Published in final edited form as:

Neuroscience. 2008 January 24; 151(2): 505–517. doi:10.1016/j.neuroscience.2007.10.025.

RETICULOSPINAL PATHWAYS IN THE VENTROLATERAL FUNICULUS WITH TERMINATIONS IN THE CERVICAL AND LUMBAR ENLARGEMENTS OF THE ADULT RAT SPINAL CORD

W. R. REED, A. SHUM-SIU, and D. S. K. MAGNUSON*

University of Louisville School of Medicine, Departments of Neurological Surgery and Anatomical Sciences and Neurobiology, 511 South Floyd Street, MDR Room 616, Louisville, KY 40292, USA

Abstract

In the mammalian spinal cord, the ventrolateral funiculus (VLF) has been identified as critical to postural control and locomotor function, in part due to the reticulospinal pathways it contains. The primary purpose of this descriptive study was to investigate the distribution of neurons in the medulla labeled retrogradely from the VLF and the intermediate gray matter of specific lumbar and cervical spinal cord segments in the adult rat.

We made discrete injections of Fluoro-Ruby (FR) into the intermediate gray matter at the cervical (C) 5/6, 7/8 or lumbar (L) 2 segmental levels followed by a single injection of Fluoro-Gold (FG) into the right VLF at T9. Double-labeled medullary neurons were found primarily in the gigantocellular group of nuclei (Gi), distributed both ipsilaterally and contralaterally following cervical or lumbar FR injections. In addition, a substantial population of neurons contained within the vestibular group of nuclei was double labeled both ipsilaterally and contralaterally. We also identified a substantial population of Gi-related neurons located ipsilateral to the VLF injections that were double labeled following left unilateral FR injections at C5/6, C7/8 or L2.

These results describe a substantial population of ipsilateral and commissural medullary neurons that project to both cervical and thoracolumbar segments. Two different populations of commissural neurons are described, one with axons that cross the midline rostral to T9, and one with axons that cross the midline caudal to T9. These observations provide strong additional evidence for a pattern of reticulo- and vestibulospinal projections that include substantial numbers of commissural neurons and project to multiple cervical and thoracolumbar levels.

Keywords

locomotion; retrograde tracing; medulla; Fluoro-Ruby; Fluoro-Gold; commissural reticulospinal neurons

Traumatic injury to the spinal cord results in a correlative loss of motor control as a consequence of the amount and location of gray and white matter tissue damage (Magnuson et al., 2005; Cao et al., 2005; Basso et al., 1996, 1995; Noble and Wrathall, 1989). While the isolated spinal cord has been shown to contain central pattern generator (CPG) circuitry capable of creating alternating rhythmic bursting activity (Ballion et al., 2001; Kjaerulff and Kiehn, 1996; Cazalets et al., 1992; Smith and Feldman, 1987), the mesencephalic locomotor region (MLR) appears

to be responsible (at least in part) for the initiation of mammalian locomotion (Shik et al., 1966; Noga et al., 1991). Output cells from the basal ganglia are thought to control MLR activity through inhibitory input, such that inhibition of these output cells results in the dis-inhibition of MLR neurons, subsequently activating pathways traveling through the pontomedullary medial reticular formation (PMRF; Garcia-Rill and Skinner, 1987) and down to the lumbar spinal cord via reticulospinal axons traveling within the ventrolateral funiculus (VLF; Steeves and Jordan, 1980, 1984; Jordan, 1986, 1991, 1998; Noga et al., 1991; Magnuson and Trinder, 1997). The PMRF is also host to reticulospinal neurons that contribute to postural control and stability during reaching (Schepens and Drew, 2004) and walking in cats (Prentice and Drew, 2001) and lordosis in rats (Kow and Pfaff, 1982; Robbins et al., 1992).

Potential targets for these descending systems have been identified and characterized by a number of authors including Jankowska and colleagues (Cavallari et al., 1987; Edgley and Jankowska, 1987a,b) as lumbar (L3–L4) spinal cord interneurons in the cat that receive input from group II muscle afferents and project onto more caudal (L7) motor neurons. Group II interneurons located in the intermediate gray matter of the L4 segment exhibit rhythmic activity during fictive locomotion and receive short-latency input from reticulospinals (Shefchyk et al., 1990).

Krutki and colleagues (2003) recently showed, in the cat, that some commissural interneurons in the lumbar enlargement, thought to play critical roles in the bilateral coordination of locomotor activity, receive convergent input from both vestibulo- and reticulospinal axons. Interestingly, these experiments uncovered a sub-group of interneurons located in the intermediate gray matter that received monosynaptic input from medullary neurons in the medial vestibular nucleus. The authors speculate that the majority of these interneurons are located in the most rostral segments of the lumbar enlargement where the majority of medial vestibulospinal neurons terminate.

Commissural interneurons located in the more rostral segments of the rat lumbar enlargement have been investigated recently using the neonatal spinal cord *in vitro* preparation. Techniques permitting the stimulation of entire hemicords simultaneous with intracellular and ventral root recordings, have allowed for the unequivocal identification of commissural interneurons with ascending, descending or bifurcating axons with either direct or indirect input onto flexor or extensor hindlimb motoneurons (Butt and Kiehn, 2003). These types of experiments have identified several different classes of commissural interneurons, based on their pharmacology, including those with direct glutamatergic or glycinergic, or indirect GABAergic input onto motoneurons (Butt and Kiehn, 2003; Quinlan and Kiehn, 2007). While the majority of these interneurons have been found to be rhythmically active during drug-induced locomotor-like activity, one class, characterized in the mouse by Zhong et al. (2006) as having bifurcating axons with ascending and descending branches, was consistently quiet. Taken together, these studies suggest that ventromedially located commissural interneurons are potential targets for descending locomotor-related axons and potential sources of short and long propriospinal pathways (Kiehn, 2006).

Schucht et al. (2002) demonstrated that as little as 5% spared ipsilateral ventral quadrant white matter can preserve sweeping movements of the hindlimb while 15–20% sparing preserves a normal body position at stance with hindlimb weight-support, interlimb coordination, and plantar placement of the hind paw during stepping. Thus, substantial postural and locomotor recovery following spinal cord injury (SCI) appears to be dependent on the sparing of reticulo-, vestibulo-, rubro- and/or long propriospinal axons descending in the ventral quadrant white matter (Schucht et al., 2002; Basso et al., 2002; Matsuyama et al., 1988; Mitani et al., 1988). However, the intermingled nature of these long descending pathways within the ventral

quadrant makes it extremely difficult to lesion or trace a specific tract and ensures that each incomplete SCI results in damage to a unique number and pattern of axons.

Further complicating our understanding of the tracts necessary to achieve significant locomotor recovery post-SCI is the fact that contusion injuries to the spinal cord have been shown to damage sub-populations of locomotor/postural-related neurons differently. Rubrospinal, vestibulospinal, and long propriospinal axons have been shown to withstand damage sustained from the clinically relevant contusion SCI model much better than either corticospinal or short propriospinal axons (Conta and Stelzner, 2004; Basso et al., 2002; Basso, 2000; Hill et al., 2001). This innate differential in sparing capacity is most likely the result of several factors which include, but are certainly not limited to, the amount of axonal collateralization known to exist in both cervical and lumbar spinal enlargements among many of these long ascending and descending ventral quadrant neurons (Wolstencroft, 1964; Abzug et al., 1973; Peterson et al., 1975; Huisman et al., 1981; Manaker et al., 1992).

Therefore, in this second part of a series of descriptive studies, we have chosen to extend our investigation to include the medullary origin of long descending axons traveling through the ipsilateral VLF at T9 in the adult rat spinal cord. In our previous report investigating inter-enlargement pathways (Reed et al., 2006), we showed that while the majority of ascending inter-enlargement pathways are commissural in nature, with axons that cross caudal to T9, there also exists a substantial ascending ipsilateral inter-enlargement cell population. We also found a substantial population of long-descending propriospinal neurons, both ipsilateral and commissural, with cell bodies located throughout the intermediate and ventral gray matter of the cervical enlargement. The VLF remains the focus of the current investigation due to its perceived importance to postural stability and locomotion in the normal and injured spinal cord (Steeves and Jordan, 1980; Robbins et al., 1990; Noga et al., 1991; Matsuyama and Drew, 2000; Prentice and Drew, 2001; Schucht et al., 2002; Matsuyama et al., 2004).

