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Reimplantation of avulsed lumbosacral ventral roots in the rat ameliorates injury-induced degeneration of primary afferent axon collaterals in the spinal dorsal columns

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Abstract

Injuries to the cauda equina/conus medullaris portion of the spinal cord can result in motor, sensory, and autonomic dysfunction, and neuropathic pain. In rats, unilateral avulsion of the motor efferents from the lumbosacral spinal cord results in at-level allodynia, along with a corresponding glial and inflammatory response in the dorsal horn of the spinal cord segments immediately rostral to the lesion. Here, we investigated the fate of intramedullary primary sensory projections following a motor efferent lesion. The lumbosacral (L6 and S1) ventral roots were unilaterally avulsed from the rat spinal cord (VRA; n=9). A second experimental group had the avulsed roots acutely reimplanted into the lateral funiculus (Imp; n=5), as this neural repair strategy is neuroprotective, and promotes the functional reinnervation of peripheral targets. A laminectomy-only group served as controls (Lam; n=7). At 8 weeks post-lesion, immunohistochemical examination showed a 42% reduction ($p<0.001$) in the number of RT97-positive axons in the ascending tracts of the dorsal funiculus of the L4-5 spinal segment in VRA rats. Evidence for degenerating myelin was also present. Reimplantation of the avulsed roots ameliorated axon and myelin degeneration. Axons in the descending dorsal corticospinal tract were unaffected in all groups, suggesting a specificity of this lesion for spinal primary sensory afferents. These results show for the first time that a lesion restricted to motor roots can induce the degeneration of intramedullary sensory afferents. Importantly, reimplantation of the lesioned motor roots ameliorated sensory axon degeneration. These data further support the therapeutic potential for reimplantation of avulsed ventral roots following trauma to the cauda equina/conus medullaris.

Keywords

Spinal cord injury; cauda equina/conus medullaris; neuroprotection; motor lesion; neurofilament; myelin

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INTRODUCTION

Injury to the cauda equina/conus medullaris (CE/CM) portion of the spinal cord may have devastating consequences for afflicted persons, including motor, autonomic, and sensory dysfunction, and neuropathic pain (Moossy et al., 1987; Sampson et al., 1995; Sindou et al., 2001). We have developed a rat model of CE/CM spinal cord injury (SCI) that approximates many of the behavioral and physiological deficits that are associated with this type of injury in humans. In this model, the lumbosacral L6 and S1 ventral motor roots are torn, or avulsed (ventral root avulsion; VRA) from the spinal cord at the central and peripheral nervous system (CNS/PNS) interface. The result is a progressive and significant death of autonomic and motor neurons at the injured spinal segments, denervation of peripheral targets, and subsequent Wallerian degeneration of the avulsed motor nerve roots (Hoang et al., 2003). Importantly, acute reimplantation of the avulsed roots into the injured spinal segments promotes cell survival, stimulates reinnervation of the implant, and facilitates some physiological restoration of bladder function (Hoang and Havton, 2006; Hoang et al., 2006b; Hoang et al., 2006a).

In light of studies that have shown that transecting a spinal motor root can result in neuropathic pain and/or sensory plasticity in the CNS (Holmberg and Kellerth, 2000; Li et al., 2002; Sheth et al., 2002; Havton and Kellerth, 2004; Obata et al., 2004), we recently decided to examine whether VRA injury might also influence sensory plasticity. As such, we showed that a unilateral L6-S1 VRA injury induces at-level allodynia, concomitant with glial and macrophage activation in the adjacent L5 deep dorsal gray matter and dorsal funiculus (DF) ipsilateral to the lesion at 8 weeks post-injury (Bigbee et al., 2007). Importantly, acute reimplantation of the avulsed lumbosacral roots into the lateral funiculus ameliorated VRA-induced neuropathic pain and inflammation in the dorsal horn (Bigbee et al., 2007). The regions of specificity of the inflammatory response in the dorsal horn of the VRA group warrants further investigation. The objective of the present experiment, therefore, was to examine the late effects of VRA or VRA combined with the reimplantation neural repair strategy (Imp) on the intramedullary sensory afferent projections in the lumbosacral spinal cord. To that end, we examined morphological features of sensory afferent collaterals in the DF of spinal segments rostral to the L6-S1 lesion and repair at 8 weeks post-lesion. The DF region is of interest, as it primarily contains large myelinated sensory afferents that carry proprioceptive information to more rostral spinal and supraspinal centers (Chung et al., 1987; Smith and Bennett, 1987; Yamamoto and Ohnishi, 1996). The present results show a significant loss of intramedullary sensory axons in the DF rostral to the lesion at 8 weeks post-VRA, concomitant with degenerating myelin. Axonal loss was restricted to the ascending sensory tracts of the DF, as axon counts were unchanged in the descending dorsal corticospinal tract (CST) region. Acute reimplantation of the lesioned roots ameliorated VRA-induced degeneration of sensory afferent projections. These data provide new insight into the surprising sensory consequences of a spinal motor root lesion, and provide further support for the promising translational therapeutic potential of nerve reimplantation following spinal nerve root avulsion injuries.

