Polysialyltransferase overexpression in Schwann cells mediates different effects during peripheral nerve regeneration

Julia Jungnickel²,*, Matthias Eckhardt¹,3,*, Kirsten Haastert-Talini²,4, Peter Claus²,4, Paul Bronzlik², Esther Lipokatic-Takacs², Helena Maier³, Volkmar Gieselmann³, and Claudia Grothe¹,2,4

²Hannover Medical School, Institute of Neuroanatomy, 30625 Hannover, Germany; ³Institute of Biochemistry and Molecular Biology, University of Bonn, Nussallee 11, 53115 Bonn, Germany; and 4Center for Systems Neuroscience (ZSN), Hannover, Germany

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The polysialic acid (PSA) moiety of the neural cell adhesion molecule (NCAM) has been shown to support dynamic changes underlying peripheral nerve regeneration. Using transgenic mice expressing polysialyltransferase ST8SiaIV under control of a glial-specific (proteolipid protein, PLP) promoter (PLP-ST8SiaIV-transgenic mice), we tested the hypothesis that permanent synthesis of PSA in Schwann cells impairs functional recovery of lesioned peripheral nerves. After sciatic nerve crush, histomorphometric analyses demonstrated impaired remyelination of regenerated axons at the lesion site and in target tissue of PLP-ST8SiaIV-transgenic mice, though the number and size of regenerating unmyelinated axons were not changed. This was accompanied by slower mechanosensory recovery in PLP-ST8SiaIV-transgenic mice. However, the proportion of successfully mono-(re)innervated motor endplates in the foot pad muscle was significantly increased in the PLP-ST8SiaIV-transgenic mice when compared with wild-type littermates, suggesting that long-term increase in PSA levels in regenerating nerves may favor selective motor target reinnervation. The combined negative and positive effects of a continuous polysialyltransferase overexpression observed during peripheral nerve regeneration suggest that an optimized time- and differentiation-dependent control of polysialyltransferase expression in Schwann cells may further improve recovery after peripheral nerves injury.

Keywords: nerve regeneration / neural cell adhesion molecule / polysialic acid / sialyltransferase / transgenic mice

Introduction

Polysialic acid (PSA), a linear homopolymer of α2,8-linked N-acetylneuraminic acid, is a post-translational modification of the neural cell adhesion molecule (NCAM) and has been shown to stimulate neurite outgrowth, migration and synaptic plasticity (for review, see Rutishauser 2008). Although NCAM is the major carrier of PSA, other molecules are also polysialylated, including the polysialyltransferases ST8SiaIV and ST8SiaII (Mühlenhoff et al. 1996; Close and Colley 1998), neuropilin-2 (Curreli et al. 2007) and SynCAM1 (Galuska et al. 2010). During the development of peripheral nerves, PSA is present on almost all growing axons (Boisseau et al. 1991), but is lost in mature, myelinated axons, and in adults, PSA is restricted to only a subset of small unmyelinated axons (Jungnickel, Bramer, et al. 2009). There is a clear time-dependent correlation between loss of axonal PSA and myelination during development (Charles et al. 2000), and remyelination after cuprizone-induced demyelination is improved in mice lacking polysialyltransferase ST8SiaIV (Koutsoudaki et al. 2010), indicating negative regulation of myelin formation by PSA. Re-expression of PSA in glial cells after chemically induced demyelination in the spinal cord (Oumesmar et al. 1995) or peripheral nerve grafts into the thalamus (Zhang et al. 1995) suggested that PSA may also positively affect nerve regeneration.

Schwann cells are critical players in the regeneration of peripheral nerves. After migrating to the lesion site, they set up a promoting environment for axonal regeneration, and finally differentiate into myelinating Schwann cells to remyelinate the regenerated axons (for review, see Fawcett and Keynes 1990). Migrating Schwann cells express PSA, and studies using transduced Schwann cells overexpressing polysialyltransferases showed improved migratory potential of Schwann cells synthesizing larger amounts of PSA (Gravvanis et al. 2005; Lavdas et al. 2006). The capability of these modified cells to remyelinate central nervous system (CNS) axons was not impaired, which correlated with the down-regulation of polysialyltransferase activity (Gravvanis et al. 2005; Lavdas et al. 2006). It has not been examined, however, whether this unexplained down-regulation of the
polysialylation capacity was required for normal remyelination or just a coincidence. In order to examine possible negative or positive effects of a sustained PSA overexpression in Schwann cells, we generated transgenic mice expressing the polysialyltransferase ST8Sial (Eckhardt et al. 1995) under the control of the glial-specific proteolipid protein (PLP) promoter (PLP-ST8Sial-transgenic mice), which is also active in myelinating and, though at a lower level, in non-myelinating Schwann cells (Puckett et al. 1987; Griffiths et al. 1989; Gupta et al. 1991; Jiang et al. 2000).