EXPERIMENTAL PROCEDURES

Dissection and tracer injections

All procedures used in this study were in strict accordance with the policies of the Association for the Assessment and Accreditation of Laboratory Animal Care, International, and were approved by the Institutional Animal Care and Use Committee at the University of Louisville. In keeping with these policies, all efforts were made to reduce the numbers of animals used in this study and to minimize the potential for pain and suffering. As described previously (Reed et al., 2006), 16 adult female Sprague-Dawley rats were anesthetized with sodium pentobarbital (50 mg/kg) and a single level laminectomy was performed at C5, C7 or T13. The dura was opened and each rat received two injections of Fluoro-Ruby (FR), separated by 1.5 mm rostrocaudally, either unilaterally (cervical (C) 5/6, FR 7; C7/8, FR 10; L2, FRL 2) or bilaterally (C5/6, FR-FG 1, 2, 3; C7/8, FR 6; L2, FRL 3, 4) into the deep intermediate gray matter (lamina VII). FR was injected as a 10% solution in 0.5 μ l volumes at each site with the exception of FR-FG 3, which received 0.25 μ l FR at each site. Three weeks later each animal received a T8 laminectomy followed by a single injection of Fluoro-Gold (FG; 0.3 μ l of 0.5%) into the right VLF at T9. After each injection, the micropipette (25 μ m diameter) remained in place for 5 min to reduce leakage of the tracer into the pipette track. After a period of 72 h, the animals were killed by an anesthetic overdose (sodium pentobarbital, 90 mg/kg) and transcardially perfused with 500 ml of 0.1 M phosphate buffer, pH 7.4, containing 4% paraformaldehyde. Seven preparations were excluded from the analysis either because the FR injections were not contained within the segmental gray matter, the unilaterally injected FR had diffused contralaterally, or the FG-injection site was outside of the ipsilateral VLF. The extent of tracer diffusion was estimated by the appearance of label in non-neuronal cells. These were restricted to the ventrolateral and lateral white matter and ventral horn gray matter following FG injection

or the gray matter following bilateral or unilateral FR injections in all preparations included in the study. Regardless of diffusion, FG uptake and transport is thought to be most robust in damaged axons at the site of tracer pressure injection (Reed et al., 2006; Burstein et al., 1990; Schmued et al., 1986).

Histochemical procedures

In all preparations, the spinal cords were removed, post-fixed overnight and transferred to 30% sucrose for 2–4 days at 4 °C. Transverse spinal cord sections were prepared at 45 μ m on a cryostat, and were mounted onto glass slides (Fisher Scientific, Pittsburgh, PA, USA) in five sets. One set of slides representing every fifth section (225 μ m apart) was hydrated and directly coverslipped with DPX medium (Sigma-Aldrich, St. Louis, MO, USA). An adjacent set of sections was stained with Cresyl Violet to aid in the determination of brainstem levels and nuclei according to the cytoarchitectural descriptions of Paxinos and Watson (1986). In total, nine animals were evaluated for the presence of labeled (single FR, single FG, double FR-FG) neurons within the caudal medulla as described previously for the cervical and lumbar enlargements (Reed et al., 2006). Photomicrographs were taken of one complete set of sections, each separated by 225 μ m, with a Spot RT CCD digital camera (Diagnostic Instruments, Sterling Heights, MI, USA) on a Nikon Eclipse microscope using ultraviolet (UV) or rhodamine epifluorescent filters and Adobe Photoshop (Adobe Systems, San Jose, CA, USA) running on a Macintosh G4 computer. Labeled cell bodies (single FR, double FR-FG) were counted and then traced using a Wacom drawing tablet (Intuos Inc., Vancouver, WA, USA). Single FG-labeled neurons were counted but not individually traced. Drawings from five sections (representing 1.125 mm of medulla), bracketing each brainstem level from –10.8 to –13.8 mm from Bregma, were then superimposed to illustrate the rostral–caudal distribution of labeled neurons. The total length of medulla exceeded 4.5 mm, extending rostral and caudal from the –10.8 and –13.8 mm levels (from Bregma). Total counts of FR-labeled neurons and the population of FR-labeled cells that also contained FG (FR-FG double-labeled) were compared using the appropriate two-way ANOVA analyses. Labeled cells from related sub-nuclei were combined for the gigantocellular (Gi, lateral paragigantocellular (LPGi), DPGi, GiA) and vestibular (VE, MVePC, MVeMC, SuVe, LVe, VeCB) nuclei. Comparisons were also made between injection sites [cervical and lumbar] and sides [right, left] of the spinal cord FR-injections.

RESULTS

Medullary cells of origin

The medulla from each preparation was evaluated for the presence and distribution of FG, FR and double FR-FG retrogradely-labeled cell bodies counted in every fifth section from approximately –9.8 mm, to just rostral to the –14.6 mm level (from Bregma; Paxinos and Watson, 1986). Counts represent approximately 4.9 mm of medulla and will be shown on schematics adapted from Paxinos and Watson (1986) representing the –10.8, –11.8, –12.8 and –13.8 mm (from Bregma). Cells that contained FG only (blue) possessed axons or axon collaterals that descended within the right VLF at least to the level of T9 and terminated outside of the region of gray matter that received the FR injections. Double FR-FG-labeled cells (pink) possessed axons or axon collaterals terminating in either the cervical (C5/6, C7/8) or lumbar (L2) intermediate gray matter and axons or collaterals that passed caudal to the T9 level in the right VLF. Neurons that were labeled with FR only (red) possessed axons or axon collaterals terminating in either the cervical (C5/6, C7/8) or lumbar (L2) intermediate gray matter (Table 1) and for the latter, axons that passed caudal to the T9 level outside the right VLF.

Fig. 1 shows the relationship between the FG-only and FR-FG double-labeled cells in the medulla divided into right and left sides. Data are presented as the total number of FR-FG

double-labeled cells (black bars) with the proportion of FG-only (A and B) shown by the gray bars. Approximately one-third ($33.4\% \pm 13$, $n=9$) of all cells labeled with FG from the right VLF are commissural in nature with axons that cross the midline rostral to T9 (Fig. 1). Fig. 1A shows that approximately 30% of the FG-labeled commissural neurons (L side) were also labeled with FR injected at C5/6 (black bars compared with gray bars) suggesting that they innervated both cervical and thoracolumbar segments. In contrast, only 14% of the commissural neurons (L side) were double-labeled following FR injections into the intermediate gray matter at C7/8 (FR 6) or L2 (FRL 3 and 4). Ipsilateral double-labeling (cell bodies on the right side) was 11 and 17% from L2, 21% from C7/8 and 28 and 34% from C5/6 injections.

Fig. 1B shows the raw FG and FR-FG counts for the remaining four preparations included in the study. Low volume, bilateral injections of FR at C5/6 ($0.5 \mu\text{l}$ total; FR-FG 3) still managed to label 19% of commissural (L side) and 25% of ipsilateral right VLF-related neurons. Of note, gray matter injections into the left side (unilateral) at C5/6 (FR 7), C7/8 (FR 10) or L2 (FRL 2) resulted in only 5% double labeling ipsilateral to the right T9 VLF injections (R side) and only 3–4% double labeling contralaterally (L side), indicating that very few medullary neurons had axons that crossed the midline caudal to T9.