EXPERIMENTAL PROCEDURES

Animals and Surgery

Animal procedures were performed according to the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals standards under protocols approved by the UCLA Chancellor's Animal Research Committee. Adult female Sprague Dawley rats (BW = 208 ± 3 g; Charles River Laboratories, Wilmington, MA) were housed in standard rat cages (12:12 light/dark cycle; 24±1° C). Experimental groups included: 1) sham operated controls receiving a hemi-laminectomy (Lam; n=7); 2) rats receiving unilateral avulsion of the L6-S1 ventral roots (VRA; n=9); and 3) rats undergoing unilateral L6-S1 VRA followed by immediate implantation of the avulsed roots into the lateral funiculus (Imp; n=5). The surgical procedures

for the unilateral VRA procedure have been previously described (Bigbee et al., 2007). Briefly, a midline incision was made over the lumbar spine under anesthesia (2% isoflurane), and an L1-3 hemi-laminectomy was performed. In all rats, the dura was opened and the spinal cord and nerve roots were gently manipulated. In VRA rats, the L6 and S1 ventral roots were unilaterally avulsed at the CNS/PNS interface using forceps to apply traction along the course of each root. Imp rats underwent VRA, and then the avulsed roots were reinserted into a longitudinal slit made into the lateral funiculus of the spinal cord. In all groups, Gelfoam® (Pharmacia & Upjohn, Kalamazoo, MI) was placed over the spinal cord, and a titanium mesh cage was secured to the vertebral processes to stabilize the spinal column (Nieto et al., 2005). Finally, the muscle and skin were sutured. Buprenorphine (0.2-0.5 mg/kg) and 0.9% saline (1 cc) were given subcutaneously after surgery, and bladders were checked twice daily and manually expressed until bladder function recovered.

Tissue preservation and immunohistochemistry (IHC)

At 8 weeks post-surgery, rats were overdosed with sodium pentobarbital and intracardially perfused with 0.1 M phosphate buffer, followed by 4% paraformaldehyde. The spinal cords were removed, post-fixed overnight, and rinsed in 0.1 M phosphate buffered saline (PBS). The injury level was anatomically verified, and only those spinal cords having the L6 and S1 ventral roots avulsed were included in the analyses (n = 7, 9, and 5 for the Lam, VRA, and Imp groups, respectively). The spinal cord tissue was cryoprotected in a 30% sucrose solution for 24 hours, preserved in OCT compound (Sakura Finetek USA, Inc. Torrance, CA), and stored at -80°C. Transverse sections were cut at 30 µm on a cryostat and collected as free-floating sections in 0.1 M PBS. Standard maps of the rat lumbosacral cytoarchitecture were used to determine the segmental location (Molander et al., 1984). It should be noted that the tissues used in these analyses are from subjects used in our recent study showing VRA-induced neuropathic pain, glial activation, and inflammation (Bigbee et al., 2007). Sections from the L4-L5 spinal segments were used for IHC analyses of axons and myelin using antibodies against the phosphorylated 200kD neurofilament variant (RT97; Developmental Studies Hybridoma Bank, Iowa City, IA), and myelin basic protein (MBP; Chemicon, Temecula, CA), respectively. Additionally, sections from the more rostral T13-L1 spinal segments were analyzed for astrocytes and microglia/macrophages using antibodies for glial fibrillary acidic protein (GFAP; Chemicon), and allograft inhibitory factor-1 (AIF-1; Abcam, Cambridge, MA), respectively, to determine whether VRA-induced cellular changes also occurred at segments multiple levels rostral to the injury. Free-floating, adjacent spinal cord sections (3-7 sections/rat) were rinsed in 0.1 M PBS, blocked for 1 hr in 5% normal donkey serum (Jackson Immuno Research Labs, Inc. Westgrove, PA), and incubated in primary antibody (anti-mouse RT97, 1:2000; anti-rat MBP, 1:400; anti-rabbit GFAP, 1:1000; anti-goat AIF-1, 1:1000) with 0.3% triton overnight at room temperature. Additionally, double labeling for neurofilament and myelin was performed using anti-mouse RT97 (1:2000) and anti-rat MBP (1:400) with 0.3% triton overnight at room temperature. Sections were then rinsed in 0.1 M PBS, incubated for 1 hr at room temperature in immunofluorescent secondary antibody for visualization (Alexa Fluor® 594 or 488, 1:500; Molecular Probes, Eugene, OR), and mounted on slides for also DAPI (1.5 µg/ml) visualization with Vectashield mounting medium (Vector Laboratories, Burlingame, CA).

