally, but with a contralateral preponderance. These projections were denser at sacral than at lumbar levels. The fibers terminating ipsilaterally had recrossed in the ventral gray commissure. At all lumbosacral levels, labeled fibers terminated in areas at the transition between white matter and lamina IX. In addition to this general distribution pattern, specific projections were found to certain motoneuronal cell groups in lamina IX.

**Motoneuronal termination sites L3** In the rostral, but not in the caudal half of L3, very dense projections were found to the lateral part of the ventral horn, which projection was equally dense at both sides of the cord (arrow a in Fig. 8). This distribution area contains motoneurons innervating the external and internal oblique and to a limited extent transverse muscles of the abdominal wall (Holstege et al., 1987; Miller, 1987). These abdominal wall motoneurons are not present in the caudal part of L3. In conclusion, in the rostral L3 segment a dense, bilateral NRA projection to motoneurons innervating abdominal wall muscles was present. In the same segment labeled fibers were also present in the medial portion of the ventral horn, but it was difficult to establish whether this area represented a termination site due to the many crossing fibers (Fig. 8 arrow b). A similar, bilateral distribution pattern to the medial portion of the ventral horn was also found in the L4-L6 segments, but was absent in segment L7 (Fig. 8 arrow b; Fig. 9 L5). These projections might terminate on motoneurons innervating the multifidus muscle (VanderHorst and Holstege, Chapter 2; Gilette et al., 1993). In segment L3 a few labeled fibers were found

![Figure 6](image-url) Darkfield photomicrographs of the L3-S1 spinal cord in cases 1471 with an injection with 'H-leucine in the NRA and medially adjacent tegmentum.)
in the area of the intermediolateral cell column, containing autonomic (sympathetic) motoneurons.

**Motoneuronal termination sites L4 and L5** In rostral L4 no specific projection to motoneurons was found, but in caudal L4-rostral L5 a specific projection to a motoneuronal cell group at the ventrolateral border of the gray matter was observed (Fig. 8 arrow c; see Fig. 10 for detail). This area contains motoneurons innervating the iliopsoas muscle (VanderHorst and Holstege, Chapter 2; see also Fig. 10). In addition, in rostral L5 labeled fibers were present in the centre of the ventral horn (Fig. 8 arrow d), in which area are located motoneurons innervating adductor muscles of the hindlimb such as m. pectineus, m. adductor longus, m. adductor femoris brevis and m. gracilis (Romanes 1951; VanderHorst and Holstege, Chapter 2). It must be emphasized that no labeled fibers terminated in the motoneuronal cell group located between the centrally located adductor group and the ventrolaterally located iliopsoas group. This group of motoneurons devoid of labeled fibers innervates the m. sartorius muscle (Romanes 1951; VanderHorst and Holstege, Chapter 2). In L5 another specific projection was found to the ventromedial corner of the ventral horn (Fig. 8 arrow e; Fig. 11 L5). It has not yet been clarified which motoneurons occupy this area.

**Motoneuronal termination sites L6** In rostral L6, except for the projection to an area in the medial portion of the ventral horn (Fig. 8 arrow b), only a limited number of labeled axons were found. At this level the ventral horn contains many motoneurons innervating the m. quadriceps (Romanes, 1951; VanderHorst and Holstege, Chapter 2). These motoneurons apparently do not receive a substantial projection from the NRA. In caudal L6, a strong projection was present to the centre of lamina IX (Fig. 8 arrow f; Fig. 9 L6). This area corresponds with the rostral portion of column 3’ of Romanes (1951, 1954), which, at this level, contains motoneurons innervating the m. semimembranosus (Romanes, 1951; VanderHorst and Holstege, Chapter 2).

**Motoneuronal termination sites L7** The dense accumulation of labeled fibers observed in caudal L6 extended into the most rostral pole of L7. In the remainder of rostral L7 such a dense accumulation was not found, but a rather diffuse distribution was present in the ventral half of the ventral horn. It was not possible to draw any conclusions about which motoneurons or motoneuronal dendrites in this area are targeted. The dorsal half of the ventral horn received only a limited number of labeled fibers at that level. In caudal L7 another accumulation of labeled fibers was found in the central part of the ventral horn (Fig. 8 arrow g). This area corresponds with columns 3’ and 3” of Romanes and contains motoneurons innervating hamstring muscles, at this level the m. semitendinosus and m. biceps femoris, respectively (Romanes, 1951; VanderHorst and Holstege, Chapter 2). Similar to the rostral L7, no labeled fibers were distributed to the dorsal portion of the ventral horn, containing moto-neurons innervating the muscles controlling the ankle joint (Romanes, 1951; Burke et al., 1977; Horcholle-Bossavit et al., 1988; VanderHorst and Holstege, Chapter 2).

