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# Level and localization of polysialic acid is critical for early peripheral nerve regeneration

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ABSTRACT

PolySia, the most striking post-translational modification of the neural cell adhesion molecule, is down-regulated during postnatal development. After peripheral nerve lesion, polySia is located on neuronal and glial cells normally not synthesizing polySia. However, structural consequences of reduced polySia content for peripheral nerve regeneration have not yet been clear. Furthermore, the contribution of sialyltransferases ST8SiaII and ST8SiaIV for the up-regulation of polySia has not been studied so far. In order to investigate the impact of polySia on regeneration processes of myelinated axons, we examined mouse mutants retaining only one functional sialyltransferase allele. In the absence of ST8SiaII, quantification of myelinated axons revealed a significant decrease in number and size of regenerated fibers without impairment of remyelination. In contrast, St8SiaIV deficiency resulted in increased fiber outgrowth and axonal maturation. Western blot analysis demonstrated that both ST8SiaII and St8SiaIV direct up-regulation of polySia. Cell-specific induction of polySia in myelinating Schwann cells and on regenerated axons in the presence of ST8SiaIV, but not ST8SiaII, indicates that not only the amount of polySia but also its cellular localization has a high impact on the regeneration progress of peripheral nerves.



## Introduction

One striking example of carbohydrate modification is polysialic acid (PSA, polySia), a linear homopolymer of α2,8-linked *N*-acetylneuraminic acid attached to the neural cell adhesion molecule (NCAM). Due to the large negatively charged and highly hydrated glycan structure, polySia increases the intermembrane space, affecting not only adhesive properties of NCAM but also other cell surface interactions. Removal of polySia by endoneuraminidase (Endo-N) has been shown to inhibit polySia-mediated dynamic cellular processes like neurite outgrowth, migration and synaptic plasticity (Seki and Arai, 1993; Rutishauser and Landmesser, 1996; Cremer et al., 2000; Rutishauser, 2008).

PolySia is synthesized on NCAM by two sialyltransferases ST8SiaII (STX) and ST8SiaIV (PST), which are differentially expressed in a tissue and cell type-specific manner (Angata and Fukuda, 2003). ST8SiaII dominates during embryonic development, whereas ST8SiaIV persists at relatively high levels in the postnatal brain (Hildebrandt et al., 1998; Ong et al., 1998). Deficiency of either enzymes resulted in mild but clearly distinct phenotype (for review see (Hildebrandt et al., 2007). Mice lacking ST8SiaIV showed a specific loss of polySia in mossy fibers accompanied by an impairment of synaptic plasticity, learning and memory (Eckhardt et al., 1995; Markram et al., 2007). Ablation of ST8SiaII is characterized by impaired basal synaptic transmission in dentate gyrus and altered fear conditioning (Angata et al., 2004; Stoenica et al., 2006). Furthermore, complete absence of polySia in mutant mice results in postnatal growth retardation, specific brain wiring defects like absence of anterior commissure, progressive hydrocephalus, and premature death during early postnatal period (Weinhold et al., 2005).

In the peripheral nervous system (PNS), polySia is homogeneously distributed on cell bodies and neurites of mouse spinal ganglia neurons on embryonic day 12 (Boisseau et al., 1991). Inhibition of polySia in chicken sensory neuron cultures increases the thickness of neurite bundles (Rutishauser et al., 1985) and reduces neurite outgrowth (Charter et al., 2002). In contrast to the central nervous system (CNS), polySia is not present on myelinating glial cells of the PNS, the Schwann cells. Although these cells express NCAM, polySia is absent on rat embryonic and perinatal Schwann cells (Thomaidou et al., 2001; Lavdas et al., 2006). Importantly, PSA-NCAM is localized and up-regulated in Schwann cells after peripheral nerve lesion, demyelination of spinal cord or graft implantation of peripheral nerve

*Abbreviations:* polySia, polysialic acid; NCAM, neural cell adhesion molecule; endo-N, endoneuraminidase.

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into thalamus (Daniloff et al., 1986; Oumesmar et al., 1995; Zhang et al., 1995) suggesting an impact of polySia on the activity of Schwann cells during the regeneration process.

Following peripheral nerve injury, Schwann cells undergo dedifferentiation and proliferation. After migration to the lesion site, they form bands of Büngner resulting in a promoting environment for axonal regeneration and ensuing remyelination (Fawcett and Keynes, 1990; Chalfoun et al., 2006). In vitro studies revealed that neonatal and adult Schwann cells cultured on polySia substrates were not influenced in their survival or proliferation (Haile et al., 2007, 2008). Genetically engineered Schwann cells with sustained polySia level exhibited enhanced migratory potential in vitro without impairment of their myelinating ability (Lavdas et al., 2006). These properties established polySia as a promising candidate for the development of new therapeutic strategies for PNS (Gravvanis et al., 2005) and CNS lesions (Papastefanaki et al., 2007; Zhang et al., 2007a,b).