Data analysis and statistics

A Spot camera (Diagnostic Instruments, Sterling Heights, MI) attached to a Nikon E600 microscope was used to capture fluorescent images of the ipsilateral (i.e., lesioned) and contralateral (i.e., uninjured internal control) DF for all groups under constant conditions. Myelin immunoreactivity and presence of DAPI-positive nuclei were examined qualitatively. Quantitative IHC analyses were performed using C-Imaging software (Compix, Inc., Brandywine, PA) to determine the total area of neurofilament (NF) immunoreactivity within

a 2500 μm^2 region of interest, as well as the number of NF-positive profiles within the same region (Fig. 1A). Statistical tests for axon quantification were performed using Student's t-test to identify differences between the ipsilateral and contralateral DF within each group. Additionally, in order to examine inter-group comparisons, the ratio of the ipsilateral to contralateral values was compared across groups using analysis of variance (ANOVA) followed by Tukey's post-hoc test. Significance for all statistical tests was determined at $p < 0.05$.

RESULTS

Ventral root avulsion-induced sensory afferent degeneration in the dorsal funiculus is ameliorated by root reimplantation

Qualitative IHC analyses in Lam rats showed intramedullary neurofilament-immunopositive (NF⁺) axons in cross-section surrounded by myelin sheaths in the DF (Fig. 1B; Fig 2A-D). At 8 weeks post-VRA, NF⁺ labeling in the ipsilateral DF was sparse and irregular (Fig. 1C; Fig. 2E-H). Additionally, many of the remaining NF⁺ profiles appeared to be unmyelinated, and myelin debris in the absence of NF was also commonly observed. We should note, however, that the most definitive way to confirm demyelination is by electron microscopy. Furthermore, rats of the VRA series also exhibited a qualitative increase in DAPI-positive nuclei in the DF (Fig. 2). This increased cellularity may correspond, at least in part, to the increased presence of glial and inflammatory cells noted here in our previous studies (Bigbee et al., 2007). In the Imp group, axon and myelin integrity were qualitatively similar to Lam rats, and no obvious myelin debris was observed (Fig. 1D; Fig. 2I-L). Within-group quantitative analyses for the total NF⁺ areas within the region of interest showed that the ipsilateral Lam and Imp were similar to their uninjured contralateral values (377 ± 42 vs. 392 ± 41 μm^2 and 276 ± 30 vs. 310 ± 30 μm^2 , respectively; $p > 0.05$) (Fig. 4A). In contrast, the ipsilateral DF in VRA rats showed a 34% reduction in the NF⁺ area relative to contralateral values (224 ± 49 vs. 337 ± 32 μm^2 ; $p < 0.05$) (Fig 4A). The number of axon profiles was also not significantly changed within groups for Lam and Imp rats (169 ± 10 vs. 159 ± 10 and 119 ± 5 vs. 144 ± 10 ; $p > 0.05$) (Fig. 4B). VRA rats, however, showed a 42% reduction in the number of axons (93 ± 13 vs. 160 ± 13) in the ipsilateral, relative to the contralateral, DF ($p < 0.001$) (Fig. 4B). Inter-group analyses of the ipsi/contra ratios were also performed using an ANOVA followed by Tukey's post-hoc test. The ipsi/contra ratio was used in order to preserve the presence of an internal control, after confirming that there were no significant differences between the contralateral values of any group. There were no significant differences between groups in the ipsi/contra ratio for NF⁺ area (0.98 ± 0.06 vs. 0.68 ± 0.17 vs. 0.85 ± 0.06 for Lam, VRA, and Imp, respectively; $p > 0.05$) (Fig. 5A). The ipsi/contra ratios for the number of axons in both VRA (0.57 ± 0.06 ; $p < 0.001$) and Imp (0.81 ± 0.07 ; $p = 0.045$), however, were significantly reduced relative to Lam (1.08 ± 0.07) (Fig. 5A).