PLP-ST8Sial-transgenic mice showed sustained elevated levels of PSA in non-myelinating and myelinating Schwann cells in intact and lesioned sciatic nerves. Elevated PSA levels in Schwann cells caused a delay in remyelination of regenerating axons in lesioned sciatic nerves, which was accompanied by a delay in sensory recovery. Furthermore, the number of non-innervated neuromuscular junctions (NMJ) was significantly increased, indicating a delay in motor target reinnervation. Unexpectedly, however, a significantly increased number of mono-innervated NMJ in transgenic mice suggests an improved selective re-occupation of motor endplates. These results suggest combined negative and positive effects of a sustained polysialyltransferase expression in Schwann cells during peripheral nerve regeneration and indicate that a better control of PSA levels may further improve recovery after peripheral nerves injury.

**Results**

*Preventing the down-regulation of polysialyltransferase expression in Schwann cells delays myelination in peripheral nerves*

Two independent transgenic mouse lines (tg246 and tg281) expressing polysialyltransferase ST8Sial under control of the PLP promoter have been described previously and showed an oligodendrocyte-specific increase in PSA (Fewou et al. 2007). We examined the possible up-regulation of PSA in peripheral nerves of these mice by western blotting, which revealed a significant increase in PSA in both transgenic lines compared with aged-matched wild-type controls (Figure 1A). NCAM-120, the predominant NCAM isoform in Schwann cells, was clearly detectable in wild-type nerves. In contrast, the NCAM-120 band was hardly detectable in both transgenic lines, indicating almost complete polysialylation of NCAM-120 in transgenic mice. The presence of NCAM-120 could be confirmed, however, by pretreating lysates with endonuclease (data not shown).

Morphometric analysis of sciatic nerves at different time points revealed that the g-ratio (ratio of the axon diameter and the outer diameter of the myelinated fiber) was slightly but significantly increased in young (P17) but not adult (P74) transgenic mice (Figure 1B), suggesting delayed myelination during post-natal development, comparable with our observations on CNS myelin formation in these mice (Fewou et al. 2007). However, the g-ratio (Figure 1B) was normal in adult transgenic mice. We therefore conclude that there was only a minor transient inhibitory effect on myelination caused by the presence of PSA in Schwann cells during development. The observation that PSA levels were strongly increased in adult transgenic mice compared with wild-type littermates (Figure 1A) is in contrast to previous reports that uses transplanted genetically modified Schwann cells overexpressing polysialyltransferases (Gravvanis et al. 2005; Lavdas et al. 2006). In these studies, an unexplained silencing of the polysialylation occurred at about the time point when the transplanted Schwann cells began to myelinate axons. In our transgenic mice, however, NCAM-120 appeared to be almost completely polysialylated, indicating that the PSA level in the transgenic mice was only limited by the NCAM-120 expression. We therefore concluded that PLP-ST8Sial-transgenic mice are a useful model system to evaluate positive or negative effects on nerve regeneration of PSA in myelinating Schwann cells that do not down-regulate their polysialylation capacity.

*After nerve crush, PSA is up-regulated in transgenic Schwann cells during remyelination*

To examine nerve regeneration capacity in transgenic mouse, the left sciatic nerve was crushed at the mid-thigh level and regenerated nerves were evaluated 0.5 mm distal to the lesion site. Seven days after sciatic nerve crush, PSA staining was unchanged in wild-type mice compared with unlesioned controls (in line with our previous observations: Jungnickel, Bramer, et al. 2009), and PSA expression was comparable distal and proximal to the lesion (Figure 2). In contrast, PSA was strongly increased distal to the lesion in transgenic mice (Figure 2).

