INVASION OF LESION TERRITORY BY REGENERATING FIBERS AFTER SPINAL CORD INJURY IN ADULT MACAQUE MONKEYS


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Abstract—In adult macaque monkeys subjected to an incomplete spinal cord injury (SCI), corticospinal (CS) fibers are rarely observed to grow in the lesion territory. This situation is little affected by the application of an anti-Nogo-A antibody which otherwise fosters the growth of CS fibers rostrally and caudally to the lesion. However, when using the Sternberger monoclonal-incorporated antibody 32 (SMI-32), a marker detecting a non-phosphorylated neurofilament epitope, numerous SMI-32-positive (+) fibers were observed in the spinal lesion territory of 18 adult macaque monkeys; eight of these animals had received a control antibody infusion intrathecally for 1 month after the injury, five animals an anti-Nogo-A antibody together with brain-derived neurotrophic factor (BDNF), and five animals an anti-Nogo-A antibody with BDNF influenced the number or the length of the SMI-32 (+) stained. In the undamaged spinal tissue, motoneurons which presumably originate from motoneurons.

Key words: spinal cord lesion, scar tissue, primate, regeneration, BDNF, Nogo-A.

INTRODUCTION

In the adult central nervous system (CNS) of mammals, axotomized nerve fibers fail to regenerate over long distances. Among the factors contributing to this failure, the development of a scar tissue at the lesion site leads to the formation of an environment hostile to nerve fibers' regrowth and acting as a barrier against axonal regeneration into and across the lesion territory. Indeed, the scar tissue contains various neurite growth-inhibiting factors produced by cells such as microglial cells, meningeal cells, astrocytes or oligodendrocytes (Reier et al., 1983; Schwab and Bartholdi, 1996; Schwab, 2002; David and Lacrroix, 2003). These last years, several growth-inhibiting molecules expressed in the scar tissue were identified such as the chondroitin sulfate proteoglycans (CSPGs), tenascin-c or semaphorins (Rudge and Silver, 1990; McKeon et al., 1991; Davies et al., 1997, 1999; Fawcett and Asher, 1999; Pasterkamp and Verhaagen, 2001). Nogo-A present in CNS myelin and oligodendrocytes inhibits the regeneration of spinal-lesioned axons (Schwab, 2004, 2010). In rodents, the application at the level of the spinal cord of an antibody neutralizing Nogo-A promotes both corticospinal (CS) fibers regeneration and functional recovery (Schnell and Schwab, 1990; Thalmai et al., 1998; Schwab, 2004; Liebscher et al., 2005; Maier et al., 2009). Experiments conducted on primates lead to similar results (Freund et al., 2006, 2007, 2009). In particular, these experiments have shown that in anti-Nogo-A antibody-treated monkeys, CS fibers sprout and regenerate rostrally, around and caudally to the spinal lesion (Fouad et al., 2004; Freund et al., 2006, 2007). However, in these animals, CS fibers were only exceptionally observed inside the lesion territory. Nevertheless, it remains possible that following spinal cord injury (SCI), neurons distinct from CS neurons show an ability to regenerate and to grow in or even through the scar tissue.

Neurofilaments constitute the main structural element of the neuronal cytoskeleton and the presence in neurons of non-phosphorylated forms of neurofilaments can be shown using the Sternberger monoclonal-incorporated...
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antibody 32 (SMI-32) (Sternberger and Sternberger, 1983; Lee et al., 1988). Inside the spinal cord, motoneurons present a strong SMI-32 immunoreactivity (Tsang et al., 2000). The goal of the present study was to investigate, using the SMI-32 antibody whether neurites can cross the scar tissue and colonize the lesion site and whether the anti-Nogo-A antibody treatment alone or combined with brain-derived neurotrophic factor (BDNF) can influence such regenerating nerve fibers.

