

Myelin contributes to the parallel orientation of axonal growth on white matter in vitro

## ABSTRACT

The present studies suggest that some of the relevant factors that constrain axonal growth on white matter are not haptotactic in nature and appear to be partly mediated by factors that are associated with myelin and may involve myelin-associated "inhibitors".

## INTRODUCTION

Background It previously was believed that axonal growth within white matter was not possible. This belief was based on the well-documented failure of injured axons to regenerate within the central nervous system (CNS) and reinforced by studies showing that neurons attach poorly to white matter in vitro. These studies, in part, supported the hypothesis that CNS myelin contains axon-growth inhibiting molecules. Additional investigations identified myelin-associated molecules, including Nogo (formerly NI-35/250), myelin-associated glycoprotein (MAG), and chondroitin sulfate proteoglycans, that inhibit neurite growth. Early studies, in which transplanted embryonic neurons extended parallel axons within white matter, appeared to be inconsistent with this hypothesis. However, successful growth was attributed to the possibility that embryonic neurons may not express receptors for myelin-associated inhibitors. Recent studies, however, demonstrated that white matter can support extensive parallel axonal growth from transplanted adult neurons. Recent tissue section culture experiments also demonstrated that white matter can support parallel neurite growth. Given the growing evidence that white matter can support axonal growth, we sought to identify the properties that mediate its parallel orientation. Physical edges and contours (haptotactic cues) can guide axonal growth independently of biochemical composition. Physical edges arranged in parallel within white matter, such as astroglial processes and axons, could theoretically guide parallel neurite growth. Alternatively, biochemical cues may guide parallel growth. Cryostat sections of rat brain were manipulated to deactivate biochemical guidance cues while preserving haptotactic cues and were then used as substrata for cultured neurons. These manipulations included prior fixation or mounting on polyornithine-coated culture dishes and, in both cases, non-parallel neurite growth occurred on white matter suggesting that biochemical cues are required for parallel growth. Additional experiments assessed the contribution of myelin to the parallel orientation of neurites. The orientation of neurites on myelin-deficient corpus callosum was assessed. Also, neurons were cultured with cAMP analogs or preincubated with nerve growth factor (NGF), treatments known to attenuate the overall inhibitory effects of myelin. Neurites extending on myelin-deficient corpus callosum or from neurons that were preincubated with NGF or treated with cAMP analogs were significantly less parallel. These results suggest that myelin contributes to the parallel orientation of neurite growth on white matter and that this effect may be mediated by its overall neurite-inhibitory properties.

## CONCLUSION

Conclusions These data suggest that the orienting influence of white matter geometry on neurite outgrowth involves myelin and, possibly, its associated neurite-growth inhibitory activity. The term "inhibitor" refers to the effects of myelin on neurites in studies that did not consider its normal geometric organization within white matter. In fact, close to the site of injury

where its geometry is disrupted, myelin may indeed be an obstacle to regeneration (see Pettigrew et al., companion paper ). It is now clear, however, that the presence alone of undisrupted myelin is insufficient to prevent regeneration. While the direct effect of myelin on growing axons in vitro is inhibitory but does little to block axonal growth within intact tracts in vivo, myelin may nevertheless play an important role in regulating axonal growth within intact tracts. Myelin may encourage directed, long-distance growth by restricting it to a parallel orientation. For example, in the spinal cord, where successful regeneration ultimately depends on axon extension in an appropriate direction over long distances, factors that promote parallel axonal growth, and discourage non-parallel growth, should enhance long-distance regeneration. In fact, several studies have been successful in promoting axonal regeneration across sites of spinal cord or optic nerve injury by blocking the myelin-associated inhibitor Nogo. However, these axons often fail to regain a parallel orientation within the distal stump. These axons "followed an irregular course" and "branching was seen quite frequently". The "inhibitory" activity of myelin may actually serve to promote axonal growth in an intact fiber tract (such as the distal stump of the injured spinal cord) by restricting this growth to an orientation that is parallel to the fiber tract while discouraging local meandering and collateral sprouting.