Experimental Neurology 213 (2008) 281–292

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

The influence of substrate curvature on neurite outgrowth is cell type dependent

Roy M. Smeal [a,*], Patrick A. Tresco [b]

[a] Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112, USA
[b] The Keck Center for Tissue Engineering, Department of Bioengineering, College of Engineering, University of Utah, Salt Lake City, Utah 84112, USA

ARTICLE INFO

Article history:
Received 20 December 2007
Revised 29 May 2008
Accepted 30 May 2008
Available online 16 June 2008

Keywords:
Neurite guidance
Regeneration
Curvature
Cytoskeleton
Dorsal root ganglion
Granule cell

ABSTRACT

Damage to axonal tracts of the central nervous system results in costly and permanent disability. The observations of aborted neurite outgrowth and disorganized scarring in injured central nervous system tissue have motivated the hypothesis that engineered bridging devices might facilitate regeneration. It is thought that both the shape and surface chemistry are important design parameters, however, their relative importance is poorly understood. Previously, we utilized smooth cylindrical surfaces to demonstrate that surfaces designed with directionally varying curvature bias in a stereotyped way postnatal dorsal root ganglion axonal regeneration in the direction of minimum curvature independent of surface chemistry. In the present study, we extend this analysis to include adult dorsal root ganglion neurons and cerebellar granule cells, cell types more representative of the challenge faced clinically. We found that axonal outgrowth of both the adult neuron and the central neuron was less sensitive to substrate curvature than the outgrowth of the postnatal neurons. These differences were quantified by constructing distributions describing the probability of outgrowth for a defined range of surface curvatures. Both the adult neuron and the central neuron exhibited a higher probability of extension in high-curvature directions compared to the postnatal neuron implying that surface geometry may not be as potent a cue in directing the regeneration of these neurons. A microtubule-stabilizing agent enhanced the sensitivity to curvature of the adult neuron, partially reversing the increased probability of growing in a high-curvature direction. The results suggest novel methods to enhance directed neuron regeneration using bridging substrates.

Introduction

Injuries to the central nervous system (CNS) are particularly costly to treat and deleterious to the quality of life of patients due to their permanence. While the neurons killed by injury and secondary cascades involved in stabilization of the wound contribute to functional deficits, a large proportion of the deficit is caused by the disruption of axonal tracts responsible for neural communication. This is most obvious in the case of spinal cord injury where tract disruption results in the loss of sensation, motor control, and in some cases, critical homeostatic control below the site of injury.

Axonal damage is a key predictor of clinical outcome for both brain and spinal cord traumas (Medana and Esiri, 2003). The failure of CNS axons to regenerate is not entirely an intrinsic property of adult CNS neurons as demonstrated by long-distance regeneration through peripheral nerves (David and Aguayo, 1981; Richardson et al., 1984, 1980). These seminal observations inspired the testing of a variety of strategies for stimulating axonal regeneration including altering the inflammatory and immune response, cell replacement, neurotrophic factor delivery, manipulation of internal neuronal properties and signaling cascades, removal of inhibitory factors, supply of attractive cues for axon growth, and the implantation of axonal bridging substrates both biological and artificial (David and Lacroix, 2003; Geller and Fawcett, 2002; Horner and Gage, 2000; Kwon and Tetzlaff, 2001).

Bridging devices with specifically designed geometry hold promise due both to their simplicity of fabrication, and numerous studies suggesting that tissue geometry is important for initial development of axonal tracts. Much of adult axonal development forms late utilizing previously formed structures (Singer et al., 1979) including oriented glia (Joosten and Gribnau, 1989; Silver et al., 1982; Silver and Ogawa, 1983), glial channels (Krayanek and Goldberg, 1981; Silver and Sidman, 1980), and the pioneering axons (Klose and Bentley, 1989; McConnell et al., 1989). The strength of the pioneering axon as a guidance cue has been demonstrated by follower growth into ectopic locations (Jellies et al., 2000) and through normally inhibitory terrain (Snow et al., 2003). More recently, a similar mechanical guidance mechanism was proposed to explain the precise reinnervation of targets by regenerating peripheral nerves (Nguyen et al., 2002).

The perceived role of substrate geometry, such as that exhibited by the pioneering axon during late development has inspired the study of specifically shaped bridging devices for axonal tract repair including filaments (Chauhan et al., 1999, Khan et al., 1991, 1990) and entubulation devices. The role of geometry relative to other cues is

---

* Corresponding author. 417 Wakara Way, Suite 3211, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84103, USA. Fax: +1 801 581 4049.
E-mail address: Roy.Smeal@m.cc.utah.edu (R.M. Smeal).

0014-4886/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2008.05.026

Table 1
Summary table listing all neuron types and pharmacological conditions tested