**Motoneuronal termination sites S1** The L7 projection to the motoneurons innervating hamstring muscles (m. semitendinosus and m. biceps femoris) continues into the rostral part of S1 (Fig. 8 arrow g). Caudally in S1 labeled fibers were present in the nucleus of Onuf (Fig. 8 arrow h; Fig. 11 S1), which contains motoneurons innervating pelvic floor muscles. The many labeled fibers terminating in the area dorsomedial to the nucleus of Onuf might represent terminations on dendrites of Onuf motoneurons (Fig. 8 arrow i; Holstege and Tan, 1987; Beattie et al., 1990). The projection to Onuf motoneurons was remarkably bilateral in contrast to the projections to somatic motoneurons in more rostral portions of the lumbosacral enlargement. The projection indicated by arrow j in Figure 8 might represent terminations on motoneurons innervating the most distal of the intrinsic hindpaw muscles (VanderHorst en Holstege, Chapter 2). In caudal S1, another projection was located in the ventromedial part of lamina IX. This projection is difficult to see in Figure 8 (arrow k), but can be distinguished in Figure 11 S1. This area contains motoneurons innervating the caudal part of the medial longissimus muscle which inserts on the base of the tail (VanderHorst and Holstege, Chapter 2).

**Motoneuronal termination sites S2 and S3** In S2 and to a limited extent in the rostral S3 another projection to the tail muscle motoneurons was found (Fig. 8 arrow l). Motoneurons innervating the proximal tail muscles are located within two regions of the ventral horn: the ventromedial nucleus located in the ventral part of the ventral horn and the nucleus commissuralis in the medial portion of the ventral horn (Rexed, 1954; Ritz et al., 1992).

The intermediolateral cell group, containing autonomic (parasympathetic) motoneurons innervating bladder and sexual organs also receives a moderate bilateral projection (Fig. 8 arrow m).

**Ipsilateral pathway in the segments L3 to S3**

**Location of descending fibers** In case 2290, with a contralateral hemisection in C2, at lumbosacral levels the bulk of the labeled fibers was present in the dorsolateral funiculus and only very few were located in the ventrolateral, lateral and ventral funiculi. The number of labeled axons gradually decreased from L1 to S3. In contrast to case 2286 with an ipsilateral hemisection, in case 2290 a considerable number of retrogradely labeled neurons was present, mainly
Figure 7  WGA-HRP injection sites in the NRA and adjoining caudal medullary lateral tegmentum. Note that the injections in cases 2286 and 2290 are combined with an ipsilateral and contralateral hemisection, respectively.

Figure 8  Schematic drawings of the labeled fibers after injection of WGA-HRP in the NRA combined with an ipsilateral hemisection (2286). Each drawing represents the labeling of 6 consecutive collected (1:4) sections. Note the accumulation of labeled fibers in distinct portions of the ventral horn motoneuronal cell groups. For reference of the arrows see text.
NRA projections to lumbosacral motoneurons: non-estrous females
contralaterally in laminae I, and V to VIII in the L7 to S3 segments. In the L3-L6 segments a much smaller number of labeled neurons was observed. Only very few labeled neurons were found in the lateral portion of laminae I and V. Due to the many retrogradely labeled neurons in this case, some of the labeled axons found in the white matter might be derived from retrogradely labeled neurons.

**Termination sites**

The bulk of the ipsilaterally descending axons terminated in laminae V to VIII, and in lamina X throughout the length of the lumbosacral cord. Only very few labeled fibers were distributed to laminae I and IX. The sympathetic intermediolateral cell column in segments L1 to L3 (Fig. 13) and to a limited extent also the sacral parasympathetic nucleus in S2 and S3 received labeled afferents. In general, all projections had a very strong ipsilateral predominance and were denser at sacral than at lumbar levels. In the contralateral gray matter, the few anterogradely labeled fibers could not be distinguished from dendrites and axons of numerous retrogradely labeled neurons. According to the findings of the retrograde study, this ipsilateral distribution is mainly derived from neurons located ventromedial to the NRA. Only a limited number of neurons in the NRA are involved in this projection.

