Available online at www.sciencedirect.com
ScienceDirect
Experimental Neurology 203 (2007) 22–33
Experimental Neurology
www.elsevier.com/locate/yexnr

Volumetric magnetic resonance imaging of dorsal root ganglia for the objective quantitative assessment of neuron death after peripheral nerve injury

Christian A. West [a,b,c], Karen A. Davies [d], Andrew M. Hart [a,b,c,e], Mikael Wiberg [b,c], Steve R. Williams [d], Giorgio Terenghi [a,*]

[a] Blond McIndoe Research Laboratories, University of Manchester, 3.102 Stopford Building, Oxford Road, Manchester M13 9PT, UK
[b] Department of Integrative Medical Biology, Section for Anatomy, Umeå University, SE-901 87 Umeå, Sweden
[c] Department of Surgical and Perioperative Science, Section for Hand and Plastic Surgery, University Hospital, Umeå, Sweden
[d] Imaging Science and Biomedical Engineering, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT, UK
[e] Canniesburn Plastic Surgery Unit, Glasgow Royal Infirmary, 84 Castle Street, Glasgow, G4 0SF, UK

Received 21 March 2006; revised 16 June 2006; accepted 19 July 2006
Available online 6 September 2006

Abstract

Prevention of neuron death after peripheral nerve injury is vital to regaining adequate cutaneous innervation density and quality of sensation, and while experimentally proven neuroprotective therapies exist, there lacks suitable clinical outcome measures for translational research. Axotomized dorsal root ganglia (DRG) histologically exhibit volume reduction in proportion to the amount of neuronal death within them. Hence, this study evaluated the validity of using magnetic resonance imaging (MRI) to quantify DRG volume as a proxy measure of cell death. A high-resolution 3D MRI sequence was developed for volumetric quantification of the L4 DRG in the rat sciatic nerve model. An unoperated "control" group ($n=4$), and a "nerve transection" group ($n=6$), 4 weeks after axotomy, were scanned. Accuracy and validity of the technique were evaluated by comparison with morphological quantification of DRG volume and stereological counts of surviving neurons (optical fractionator). The technique was precise (coefficient of variation=4.3%), highly repeatable (9% variability), and sensitive (mean 15.0% volume reduction in axotomized ganglia detected with statistical significance: $p<0.01$). MRI showed strong and highly significant correlation with morphological measures of DRG volume loss ($r=0.90$, $p<0.001$), which in turn correlated well with neuron loss ($r=0.75$, $p<0.05$). MRI similarly exhibited direct correlation with neuron loss ($r=0.67$, $p<0.05$) with consistent agreement. MRI volumetric quantification of DRG is therefore a valid in vivo measure of neuron loss. As a non-invasive, objective measure of neuronal death after nerve trauma this technique has potential as a diagnostic modality and a quantitative tool for clinical studies of neuroprotective agents.

Keywords: Axotomy; Sensory neuron death; Magnetic resonance imaging; Volumetry; Neuroprotection; Dorsal root ganglia; Sciatic nerve; Peripheral nerve injury; Quantification; Optical fractionator

Introduction

Functional outcome following peripheral nerve injuries remains disappointingly poor despite meticulous microsurgical repair, so attention is now focused upon understanding and modulating the neurobiology of nerve injury and regeneration (Lundborg, 2000; Terzis et al., 1997). However, lack of suitable quantificative tools for use in translational research restricts the transfer of promising experimental developments from the laboratory to clinical practice. Presently available measures of outcome following nerve injury provide little evidence of validity or reliability (Jerosch-Herold, 2005) and can lack the objectivity and sensitivity required to demonstrate the clinical efficacy of novel therapeutic interventions in comparison to the current standards of clinical care. Electrophysiological studies are the gold standard for assessment of nerve lesions in vivo, but are of limited value in the early post-injury phase (Krarup et al., 2002), and are frequently poorly tolerated by patients due to their invasive nature. Furthermore, these tests fail to distinguish

* Corresponding author.
E-mail address: giorgio.terenghi@manchester.ac.uk (G. Terenghi).

0014-4886/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2006.07.013

the individual peripheral elements (neuron survival, axonal regeneration, target organ reinnervation) that are necessary for restoration of sensorimotor function.

Magnetic resonance imaging (MRI) has provided non-invasive assessment of structural, metabolic and functional aspects of the peripheral nervous system following injury (Aagaard et al., 2003; Baldassarri et al., 1995; Bendszus and Stoll, 2003; Bendszus et al., 2004; Cudlip et al., 2002; Stanisz et al., 2001; Wessig et al., 2004), and has potential to be developed to deliver the desired information. This study uses MRI to focus on the assessment of neuronal death, which is a major factor explaining poor sensory functional outcome after peripheral nerve trauma. As a result of neuron loss, cutaneous innervation density and quality of sensation cannot return to normal (Wiberg et al., 2003), regardless of how well the individual surviving neurons regenerate, although potentially clinically applicable treatments have now been identified that provide complete sensory neuroprotection (Hart et al., 2004; Wilson et al., 2003).