EXPERIMENTAL PROCEDURES

Animals

Tissue material derived from 18 young adult macaques (3.5–6.9 years old; weight ranging from 3.0 to 5.0 kg), subjected to an incomplete spinal cord hemisection performed at cervical level C7/C8, was analyzed histologically. These animals were part of several studies on the consequences of anti-Nogo-A antibody treatment on the regeneration of CS axons as well as on behavioral recovery after cervical cord injury. Separate data from 10 of these animals have appeared in previous reports (Freund et al., 2006, 2007, 2009; Beaud et al., 2008; Wannier-Morino et al., 2008). Surgery and care of the animals were in conformity to the Guide for the Care and Use of Laboratory Animals (ISBN 0-309-05377-3; 1996) and authorized by local Swiss veterinary authorities. The housing conditions as well as the surgical procedures were reported in detail in earlier reports (Schmidlin et al., 2004, 2005; Freund et al., 2006, 2007, 2009). The experimental protocol can be summarized as follows. In a first stage, the animals were trained to perform behavioral (manual dexterity) tasks until they reached a stable behavioral score (pre-lesion plateau). In the second stage, an incomplete unilateral cervical cord hemisection was performed and the tip of a catheter attached to an osmotic pump was inserted intrathecally at the lesion site. A group of eight animals received a control antibody (14–80 mg/animal), a second group of five animals received an anti-Nogo-A antibody treatment (total: 14.6–36 mg/animal), and a last group of five animals received a combined treatment of anti-Nogo-A antibody (36 mg/animal) and BDNF (1.4 mg/animal). The treatments were initiated immediately after the lesion and lasted 1 month.

In a third stage, starting immediately after the lesion, each animal was assessed behaviorally until it reached a stable score (post-lesion plateau). In the fourth stage, the anterograde tracer biotin dextran amine (BDA) was injected into the contralesional motor cortex. In some animals, a dextran–fluorescein tracer was injected in the ipsilesional motor cortex. After about 2 months, delay to allow anterograde transport of the tracers, the animals were sacrificed. In this report, the code identifying each animal is built in two parts: the first letters of the code indicate the treatment given (Mk-C for the control antibody treatment, Mk-AB for the combined anti-Nogo-A antibody/BDNF treatment) and the additional letter(s) individuate the corresponding animal. The experimenter was blind as to the treatment of the animals and a different identification code was used during the whole duration of the experimental period. The final identification code was set after the sacrifice of the animals when the lesion was reconstructed and some of the behavioral and anatomical data analyzed.

Histology (BDA/SMI-32/dextran–fluorescein staining)

When the animals had reached a stable behavioral score post-lesion, approximately 100 days following the lesion, the BDA tracer was injected unilaterally in the contralesional motor cortex (hand territory) using Hamilton syringes (Table 1). After
transport time (Table 1), the animals were sacrificed as follows. First, the monkeys were sedated with ketamine. Then, they received a lethal injection of sodium pentobarbital (90 mg/kg) intraperitoneally. They were then perfused transcardially with 0.4 l of saline (0.9%), followed by 3 l of a fixative solution (4% of paraformaldehyde in 0.1 M of phosphate buffer, pH 7.6). The perfusion continued subsequently with two solutions of similar fixative containing sucrose of increasing concentrations (10%, 20%) and ended with a 30% sucrose solution in phosphate buffer (pH 7.6). At the end of the perfusion, the entire CNS was isolated and placed during few days in a 30% solution of sucrose in phosphate buffer (pH 7.6) for cryoprotection. A spinal cord segment (C3-T4) comprising the lesion was cut parasagittally in either three or five series of respectively 50 or 30-μm sections and processed to visualize either BDA or SMI-32 staining. More rostral and caudal spinal cord segments were cut in 50-μm-thick coronal sections and also collected in series. The SMI-32 staining was carried out according to the following protocol: first, to remove the endogenous peroxidase activity, the free-floating sections were preincubated during 10 min in 1.5% H2O2 in phosphate-buffered saline (PBS; pH 7.2) and incubated overnight at 4 °C in SMI-32 monoclonal antibody (Sigma, dilution 1:3000) in addition to 2% normal horse serum and 0.2% Triton X-100; then the sections were rinsed several times in PBS. After that, the sections were rinsed several times again and incubated in a biotinylated secondary antibody (1:200, Vector Burlingame, CA, USA) during 30–60 min at room temperature. Finally, the sections were stained with the avidin–biotin complex (ABC) immunoperoxidase method (Vectorstain Elite kits, Vector, Burlingame, CA, USA). The reaction was visualized with 3,3′-diaminobenzidine tetrahydrochloride (DAB 0.05%) as the chromogen, diluted in Tris–saline with 0.001% H2O2. The sections were then washed, mounted on gelatin-coated slides, dehydrated, and coverslipped. In the present investigation, the number and the cumulative length of the SMI-32 (+) fibers visible in the lesion site were assessed at 400× magnification using a light microscope (Olympus) and the software Neurolucida (MicroBrightField, Williston, VT, USA). The sections analyzed in the present study were used previously in some of the monkeys to reconstruct the location and the extent of the cervical lesion in previous reports (Schmidlin et al., 2004; Wannier et al., 2005; Freund et al., 2006). The question of whether the two types of anti-Nogo-A antibodies present a difference in efficacy was not addressed in the present study because only one animal was treated with the 11C7 type of anti-Nogo-A antibody. Five animals (Mk-ABBo, Mk-ABMa, Mk-ABMx, Mk-ABP and Mk-ABS) have received the anti-Nogo-A antibody treatment (36 mg in 4 weeks), combined to 1.4 mg of the neurotrophic factor BDNF (Peprotech, UK) diluted in artificial CSF (150 mM Na, 3 mM K, 1.4 mM Ca, 0.8 mM Mg, 1 mM P, 155 mM Cl) and delivered intrathecally from a second similar osmotic pump caudally to the lesion site during the same period of 4 weeks (Table 1).

