Available online at www.sciencedirect.com

SCIENCE DIRECT

Experimental Neurology 194 (2005) 221–229

Experimental Neurology

www.elsevier.com/locate/yexnr

Electrical stimulation restores the specificity of sensory axon regeneration

Thomas M. Brushart[a,*], Rajesh Jari[a], Valerie Verge[b], Charles Rohde[c], Tessa Gordon[d]

[a]*Department of Orthopaedic Surgery, Johns Hopkins Medical Institutions, 601 N. Caroline Street, Baltimore, MD 21287, USA*
[b]*Department of Anatomy and Cell Biology, Cameco MS Neuroscience Research Center, University of Saskatchewan, Canada*
[c]*Department of Biostatistics, The Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD 21287, USA*
[d]*Department of Pharmacology, Division of Neuroscience, University of Alberta, Alberta, Canada, T6G 2S2*

Received 19 January 2005; revised 7 February 2005; accepted 17 February 2005
Available online 6 April 2005

Abstract

Electrical stimulation at the time of nerve repair promotes motoneurons to reinnervate appropriate pathways leading to muscle [Al-Majed, A.A., Neumann, C.M., Brushart, T.M., Gordon, T., 2000. Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. J. Neurosci. 20, 2602–2608] and stimulates sensory neurons to regenerate [Geremia, N.M., Gordon, T., Al-Majed, A.A., Brushart, T.M., Verge, V.M., 2002. Brief electrical stimulation promotes regeneration of sensory fibers into cutaneous and muscle branches of femoral nerve. Neurosci. Abstr. 535.14]. The present experiments examine the effects of electrical stimulation on the specificity of sensory axon regeneration. The unoperated rat femoral cutaneous branch is served by 2–3 times more DRG neurons than is the muscle branch [Brushart, T.M., 1988. Preferential reinnervation of motor nerves by regenerating motor axons. J. Neurosci. 8, 1026–1031]. After transection and repair of the femoral trunk, equal numbers of DRG neurons project to both branches. However, 1 h of electrical stimulation restores the normal proportion of DRG neurons reinnervating skin and muscle. To ask if the redistribution of stimulated neurons results from enhanced specificity of target reinnervation, we developed a new technique of sequential double labeling. DRG neurons projecting to the femoral muscle branch were prelabeled with Fluoro Gold 2 weeks before the nerve was transected proximally and repaired with or without 1 h of 20-Hz electrical stimulation. Three weeks after repair, the muscle nerve was labeled a second time with Fluororuby. The percentage of regenerating neurons that both originally served muscle and returned to muscle after nerve repair increased from 40% without stimulation to 75% with stimulation. Electrical stimulation thus dramatically alters the distribution of regenerating sensory axons, replacing normally random behavior with selective reinnervation of tissue-specific targets. If the enhanced regeneration specificity resulting from electrical stimulation is found to improve function in a large animal model, this convenient and safe technique may be a useful adjunct to clinical nerve repair.

Keywords: Afferent; Reinnervation; Axonal transport; Dorsal root ganglion; Muscle; Peripheral nerve

Introduction

Repair of transected peripheral nerve is followed by permanent functional compromise in up to 90% of adults (Brushart, 1998). This therapeutic failure often results from misdirection of regenerating axons to functionally inappropriate end organs (Brushart, 1991). Refinement of microsurgical techniques in the 1960s and 1970s engendered the hope that accurate alignment of neural components would improve outcomes. Microsurgical nerve repairs indeed proved superior to those performed macroscopically (Brushart, 1998). Nevertheless, further refinement of technique by selective re-union of individual nerve fascicles added little additional benefit (Gaul, 1986; Jabaley, 1991; Kato et al., 1998). Limits to the surgical approach are probably imposed by the lateral wandering of regenerating axons (Witzel et al., in press) and the complex organization of axons within fascicles (Hallin, 1990). A physiologically based therapy to enhance the specificity of axon regeneration is thus clearly needed.

Recently, 1 h of electrical stimulation at the time of nerve repair was found to promote correct reinnervation of muscle nerve and muscle by motoneurons (Al-Majed et al., 2000b;

* Corresponding author. Fax: +1 410 502 6816.
E-mail address: tbrusha@jhmi.edu (T.M. Brushart).

0014-4886/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2005.02.007

Brushart et al., 2002a). These studies were performed in the rat femoral nerve model (Brushart, 1988). Proximally, at the site of nerve transection and repair, axons destined for skin and muscle intermingle. Regenerating axons that contact the distal stump will thus have access to Schwann cell tubes that lead either to muscle or to skin. Distally, the nerve bifurcates into a muscle branch to the quadriceps, containing both motor efferent and muscle afferent fibers, and a purely afferent cutaneous branch.