**DISCUSSION**

The present paper is the first to precisely describe nucleus retroambiguus (NRA) projections to lamina IX in the lumbar enlargement. The projection does not involve all lumbar motoneuronal cell groups, but a distinct set innervating a certain group of hindlimb and back muscles. In earlier studies, the NRA has been described to project to other distinct motoneuronal cell groups. Table 2 gives an overview of all the NRA-motoneuronal projections and their possible function.

**NRA projections to motoneurons other than in the lumbosacral cord**

The dorsal group of the nucleus ambiguus, containing motoneurons innervating soft palate and pharynx muscles, is known to receive strong projections from
Physiologically, NRA-laryngeal motoneuron projections have been demonstrated by Zhang et al. (1992). These NRA-pharynx/larynx motoneuron projections play a role in expiration (Feldman, 1986) and in vocalization (Holstege, 1989; Zhang et al., 1992). Large portions of the NRA have been shown to project to the phrenic nucleus (Holstege and Kuypers, 1982; Feldman et al., 1985), but the most caudal portion of the NRA is not involved in this projection (Holstege, 1989). The NRA has also been shown to project to pectoral muscle motoneurons in the C7 segment (Holstege and Kuypers, 1982). These muscles serve as adductors of the shoulder (Crouch, 1969). Another NRA projection is to the cutaneus trunci muscle motoneurons in the C8-T1 segments (Holstege and Blok, 1989). Throughout the entire length of the thoracic cord, the NRA projects to motoneuronal cell groups in the ventral horn (Holstege and Kuypers, 1982; Feldman et al., 1985). The first group of motoneurons at these levels innervates intercostal muscles, to which belong the intercartilaginous, triangularis sterni, internal and external intercostal muscles. The external and internal intercostal muscles, have a role in postural function, with the exception of the caudal internal intercostal muscles, which are involved in expiration as well (Duron, 1973). The second group consists of the transverse, internal oblique, and external oblique abdominal wall motor nuclei. Their motoneurons are located laterally in the T5-L3 ventral horn (Holstege et al., 1987; Miller, 1987) and receive bilateral projections from the NRA, with a contralateral predominance (Holstege and Kuypers, 1982; Feldman et al., 1985; Holstege, 1989). The abdominal wall muscles have a function in expiration and expiration related activities (Campbell and Green, 1953; McCarthy and Borison, 1974).

**NRA projections to motoneurons in the lumbosacral cord**

**hindlimb motor nuclei**

**iliopsoas muscles** At the level L4-L5, the NRA projects to the motoneuronal cell group innervating the m. iliopsoas. This muscle consists of two parts, the m. iliacus and the m. psoas. The iliacus muscle originates from the ventral border of the ilium, and the psoas muscle from the 5 most caudal vertebrae. Both parts insert to the the lesser trochanter of the femur. Their
Chapter 3

Figure 11  On the left a combined polarized light and darkfield photomicrograph of the ventral part of the ventral horn in L5 in case 2286 (WGA-HRP injection in the NRA and adjoining tegmentum combined with an ipsilateral C2 hemisection). Note the specific projection to the ventromedial nucleus (see also Fig. 8 arrow e). On the right a combined polarized light and darkfield photomicrograph of the S1 ventral horn in case 2286. Note in S1 the anterograde labeling in the nucleus of Onuf (see also Fig. 8 arrows h and k). Bar represents 200 µm.

Figure 12  Schematic drawings of the labeled fibers after injection of WGA-HRP in the NRA and adjoining tegmentum combined with a contralateral hemisection (2290). Each drawing represents the labeling of 3 consecutive collected (1:4) sections.
function is fixation of the spine and flexion of the hip (Crouch, 1969).