In transgenic, but not in wild-type, mice, PSA was detectable on non-myelinating [i.e. growth-associated protein (GAP)-43-positive] Schwann cells, proximal (Figure 3A) and
To address the question whether the Schwann cell-specific up-regulation of PSA interferes with the early regeneration and/or remyelination process, the total number and size of myelinated axons were determined 1 week after peripheral nerve crush injury in regenerating sciatic nerves. We found significantly fewer myelinated regenerated axons 0.5 mm distal to the lesion site in transgenic mice (line tg281: 11%; line tg246: 39%) compared with wild-type littermates (Figure 4A and B). However, the g-ratio of myelinated axons in transgenic fibers was not significantly different from wild-type controls, as was the mean axonal diameter of regenerated axons (Figure 4B). To further discriminate between reduced neurite outgrowth and delayed remyelination of regenerating axons in transgenic mice, number and diameter of unmyelinated axons in Remak bundles were determined. Although there was no significant difference concerning number (Figure 4C) and diameter (data not shown) of unmyelinated axons in transgenic mice compared with wild-type littermates, transgenic mice had a greater proportion of Schwann cell...
units containing a larger number of unmyelinated axons resulting in a significant change in frequency distribution (Figure 4C).

Thus, though the number of non-myelinated regenerated axons and the g-ratio of remyelinated axons were not significantly altered in transgenic mice compared with wild-type controls, there was a strong and significant reduction in the number of regenerated myelinated axons in transgenic mice. Therefore, we conclude that initiation of remyelination by the transgenic Schwann cells was disturbed, but that the myelination process thereafter appeared to precede normally.
Altered motor target reinnervation in transgenic mice

Myelinated axons regrowing into the motor target were evaluated by estimating the density of nerve bundles in semi-thin sections of the flexor plantaris muscle 4 weeks after sciatic nerve crush (Figure 5A). The number of myelinated axons in transgenic mice was strongly reduced and in fact only 50% of nerve bundles examined contained myelinated axons. In contrast, myelinated fibers were present in all examined wild-type samples.

Reinnervation of NMJ was examined by histological analysis of motor endplates of plantar muscles 4 weeks after nerve injury. The percentage of non-innervated endplates was clearly higher (29 ± 3%) in transgenic mice compared with wild-type controls (7 ± 2%) (Figure 5B) and could potentially indicate delayed ingrowth of regenerating axons into the muscle. Whereas a minority of endplates in the plantaris muscle (20 ± 7%) was mono-innervated in wild-type mice, the percentage of mono-innervated endplates was significantly increased (50 ± 5%) in transgenic mice (Figure 5B). The small percentage of mono-innervated endplates in wild-type mice is in contrast to previous studies evaluating motor endplates of the tibialis and soleus muscle (Wang et al. 2004; Magill et al. 2007), but may be explained by the rather distal location of the plantaris muscle. The majority of endplates in wild-type mice (72 ± 10%) showed a small post-synaptic area, possibly due to permanent loss of acetylcholine receptor (AChR) and/or lack of insertion of new receptor underneath the eliminated nerve terminal, which is typical for this stage of regeneration (Kawabuchi et al. 2001). Receptors begin to be eliminated only after axons reoccupy an endplate site (Rich and Lichtman 1989). In contrast, the percentage of NMJ population with a (partial) loss of AChR was significantly lower (15 ± 7%) in transgenic mice (Figure 5B).

Delayed mechanosensory recovery in transgenic mice

Pressure and pain sensitivity was tested by pinching the three lateral digits (toes 3, 4 and 5) of uncrushed and nerve crushed hind limbs. A pinch reliably induced a rapid retraction of the lateral digits (toes 3, 4 and 5) of uncrushed and nerve crushed hind limbs. Pinch tests were always done by the same person and blinded for each genotype. The sensitivity was evaluated in the lateral digits was slower. The pinch test demonstrated a significantly slower (+6 days) recovery of pressure/pain sensation of the third digit of PLP-ST8SiaIV-transgenic mice (line tg281) when compared with wild-type littermates (Figure 6).

Discussion

PSA significantly affects regeneration processes in the CNS and PNS. Thereby, positive and negative effects can be observed, depending on the time point of the regeneration process and the cell types synthesizing PSA. While migration of neuronal and glial precursor cells and neurite outgrowth are positively influenced by PSA (for review, see Rutishauser 2008), a number of studies indicated that axonal PSA is a negative regulator of myelination (Bartsch et al. 1990; Oumesmar et al. 1995; Jakovcevski et al. 2007). In line with this, remyelination after cuprizone-induced demyelination was improved in polysialyltransferase ST8SiaIV-deficient mice, due to better oligodendrocyte precursor cell recruitment and differentiation (Koutsoudaki et al. 2010). In contrast, myelin formation was impaired in transgenic mice overexpressing ST8SiaIV in oligodendrocytes (Fewou et al. 2007), indicating that PSA may also impair myelination if present on the myelin forming glial cells in the CNS. We show here that the inhibitory effect of PSA on myelination is also found in the PNS, as indicated by an increased g-ratio during post-natal development. However, in contrast to CNS myelin (Fewou et al. 2007), reduced myelin content in the PNS appear to be transient, as indicated by the normal g-ratio in adult transgenic mice.

