# Disruption of spinal cord white matter and sciatic nerve geometry inhibits axonal growth in vitro in the absence of glial scarring


## ABSTRACT

These observations suggest that glial scar-associated factors are not necessary to block axonal growth at sites of injury. Disruption of fiber tract geometry, perhaps involving myelin-associated neurite-growth inhibitors, may be sufficient to pose a barrier to regenerating axons in spinal cord white matter and peripheral nerves.


## INTRODUCTION

Background Axonal regeneration is limited in the central nervous system (CNS) following injury. This regeneration failure appears not to be due to intrinsic limitations of mature neurons to grow axons but, rather, to nonpermissive properties of the CNS environment. One theory is that white matter contains putative inhibitors of axonal growth associated with myelin. However, extensive axonal growth occurs in vivo from neurons transplanted into white matter, providing that disruption of tissue organization and glial scarring are minimized. In contrast, when tissue disruption was significant and accompanied by glial scarring, including astrogliosis and the upregulation of chondroitin sulfate proteoglycans (CSPGs), axonal growth stopped in areas of CSPG expression. These studies have questioned the role of myelin-associated inhibitors in preventing axonal regeneration and, together with in vitro studies showing inhibition of neurite growth by CSPGs, implicate the expression of CSPGs at injury sites in causing regeneration failure. However, these transplantation studies involved survival periods of over two days so that the contributions of glial scarring and disruption of the organization of cells and molecules that were present prior to injury (disrupted geometry) cannot be evaluated separately. This limitation can be overcome by culturing neurons on cryostat sections where both the success and orientation of neurite growth on white matter have been shown to depend on the geometry of the underlying fiber tract. Neurites on white matter are restricted to a parallel orientation, consistent with successful axonal growth in vivo from neuronal transplants. Given the dependence of neurite growth on tissue geometry, we sought to determine whether disruption of this geometry is sufficient to inhibit neurite growth in the absence of glial scarring. Adult rat spinal cord or sciatic nerve was crushed with forceps ex vivo and immediately frozen to prevent additional changes within the tissue, such as glial scarring, Wallerian degeneration and formation of bands of Büngner. Neurite growth on the uncrushed portions of spinal cord white matter or nerve was extensive and mostly parallel to the tract but significantly inhibited by crushed white matter or nerve. In contrast, neurites were unimpeded by crushed gray matter. These data suggest that disruption of CNS white matter and peripheral nerve geometry is sufficient to prevent axonal regeneration. The disrupted tissue elements involved in white matter may be oligodendrocytes and/or myelin since neurites were not inhibited by crushed gray matter. Therefore, regeneration failure in CNS white matter may be partly due to the persistence of disrupted myelin. In peripheral nerves, acute injury may also prevent regeneration but successful regeneration may occur following chronic changes involving clearance of putative inhibitors.

## CONCLUSION

Conclusions These results support a surprising hypothesis. Both white

matter and peripheral nerves may contain inhibitory factors whose normal role is to constrain axons to a parallel orientation by preventing collateral sprouting. In both situations, disruption of tissue geometry would result in a substrate that is less navigable by growing axons. However, whereas in peripheral nerves the appropriate geometry is reconstructed, in white matter, such reconstruction apparently fails or does not succeed in sufficient time to permit regeneration. In addition, glial scarring has long been suspected to contribute to the non-permissive environment encountered by regenerating axons in the CNS. What may then distinguish white matter and peripheral nerves with regard to regenerative potential is the rate of degeneration and/or reconstruction of the peritraumatic region and distal stump. Wallerian degeneration normally occurs much faster in peripheral nerves than in white matter. Although myelin clearance within the peritraumatic region of central fiber tracts begins soon after injury, this clearance proceeds slowly. Myelin debris have been reported within peritraumatic regions of white matter as late as 52 to 60 days following injury. Even if myelin debris are ultimately cleared from the peritraumatic region, this may not be sufficient to permit axonal regeneration. Although neurons appear to possess the intrinsic capacity to regenerate their axons, this capacity may not be retained indefinitely following injury. In the mutant Ola mouse, Wallerian degeneration is significantly delayed following injury to peripheral nerves but nevertheless occurs. However, nerve regeneration in these mutants never reaches the capacity observed in injured nerves of wild-type mice. If a similar time-constraint exists in white matter then degeneration of the distal stump must occur sufficiently within a critical time period to allow regeneration. The slow rate of Wallerian degeneration and reduced restoration of white matter geometry following injury, compared with that in peripheral nerves, may be a key reason why axonal regeneration in the CNS is impaired.