**adductor muscles** Motoneurons innervating different adductor muscles of the thigh are located in column 3 of Romanes (1951), which extends from rostral L5 to caudal L6. The NRA projection to column 3 did not involve its entire rostrocaudal extent, but was present only in the L5 and most rostral L6 segments, which matches the rostrocaudal distribution of motoneurons innervating the m. pectineus, adductor longus and brevis, and gracilis (Romanes, 1951; VanderHorst and Holstege, Chapter 2). The m. pectineus and adductor longus originate from the os pubis, insert on the femur and adduct the thigh. The m. adductor brevis has its origo on the symphysis, inserts on the femur and functions as adductor and extensor of the hip. The m. gracilis, with its origo on the symphysis and insertion on the proximal tibia, is not only an adductor of the thigh, but can also act as an extensor of the hip and flexor of the knee (Crouch, 1969; Zajac, 1985; Pratt et al., 1991).

**hamstring muscles** Motoneuronal cell groups innervating hamstring muscles (semimembranosus, semitendinosus and biceps femoris) also receive NRA afferents. The m. semimembranosus motoneurons are located in column 3’ of Romanes (1951, 1954) at the level caudal L6-rostral L7. The semimembranosus can be devided into two functionally different parts, both originating from the ischium. The anterior part inserts on the distal femur and acts as an extensor of the hip. The posterior part inserts on the proximal tibia and functions as an extensor of the hip and as a flexor of the knee (Crouch, 1969; Zajac, 1985; Pratt et al., 1991).

The motoneurons of the m. semitendinosus are located in column 3’ of Romanes (1951, 1954) at the level caudal L7/rostral S1 and caudal to those innervating the m. semimembranosus. Similar to the posterior part of the semimembranosus and this muscle also has its origin on the ischium and inserts on the proximal tibia. It is a strong flexor of the knee and helps to extend the hip (Crouch, 1969; Zajac, 1985; Pratt et al., 1991).

The motoneurons innervating the m. biceps femoris are located at the same level, but laterally to those of the semitendinosus (column 3’ of Romanes, 1951). The biceps femoris muscle can be devided into an anterior and a posterior part. Both parts originate from the ischium, and the anterior part inserts on the distal femur and the posterior part on the proximal tibia. Both muscle compartments function as extensors of the hip, while the posterior biceps femoris also serves as a flexor of the knee (Crouch, 1969; Zajac, 1985; Pratt et al., 1991; Chanaud et al., 1991a,b).

**nucleus of Onuf** Holstege and Kuypers (1982) were the first to demonstrate that the NRA projects to the level of the sacral cord. In a later study, the nucleus of Onuf was found to be the main target of these NRA fibers (Holstege and Tan, 1987). In the cat, the nucleus of Onuf innervates pelvic floor muscles including the external anal and urethral sphincters (Sato et al., 1978). Pelvic floor muscles play a role in various activities such as abdominal straining, mating behavior, micturition and defecation.

**ventromedial nucleus of the lumbosacral cord**

The ventromedial nucleus at lower lumbar levels contains motoneurons innervating the lateral (Holstege et al., 1987) and medial components of the longissimus dorsi, which serve as extendors of the back (Brink et al., 1979, in the rat). The most caudal portion of the medial longissimus deviates the tail when activated unilaterally. Motoneurons of this part of the muscle are located in the ventromedial nucleus at the level of S1. At the level of S2, the ventromedial nucleus contains motoneurons innervating intrinsic muscles of the proximal tail (Ritz et al., 1992). NRA projections have been observed to the ventromedial nucleus at the levels L5-L6 and S1-S2.

**What is the function of the NRA-lumbosacral motoneuronal projection?**

In only a few electrophysiological studies, the presence of NRA axons caudal to the level of L3 was determined. Miller et al. (1985) reported that of all investigated expiratory NRA neurons projecting to the L1 segment, one third could also be antidromically activated from the white matter in L4-L5. Most expiratory neurons projecting to L4-L5 were located caudal to the expiratory neurons which projected to L1. Another physiological study (Sasaki et al., 1991, 1994) showed that expiratory neurons in the region of the NRA have axonal branches terminating in the lumbosacral gray matter, but the authors did not reveal the exact targets.