In previously reported model systems using transplanted Schwann cells overexpressing polysialyltransferase ST8SiaIV (Gravvans et al. 2005; Lavdas et al. 2006) or soluble PSA mimicking peptides (PSA mimetic) (Mehanna et al. 2009, 2010), significant improvements of Schwann cell migration, remyelination and functional recovery have been observed. In these model systems, the presence of PSA or the PSA mimetic was (or was expected to be) only transient, because of an unexplained loss of polysialylation capability of the transduced Schwann cells (or the expected short half-life of the PSA mimetic) (Mehanna et al. 2009). Whereas PSA stimulated migration of transplanted Schwann cells without affecting remyelination capacity (Gravvans et al. 2005; Lavdas et al. 2006), remyelination was improved in the presence of the PSA mimetic (Mehanna et al. 2009, 2010). Loss of PSA from the genetically modified Schwann cells at about the time point, when remyelination occurred, could explain normal myelination capacity of these cells, though it was not shown that this PSA down-regulation was indeed necessary for successful remyelination.

Our results indicate that the permanent up-regulation of PSA synthesis in Schwann cells delays remyelination of regenerated axons. Because the g-ratios of remyelinated axons and the size and number of unmethylated axons in regenerating nerves were not significantly affected in the transgenic mice compared with wild-type littermates, it appears most likely that the initiation of remyelination was impaired. This could be due to impaired recruiting of transgenic Schwann...
cells to the lesion site, which, however, seems unlikely, as the presence of PSA on the surface of these cells has been shown to stimulate migration and nerve regeneration (Lavdas et al. 2006). Most likely, reduced numbers of remyelinated axons 1 week after the lesion, are caused by an inhibitory effect of PSA-carrying NCAM, and possibly neuropilin-2 and SynCAM1, present on myelinating transgenic Schwann cells, disturbing trans-interactions with the regenerated axons. Delayed mechanosensory recovery in transgenic mice indicates a mild impairment in the functional recovery caused by the presence of PSA in Schwann cells.

Polysialylated molecules synthesized by Schwann cells, however, may also exhibit positive effects on the functional motor neuron regeneration. Successful re-occupation of motor endplates correlates well with the recovery of motor function (Guntinas-Lichius et al. 2005; Magill et al. 2007). Our data show that up-regulation of PSA in Schwann cells increased the number of mono-innervated NMJ. A prerequisite for successful reinnervation of NMJ is a sufficient number of outgrowing axons occupying their original endplates after entering the correct muscle fiber. The number of ingrowing axons can be increased by selective outgrowth of original fibers or by unsppecific branching of collaterals (at the node of Ranvier) or terminals (at the endplate) resulting in polyinnervated motor endplates. It is well established that the intrinsic property of different pools of motor neurons to re-express PSA after peripheral nerve transection and repair is necessary for selective motor axon targeting (Franz et al. 2005, 2008). In mice lacking PSA, the number of collateral sprouts, their field of arborization and the withdrawal of misprojected axons were attenuated, indicating that PSA promotes these processes (Franz et al. 2005). In our crush model, the number of polyinnervated NMJ was not significantly altered in transgenic mice suggesting that selective reinnervation of original endplates but not increased sprouting is responsible for the increased number of mono-innervated NMJ in transgenic mice. How could the presence of PSA in Schwann cells promote selective neurite outgrowth? One possibility is that Schwann cell-derived PSA serves as a growth stimulus in perineural tubes (bands of Büngner). Furthermore, PSA in terminal Schwann cells could promote sprouting and specifically guide the lesioned axons to their destination. Interestingly, the number of non-innervated NMJ with complete or partial loss of AchR was reduced in transgenic mice.

