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

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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.

Key words: 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

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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 paragigantocellular nucleus; lRt, 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.

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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 μl volumes at each site with the exception of FR-FG 3, which received 0.25 μl FR at each site. Three weeks later each animal received a T8 laminectomy followed by a single injection of Fluoro-Gold (FG; 0.3 μl of 0.5%) into the right VLF at T9. After each injection, the micropipette (25 μm 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 μm 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 μm 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