**Are NRA motoneuronal projections monosynaptic?**

In the present paper it is demonstrated that NRA neurons project to motoneuronal cell groups innervating specific hindlimb muscles such as the iliopsoas, adductor, and hamstring muscles, as well as axial muscles such as the caudal medial longissimus and proximal tail muscles. The location of many labeled NRA axons within the confines of distinct motoneuronal cell groups indicates that the NRA might form direct connections with lumbosacral motoneurons. Preliminary results of an electronmicroscopic double-labeling study (VanderHorst and Holstege, 1994) show that NRA terminals form asymmetric, presumably excitatory contacts with lumbosacral motoneurons.
NRA motoneuronal projections belong to the basic motor system

In general, motoneurons receive their main input from interneurons in the spinal intermediate zone. Usually these projections are called propriospinal pathways. Supraspinal structures as well as afferent input from the periphery make use of these interneurons to influence the motoneurons. The propriospinal projections take part in the basic motor system in the concept of Holstege (Holstege, 1991, 1994).

Specific, direct projections to distinct motoneuronal cell groups, bypassing the spinal interneurons, are rare; in the cat examples are Ia afferents from muscle spindles and a very few rubrospinal fibers (Holstege, 1987).

In the framework of the NRA motoneuronal projections, the NRA neurons might be considered as interneurons, similar to the ones in the spinal intermediate zone. The interneurons in the NRA do not receive afferents from the usual sources such as the peripheral Ia afferents and the rubro- and corticospinal tracts. Their afferents come from different sources, such as the PAG. This might be the reason that they are located at a different site. In this concept the NRA motoneuronal pathway belongs to the basic motor system, which consists of premotor interneurons (Holstege, 1994). The same is true for the M- and L-region neurons in the dorsolateral pons, which project to sacral parasympathetic and Onuf motoneurons, respectively, and coordinate micturition (Holstege et al., 1986).

The fact that the NRA-motoneuronal pathway is so different from other pathways to motoneurons suggests that the former projection system is involved in a specific motor activity. Candidates are synchronization of respiratory and locomotor rhythms or respiration independent behavioral patterns, as defecation, parturition, jumping or lordosis. The latter two activities form examples of specific emotional behavior which can be elicited by stimulation in the PAG. The various behavioral patterns will be discussed below.

Synchronization of respiratory and locomotor rhythms

It has been shown in man and other mammals that breathing and locomotion rhythms become synchronized during exercise (e.g., Bramble and Carrier, 1983). In decerebrated, vagotomized and immobilized cats, respiration and locomotion are still coupled (Kawahara 1989, 1990). These studies report that diaphragmatic and gastrocnemius muscle activities are synchronized, depending on the strength of the locomotor pattern and end-tidal pCO2. The central nervous system mechanisms responsible for this coupling are unknown. The NRA might play a role in the synchronization of locomotion and respiratory rhythms, because it projects to both respiratory and hindlimb muscle motoneurons. However, the motoneuronal cell group innervating the m. gastrocnemius does not seem to receive afferent projections from the NRA.

Defecation and parturition

Since the NRA is known to be involved in forced expiration, the question arises whether NRA neurons take part in other straining related activites, such as defecation and parturition. During defecation, distension of the rectal wall initiates weak peristaltic waves in the descending colon, sigmoid and rectum as well as relaxation of the internal anal sphincter. This reflex is called the intrinsic defecation reflex and is mediated by the m. rectus abdominis. Stimulation of afferents from the rectum to the sacral cord initiates a parasympathetic defecation reflex, which intensifies the intrinsic reflex (Guyton, 1986). However, for actual defecation the tonically activated external anal sphincter, innervated by Onuf motoneurons, must be relaxed (for review see Dubrovsky and Filipini, 1990). The exact mechanism by which the external anal sphincter motoneurons are inhibited is not yet known.

Defecation also has an abdominal wall straining component. For example, Kufuda and Fukai (1986a) found simultaneous activity of the abdominal wall muscles (rectus and obliquus externus), internal intercostal muscles and the diaphragm after distension of the vagina orrectum in decerebrate dogs. The question is whether this straining component is an integral part of the defecation reflex. In patients and animals with transection of the spinal cord rostral to the sacral cord, defecation is still possible, but relaxation of the external anal sphincter and the abdominal wall straining component are lacking. Possibly, the NRA-abdominal muscle motoneuronal pathway is involved in the straining component (Fukuda and Fukai, 1986a,b), although the rectus abdominis muscle, which is active during straining, is reported not to receive NRA afferents.
The NRA-lumbosacral motoneuronal projection might play a role in the specific posture that accompanies defecation (Kufuda and Fukai, 1986a). An argument against such an involvement is that only part of the motoneurons innervating muscles the defecation-posture muscles receive NRA afferents. In the same way, the NRA might be involved in parturition, which also is accompanied by abdominal wall straining as well as a specific posture.