Though the reason for this improved selective reinnervation of motor endplates are not clear at present, these results have implications for the development of strategies using polysialyltransferase overexpression in (e.g. lentiviral transduced) transplanted Schwann cells or PSA mimetics to improve functional recovery after peripheral nerve injury. Although the persisting PSA increase in Schwann cells can disturb axon–glial interactions and therefore delay remyelination, suggesting it may be important to ensure the down-regulation of polysialyltransferase expression after the cells have reached their destination (Gravvanis et al. 2005; Lavdas et al. 2006), persistent polysialyltransferase overexpression have also positive effects on the regeneration process. Therefore, an optimized time- and differentiation-dependent control of polysialyltransferase overexpression and down-regulation in genetically modified Schwann cells (and possibly also oligodendrocyte precursor cells) used to improve functional recovery in PNS (and possibly also in CNS) may even further improve the regeneration capacity of these cells.

Materials and methods

Characterization of PLP-ST8SiaIV-transgenic mice

Transgenic mice expressing ST8SiaIV in myelinating glia cells under control of the PLP promoter (PLP-ST8SiaIV) were generated and characterized previously, regarding the specific overexpression of ST8SiaIV in oligodendrocytes and its impact on CNS myelination (Fewou et al. 2007). Several founder mice were obtained from which two independent transgenic lines (tg281 and tg246) were established and maintained on a mixed C57BL6xCBA genetic background. Genotyping of mice was done by polymerase chain reaction on genomic tail DNA using oligonucleotides 5’-CCATGCCGCTCAATT AGAAAACG-3’ and 5’-TTATGCTTCTGACACTTTC-3’, which resulted in a 1090 bp product in the case of transgenic mice.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and western blotting

Sciatic nerve samples from transgenic and wild-type controls from both transgenic lines were homogenized in 20 mM Tris–HCl (pH 7.4), 150 mM NaCl (tris-buffered saline, TBS), 5 mM ethylenediaminetetraacetic acid, 1 mM phenylmethylsulfonyl fluoride using an Ultraturrax homogenizer (Janke & Kunkel, Staufen, Germany). Homogenates were centrifuged at 1000 × g for 10 min and the supernatants were centrifuged again at 100,000 × g for 1 h (4°C) to isolate membranes. The membrane pellet was resuspended in TBS. Protein concentrations were determined using the Biorad DC assay (Biorad, Munich, Germany). The final pellet was dissolved in TBS and separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) in 10% polyacrylamide gels by using the standard procedures. Protein transfer onto nitrocellulose membranes ( pore size 0.45 µm; Schleicher&Schuell, Dassel, Germany) was done with 48 mM Tris, 39 mM glycine as transfer buffer using semi-dry blotting. Non-specific binding sites were blocked with 3% (w/v) nonfat dry milk in TBS and antibodies were diluted in the same buffer. Blots were stained with anti-PSA antibody 735 (mouse IgG; gift from R. Gerardy-Schahn, Hannover Medical School, Germany), rat-anti-NCAM clone H28 (Chemicon Europe, Hoenheim, Germany) and anti-β tubulin (clone B-5-1-2, mouse IgG; Sigma, Taufkirchen, Germany) dissolved in blocking solution. Unbound antibodies were removed by washing membranes in TBS/0.3% Tween-20. Bound primary antibodies were detected using the appropriate peroxidase-conjugated secondary antibodies (Dianova, Hamburg, Germany) followed by chemiluminescence detection, as described (Eckhardt et al. 2002).

Surgery

Sciatic nerve crush was done in transgenic and wild-type mice as described before (Jungnickel et al. 2006). Briefly, mice were anesthetized by intraperitoneal injection (10 mL/kg) of a
mixture of ketamine (1%) and xylazine (0.1%). The left sciatic nerve was crushed at the mid-thigh level with a flattened forceps for 30 s. Nerves from unlesioned mice were used as control samples. One and 4 weeks after peripheral nerve crush, experimental animals and age-matched control animals were sacrificed for tissue harvest. Experimental protocols were done in accordance to the German law for protection of animals with a permit by the local authority.

Functional tests
Recovery of mechanical sensitivity was tested as described previously (Siconolfi and Seeds 2001; Lino et al. 2007). Foot withdrawal and vocalization were recorded as positive responses after pinching the most distal portion of the digits on lesioned and unlesioned hind limbs with a flattened forceps (the pinch test). Because the activated saphenous nerve sprouts in the denervated most medial digits of the hind paw (De Koning et al. 1986), only the three lateral digits were tested before and after sciatic nerve crush for up to 28 days.

