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LONG-TERM EFFECTS OF A LUMBOSACRAL VENTRAL ROOT AVULSION INJURY ON AXOTOMIZED MOTOR NEURONS AND AVULSED VENTRAL ROOTS IN A NON-HUMAN PRIMATE MODEL OF CAUDA EQUINA INJURY ☆

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Abstract—Here, we have translated from the rat to the non-human primate a unilateral lumbosacral injury as a model for cauda equina injury. In this morphological study, we have investigated retrograde effects of a unilateral L6–S2 ventral root avulsion (VRA) injury as well as the long-term effects of Wallerian degeneration on avulsed ventral roots at 6–10 months post-operatively in four adult male rhesus monkeys. Immunohistochemistry for choline acetyl transferase and glial fibrillary acidic protein demonstrated a significant loss of the majority of the axotomized motoneurons in the affected L6–S2 segments and signs of an associated astrocytic glial response within the ventral horn of the L6 and S1 spinal cord segments. Quantitative analysis of the avulsed ventral roots showed that they exhibited normal size and were populated by a normal number of myelinated axons. However, the myelinated axons in the avulsed ventral roots were markedly smaller in caliber compared to the fibers of the intact contralateral ventral roots, which served as controls. Ultrastructural studies confirmed the presence of small myelinated axons and a population of unmyelinated axons within the avulsed roots. In addition, collagen fibers were readily identified within the endoneurium of the avulsed roots. In summary, a lumbosacral VRA injury resulted in retrograde motoneuron loss and astrocytic glial activation in the ventral horn. Surprisingly, the Wallerian degeneration of motor axons in the avulsed ventral roots was followed by a repopulation of the avulsed roots by small myelinated and unmyelinated fibers. We speculate that the small axons may represent sprouting or axonal regeneration by primary afferents or autonomic fibers.

Key words: rhesus macaque, spinal cord, Wallerian degeneration, electron microscopy.

INTRODUCTION

Conus medullaris and cauda equina injuries commonly result in a complex syndrome of motor, sensory, and autonomic impairments (Harrop et al., 2004; Havton and Carlstedt, 2009). Cauda equina injuries following trauma to the thoracolumbar spine may present with lesions also to the sacral spinal cord and result in a conus medullaris syndrome (Maynard et al., 1997). A cauda equina injury or an anatomically complete conus medullaris syndrome results in a lower motor neuron syndrome with denervation of peripheral targets, including skeletal muscle and autonomic ganglia.

Both the ventral and dorsal lumbosacral roots of the cauda equina may be affected by trauma to the lower spine. Transection, laceration, crush, or avulsion injuries to the ventral roots result in denervation of peripheral targets and lead to characteristic peripheral denervation and flaccid weakness of the affected end organ (Pavlakis et al., 1983; Hoang and Havton, 2006; Mauffrey et al., 2008). In addition, following a motor nerve or ventral root injury, motoneuron somata undergo a retrograde reaction and the distal segment of the motor axon undergoes Wallerian degeneration (Koliatsos and Price, 1996; Griffin, 2007).

Earlier studies in rats have demonstrated that a proximal ventral root avulsion (VRA) injury mimics many of the deficits encountered in a clinical conus medullaris syndrome and results in progressive and severe loss of motoneurons in the spinal cord (Koliatsos et al., 1994; Hoang et al., 2003, 2006). However, information on the response to a proximal ventral root injury in large mammalian models is sparse. Retrograde effects to injury may show differences between species, as e.g. immune and inflammatory reactions to nerve and spinal cord injury differ between different strains of rats and

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Abbreviations: ChAT, choline acetyltransferase; GFAP, glial fibrillary acidic protein; PBS, phosphate-buffered saline; VRA, ventral root avulsion.

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between species (Popovich et al., 1997; Sroga et al., 2003; Kigerl et al., 2006). Here, we investigated the long-term effects of a unilateral lumbosacral VRA injury in rhesus macaques. Our principal aim was to determine the response of axotomized motor neurons to a proximal ventral root lesion and late effects of Wallerian degeneration of avulsed ventral roots in a clinically relevant cauda equina injury model. An additional goal was to provide anatomical information about the potential suitability of avulsed ventral roots for delayed replantation procedures in the non-human primate.

We demonstrate that a lumbosacral VRA injury in the non-human primate resulted in a marked loss of axotomized motoneurons at 6–10 months post-operatively. An intramedullary astrocytic glial response was also detected in the ventral horn. The distal denervated segment of the ventral roots had undergone Wallerian degeneration. However, combined light and electron microscopic studies showed that the avulsed ventral root segments have been repopulated by numerous small myelinated and unmyelinated axons. It is possible that the small caliber fibers in the avulsed ventral roots may be of primary afferent or autonomic origin.