**Jumping**

Jumping is one of the components of survival behavior which can be elicited by stimulation in the lateral part of the substantiolar PAG (Bandler et al., 1991). During jumping, the hamstrings, quadriceps, and plantar flexors of the ankle are activated, but the iliopea muscles is not (Zajac, 1985; Loeb, 1993). The NRA-lumbosacral projection indeed includes projections to hamstring muscle motoneurons, but the motoneurons innervating extensor muscles of the knee (quadriceps) and plantar flexors of the ankle (e.g. medial and lateral gastrocnemius and plantaris) do not receive NRA afferents.

The PAG is essential for the control of lordosis (see Ogawa et al., 1991 for review). Stimulation of the lateral and dorsal PAG facilitates lordosis (Sakuma and Pfaff, 1979a), whereas lesions suppress it in estrogen primed rats (Sakuma and Pfaff, 1979b).

**Lordosis**

Lordosis is species specific, female receptive behavior and has been studied extensively in rat, hamster, guinea pig and cat (see Pfaff and Schwartz-Giblin, 1988 for review). In the cat the full receptive posture consists of crouching (forelegs collapsed), lowering of the head, perineal elevation, tail deviation, and treading, often in combination with calling and vulval excretion (Michael, 1961).

The NRA-lumbosacral projection involves a combination of muscles which may be activated specifically during lordosis behavior. Axial muscles extend the lower back, iliopea muscles fixate the lower spine to the pelvis, hamstring muscles cause extension of the hip and flexion of the knee, and adductor muscles stabilize the posture. Moreover, the medial longissimus muscle, when unilaterally activated, produces the typical deviation of the tail, and pelvic floor muscles contract rhythmically. Quadriceps (extensor of the knee) and triceps surae muscles (plantar flexors of the ankle) are not involved in this behavior. In the rat it has been shown that mechanical stimulation of the cervix, but not the vagina, activates the iliopeas muscles (Martinez-Gomez et al., 1992). In the cat, the m. semitendinosus is rhythmically active during mechanical stimulation of the cervix, whereas triceps surae muscles showed sustained EMG activity during and after cervical stimulation (Cueva-Rolon et al., 1993).

The PAG does not abolish PAG-facilitated lordosis in estrogen primed rats (Sakuma and Pfaff, 1979a). Lesions in the VMH do not abolish PAG-facilitated lordosis in estrogen primed rats (Sakuma and Pfaff, 1979b).

The NRA projections to lumbosacral motoneurons: non-estrous females

<table>
<thead>
<tr>
<th>Motoneuronal cell group</th>
<th>Location in CNS</th>
<th>Muscle innervated</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral rectus abdominis</td>
<td>T2-T3</td>
<td>external oblique</td>
<td>Inspiration</td>
<td>Kufuda and Fukai, 1986</td>
</tr>
<tr>
<td>Erector spinae</td>
<td>T12-L3</td>
<td>intercostal</td>
<td>Vomiting, coughing, and eructation</td>
<td>Holstege et al., 1987; Miller, 1987/89</td>
</tr>
<tr>
<td>Iliopsoas</td>
<td>L4-L5</td>
<td>psoas</td>
<td>Flexion of the leg</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
<tr>
<td>Coccygeus</td>
<td>S1</td>
<td>coccygeus</td>
<td>Pelvic floor contraction</td>
<td>Holstege and Logan, 1982</td>
</tr>
<tr>
<td>Quadratus lumborum</td>
<td>T12-L4</td>
<td>quadratus lumborum</td>
<td>Extension of the spine</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
<tr>
<td>Pectineus</td>
<td>L5-S3</td>
<td>pectineus</td>
<td>Adduction of the thigh</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
<tr>
<td>Semimembranosus</td>
<td>L5-S4</td>
<td>semimembranosus</td>
<td>Extension of the knee and hip</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
<tr>
<td>Semitendinosus</td>
<td>L5-S4</td>
<td>semitendinosus</td>
<td>Extension of the knee and hip</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
<tr>
<td>Biceps femoris</td>
<td>L5-S4</td>
<td>biceps femoris</td>
<td>Extension of the knee and hip</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
<tr>
<td>Brachialis</td>
<td>C5-C6</td>
<td>brachialis</td>
<td>Flexion of the elbow</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
<tr>
<td>Triceps brachii</td>
<td>C5-C6</td>
<td>triceps brachii</td>
<td>Extension of the elbow</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
<tr>
<td>Brachioradialis</td>
<td>C5-C6</td>
<td>brachioradialis</td>
<td>Flexion of the wrist</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
<tr>
<td>Deltoid</td>
<td>C5-C6</td>
<td>deltoid</td>
<td>Abduction of the arm</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
<tr>
<td>Sciaticus</td>
<td>L4-S2</td>
<td>sciaticus</td>
<td>Extension of the leg</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
<tr>
<td>Sartorius</td>
<td>L4-S2</td>
<td>sartorius</td>
<td>Flexion of the knee</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
<tr>
<td>Adductor magnus</td>
<td>L4-S2</td>
<td>adductor magnus</td>
<td>Adduction of the thigh</td>
<td>Holstege and Kuypers, 1982</td>
</tr>
</tbody>
</table>