Axon degeneration in the DF is specific to afferent axon collaterals and ascends multiple segments rostral to the lesion

The dorsal CST region of the DF was then examined in order to assess the specificity of the axonal loss induced by VRA. NF⁺ axons are clearly observed in the dorsal CST (Fig. 3). These axons, as expected (Smith and Bennett, 1987) were smaller than those observed in the more dorsal aspect of the DF and appeared primarily thinly myelinated. There were no significant differences in the NF⁺ area or the number of NF⁺ axon profiles in the dorsal CST either within or between groups (Fig. 3; Fig. 4C, D). Furthermore, there were no significant differences between the Lam, VRA, and Imp groups with regards to the ipsi/contra ratios for NF⁺ area or number of axons in the dorsal CST (Fig. 5B).

Finally, in order to determine whether the axonal loss in the DF represented a local intraspinal phenomenon or a more extensive one that extended rostral to the lesion, we qualitatively examined sections from the T13-L1 spinal segments in VRA rats for NF⁺ axons and markers of glial activation (Fig. 6). The DF showed a qualitative loss of NF⁺ axons concomitant with the prominent activation of astrocytes and microglia/macrophages ipsilateral to the lesion, suggesting that the sensory afferent degeneration extends rostrally at least multiple segments along the ascending dorsal column tract. As in the L4-5 segments, the corticospinal tract region of the T13-L1 DF were unaffected by the VRA lesion (Fig. 6).

DISCUSSION

VRA induces degeneration of primary afferent collaterals in the dorsal funiculus in the absence of a direct injury to sensory axons

While sensory axon loss in the DF has previously been shown following spinal cord or peripheral nerve injuries in which there is direct trauma to the primary sensory afferents (Aldskogius et al., 1985; Blight and Decrescito, 1986; Smith and Bennett, 1987; George and Griffin, 1994; Wroblewski et al., 2000; Baker and Hagg, 2005), the present results show for the first time that a lesion to just the lumbosacral motor efferents results in the degeneration of intramedullary sensory axon collaterals in the absence of direct injury to the primary afferents. These results were indeed surprising, and while one might speculate that the extensive surgical procedures required in performing the laminectomy may have played a role in the present results, we reject that this is the underlying cause for the observed results in the VRA group. All groups, including the sham control, underwent a hemilaminectomy and similar manipulation of the spinal cord and dorsal roots. Further, even though the implantation procedure is even more invasive than VRA alone, the Imp group was spared the degree of axon loss induced by VRA, and did not exhibit the characteristic myelin degradation observed in VRA rats. Also, the VRA-induced axon loss was restricted to the ascending white matter tract region of the DF, supported by the combined absence of axonal loss and the lack of a glial/inflammatory response within the dorsal CST. This is in contrast to the intramedullary axonal loss induced by a spinal contusion injury, for example, where the descending dorsal CST is also affected (Hains et al., 2004; Collazos-Castro et al., 2005). The regions of axonal loss, combined with the previously observed glial response pattern (Bigbee et al., 2007) are consistent with the location of proprioceptive sensory tracts (Aldskogius et al., 1985; Smith and Bennett, 1987; Yamamoto and Ohnishi, 1996). We considered that the reduction in RT97⁺ axons might reflect a phenotypic change in NF isoform. Although we cannot completely rule out a phenotypic change in NF, the concomitant degradation of myelin sheaths observed in VRA rats suggests a breakdown of the entire axonal functional unit, rather than simply a phenotypic switch in axonal components. Also, while the degenerating axons are spatially located within regions of prominent glial activation (Bigbee et al., 2007), it is presently unknown whether the local inflammatory response causes, or results from, axonal degeneration. However, the current results are consistent with the presence of allodynia in these rats as documented in our previous study using this injury model (Bigbee et al., 2007).