The present experiments examine the effects of electrical stimulation on the specificity of sensory axon regeneration. Early work in the femoral nerve model established that the number of DRG neurons normally projecting to the cutaneous branch was 2–3 times that projecting to the muscle branch (Brushart, 1988). After femoral nerve transection and repair, however, these numbers were usually equal, suggesting that reinnervation of the distal pathways was not specific as to pathway or end organ. Repeating these experiments with more modern tracing and counting techniques confirms that the branch distribution of DRG neurons is equalized after nerve repair. Furthermore, this distribution can be altered significantly by electrical stimulation at the time of surgery (Geremia et al., 2002). These experiments, evaluated by simultaneously labeling the femoral muscle and cutaneous branches, could not determine whether axons were redistributed to their correct pathways, or just reshuffled. To differentiate between these possibilities, we developed a new technique of sequential double labeling. DRG neurons normally projecting to the femoral muscle branch are prelabeled before nerve repair. A second label is then applied to the same nerve 3 weeks after repair to determine which DRG neurons have correctly reinnervated the muscle pathway. After routine nerve suture, a mean of only 40% of the original muscle afferent neurons reinnervate muscle nerve; with stimulation, 75% return to their correct destination. One hour of electrical stimulation at the time of nerve repair thus promotes the return of sensory axons to their tissue of origin. Correlation of these findings with improved function in a clinically relevant model would be required, however, before this technique could be recommended for clinical use.

Materials and methods

Experimental groups

Experiments were evaluated by two distinct labeling techniques. Simultaneous double labeling involves application of one tracer to one distal femoral branch (cutaneous or muscle) and, during the same surgery, application of a second tracer to the other branch. This technique identifies the destination of all regenerating axons. Since DRG neurons normally project to both cutaneous and muscle branches, it cannot determine whether a regenerating sensory axon has returned to its correct pathway. Sequential double labeling, in contrast, requires labeling one branch before nerve repair and then again, with a different tracer, after repair and regeneration. This technique identifies neurons that have returned correctly to their original pathway, but does not account for all neurons in the system. Four groups of animals were prepared: two experimental groups, and controls for both simultaneous and sequential labeling techniques.

Simultaneous Double-Labeling Controls evaluated the efficacy of tracer application, and established the number of DRG neurons projecting to the femoral muscle and cutaneous branches in unoperated animals. In 3 rats, both right and left femoral muscle branches were labeled with Fluoro Gold (FG) and, during the same procedure, both cutaneous branches were labeled with Fluororuby (FR). The Simultaneous Double-Labeling-Experimental group (Fig. 1) evaluated the distribution of DRG neurons to femoral muscle and cutaneous branches after nerve repair, with and without electrical stimulation. Both femoral nerves of 6 rats were transected and repaired proximal to the iliacus branch. On one side of the animal, randomly determined, the nerve was stimulated for 1 h during surgery. Three weeks later, DRG neurons projecting to femoral cutaneous and muscle nerves were labeled with FG and FR. The matching of branch (muscle or cutaneous) with tracer (FG or FR) was determined randomly and coded to facilitate unbiased neuron counting.

Sequential Double-Labeling Controls (Fig. 3) were necessary to define: (1) the ability of the FG–FR label combination to double label the same neurons when sequentially applied in an experimental sequence that includes nerve injury and repair, (2) the degree to which FG might remain in the muscle branch and label regenerating

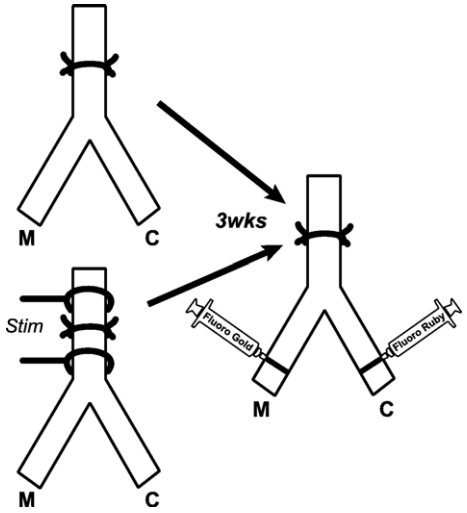

Fig. 1. Simultaneous double labeling is performed after nerve repair and regeneration to identify the destination of regenerating axons. Here, the proximal femoral nerve is transected and repaired, with or without electrical stimulation. Three weeks later, the femoral muscle (M) and cutaneous (C) branches are labeled with Fluoro Gold and Fluororuby, respectively. The labels are applied within minutes of one another, and thus identify neurons that project to either or both labeling sites at a single, defined interval after surgery. This technique cannot identify the original, pre-operative destination of these axons.