* Larmicol did not investigate the location of intercostal motoneurons caudal to T8. Probably they are present in T9-T12 as well.
and is known to respond to lordosis relevant somatosensory stimulation (Pfaff and Schwartz-Giblin, 1988 for review). Lordosis can still be elicited by somatosensory and vaginocervical stimulation in ovariectomized rats after precollicular decerebration (Rose and Flynn, 1993). In freely moving animals, lordosis behavior is initiated easily by applying tactile stimuli to the skin of the flanks, posterior rump, tailbase, and perineum (e.g., for the rat: Kow et al., 1979; and for the cat: Michael, 1961).

It is not known how lordosis behavior is mediated from the mesencephalic region to motoneurons that innervate the muscles involved in this behavior. Direct projections from the PAG to the spinal cord have been shown in the cat by Mouton and Holstege (1994). These projections terminate in the medial ventral horn throughout the length of the spinal cord, and not specifically on lordosis motoneurons, many of which are located in lateral portions of the ventral horn. The PAG has been proposed to have a tonic effect on the gigantocellular and the lateral vestibular nuclei, which in turn control the spinal cord (Pfaff and Schwartz-Giblin, 1988; Ogawa et al., 1991). Lesions in these areas affected lordosis (Modianos and Pfaff, 1976), whereas stimulation activated deep lumbar axial muscles involved in lordosis (Schwartz-Giblin et al., 1984; Cottingham et al., 1988). The lateral vestibular nucleus and the paragigantocellular nucleus project to the ventromedial parts of the intermediate zone throughout the entire length of the spinal cord, but not specifically to lumbar levels (Holstege and Kuypers, 1982). Furthermore, lordosis behavior not only involves the activation of axial, but also of hindlimb muscles (Martinez-Gomez et al., 1992; Cueva-Rolon et al., 1993). The specific PAG-NRA-lumbosacral motoneuronal pathway presented in this study, involves both axial and specific hindlimb muscles and therefore might form the final common pathway for lordosis behavior.

**Epilogue**

In the present study, both retro- and anterograde WGA-HRP experiments were done in female cats, which showed no signs of estrus behavior. The anterogradely labeled NRA fibers of six sections had to be condensed into one drawing in order to produce the pattern as presented in Figure 8. Since female cats only display lordosis behavior in pro-estrus or estrus (Michael, 1961), in a new series of experiments the NRA-lumbosacral projections was compared in estrus versus anestrus cats. Preliminary results showed marked differences in the density of the NRA-lumbosacral projection in estrus and anestrus cats; in the estrus cat NRA fibers were heavily labeled and could be easily observed in a single section (VanderHorst and Holstege, in preparation). These results present further evidence for the concept that the PAG-NRA-lumbosacral pathway is the final common pathway for lordosis. The NRA in this concept would serve as a relay within the final common pathway from the PAG to motoneuronal cell groups for vocalization as well as lordosis (Fig. 14).

![Figure 14](image_url)  
Schematic representation of the concept for the final common pathways for vocalization and lordosis.