Tables 1, 2 and 3 show the entire data set represented as the ratios of double FR-FG-labeled neurons over the total number of FR-labeled neurons that were identified in medullary nuclei. The percentages of double-labeled neurons for each group of nuclei are shown beneath each column (double FR-FG labeled over FR labeled $\times 100$). Following bilateral injections of FR into the cervical or lumbar spinal cord (Tables 1, 2 and 3) there were no right–left differences in the numbers of FR-labeled cells in any nucleus or group of nuclei assessed (vestibular, paramedian reticular (PMn), MdD or medullary reticular ventral (MdV), Gi-related; $P>0.05$), or with all nuclei combined. However, there were more FR-labeled cells within the medulla following cervical FR injections ($n=6$) than following lumbar FR-injections ($n=3$; $P<0.05$). The number of labeled neurons tended to be low at the spinal cord–medulla junction and increased through the -12.8 and -11.8 mm (mid-medulla) levels. Large numbers of FR-labeled neurons were found in the Gi-related nuclei throughout the mid-medulla and in the vestibular group of nuclei in the most rostral sections assessed (just caudal to the -9.8 mm level, represented on the -10.8 mm schematic). The rostrocaudal distribution of FR-labeled neurons following cervical or lumbar bilateral injections can be inferred from Tables 1–3, and examples are shown in Figs. 2 and 3.

Following cervical FR-injections, double FR-FG-labeled neurons had to possess axon collaterals terminating in the intermediate gray matter of C5/6 or C7/8 in addition to long descending axons or axon collaterals that extended caudal to T9 in the right VLF (Figs. 2 and 3). Only small numbers of double-labeled cells were found at the spinal cord–medulla transition zone in these preparations, with most of these being located ipsilateral to the T9 FG injection site. Noticeably more double-labeled cells were found at the -10.8 mm level following the C5/6 injections than in the single preparation that received bilateral injections into C7/8, and the latter resulted in only sparse labeling of the vestibular group of nuclei. These differences between C5/6 and C7/8 will need to be confirmed in a larger study. Significant numbers of commissural neurons (double-labeled, located on the left) were observed in the Gi-related nuclei following C5/6 or C7/8 FR injections. As mentioned earlier, the number of neurons labeled from FR injections into the intermediate gray matter at C5/6 or C7/8 exceeded that following injections into L2 (Tables 1, 2 and 3), however, when counts of double-labeled neurons within the Gi-related nuclei only were compared, there were no significant differences between cervical and lumbar preparations ($P>0.05$).

As anticipated, there were more double-labeled cells ipsilateral (right) to the FG-injection site within the Gi-related nuclei ($P<0.05$; Tables 1, 2 and 3). While the majority of double-labeled cells were located within the Gi nucleus itself, smaller numbers of double-labeled cells were also found within the LPGi, gigantocellular reticular ventral (GiV), PMn, MdV, and vestibular nuclei (Tables 1, 2 and 3; Figs. 2 and 3). Since there were negligible numbers of double-labeled cells among the mid-line raphe-related nuclei, no statistical analyses were performed on these nuclei.

Animals receiving unilateral (left) cervical or lumbar FR-injections (FR 7,10, FRL 2) along with subsequent right thoracic (T9) FG-injections showed significant numbers of double-labeled neurons in the Gi-group of nuclei, ipsilateral to the FG injection site (Tables 2 and 3). Very few double-labeled neurons were found contralaterally. In addition, left unilateral injections of FR at L2 (FRL 2), but not at C5/6 or 7/8, resulted in a number of double-labeled cells being found in the MVeMC nucleus (Table 2).

Large numbers of double-labeled neurons were observed throughout the entire length of medulla following C5/6 FR injections (Figs. 1 and 2). Following L2 FR injections, however, very few double-labeled neurons were found at the more caudal levels (−13.8 and −12.8 mm; Fig. 3). These patterns suggest that the differences between C5/6 and L2 are substantial in the caudal medulla and less so in the mid- and rostral medulla. The one preparation that received bilateral injections into C7/8 (FR-6) was found to have substantial numbers of double-labeled neurons located ipsilaterally at −12.8, but significant numbers of commissural neurons were not seen until the −11.8 and −10.8 levels (Table 3). This observation will need to be confirmed in a larger study.

DISCUSSION

The distribution of reticulospinal neurons in the medulla was nicely described for the cat by Torvik and Brodal (1957), and details of this major descending system have been added by numerous authors over the last 50 years. For example, we now know that sub-populations of raphe-, vestibulo-, rubro- and reticulospinal neurons have axons or collaterals that terminate in both the cervical and lumbar spinal enlargements (Wolstencroft, 1964; Abzug et al., 1974, 1973; Peterson et al., 1975; Huisman et al., 1981; Manaker et al., 1992) and that many of the reticulospinal neurons innervating both enlargements do so at multiple cervical and lumbar segmental levels (Takakusaki et al., 1994; Kakei et al., 1994). In addition, Giovanelli Barilari and Kuypers (1969) compared the terminal distributions of long propriospinal fibers interconnecting the enlargements with those of various descending supraspinal pathways and found substantial overlap with reticulospinal fibers along with a significant but more varied overlap with terminals of vestibulo-, tecto-, and interstitiospinal fibers.

Functionally, the VLF contains the majority of reticulospinal axons, and has been implicated as a descending locomotor command pathway in a number of mammalian preparations (for review see Jordan, 1986, 1991, 1998). Jankowska and colleagues (Jankowska et al., 2006, 2005b, 2003; Cabaj et al., 2006; Krutki et al., 2003) have demonstrated both direct and indirect coupling of commissural and ipsilaterally projecting vestibulospinal and reticulospinal neurons with identified commissural interneurons in the lumbar enlargement of the cat. These authors suggest that reticulospinal and vestibulospinal inputs are involved in the bilateral control of locomotion and postural control. The retention of coordinated flexor-extensor rhythmic activity following thoracic lateral hemisections (Bonnot et al., 2002; Kjaerulff and Kiehn, 1997) or incomplete thoracic laceration spinal cord injuries (Schucht et al., 2002) indicates that sparing of only 15–20% of the ipsilateral ventral/ventrolateral white matter can mediate recovery of normal posture at stance and weight-supported plantar stepping with some interlimb coordination. Thus, the relationship between the ventral white matter at T9 (a standard location

used in many animal models of SCI), and the reticulospinal and long propriospinal (inter-enlargement) pathways contained therein remains the subject of our current investigation (Reed et al., 2006).

For these studies (Reed et al., 2006) we chose to use small volumes of the retrograde tracers FG (0.3 μ l) and FR (0.5–2.0 μ l) to identify and locate propriospinal and reticulospinal neurons with axons/collaterals or terminals in specific regions of spinal cord gray matter and the VLF. This strategy was chosen in an effort maximize FR uptake and transport by terminals at the respective segmental levels, and to minimize both FR and FG labeling of undamaged axons of passage (Schmued and Fallon, 1986; Burstein et al., 1990). The intent of the FG injections was to limit labeling to VLF fibers. As a result, we anticipate that the labeled neurons represent only a subset of the total and that some variability is inherent in this design. The approach did allow us to easily identify and exclude preparations in which the FG-injection sites were located outside the VLF or where labeling of non-neuronal cells indicated significant diffusion of FG to outside the target area. Using this strategy we showed previously (Reed et al., 2006) that a substantial population of lumbar neurons, located in the intermediate and ventral gray matter and likely numbering in the thousands, has axons that ascend in the right VLF at least to the level of T9. Approximately 60% of these neurons are commissural in nature with axons that cross the midline caudal to T9, sharing characteristics of the lumbar commissural neurons that receive direct reticulospinal input described by Matsuyama et al. (2004). A smaller population of VLF-related neurons was described in the cervical enlargement, and again, the majority of these (~65%) were found to be commissural in nature having axons that crossed the midline rostral to T9. Importantly, 13% of the cervical neurons and 3.5% of the lumbar neurons were also retrogradely labeled with FR that was injected into the intermediate gray matter at L2, C5/6 or C7/8 (as appropriate), indicating that an overall population of 500 or more neurons in each enlargement has axons or collaterals that pass through the right VLF and axons or terminals in the intermediate gray matter of a restricted region of the corresponding enlargement. For the present study, we have analyzed the populations of labeled neurons in the caudal and mid-medulla from the same preparations described earlier (Reed et al., 2006) allowing us to relate the numbers and distributions of VLF and enlargement-related reticulospinal neurons found to the previously identified populations of long propriospinal (inter-enlargement) neurons.