Morphometric analyses
Preparation of sciatic nerves, sectioning and quantification of myelinated axons and myelin thickness were done as described previously (Jungnickel et al. 2004; Haastert et al. 2006). Briefly, sciatic nerves from 12 transgenic animals and 6 wild-type mice were removed and fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in sodium cacodylate buffer overnight at 4°C. Samples were osmicated (90 min) and stained with 1% potassium dichromate followed by 0.5% hematoxylin/70% ethanol. After epon-embedding, semi-thin transverse sections (1 µm) were cut with glass knives and additionally stained with toluidine blue prior to analysis by light microscopy (BX60, Olympus, Germany). Quantification of myelinated axons (large diameter fibers wrapped by a Schwann cell forming a myelin sheath) was performed with a semi-automatic program on the basis of AnalySIS as described before (Timmer et al. 2003). At a distance of 0.5 mm distal to the crush site, the total fiber numbers and axonal areas were determined on entire cross sections. The crush site, i.e. the transition from intact to lesioned axons, was identified histologically on semi-thin sections as described (Jungnickel et al. 2004, 2006). The g-ratio (ratio of the axon diameter and the outer diameter of the myelinated fiber) and myelin thickness were evaluated for 100 axons of each section. All morphometric measurements were conducted in a blinded manner using coded sections. Ultrathin sections (50 nm) were cut with a diamond knife, collected and stained with lead citrate and uranyl acetate. Four fields with unmyelinated fibers (variable number of small diameter axons simply enveloped by the cytoplasm of Schwann cells) were photomicrographed with an EM10 electron microscope (Zeiss, Germany) at a magnification of 6,300×, and the number of Remak bundles including the number and size of unmyelinated axons was determined by using AnalySIS software. For comparison with previous studies (Funakoshi et al. 1998), the most distal flexor plantaris muscles from four transgenic and four wild-type mice were dissected and embedded in epon. The number of intramuscular axon bundles was estimated in two semi-thin sections of flexor plantaris muscles 4 weeks after sciatic nerve injury.

Immunocytochemistry
Sciatic nerves of three additional transgenic and three wild-type animals were removed and fixed in 4% paraformaldehyde. Semi-thin transverse sections (1 µm) were cut with glass knives after epon-embedding. Corrosion of the section surface was obtained by etching the section in a solution of saturated sodium hydroxide diluted in absolute ethanol for 15 min. After rehydration, non-specific binding sites were blocked with 5% horse serum in phosphate-buffered saline. Sections were incubated overnight at 4°C with the following antibodies: mouse anti-PSA (1:175), rabbit anti-GAP-43 (1:300; Chemicon Europe), rabbit anti-S100 (1:500; Dako, Hamburg, Germany) and mouse anti-myelin protein zero (P0, 1:1000, gift from J. J. Archelos). Bound primary antibodies were detected with Alexa Fluor 555 goat anti-mouse IgG and Alexa Fluor 488 goat anti-rabbit IgG (1:400, Life Technologies, Darmstadt, Germany). Longitudinal sections of skin foot pad 40 µm in thickness were stained with an antibody directed against neurofilament (pan cocktail; Biotrend, Cologne, Germany). Longitudinal sections of plantaris muscle 40 µm in thickness were labeled with Alexa Fluor 555-conjugated α-bungarotoxin (Life Technologies) and co-stained with antibodies directed against synaptophysin (Dako), neurofilament (pan cocktail; Biotrend) and S100 (Dako), using a standard protocol as described previously (Li et al. 2007).

Counting and classification of NMJ
All endplates were counted in every 15th section (total of 6–7 sections per animal) by one investigator in a blinded fashion using a fluorescence microscope (objective 20×, BX70, Olympus). Endplates were classified as mono-innervated (one axon with presynaptic compartment), poly-innervated (two or more axons), non-innervated (no axon and no presynaptic compartment) and endplates with substantial loss of postsynaptic AChR (no pretzel-like shape, more than 50% smaller post-synaptic area and no presynaptic compartment). Photographic documentation was made on a SP2 confocal microscope (Leica, Wetzlar, Germany). Images were taken in z-series with maximum projection.

Statistics
Data were expressed as the mean ± SD (western blot densitometry), mean ± SEM (morphometry and behavioral analysis) or percentage of the evaluated population (number of axons per Remak bundle) and evaluated by an unpaired Student’s t-test or by χ² test depending on the experimental design. Differences among groups were considered significant if P < 0.05.

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Conflict of interest
None declared.

Abbreviations
AChR, acetylcholine receptor; CNS, central nervous system; GAP, growth-associated protein; NCAM, neural cell adhesion molecule; NJM, neuromuscular junctions; P/LP, proteolipid protein; PSA, polysialic acid; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TBS, tris-buffered saline.

References