Possible mechanisms underlying axonal degeneration in the dorsal funiculus

There are a number of possible mechanisms through which VRA-induced sensory axon degeneration may occur. Wallerian degeneration, for example, occurs within a distal nerve segment following a mechanical separation between nerve cell bodies and their targets (Aldskogius et al., 1985; Smith and Bennett, 1987; Hill et al., 2001). Indeed, this is the case for the L6 and S1 motor efferents in the VRA injury model. However, the sensory axon degeneration observed here does not fit within the context of Wallerian degeneration, as neither the afferent cell bodies nor collaterals have undergone any primary mechanical lesion. A second possibility is that the axon collaterals central to the dorsal root ganglia (DRG) undergo branch

elimination in response to the loss of target cells, similar to the axon retraction processes that occur during development (Raff et al., 2002). Elimination of intramedullary motor axon collaterals may also take place in axotomized motoneurons after a peripheral nerve injury (Havton and Kellerth, 1990). A third possibility builds upon the idea that degeneration of motor efferents in mixed nerve bundles may affect the cellular and electrophysiological properties of adjacent, uninjured sensory DRG neurons and spinal segments (Li et al., 2000; Wu et al., 2001; Sheth et al., 2002). As such, the biochemical by-products of degeneration, such as cytokines, have previously been identified as possible contributors to the development of pain and sensory plasticity after motor root transection (Li et al., 2000; Wu et al., 2001). Further, the degree and anatomical location of the inflammatory response within the DF of the T13-L1 segments is reminiscent of the somatotopy of axonal degeneration seen in the L1 DF following a sciatic (i.e., L4-6) nerve transection (Aldskogius et al., 1985), supporting the possibility that sensory afferents from injured and uninjured spinal levels may also undergo central degeneration following an L6-S1 VRA. Future experiments, including studies at different time points after the VRA injury, are needed to determine the mechanisms for afferent fiber degeneration in the DF.

Reimplantation of avulsed motor roots exerts a prominent neuroprotective effect on intramedullary sensory afferent projections

When examining the feasibility of a neural repair strategy for spinal cord injury, it is vital to ensure that the repair strategy does not inflict unintended negative physiological consequences, such as pain. To that end, we have shown that the acute reimplantation repair strategy following a lumbosacral VRA injury facilitates regeneration of efferent axons (Hoang et al., 2006b; Hoang et al., 2006a), reduces neuropathic pain (Bigbee et al., 2007), and here, ameliorates the degeneration of intramedullary afferent projections. By no means, however, is the Imp group completely impervious to the effects of VRA. We know that early allodynia does exist in the Imp group, and some evidence for a glial and inflammatory response remains in the deep dorsal gray matter and DF at 8 weeks post-VRA (Bigbee et al., 2007). The current data showed that, while there were no differences in Imp NF+ area or axon number relative to internal, contralateral control data, the ipsi/contra ratio was significantly reduced relative to Lam ($p=0.045$). This could be interpreted in a couple of ways. One might speculate that there is a contralateral effect of the unilateral lesion. However, there were no quantitative differences between groups when comparing the contralateral sides only, nor were there any signs of inflammation or degenerative processes contralateral to the lesion in any group (Bigbee et al., 2007). As there is a normal degree of variability between animals with regards to the location of the motor columns (Romanes, 1951; Rexed, 1954; Vanderhorst and Holstege, 1997), we consider uninjured contralateral DF within each animal to be the most appropriate control for each group. Regardless, qualitative morphological observations showed that axonal integrity was well maintained in Imp relative to VRA rats, and Imp also greatly ameliorates pain and the glial response by 8 weeks post-lesion relative to VRA alone (Bigbee et al., 2007). Combined, these data are consistent with evidence that nerve repair following brachial plexus injuries in humans positively influence the recovery of motor function and may reduce neuropathic pain (Carlstedt et al., 2000; Htut et al., 2006; Kato et al., 2006). Overall, the current data add to a growing body of literature demonstrating sensory responses to efferent injuries (Li et al., 2002; Sheth et al., 2002; Obata et al., 2004; Bigbee et al., 2007), and the beneficial therapeutic effects of neural repair of spinal cord efferents (Carlstedt et al., 2000; Hoang et al., 2006b; Hoang et al., 2006a; Htut et al., 2006; Kato et al., 2006; Bigbee et al., 2007).