The major findings of the current investigation can be divided into three primary areas of interest. First of all, large populations of reticulospinal neurons were labeled from single, discrete injections of FG into the right VLF at T9, with up to 30% being commissural in nature. Secondly, large numbers (20–35%) of reticulospinal neurons with axons/terminals in the intermediate gray matter at C5/6 or C7/8, also have axons that descend in the VLF at least to T9. Finally, while the groups of medullary nuclei labeled from C5/6, C7/8 or L2 involve substantial overlap, a few nuclei with significant numbers of labeled neurons appear to be unique to each of the segmental levels examined.

Reticulospinal neurons labeled from the VLF at T9

As anticipated, we found large numbers of neurons throughout the medulla that were retrogradely labeled with FG from the right VLF at T9. Overall, between 6000 and 8500 neurons were labeled from single VLF injections of FG, estimated as five times the totals we counted in every fifth section taken from the region of medulla examined that extended from approximately –9.8 mm from Bregma to the spinal cord–medulla junction. Fehlings and Tator (1995) estimated the total number of myelinated axons at the T1 spinal cord segment in adult Wistar rats to be approximately 375,000, which is roughly in agreement with earlier studies by Blight (Blight, 1983; Blight and Decrescito, 1986) in cats and guinea pigs. These data allow us to speculate that the ventrolateral quadrant of thoracic spinal cord should consist of

approximately 100,000 myelinated axons. Thus, in the present study, the successfully FG-labeled reticulospinal axons, assuming they are myelinated (Noga et al., 1995), should comprise somewhat less than 10% of the total number of myelinated axons present in the ventrolateral quadrant. Care was taken during counting to include only those cells with well-delineated neuronal profiles in non-consecutive sections (separated by 225 μ m), however, for this estimation we made no effort to correct for this potential source of error. Approximately 30% of the total number of FG-labeled neurons were found to be commissural, allowing us to speculate that approximately 30% of the axons in the ventrolateral white matter arise from commissural reticulospinal neurons. The existence of such pathways is supported by Jankowska et al. (2006) who describe double-crossed pathways involving commissural reticulospinal neurons along with their ability to activate hindlimb motoneurons caudal to an ipsilateral thoracic spinal cord hemisection. Matsuyama et al. (2004) also emphasized the point that monosynaptic reticulospinal inputs (originating from either side of the brainstem) can coordinate bilateral CPG-related activity via spinal commissural interneuronal circuits in the cat spinal cord.

Reticulospinal-VLF neurons labeled from C5/6 or C7/8

Injections of FR at C5/6 and C7/8 labeled from 2500–3700 reticulospinal neurons. Of these populations, just under 50% had ipsilateral axons that descended in the VLF at least to the T9 level allowing them to be double-labeled with FG (Tables 1 and 3). For the C5/6 preparations, approximately 30% of the commissural reticulospinal neurons also had axons that extended to T9 and were FG labeled. However, only 13% of the commissural neurons were labeled in the lone C7/8 preparation that received bilateral FR injections. In contrast to long descending commissural propriospinal neurons (Reed et al., 2006), which included a population with axons that crossed the midline caudal to T9, very few double-labeled neurons were found following unilateral injections of FR into the left intermediate gray matter at C5/6 or C7/8. This finding indicates that few, if any commissural reticulospinal neurons have axons that re-cross the midline caudal to T9. This agrees nicely with the description of long descending, commissural reticulospinal neurons (Jankowska et al., 2006), which have axons that cross the midline rostral to a T12 hemisection and innervate lumbar interneurons, which in turn synapse directly onto motoneurons. In the present study, the vast majority of neurons labeled from C5/6 and C7/8 were contained in the gigantocellular group of nuclei. Overall, these data suggest that a substantial minority of all reticulospinal neurons originating from the gigantocellular group of nuclei, whether they be ipsilaterally or contralaterally projecting, innervates both cervical and thoracolumbar spinal cord segments. Our data are in good general agreement with those of many earlier papers (Wolstencroft, 1964; Abzug et al., 1973; Peterson et al., 1975; Huisman et al., 1981; Manaker et al., 1992) but provide additional details on the ratio of commissural to ipsilateral neurons and their relationship with the VLF at T9, a common injury site for rat models of SCI.

Reticular nuclei labeled from C5/6 or L2

The distributions of double-labeled reticulospinal neurons with terminals at C5/6 or L2 have many similarities, in particular with respect to the gigantocellular group of nuclei. Interestingly, however, both levels have some unique relationships with nuclei outside the gigantocellular group. Labeling from C5/6 included substantial populations of ipsilateral neurons within two of the vestibular nuclei (LVe and MVeMC) and a significant number of LVe neurons, contralaterally. These nuclei were also represented following FR injection at L2. The single preparation that received bilateral injections of FR at C7/8 (FR-6; Table 1) had few double-labeled neurons in these nuclei. Using *Phaseolus vulgaris*-leucoagglutinin tracing techniques, Kuze et al. (1999) found segment-specific termination patterns of vestibulospinal fibers within the cervical, thoracic, and lumbar spinal cords, suggesting a functional relationship with segmental spinal circuitry. Comparing the labeling of C5/6 preparations with the single C7/8

preparation in the present study supports the Kuze et al. findings, however a larger study will need to be completed to confirm these observations. Nonetheless, both vestibulo- and reticulospinal pathways appear to be able to mediate coordinated right-left limb movements, with similar actions resulting from stimulation of either nucleus (Liu and Jordan, 2005; Krutki et al., 2003; Jankowska et al., 2003; Gossard et al., 1996; Grillner et al., 1971, 1970).

In addition to the vestibulospinal pathway, two of the three bilateral L2 preparations had double-labeled neurons located in the contralateral intermediate reticular nucleus which was only sparsely labeled in all six preparations that received cervical injections. Notably, the lateral reticular nucleus (IRt) had only three double-labeled neurons in one C5/6 preparation and none in any of the L2 preparations, despite expectations that the VLF at T9 carries long descending axons originating in the IRt (Antonino-Green et al., 2002). The LPGi, that lies just rostral to the IRt, had significant numbers of double-labeled neurons in the bilateral L2 preparations and confusion between those two nuclei in the neonatal brainstem/spinal cord preparation may have contributed to the different expectations.

Relationship to propriospinal pathways

The distribution of double-labeled neurons following bilateral FR injections at cervical or lumbar levels indicates that the reticulospinal axons traveling in the VLF are in many respects similar to the long (inter-enlargement) propriospinal system (Giovannelli Barilari and Kuypers, 1969; Reed et al., 2006). Jankowska et al. (2003) demonstrated that lumbar lamina VIII commissural neurons mediate crossed di-synaptic reticulospinal actions on contralateral hindlimb motoneurons while Matsuyama et al. (2004) recorded rhythmic activity from all lamina VIII commissural interneurons with bifurcating or descending axons during MLR-evoked fictive locomotion. In the present study, the vast majority of double-labeled reticulospinal neurons appear to be either entirely ipsilateral with respect to the T9 FG injection site or commissural with axons that cross the midline rostral to T9. However, our data have highlighted two additional populations of commissural reticulospinal neurons. The first is represented by a group of several hundred neurons that were double-labeled following left unilateral injections of FR into the intermediate gray matter at C5/6 or C7/8 and the standard right VLF injections of FG at T9. By definition these neurons must have an axon or collateral that crosses the midline in the cervical enlargement and an axon or collateral that descends in the right VLF at least to T9. The second, and possibly overlapping group was double-labeled following left unilateral injections of FR into the intermediate gray matter at L2 and the standard right VLF injections of FG. Again, by definition, these neurons must have axons that descend in the right VLF at least to the T9 level and then cross the midline rostral to, or at the L2 level. These same preparations revealed a class of double commissural propriospinal neurons with an axon or collateral that crosses the midline rostral to T9 and re-crosses the midline caudal to T9 (Reed et al., 2006). In the current study, only three such neurons were identified in the single unilateral L2 preparation and a total of 17 in the two unilateral cervical preparations. While we cannot rule out the possibility that our unilateral FR-injections were not completely restricted to the side of the injection, we used strict inclusion criteria and saw no evidence of direct FR labeling contralateral to the injection.