In the rat sciatic nerve transection model, a large proportion of the sensory neuronal population is progressively lost over the first 2 months following nerve injury, with cell death reaching statistical significance after 1 week (McKay et al., 2002). This neuron loss strongly correlates with a significant reduction in volume of the neuron-containing region of axotomized dorsal root ganglia (DRG) when studied morphologically (McKay et al., 2002). Hence, the aim of this study was to examine the efficacy of MRI-based quantification of DRG volume as a proxy measurement of neuron death/survival. The development of high-resolution (micro) MRI has allowed visualization of a variety of very small structures in the nervous system (Natt et al., 2002), and is reported to be a reliable tool for the evaluation of anatomical volumes (Gadeberg et al., 1999; Marziali et al., 2004; McDaniel et al., 2001; Moats et al., 2003). Imaging of dorsal root ganglia has been previously described in human subjects (Aota et al., 2001; Castillo and Mukherji, 1996; Hamanishi and Tanaka, 1993; Hasegawa et al., 1996), but to date the volumetric analysis of these structures has not been performed. We have developed a high-resolution 3D MRI protocol for the volumetric quantification of DRG in the rat sciatic nerve model. The accuracy, repeatability and validity of this MRI technique have been evaluated through comparison with morphological quantification of DRG volume and stereological counts (optical disection) of the number of surviving neurons contained within the DRG.

Materials and methods

All work was performed in accordance with the terms of the Animals (Scientific Procedures) Act 1986 (UK) and reflected the principles of reduction and welfare optimization.

Experimental design

This study comprised two groups of female Sprague-Dawley rats, one unoperated "control" group ($n=4$), and one "nerve transection" group ($n=6$) that underwent unilateral sciatic nerve division at 5 weeks of age. A 4-week post-axotomy time point was employed, with MRI scanning and harvest of L4 DRG performed at the age of 9 weeks in all animals. At harvest the weights of the animals were similar in both the control group (mean weight 231.5 g, SD 9.81) and the nerve transection group (mean weight 233.67 g, SD 8.45).

Operative procedure

The surgical procedure was similar to previously published studies, employing an established rat sciatic nerve transection model of nerve injury (Hart et al., 2004; Wilson et al., 2003). Under halothane anesthesia (maintenance 2% in 500 ml/min oxygen) the sciatic nerve was divided unilaterally at the upper border of quadratus femoris (mid-thigh), with resection of a 3-mm segment from the distal stump. Proximal and distal nerve stumps were ligated with 6/0 polypropylene (Prolene™) and secured into silicone caps with a single 9/0 polyamide (Ethilon™) suture to prevent spontaneous regeneration. Post-operative analgesia was provided as a single dose of buprenorphine (0.03 mg/kg).

Magnetic resonance image (MRI) acquisition

Immediately prior to scanning, animals underwent Schedule 1 termination by cervical dislocation following CO_2 narcosis. Scans were performed on a 7.0 T, 15-cm bore horizontal actively shielded magnet (Magnex UK Ltd., Abingdon) integrated to a SMIS console (Magnetic Resonance Research Systems, Guildford, UK) using a custom made 2-cm diameter transmit–receive surface radio-frequency coil. A series of pilot sequences in the sagittal and coronal (Fig. 1) directions were initially performed to guide the positioning of a transverse 3D imaging slab that spanned the L4 DRG bilaterally. This slab was orientated perpendicular to the long axis of the spinal cord to generate image slices of the DRG in cross-section (Fig. 2). The 3D data acquisition (TR 17 ms, TE 6 ms) was based on a field of view of $30\times30\times8$ mm and in plane data matrices of 256×128, which after interpolation to 256×256 (zero filling) yielded 32 image slices of 117×117-μm resolution and 0.25-mm thickness. Scan time was 45 min, although taking the experimental setup into account, i.e., the instrumental hardware, animal arrangement, RF surface coil positioning and pilot sequences, the total image acquisition time was 1–1.5 h per animal.

This sequence was chosen to provide high spatial resolution with the best signal-to-noise in a reasonable data acquisition time. A 3D gradient echo volume acquisition is the most time efficient sequence to acquire these data, and pilot experiments showed that it gave good definition of the DRG. The pulse angle was adjusted to give maximum signal in a slice containing the DRG parallel to the plane of the surface coil. A single slice 2D gradient echo acquisition was used to optimize the pulse at the same TE/TR as the 3D acquisition. As a result of this choice of pulse angle, the images have T1 as well as proton density weighting, but we did not optimize for any particular contrast. Exploitation of other contrast mechanisms would need longer acquisitions to achieve the same signal-to-noise and resolution.