Cerebellar granule cell						
Filament diameter	30 µm	40 µm	60 µm	100 µm	220 µm	500 µm
Total neurite length (µm)	1887	1034	6883	4715	16,670	16,432
Postnatal DRG						
Filament diameter	35 µm	54 µm	95 µm	200 µm	300 µm	500 µm
Total neurite length (µm)	1769	3716	4130	17,087	14,279	11,189
Adult DRG						
Filament diameter	35 µm	65 µm	100 µm	215 µm	375 µm	550 µm
Total neurite length (µm)	14,413	13,005	19,587	16,269	11,701	29,010
Postnatal DRG DMSO						
Filament diameter	25 µm	65 µm	100 µm	200 µm	360 µm	550 µm
Total neurite length (µm)	16,513	9394	19,903	34,801	12,562	10,026
Postnatal DRG cyto						
Filament diameter	35 µm	65 µm	100 µm	215 µm	375 µm	
Total neurite length (µm)	4110	8867	7013	3573	3714	
Postnatal DRG taxol						
Filament diameter	35 µm	65 µm	100 µm	215 µm	375 µm	550 µm
Total neurite length (µm)	9534	5776	12,088	13,589	14,840	3228
Adult DRG DMSO						
Filament diameter	25 µm	65 µm	100 µm	200 µm	360 µm	550 µm
Total neurite length (µm)	12,546	8367	19,636	12,774	9523	11,203
Adult DRG cyto						
Filament diameter	25 µm	40 µm	65 µm	100 µm	200 µm	360 µm
Total neurite length (µm)	56,403	14,797	14,483	20,485	40,761	39,670
Adult DRG taxol						
Filament diameter	25 µm	40 µm	65 µm	100 µm		360 µm
Total neurite length (µm)	7090	6775	14,390	18,337		39,670

For each condition, all filament sizes examined and the total neurite length measured for each filament size are listed.

difficult to investigate *in vivo* due to an inability to independently test the individual contribution of the physical and the molecular adhesive features of extra-cellular matrix and cellular structures. We addressed this issue in previous work that examined axonal outgrowth from postnatal dorsal root ganglion (DRG) neurons on cylindrical substrates of varying diameters (Smeal et al., 2005). The quantification of neurite outgrowth demonstrated a strong bias in the direction of minimum curvature that increased as the filament diameter became more similar to structures found *in vivo*. A Boltzmann model suggested that the intrinsic stiffness of the axonal cytoskeleton was an important determinant of directional outgrowth on the curved substrate, that is, it appeared that the axon was too stiff to bend around the curved substrate. This study suggested that surfaces presenting adhesive cues impose additional constraints on growth choice due to the surface geometry. Since geometry can be easily engineered into bridging devices we sought to understand whether this type of curvature sensitivity was a general principle applicable to different ages and types of neurons given that a guidance cue for regeneration can only be effective if the target cell can sense and respond appropriately. For these reasons, we studied the behavior of the cerebellar granule neuron and the adult DRG neuron. The examination of the cerebellar granule neuron generalizes our previous observations of postnatal DRG neuron outgrowth behavior to a central nervous system neuron while examination of the adult DRG neuron allows comparison of the outgrowth behavior of the same neuronal type at two different ages. Study of these different neuron types will help determine if surface curvature is a generally effective cue for directing axonal outgrowth.

Methods

Substrate fabrication and curvature determination

Substrates of isotactic polypropylene (M_w=250,000; Aldrich; Milwaukee) were manufactured as previously described (Smeal et al., 2005). Filament diameters were visualized and measured using light microscopy and Image Pro software (Media Cybernetics; Silver Spring, MD). Filaments of diameters ranging from 25 µm to 550 µm were fabricated (Table 1) and the maximum standard deviation within a particular filament size was less than 3% of the diameter. While within batch variation is quite small, between batches it is difficult to recreate the exact diameter of previous batches due to the high sensitivity of the extruder to initial conditions. Surface topography was characterized using atomic force microscopy (Topometrix; Santa Clara, CA). Images of filament sizes revealed a nodular nanotopography with surface roughness averages varying between 18–55 nm, a level of topography that we found was insufficient to bias the directionality of neurite outgrowth even when intentionally patterned (Smeal et al., 2005). This surface characterization was important because only once it is established that curvature of the surface is indeed the only variable changing as the direction of neurite outgrowth changes can we say anything about the standard

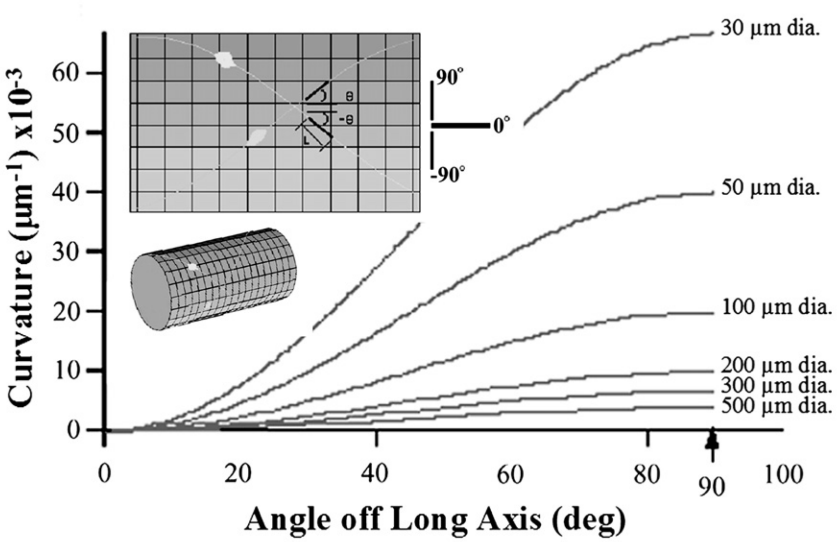

Fig. 1. Graphical representation of how curvature varies with respect to direction relative to the long axis of cylindrical structures. The long axis always has zero curvature while maximum curvature is always achieved at 90° relative to the direction of the cylinder's long axis. The maximum curvature for each filament size increases with decreasing filament diameter. (Inset) A graphic demonstrating how the angle of growth was measured. Angles were measured relative to the long axis and had a maximum value of 90°, a direction perpendicular to the long axis. The handedness of growth was initially preserved (negative or positive angles) in order to determine if any biases existed.