Implications for SCI

Numerous authors have attempted to relate functional outcomes following SCI to spared white matter, but very few have specifically addressed how the pattern of sparing of long descending axons may relate to functional recovery. Basso et al. (2002) compared the retrograde labeling patterns of supraspinal neurons following contusion SCI in adult rats to identify, by subtraction, descending systems that may play a role in mediating locomotor recovery in mild compared with moderate injury severities. They reported that while corticospinal and propriospinal axons were especially vulnerable to SCI and non-essential to HL-FL recovery, extensive locomotor

recovery was observed and likely attributable to the amount of sparing of long descending axons originating in the GiV, GiA, and PnV (pontine ventralis) nuclei. They went on to suggest that recovery of HL-FL coordination was most likely related to the sparing of reticulospinal axons within the ventral medulla in particular. In their study, they further reported that animals with moderate contusion injuries retained only 2–7% of FG-labeled lumbar-related neurons among the Gi, DPGi and GiA nuclei along with a much smaller decrease (40% of normal) in GiV neurons. Even among the mildly injured animals, the largest decrease (20% of normal) of brainstem-labeled neurons occurred in the Gi and DPGi nuclei (with little to no loss of GiV or GiA neurons). The results from the present study illustrate that surviving gigantocellular-related neurons include those with ipsilateral and commissural projections that likely innervate multiple targets at both cervical and lumbar levels. At least a proportion of these axons likely occupies the spared peripheral rim of tissue in the VLF and thus can subserve significant functional recovery despite the loss of the bulk of cortico-, rubro- and vestibulospinal axons (Basso et al., 2002), including those descending axons that mediate trans-cranial magnetic motor evoked potentials (Loy et al., 2002a,b; Magnuson et al., 1998).

CONCLUSION

The goal of this series of studies (Reed et al., 2006) was to further characterize the anatomical relationships between the intermediate gray matter of C6–8 and L2 with the reticulospinal and long propriospinal axons traveling in the VLF at T9. These regions are of interest due to their documented involvement in mammalian locomotion, and their potential importance to functional recovery following SCI. Our findings illustrate that the generic “reticulospinal” pathway includes a substantial population of neurons that project to both cervical and thoracolumbar segments, that it includes a substantial population of commissural neurons with axons that cross the midline rostral to T9, as well as a modest but significant number of commissural neurons with axons that cross the midline caudal to T9 and terminate in the intermediate gray matter at L2. The multisegmentally-specific innervation patterns of reticulo- and vestibulospinal neurons are well-suited to influence the output of several types of interneurons throughout the spinal cord to coordinate head, trunk, and limb movements during a variety of motor activities including locomotion. Based on these and other recent supportive findings we hypothesize that the substantial improvement in locomotor function observed following moderately-severe and severe contusive spinal cord injuries is due, in large part, to the redundant and bilaterally distributed nature of the reticulospinal system. Further, we hypothesize that the substantial numbers of reticulospinal neurons that project to multiple spinal cord segments and/or that cross the midline caudal to T9 represent heretofore under-utilized therapeutic targets for neuroprotective, regenerative and remodeling strategies following SCI.

Abbreviations

C (plus number)	cervical
CPG	central pattern generator
FG	Fluoro-Gold
FR	Fluoro-Ruby
GiV	gigantocellular reticular ventral nucleus
L (plus number)	lumbar
LPGi	lateral paragiganto-cellular nucleus
IRt	lateral reticular nucleus

MdV	medullary reticular ventral nucleus
MLR	mesencephalic locomotor region
PMn	paramedian reticular nucleus
PMRF	pontomedullary medial reticular formation
SCI	spinal cord injury
VLF	ventrolateral funiculus

REFERENCES

- Abzug C, Maeda M, Peterson BW, Wilson VJ. Cervical branching of lumbar vestibulospinal axons. *J Physiol* 1974;243:499–522. [PubMed: 4449072]
- Abzug C, Maeda M, Peterson BW, Wilson VJ. Branching of individual lateral vestibulospinal axons at different spinal cord levels. *Brain Res* 1973;56:327–330. [PubMed: 4715625]
- Antonino-Green DM, Cheng J, Magnuson DS. Neurons labeled from locomotor-related ventrolateral funiculus stimulus sites in the neonatal rat spinal cord. *J Comp Neurol* 2002;442:226–238. [PubMed: 11774338]
- Ballion B, Morin D, Viala D. Forelimb locomotor generators and quadrupedal locomotion in the neonatal rat. *Eur J Neurosci* 2001;14:1727–1738. [PubMed: 11860467]
- Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma* 1995;12:1–21. [PubMed: 7783230]
- Basso DM, Beattie MS, Bresnahan JC. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. *Exp Neurol* 1996;139:244–256. [PubMed: 8654527]
- Basso DM, Beattie MS, Bresnahan JC. Descending systems contributing to locomotor recovery after mild or moderate spinal cord injury in rats: experimental evidence and a review of the literature. *Restor Neurol Neurosci* 2002;20:189–218. [PubMed: 12515895]
- Basso DM. Neuroanatomical substrates of functional recovery after experimental spinal cord injury: implications of basic science research for human spinal cord injury. *Phys Ther* 2000;80:808–817. [PubMed: 10911417]
- Blight AR. Cellular morphology of chronic spinal cord injury in the cat: Analysis of myelinated axons by line-sampling. *Neuroscience* 1983;10:521–543. [PubMed: 6633870]
- Blight AR, Decrescito V. Morphometric analysis of experimental spinal cord injury in the cat: The relation of injury intensity to survival of myelinated axons. *Neuroscience* 1986;19:321–341. [PubMed: 3785669]
- Bonnot A, Whelan PJ, Mentis GZ, O'Donovan MJ. Locomotor-like activity generated by the neonatal mouse spinal cord. *Brain Res Brain Res Rev* 2002;40:141–151. [PubMed: 12589913]
- Burstein R, Cliffer KD, Giesler GJ. Cells of origin of the spinohypothalamic tract in the rat. *J Comp Neurol* 1990;291:329–344. [PubMed: 2298937]
- Butt SJ, Kiehn O. Functional identification of interneurons responsible for left-right coordination of hindlimbs in mammals. *Neuron* 2003;38:953–963. [PubMed: 12818180]
- Cabaj A, Stecina K, Jankowska E. Same spinal interneurons mediate reflex actions of group Ib and group II afferents and crossed reticulospinal actions. *J Neurophysiol* 2006;95:3911–3922. [PubMed: 16554505]
- Cao Q, Zhang Y, Iannotti C, DeVries W, Xu X, Shields C, Whittemore S. Functional and electrophysiological changes after graded traumatic spinal cord injury in adult rat. *Exp Neurol* 2005;191:S3–S16. [PubMed: 15629760]
- Cavallari P, Edgley SA, Jankowska E. Post-synaptic actions of midlumbar interneurons on motoneurons of hind-limb muscles in the cat. *J Physiol* 1987;389:675–689. [PubMed: 3681740]
- Cazalets JR, Squalli-Houssaini Y, Clarac F. Activation of the central pattern generators by serotonin and excitatory amino acids in the neonatal rat. *J Physiol* 1992;455:187–204. [PubMed: 1362441]