However, the physiological role of polySia during early regeneration of myelinated axons in the peripheral nervous system is not completely understood. In addition, the importance of ST8SiaII and ST8SiaIV for polysialylation throughout this process has not been explored yet. Regenerating nerves from mutant mice with reduced polySia content showed differences in number and size of outgrowing axons depending on the presence of the appropriate polySia-synthesizing enzyme. In the absence of ST8SiaII, polySia is exclusively located on myelinating Schwann cells and regenerating axons. This study demonstrates the modulating function of polySia on neurite outgrowth and maturation during the early regeneration process and indicates that the level and localization of polySia is critical for the timing of regeneration.

## Results

### *Absence of polySia has no influence on the postnatal development of the sciatic nerve*

To examine whether polySia is important for the neurite outgrowth and myelination of developing peripheral nerve fibers, we quantified the number and size of myelinated axons in sciatic nerves of polySia-deficient mice (ST8SiaII$^{-/-}$ST8SiaIV$^{-/-}$) at postnatal day 10. At this time, nearly all NCAM is polysialylated (Oltmann-Norden et al., 2008). The density of myelinated fibers (calculated by dividing the total number of axons per nerve diameter) was similar in wildtype mice and mouse mutants (Figs. 1A, B). Because several lines of evidence indicate that polySia has a negative effect on myelination in CNS (Bartsch et al., 1990; Oumesmar et al., 1995; Charles et al., 2000, 2002; Fewou et al., 2007; Jakovcevski et al., 2007), we measured the myelin thickness and calculated the *g*-ratio, an indicator for myelination extent. The *g*-ratio was unaffected in the absence of polySia (Fig. 1C). Also the size of axons and myelin sheaths was not different between both genotypes, independent of specific axonal subpopulations (Figs. 1D, E). These results indicate that the presence of polySia is not essential for the structural development of peripheral nerves.

### *Reduced polySia level altered the number and size of regenerated fibers*

The early postnatal death of mice lacking both polySia transferases constrained us in examining the relevance of polySia in the regeneration process in the adult peripheral nervous system of mice with a normal life expectancy. Therefore we used mouse mutants with one functional polysialyltransferase allele demonstrating the lowest

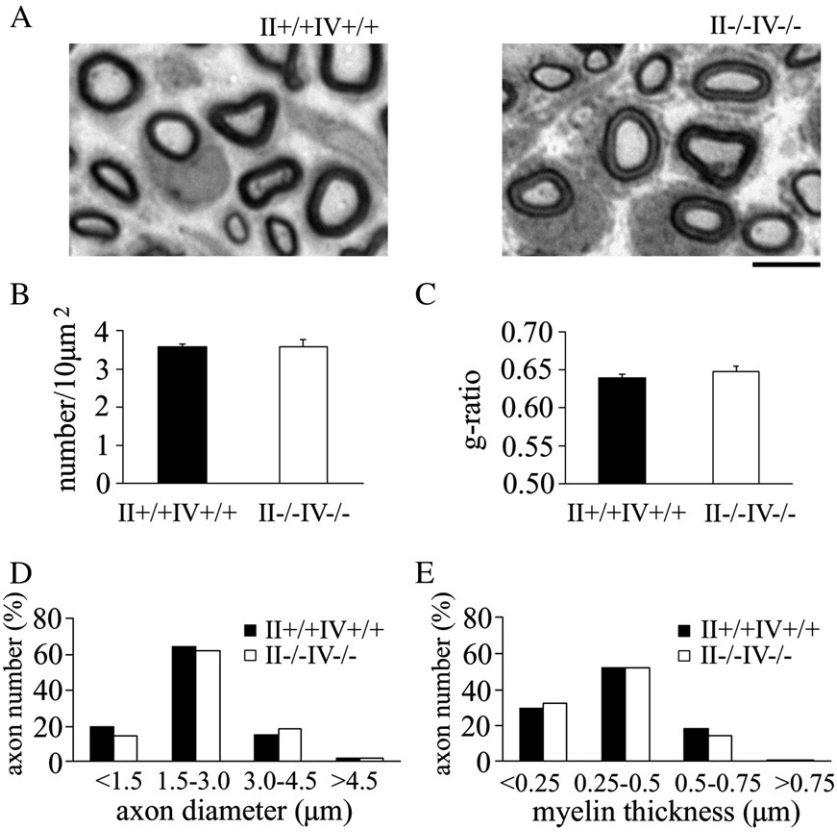


**Fig. 1.** No difference in number and size of myelinated axons in sciatic nerve from polySia-deficient mice at postnatal day 10. (A) Note the same distribution of myelinated axons for wildtype and polySia-deficient (St8SiaII$^{-/-}$St8SiaIV$^{-/-}$) mice. Scale bar: 10 µm. Quantification of fiber density (B) and calculation of *g*-ratio (C) revealed no change in polySia-deficient mice compared to wildtype. In addition, distribution of axon diameter (D) and myelin sheaths thickness (E) was unaffected. Means (±SEM) of wildtype (II$^{+/+}$IV$^{+/+}$; $n=4$) and polySia-deficient mice (II$^{-/-}$IV$^{-/-}$, $n=4$) are presented.