For statistics, we compared the diameter distribution of fibers in the lesion territory with that of motoneurons’ axons using the Student’s t-test. For assessing whether the number or the length of the fibers inside the lesion site were affected in one of the groups that received growth-promoting substances, we performed multivariate nonparametric analysis using the R program (R Development Core Team, 2008) with the MNM package (Klaus Nordhausen et al., 2009). This approach uses a nonparametric multivariate rank test (Oja and Randles, 2004) and is equivalent to the approach we used in a previous report (Freund et al., 2009).

RESULTS

Cervical cord lesion

All the animals were subjected to an incomplete spinal cord hemisection at the level of the transition between the segments C7 and C8. The lesions sites were clearly identifiable in all animals and reconstructed from the histological sections. Their relative size was determined as a proportion (in percent) of the extent of the hemi-cord cross-sectional surface (Table 1) and was always clearly identifiable. The lesion extent ranged between 38% and 95% of the hemisected surface. In nearly all monkeys, the lesion completely interrupted the dorsolateral funiculus, spared large portions of the dorsal columns and spread in the ventral quadrant. Two representative histological reconstructions of the spinal cord lesion are shown in Fig. 2.

Which spinal elements are labeled by SMI-32 staining?

The SMI-32 antibody recognizes an epitope on non-phosphorylated neurofilaments, which is only expressed by specific categories of neurons (Sternberger and Sternberger, 1983; Campbell and Morrison, 1989). We identified and analyzed thin and large SMI-32 (+) fibers in all animals, without any particular individual difference of distribution in the lesion site. Cross sections from intact spinal cord tissue segments located rostrally to the spinal cord lesion and stained for SMI-32 revealed that among spinal neurons, motoneurons were the most heavily stained (Fig. 1A, F, white arrowheads). Other SMI-32-stained neurons are scattered across the entire gray matter but only few of them were as heavily stained as motoneurons (Fig. 1E, white arrowhead). In the white matter, lightly stained axons were often visible, as for instance in the dorsal funiculus (Fig. 1B), in the dorsal root entry zone (Fig. 1C) or in the dorsolateral funiculus (Fig. 1D), whereas heavily stained axons were rare, with the exception of axons present in the spino-cervical tract (Fig. 1C). Motoneuronal axons in

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the white matter were also heavily stained (Fig. 1A, F, white arrows).