- Conta AC, Stelzner DJ. Differential vulnerability of propriospinal tract neurons to spinal cord contusion injury. *J Comp Neurol* 2004;479:347–359. [PubMed: 15514981]
- Edgley SA, Jankowska E. Field potentials generated by group II muscle afferents in the middle lumbar segments of the cat spinal cord. *J Physiol* 1987a;385:393–413. [PubMed: 3656164]
- Edgley SA, Jankowska E. An interneuronal relay for group I and group II muscle afferents in the midlumbar segments of the cat spinal cord. *J Physiol* 1987b;389:647–674. [PubMed: 3681739]
- Fehlings MG, Tator CH. The relationships among the severity of spinal cord injury, residual neurological function, axon counts and counts of retrogradely labeled neurons after experimental spinal cord injury. *Exp Neurol* 1995;132:220–228. [PubMed: 7789460]
- Garcia-Rill E, Skinner RD. The mesencephalic locomotor region. I. Activation of a medullary projection site. *Brain Res* 1987;411:1–12. [PubMed: 2440511]
- Giovanelli Barilari M, Kuypers HG. Propriospinal fibers interconnecting the spinal enlargements in the cat. *Brain Res* 1969;321:30.
- Gossard JP, Floeter MK, Degtyarenko AM, Simon ES, Burke RE. Disynaptic vestibulospinal and reticulospinal excitation in cat lumbosacral motoneurons: modulation during fictive locomotion. *Exp Brain Res* 1996;109:277–288. [PubMed: 8738376]
- Grillner S, Hongo T, Lund S. Convergent effects on α -motoneurons from the vestibulospinal tract and a pathway descending in the medial longitudinal fasciculus. *Exp Brain Res* 1971;12:457–479. [PubMed: 5093725]
- Grillner S, Hongo T, Lund S. The vestibulospinal tract. Effects on α -motoneurons in the lumbosacral spinal cord in the cat. *Exp Brain Res* 1970;10:94–120. [PubMed: 5411977]
- Hill CE, Beattie MS, Bresnahan JC. Degeneration and sprouting of identified descending supraspinal axons after contusive spinal cord injury in the rat. *Exp Neurol* 2001;171:153–169. [PubMed: 11520130]
- Huisman AM, Kuypers HGJM, Verburgh CA. Quantitative differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in rat as demonstrated with multiple fluorescent retrograde tracer technique. *Brain Res* 1981;209:271–286. [PubMed: 7225794]
- Jankowska E, Stecina K, Cabaj A, Pettersson LG, Edgley SA. Neuronal relays in double crossed pathways between feline motor cortex and ipsilateral hindlimb motoneurons. *J Physiol* 2006;575:527–541. [PubMed: 16740611]
- Jankowska E, Edgley SA, Krutki P, Hammer I. Functional differentiation and organization of feline midlumbar commissural interneurons. *J Physiol* 2005b;565:645–658. [PubMed: 15817636]
- Jankowska E, Hammar I, Slawinska U, Maleszak K, Edgley SA. Neuronal basis of crossed actions from the reticular formation on feline hindlimb motoneurons. *J Neurosci* 2003;23:1867–1878. [PubMed: 12629191]
- Jordan LM. Initiation of locomotion in mammals. *Ann N Y Acad Sci* 1998;860:83–93. [PubMed: 9928303]
- Jordan, LM. Brainstem and spinal cord mechanisms for the initiation of locomotion. In: Shimamura, M.; Grillner, S.; Edgerton, V., editors. *Neurobiological basis of human locomotion*. Japan Scientific Series; Tokyo: 1991. p. 3–20.
- Jordan, LM. Initiation of locomotion from the mammalian brainstem. In: Grillner, S.; Stein, PSG.; Stuart, DG.; Forssberg, H.; Herman, RM., editors. *Wenner-Gren Centre international symposium series, Vol. 45, Neurobiology of vertebrate locomotion*. Macmillan; London: 1986. p. 21–37.
- Kakei S, Muto N, Shinoda Y. Innervation of multiple neck motor nuclei by single reticulospinal tract axons receiving tectal input in the upper cervical spinal cord. *Neurosci Lett* 1994;172:85–88. [PubMed: 8084543]
- Kiehn O. Locomotor circuits in the mammalian spinal cord. *Ann Rev Neurosci* 2006;29:279–306. [PubMed: 16776587]
- Kjaerulff O, Kiehn O. Distribution of networks generating and coordinating locomotor activity in the neonatal rat spinal cord in vitro: a lesion study. *J Neurosci* 1996;16:5777–5794. [PubMed: 8795632]
- Kjaerulff O, Kiehn O. Crossed rhythmic synaptic input to motoneurons during selective activation of the contralateral spinal locomotor network. *J Neurosci* 1997;17:9433–9447. [PubMed: 9390999]

- Kow LM, Pfaff DW. Responses of medullary reticulospinal and other reticular neurons to somatosensory and brainstem stimulation in anesthetized or freely-moving ovariectomized rats with or without estrogen treatment. *Exp Brain Res* 1982;47:191–202. [PubMed: 7117444]
- Krutki P, Jankowska E, Edgley SA. Are crossed actions of reticulospinal and vestibulospinal neurons on feline motoneurons mediated by the same or separate commissural neurons? *J Neurosci* 2003;23(22):8041–8050. [PubMed: 12954866]
- Kuze B, Matsuyama K, Matsui T, Miyata H, Mori S. Segment-specific branching patterns of single vestibulospinal tract axons arising from the lateral vestibular nucleus in the cat: a PHA-L tracing study. *J Comp Neurol* 1999;414:80–96. [PubMed: 10494080]
- Liu J, Jordan LM. Stimulation of the parapyramidal region of the neonatal rat brain stem produces locomotor-like activity involving spinal 5-HT₇ and 5-HT_{2A} receptors. *J Neurophysiol* 2005;94:1392–1404. [PubMed: 15872068]
- Loy DN, Magnuson DS, Zhang YP, Onifer SM, Mills MD, Cao QL, Darnall JB, Fajardo LC, Burke DA, Whittemore SR. Functional redundancy of ventral spinal locomotor pathways. *J Neurosci* 2002a;22:315–323. [PubMed: 11756515]
- Loy DN, Talbott JF, Onifer SM, Mills MD, Burke DA, Dennison JB, Fajardo LC, Magnuson DS, Whittemore SR. Both dorsal and ventral spinal cord pathways contribute to overground locomotion in the adult rat. *Exp Neurol* 2002b;177:575–580. [PubMed: 12429203]
- Magnuson DSK, Trinder T. Locomotor rhythm evoked by ventrolateral funiculus stimulation in the neonatal rat spinal cord *in vitro*. *J Neurophysiol* 1997;77:200–206. [PubMed: 9120561]
- Magnuson DSK, Green DM, Sengoko T. Lumbar spinoreticular neurons in the rat: part of the central pattern generator for locomotion? *Ann N Y Acad Sci* 1998;860:436–440. [PubMed: 9928332]
- Magnuson DSK, Lovett R, Coffee C, Gray R, Han Y, Zhang YP, Burke DA. Functional consequences of lumbar spinal cord contusion injuries in the adult rat. *J Neurotrauma* 2005;22:529–543. [PubMed: 15892599]
- Manaker S, Tischler LJ, Morrison AR. Raphespinal and reticulospinal axon collaterals to the hypoglossal nucleus in the rat. *J Comp Neurol* 1992;322:68–78. [PubMed: 1385487]
- Matsuyama K, Nakajima K, Mori F, Aoki M, Mori S. Lumbar commissural interneurons with reticulospinal inputs in the cat: morphology and discharge patterns during fictive locomotion. *J Comp Neurol* 2004;474:546–561. [PubMed: 15174072]
- Matsuyama K, Drew T. Vestibulospinal and reticulospinal neuronal activity during locomotion in the intact cat. II. Walking on an inclined plane. *J Neurophysiol* 2000;84:2257–2276. [PubMed: 11067970]
- Matsuyama K, Ohta Y, Mori S. Ascending and descending projections of the nucleus reticularis gigantocellularis in the cat demonstrated by the anterograde neural tracer, Phaseolus vulgaris leucoagglutinin (PHL-A). *Brain Res* 1988;460:124–141. [PubMed: 2464400]
- Mitani A, Ito K, Mitani Y, McCarley RW. Descending projections from the gigantocellular tegmental field in the cat. Cells of origin and their brainstem and spinal cord trajectories. *J Comp Neurol* 1988;268:546–566. [PubMed: 2451685]
- Noble LJ, Wrathall JR. Correlative analyses of lesion development and functional status after graded spinal cord contusive injuries in the rat. *Exp Neurol* 1989;103:34–40. [PubMed: 2912748]
- Noga BR, Kriellaars DJ, Jordan LM. The effect of selective brainstem or spinal cord lesions on treadmill locomotion evoked by stimulation of the mesencephalic or pontomedullary locomotor region. *J Neurosci* 1991;11:1691–1700. [PubMed: 2045881]
- Noga BR, Fortier PA, Kriellaars DJ, Dai X, Detillieux GR, Jordan LM. Field potential mapping of neurons in the lumbar spinal cord activated following stimulation of the mesencephalic locomotor region. *J Neurosci* 1995;15:2203–2217. [PubMed: 7891162]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Academic Press; San Diego: 1986.
- Peterson BW, Maunz RA, Pitts NG, Mackel RG. Patterns of projection and branching of reticulospinal neurons. *Exp Brain Res* 1975;23:333–351. [PubMed: 1183508]
- Prentice SD, Drew T. Contributions of the reticulospinal system to the postural adjustments occurring during voluntary gait modifications. *J Neurophysiol* 2001;85:679–698. [PubMed: 11160503]
- Quinlan KA, Kiehn O. Segmental, synaptic actions of commissural interneurons in the mouse spinal cord. *J Neurosci* 2007;27:6521–6530. [PubMed: 17567813]