EXPERIMENTAL PROCEDURES

All animal procedures were carried out at the California National Primate Research center at UC Davis. The facility is AAALAC-accredited, and all animals were maintained in accordance with provisions of the Animal Welfare Act (as Amended 2007.7 USC §2131–2159) and the Guide for Care and Use of Laboratory Animals (National Institutes of Health Publications No. 86-23, revised 1985). All procedures were approved by the Institutional Animal Care and Use Committee at UC Davis. A total of four male rhesus monkeys (*Macaca mulatta*) were included in the study. The subjects were adults and weighed 8.8–11.7 kg at the time of surgery at the onset of the study.

Surgical procedures

All surgical procedures were performed under general anesthesia and sterile surgical conditions. All subjects were fasted overnight prior to surgery. Initial sedation and immobilization was obtained by administration of ketamine (10–15 mg/kg im) followed by 1–2% isoflurane (Abbott, North Illinois, IL, USA) in 100% O_2 via an inserted endotracheal tube for surgical anesthesia.

A longitudinal mid-line cutaneous incision was made over the L1–L5 spinous processes. The lumbar fascia was identified and cut slightly to the left of the midline. Using electrocautery as well as sharp and blunt subperiosteal dissection, the paraspinous muscles on the left side were gently separated to expose the dorsolateral surface of the laminae, facet joints, and pedicles of the spinal vertebrae. A left-sided hemi-laminectomy was performed using rongeurs and a high-speed diamond-bit drill under continuous saline irrigation. The hemi-laminectomy spared the spinous processes and extended from the caudal aspect of the L1 vertebra to the rostral aspect of the L3 vertebra.

A longitudinal incision was made to open the dura using a 15-blade dural scalpel and microscissors. Guided by vertebral landmarks and normal variations in ventral root caliber, the left L6–S2 ventral roots were identified by gentle micro-dissection using blunt instruments and a surgical microscope. The S2, S1, L7, and L6 ventral roots were individually avulsed from the surface of the spinal cord by applying traction along the normal course of the roots with a pair of fine forceps. The proximal stump of the avulsed roots were trimmed and left within the subdural space. The dura was closed using continuous Ethilon® 6-0 (Ethicon, New Brunswick, NJ, USA) sutures. The muscle layer was closed over the laminectomy site. The skin was closed using intradermal resorbable Vicryl® 4-0 (Ethicon) sutures. All animals recovered well and received oxymorphone (0.15 mg/kg IM TID) for 3 days post-operatively.

At 6–10 months after the unilateral L6–S2 VRA injury, the subjects were placed under deep surgical anesthesia and transcardially perfused with saline followed by a 4% paraformaldehyde solution. After the perfusion procedure, the spinal cord and associated nerve roots were removed for morphological studies.

Immunohistochemical studies of spinal cord sections

The removed lumbosacral spinal cord segment was examined and the segmental levels for the L6–S2 VRA injuries were confirmed in all subjects. The L6, L7, S1, and S2 spinal cord segments were blocked and frozen individually. Serial 14-μm cryostat sections were cut and mounted on glass slides (Menzel-Gläzer, Braunschweig, Germany), air-dried and stored at −20 C°. For immunohistochemistry, commercially available primary antibodies were used for the detection of choline acetyltransferase (ChAT) and glial fibrillary acidic protein (GFAP) (Table 1).

A goat polyclonal primary antibody against ChAT (AB144P; Millipore, Bedford, MA, USA) was used to detect cholinergic neurons in the primate spinal cord. According to the manufacturer's information, Western blot analysis using a 1:1000 dilution of this lot detected ChAT on 10 μg of mouse brain lysates. Immunohistochemical studies of the primate spinal cord

Table 1. Antibodies used in the present study

Name	Immunogen	Manufacturer	Species and type	Dilution
ChAT	Choline acetyltransferase	Millipore #AB144P	Goat polyclonal	1:100
GFAP	Glial fibrillary acidic protein	Millipore #AB5804	Rabbit polyclonal	1:2000

References:
ChAT: JCN Antibody Database: Enjin et al. (2010), Puller et al. (2011). <http://antibodyregistry.org/AB_2079751/>.
GFAP: JCN Antibody Database: Talos et al. (2006), Kim et al. (2008), Chung et al. (2008). <http://antibodyregistry.org/AB_92037/>.