Potential mechanisms for sensory axon protection provided by ventral root reimplantation

Here, reimplantation of lesioned ventral roots into the spinal cord greatly ameliorated intramedullary degeneration of sensory axons, despite Wallerian degeneration of the motor efferents in both the VRA and Imp groups. The question remains as to how this protective

effect might occur. One source of potential trophic support is represented by the intramedullary scar that develops following a VRA injury and acute surgical implantation of lesioned roots into the spinal cord, as a traumatic injury to the ventral funiculus produces a permissive substrate, which allows for regenerating motor axons to extend into the scar tissue (Risling et al., 1983; Lindå et al., 1992). In addition, the ventral root implantation procedure introduces into the spinal cord the lesioned ventral root segment containing severed axons and their associated Schwann cells. However, Schwann cells do not appear to invade the spinal cord spontaneously following traumatic lesions to the ventral funiculus (Risling et al., 1983; Cullheim et al., 1999). It is possible that the implanted roots with associated Schwann cells may provide trophic support, as there is evidence that a peripheral nerve injury induces the proliferation of Schwann cells and increased production of brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), and nerve growth factor (NGF) (Salzer and Bunge, 1980; Meyer et al., 1992; Funakoshi et al., 1993; Naveilhan et al., 1997). Neurotrophic properties of lesioned ventral roots may be detected over long distances in the rat spinal cord, as a multilevel VRA injury followed by an acute implantation of a single lesioned ventral root into the conus medullaris attracts the regenerative growth of autonomic and motor axons from several adjacent segments (Hoang and Havton, 2006). In this context, we hypothesize that a root implantation may also protect intramedullary sensory axons against degeneration in the DF by providing trophic support to the spinal cord. Interestingly, recent studies showed that Schwann cells exhibit both motor and sensory phenotypes that respond to injury by differentially up-regulating growth factors (Höke et al., 2006). It was also shown that such distinct phenotypic properties of Schwann cells are important to take into consideration for neural repair studies, as cutaneous nerve and ventral root grafts preferentially supported sensory and motor axon regeneration, respectively (Höke et al., 2006). It is interesting to note in the current study that the implantation of the L6 and S1 ventral roots, which contain both skeletal motor and parasympathetic efferents with associated Schwann cells exert a very marked protective effect on the ascending primary sensory projections of likely proprioceptive modalities in the DF portion of the spinal cord. The phenotypic expression of growth factors by supporting cells in these lumbosacral roots following ventral root injuries therefore warrants further examination.

CONCLUSIONS

The current study shows that a lesion restricted to motor efferents can have significant negative consequences for primary afferents that were never directly injured. These data also showed that the acute reimplantation of avulsed ventral roots protects intramedullary projections of primary afferents against degenerative processes. We conclude that the root reimplantation data presented here, combined with the neuroprotective and regenerative effects reported previously in this experimental model, support the concept that repair of the motor roots following an avulsion injury may also be of interest for future clinical translation to facilitate functional recovery after CE/CM SCI.

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Abbreviations

AIF-1, allograft inhibitory factor-1

ANOVA, analysis of variance
 CE/CM, cauda equina/conus medullaris
 CNS, central nervous system
 contra, contralateral
 CST, corticospinal tract
 DF, dorsal funiculus
 DRG, dorsal root ganglia
 GFAP, glial fibrillary acidic protein
 GM, gray matter
 IHC, immunohistochemistry
 Imp, implanted
 ipsi, ipsilateral
 Lam, laminectomy
 MBP, myelin basic protein
 NF, neurofilament
 PNS, peripheral nervous system
 PBS, phosphate buffered saline
 SCI, spinal cord injury
 VRA, ventral root avulsion
 VRT, ventral root transection

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Fig. 1.