- Reed WR, Shum-Siu A, Onifer SM, Magnuson DS. Inter-enlargement pathways in the ventrolateral funiculus of the adult rat spinal cord. *Neuroscience* 2006;142:1195–1207. [PubMed: 16938403]
- Robbins A, Pfaff DW, Schwartz-Giblin S. Reticulospinal and reticuloreticular pathways for activating the lumbar back muscles in the rat. *Exp Brain Res* 1992;92:46–58. [PubMed: 1486954]
- Robbins A, Schwartz-Giblin S, Pfaff DW. Ascending and descending projections to medullary reticular formation sites which activate deep lumbar back muscles in the rat. *Exp Brain Res* 1990;80:463–474. [PubMed: 2387348]
- Schepens B, Drew T. Independent and convergent signals from the pontomedullary reticular formation contribute to the control of posture and movement during reaching in the cat. *J Neurophysiol* 2004;92:2217–2238. [PubMed: 15175364]
- Schmued LC, Fallon JH. Fluoro-Gold: a new fluorescent retrograde axonal tracer with numerous unique properties. *Brain Res* 1986;377:147–154. [PubMed: 2425899]
- Schucht P, Raineteau O, Schwab ME, Fouad K. Anatomical correlates of locomotor recovery following dorsal and ventral lesions of the rat spinal cord. *Exp Neurol* 2002;176:145–153.
- Shefchyk S, McCrea D, Kriellaars D, Fortier P, Jordan L. Activity of interneurons within the L4 spinal segment of the cat during brainstem-evoked fictive locomotion. *Exp Brain Res* 1990;80(2):290–295. [PubMed: 2358044]
- Shik ML, Severin FG, Orlovsky GN. Control of walking and running by means of electrical stimulation of the midbrain. *Biophysics* 1966;11:756–765.
- Smith JC, Feldman JL. *In vitro* brainstem-spinal cord preparations for the study of motor systems for mammalian respiration and locomotion. *J Neurosci Methods* 1987;21:321–333. [PubMed: 2890797]
- Steeves JD, Jordan LM. Localization of a descending pathway in the spinal cord which is necessary for controlled treadmill locomotion. *Neurosci Lett* 1980;20:283–288. [PubMed: 7443078]
- Steeves JD, Jordan LM. Autoradiographic demonstration of the projections from the mesencephalic locomotor region. *Brain Res* 1984;307:263–276. [PubMed: 6466996]
- Takakusaki K, Shimoda N, Matsuyama K, Mori S. Discharge properties of medullary reticulospinal neurons during postural changes induced by intrapontine injection of carbachol, atropine and serotonin, and their functional linkages to hindlimb motoneurons in cats. *Exp Brain Res* 1994;99:361–374. [PubMed: 7957716]
- Torvik A, Brodal A. The origin of reticulospinal fibers in the cat. An experimental study. *Anat Rec* 1957;128:113–137. [PubMed: 13458831]
- Wolstencroft JH. Reticulospinal neurons. *J Physiol* 1964;174:91–108. [PubMed: 14228614]
- Zhong G, Diaz-Rios M, Harris-Warrick RM. Intrinsic and functional differences among commissural interneurons during fictive locomotion and serotonergic modulation in the neonatal mouse. *J Neurosci* 2006;26:6509–6517. [PubMed: 16775138]

Fig. 1.

Total numbers of medullary neurons retrogradely labeled following injection of FG into the right VLF at T9. Counts were made in every 5th, 45 μ m section taken from rostral to the -10.8 level through to just caudal to the -13.8 mm level (from Bregma) as described by Paxinos and Watson (1986). The black portion of each bar represents the number of neurons that were also labeled with FR injected into the intermediate gray matter, at C5/6, C7/8 or L2. In A, five preparations that received similar amounts of FR (2.0 μ l) are compared directly. These injections were made bilaterally into the intermediate gray matter at C5/6, C7/8 or L2. Cell counts from the remaining four preparations are shown in B. One received a low-volume (0.5 μ l) of FR bilaterally at C5/6 and the others received 1.0 μ l injections of FR unilaterally into the intermediate gray matter on the left side at C5/6, C7/8 or L2.

C5-6 Bilateral FR Injections

Fig. 2.

Shown are single FR- (gray) and double FR-FG- (black) labeled brainstem neurons following bilateral injections of FR at C5/6 and a single injection of FG into the right VLF at T9 (preparation FR-FG2, Fig. 1). Labeled cells from five sections, each 225 μ m apart, were traced onto brainstem templates modified from Paxinos and Watson (1986).

L2 Bilateral FR Injections

Fig. 3.

Shown are single FR- (gray) and double FR-FG- (black) labeled brainstem neurons following bilateral injections of FR at L2 and a single injection of FG into the right VLF at T9 (preparation FRL 3, Fig. 1). Labeled cells from five sections, each 225 μ m apart, were traced onto brainstem templates modified from Paxinos and Watson (1986).

Table 1

Brainstem labeling from bilateral injections at C5/6 and right T9 VLF

Level	Nucleus	FR-FG 1		FR-FG 2		FR-FG 3	
		Left	Right	Left	Right	Left	Right
-10.8	VeCb	0/0	0/0	0/0	0/0	0/0	0/0
	MVePC	0/4	0/0	0/0	0/0	0/0	0/0
	MVeMC	3/9	0/0	1/16	11/22	1/10	1/8
	Lve	15/36	19/42	8/34	9/27	0/0	0/0
	SuVe	6/14	1/3	0/5	2/4	0/3	0/0
		38%	44%	16%	42%	8%	15%
	PCRtA	0/7	0/4	0/7	3/8	2/4	0/0
	Irt	9/13	8/11	2/4	0/4	6/11	5/8
	DPGi	4/7	1/3	0/3	4/8	0/2	3/5
	Gi	12/26	32/40	9/26	26/37	8/36	23/42
-11.8	LPGi	0/0	2/2	0/0	0/0	0/1	1/2
	GiA	0/2	0/0	0/2	4/5	0/0	0/0
		52%	77%	31%	63%	28%	58%
	MVePC	0/1	1/2	0/1	0/0	1/2	0/0
	Pr	0/0	0/0	0/0	0/0	0/0	0/0
	MVeMC	0/0	0/1	5/5	0/3	1/2	0/0
	SpVe	0/12	0/3	0/5	0/5	0/5	0/0
		0%	17%	45%	22%		
	PCRt	0/2	0/2	0/2	0/4	0/0	0/0
	Irt	0/1	1/1	0/1	0/3	0/0	0/1
-12.8	DPGi	2/20	0/5	4/10	0/4	3/16	1/15
	Gi	18/99	18/37	15/55	43/81	20/68	36/63
	LPGi	0/1	0/0	0/1	0/2	0/0	0/0
	GiV	3/13	8/10	0/7	9/13	0/1	3/8
		17%	49%	25%	49%	27%	46%
	Mve	0/0	0/2	0/0	0/0	0/0	0/0
	Cu	0/4	0/1	2/4	0/3	0/0	0/0

Level	Nucleus	FR-FG 1		FR-FG 2		FR-FG 3	
		Left	Right	Left	Right	Left	Right
		0%	0%	50%	0%		
	PCRt	2/2	0/3	0/2	0/0	0/0	0/0
	Irt	0/1	0/0	2/6	1/7	0/4	0/4
	DPGi	0/1	0/0	0/0	0/0	0/1	0/1
	Gi	1/17	6/17	0/27	11/31	3/47	21/47
	PMIn	9/25	12/22	2/10	17/32	1/7	26/32
	LPGi	0/0	2/7	0/0	6/10	4/5	0/2
	GiV	0/4	17/23	4/8	13/16	0/6	4/4
	24%		51%	15%	50%	11%	57%
-13.8	MdD	0/2	3/8	0/13	2/6	0/4	0/5
	IRt	1/4	2/5	1/11	1/8	0/7	0/3
	MdV	9/25	45/82	0/26	6/20	3/21	16/24
	LRt	0/0	3/3	0/0	0/0	0/0	0/0
	PMIn	10/18	20/35	0/5	12/17	1/5	4/6
	49%		55%	2%	38%	11%	53%

Table 2

Brainstem labeling from uni- and bilateral injections at L2 and right T9 VLF

Level	Nucleus	FRL 2 (Uni)		FRL 3 (Bi)		FRL 4 (Bi)	
		Left	Right	Left	Right	Left	Right
-10.8	VeCb	0/2	0/0	0/1	0/0	1/2	3/3
	MVePC	0/1	1/2	0/1	0/0	1/2	0/0
	MVeMC	0/15	14/19	0/2	1/4	15/17	7/8
	Lve	0/11	0/0	2/15	4/5	3/4	18/21
	SuVe	0/3	0/5	0/12	0/1	0/0	4/4
		0%	58%	6%	50%	80%	89%
	PCRtA	0/0	0/0	0/3	0/0	0/0	6/6
	Irt	0/3	0/0	0/12	0/0	1/1	1/1
	DPGi	1/8	3/6	0/0	0/1	3/3	0/0
	Gi	1/9	11/22	5/40	10/22	17/27	6/16
	LPGi	0/2	0/0	0/2	0/0	0/0	0/0
	GiA	0/2	1/2	1/33	11/13	2/13	5/13
		9%	50%	7%	58%	53%	40%
-11.8	MVePC	0/0	0/0	0/0	0/0	0/0	0/0
	Pr	0/0	0/0	0/0	0/0	0/0	0/0
	MVeMC	0/0	0/0	0/0	0/0	0/0	0/0
	SpVe	0/0	0/0	0/2	0/1	0/0	0/0
				0%	0%		
	PCRt	0/0	0/1	0/0	0/0	0/0	0/1
	Irt	0/0	0/0	0/2	0/0	0/2	3/3
	DPGi	2/2	0/2	2/2	1/1	0/0	0/1
	Gi	1/14	9/17	2/21	36/53	1/16	22/33
	LPGi	0/0	0/0	0/0	1/2	0/0	11/13
	GiV	0/4	1/1	0/8	5/8	0/7	2/2
	ts	0/2	1/3	2/9	4/5	0/2	0/3
		14%	46%	14%	68%	4%	68%
-12.8	Mve	0/0	0/0	0/0	0/1	0/0	0/0

Level	Nucleus	FRL 2 (Umi)		FRL 3 (Bi)		FRL 4 (Bi)	
		Left	Right	Left	Right	Left	Right
	Cu	0/0	0/0	0/0	0/0	0/0	0/0
	PCRt	0/0	0/0	0/13	0%	0/0	0/0
	Irt	0/0	0/0	0/0	0/0	0/1	1/2
	DPGi	0/0	0/0	0/0	0/0	0/0	0/0
	Gi	1/12	2/3	3/13	2/4	1/9	7/9
	PMn	1/3	8/11	0/4	3/3	0/1	0/1
	LPGi	0/1	0/0	0/0	0/2	1/1	7/7
	GiV	0/1	0/0	1/2	1/1	3/5	0/0
		12%	71%	20%	55%	29%	79%
-13.8	MdD	0/0	0/0	0/1	0/0	0/0	1/1
	IRt	0/0	0/1	9/19	0/4	8/9	3/15
	MdV	0/1	0/1	0/4	1/8	0/2	1/4
	LRt	0/0	0/0	0/0	0/1	0/0	0/0
	PMn	0/1	0/0	0/1	0/0	0/0	0/0
		0%	0%	38%	8%	73%	25%

Table 3
Brainstem labeling from uni- and bilateral injections at C5/6 or C7/8 and right T9 VLF

Level	Nuclei	FR 6 (Bi C7/8)		FR 7 (Uni C5/6)		FR 10 (Uni C7/8)	
		Left	Right	Left	Right	Left	Right
-10.8	VeCb	0/0	0/0	0/0	0/0	0/0	0/0
	MVePC	0/5	0/0	0/3	0/0	0/0	0/3
	MVeMC	0/12	3/11	0/29	1/4	1/15	0/12
	Lve	0/8	6/15	4/18	3/9	0/22	1/2
	SuVe	0/0	0/1	0/0	0/0	0/4	0/0
		0%	33%	8%	31%	2%	6%
	PCRtA	0/2	1/6	0/5	0/3	0/6	0/2
	Irt	0/1	0/0	0/2	0/2	0/2	0/0
	DPGi	1/7	1/2	0/0	0/2	0/2	1/2
	Gi	9/31	12/25	1/32	6/20	0/8	4/13
-11.8	LPGi	0/1	3/5	0/0	0/2	0/2	0/1
	GiA	0/9	6/12	0/11	2/12	0/4	0/1
		20%	51%	2%	20%	0%	42%
	MVePC	0/1	0/0	0/1	0/0	0/3	0/0
	Pr	0/0	0/0	0/0	0/1	0/0	0/1
	MVeMC	0/1	0/0	0/1	0/3	0/0	0/2
	SpVe	0/9	0/5	0/0	0/1	0/11	0/4
		0%	0%	0%	0%	0%	0%
	PCRt	0/0	0/0	0/2	0/2	0/0	0/5
	Irt	0/1	0/0	0/0	1/3	0/0	0/2
-12.8	DPGi	4/13	3/8	0/0	2/11	0/6	1/8
	Gi	14/49	65/91	6/80	19/58	2/41	20/45
	LPGi	0/0	6/9	0/6	1/1	0/0	0/0
	GiV	0/4	11/22	0/4	4/5	0/0	0/0
		25%	76%	7%	34%	4%	35%
	Mve	0/0	0/0	0/0	0/0	0/0	0/0
	Cu	1/4	0/0	0/0	0/0	0/0	0/0

Level	Nuclei	FR 6 (Bi C7/8)		FR 7 (Uni C5/6)		FR 10 (Uni C7/8)	
		Left	Right	Left	Right	Left	Right
		25%					
	PCRt	0/0	0/1	0/1	1/1	0/0	0/0
	Irt	0/4	0/1	0/5	0/9	0/0	0/5
	DPGi	0/0	0/0	0/0	0/1	0/0	1/1
	Gi	1/23	11/25	1/26	1/12	1/28	12/21
	PMIn	1/14	2/5	0/5	0/14	0/13	1/11
	LPGi	0/1	2/3	0/0	0/3	0/0	1/2
	GiV	1/1	1/2	0/1	0/0	0/5	4/4
		5%	64%	3%	5%	2%	43%
-13.8	MdD	0/7	0/3	0/4	0/7	0/8	1/2
	IRt	0/6	0/7	0/1	4/15	0/7	3/6
	MdV	1/22	1/17	0/26	2/20	1/13	8/16
	LRt	0/0	0/0	0/0	0/0	0/1	0/0
	PMIn	0/6	0/0	0/2	0/2	0/2	1/2
		2%	4%	0%	14%	3%	50%