VRA-induced sensory afferent degeneration in the DF is ameliorated by reimplantation. Schematic shows the regions of the dorsal funiculus (DF) and corticospinal tract (CST) that were quantitatively evaluated for neurofilament-positive axons (A). Transverse sections of the L5 dorsal horn from a Lam control shows axons (200 kD neurofilament or NF; red) and myelin (MBP; green) in the contralateral and ipsilateral DF (B). At 8 wks post-ventral root avulsion (VRA), the presence of axons is sparse ipsilateral to the lesion, concomitant with a visible increase in the number of DAPI-positive nuclei (C). Rats which underwent ventral root avulsion plus reimplantation of the avulsed roots (Imp) exhibited a qualitatively more symmetrical

distribution of axons between the contralateral and ipsilateral DF, similar to that exhibited in Lam control rats (D). Calibration bar = 100 μ m.

Fig. 2.

Morphological characteristics of the ipsilateral DF at 8 weeks post-lesion. The Lam DF (A-D), showed neurofilament positive axons (red; A) that were commonly surrounded by myelin sheaths (green; B). Some Dapi-positive nuclei were also observed (C). At 8 weeks post-VRA, NF labeling was sparse (E), and remaining axons were often observed without a visible myelin sheath (arrow in E-H; however, see Results section for caveat). There was also a distinct reduction in the presence of ring-like myelin-positive structures (compare F to B), while some remaining myelin-positive structures were not associated with any visible neurofilament (arrowheads in E-H). VRA rats also exhibited a qualitative increase in the number of Dapi-positive nuclei (G). Imp rats exhibited neurofilament and myelin-positive structures in the ipsilateral DF that are qualitatively similar to Lam controls. Arrows highlight the location of NF-positive structures, while arrowheads highlight myelin-positive structures in the absence of NF, which exhibit characteristics of degradation. Calibration bar = 30 μ m.

Fig. 3. NF-positive axons in the corticospinal tract. The contralateral (Contra) and ipsilateral (Ipsi) dorsal corticospinal tracts (CST) are shown for Lam (A-C), VRA (D-F), and Imp rats (G-I). NF and MBP are shown in red and green, respectively. Qualitatively, Contra and Ipsi NF and MBP immunopositive labeling is symmetrical. Calibration bar = 50 μ m.

Fig. 4.

Using the contralateral dorsal horn as an unlesioned, internal control, the ipsilateral DF of VRA rats exhibited a significant reduction in the total NF+ area (A) and the number of neurofilament-positive axons (B) within a 2500 μm^2 region. On the contrary, ipsilateral Lam and Imp NF values did not differ from their respective contralateral sides. In the same sized region of the corticospinal tract, the ipsilateral NF+ area and the number of NF+ structures were unaffected in all groups relative to their contralateral, internal control levels (C and D). Data shown are the mean values $\pm$ S.E.M. *, significantly different at $p < 0.05$

Fig. 5.

The relationship between the number of remaining NF⁺ profiles and the NF⁺ area are shown for the dorsal funiculus (A) and the corticospinal tract (B). Values presented are the ratio of the ipsilateral divided by the contralateral values in a 2500 μm^2 region. Individual subject values (closed symbols) are shown, as are the group mean values (open symbols) $\pm$ S.E.M. •, Lam; ■, VRA; ▲, Imp. *, significantly different from Lam at $p < 0.05$; ^, significantly different from Lam at $p < 0.001$.

Fig. 6.

VRA affects ascending sensory afferent pathways multiple segments rostral to the lesioned spinal segments. Adjacent spinal segments from the T13-L1 segments of the spinal cord at 8 wks post-L6-S1 VRA show a somatotopic loss of NF⁺ axons in the ipsilateral DF (red; A), concomitant with an increased activation of astrocytes (GFAP, green; B) and microglia (AIF-1, pseudo-colored blue; C), suggesting that VRA-induced sensory afferent degeneration extends rostrally from the segmental level of the lesion. The arrows point to the affected portion of the DF. Note the absence of a glial response in the dorsal CST regions. Contra and Ipsi refer to the contralateral and ipsilateral sides of the spinal cord relative to the VRA, respectively. Calibration bar = 50 μ m.