SMI-32-stained fibers in the lesion territory

The number of SMI-32-stained fibers in the lesion territory varied from a total of 129 counted fibers in Mk-AF to a maximal value of 2539 fibers in Mk-CH (Table 1). Fig. 2 depicts two SMI-32-stained longitudinal sections of the spinal cord at the lesion site. In the first section, the lesion extends through the entire dorso-ventral axis (Fig. 2A). In the gray matter adjacent to the lesion, numerous fibers and many neurons can be seen (Fig. 2A). Heavily stained neurons with a large cell body are concentrated in the adjacent ventral gray matter, most of them are most likely corresponding to motoneurons (Fig. 2A, arrowheads). Numerous large caliber SMI-32 (+) fibers grow into and are present in the lesion site (Fig. 2B, black arrowheads), some of which can unequivocally be recognized as motoneurons axons, as they are prolongations of motoneuronal axon bundles from the intact white matter (Fig. 2B, arrows). Fig. 2C and D depicts a section from another animal located at a more medial position than in the upper panels of Fig. 2. In this section, the lesion occupies only the ventral part of the spinal cord (Fig. 2C). Here too, numerous SMI-32 (+) fibers are present in the lesion territory. In all animals, the fiber growth did not follow a specific orientation (Fig. 2B, D) and only few exceptions were observed to bridge the rostro-caudal extent of the lesion (Fig. 2D, black arrowhead).

Do CS fibers contribute to the SMI-32 (+) fibers present in the lesion site?

To clarify whether CS fibers are present and common among the SMI-32 (+) fibers detected in the lesion, SMI-32-stained sections were compared to adjacent

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**Fig. 1.** Distribution of SMI-32-stained elements inside the spinal cord. (A) SMI-32-stained coronal section from a level rostral to the cervical lesion. The gray matter is easily distinguishable from the white matter and presents numerous sites with darkly stained elements. In particular, in the ventral gray matter, strongly stained pools of motoneurons are well recognizable (white arrowheads; F). In the white matter, apart from bundles of fiber spreading in the plane of the section (white arrows), no conspicuous elements are seen. (B) At higher magnifications, lightly stained SMI-32 (+) fibers can be seen in the left portion of this microphotograph in the dorsal columns. This portion is in the region containing ascending axons which have not been sectioned by the lesion. On the right of the microphotograph, which corresponds to a region where axons have degenerated after the lesion, no such staining is found. (C) Numerous well-stained SMI-32 (+) fibers are present in the posterior spinocerebellar tract. (D) Moderately stained SMI-32 (+) fibers are present in the dorsolateral funiculus. (E) The gray matter is filled with a fine mesh of SMI-32 (+) neuropile components in which few large (white arrowhead) and frequent small (white arrows) positively stained cell bodies are disseminated. (F) The motoneurons pools (white arrowheads) build the only sites where numerous heavily stained SMI-32 (+) neurons are grouped. Scale bars = (A) 500 μm and (B–F) 100 μm.
sections processed for BDA only, the tracer that was injected before sacrifice of the animals into the primary motor cortex. Numerous darkly BDA-stained CS fibers are visible in the dorsolateral funiculus rostrally to the cervical lesion (Fig. 3B). Thus, the progression of the CS fibers is clearly stopped at the rostral border of the lesion site (Fig. 3C). As described before, numerous SMI-32 (+) fibers were seen in the lesion site (Fig. 3D), particularly in its ventral portion (Fig. 3F). In contrast, BDA (+)-stained fibers were not found in the corresponding regions of the adjacent BDA-processed sections (Fig. 3E, G). These data show that the overwhelming majority of the SMI-32 (+) fibers observed in the lesion territory do not comprise CS axons.

What is the origin of the SMI-32 (+) fibers visible in the lesion site?

As described above, SMI-32 (+) motoneuronal axons are sometimes clearly seen to penetrate in the scar tissue (Fig. 2B) but the question remains as to whether other fiber populations also significantly contribute to the population of SMI-32 densely labeled fibers found in the lesion territory. We measured the diameter of the SMI-32 (+) axons in the lesion site and compared it to that of the SMI-32 (+) motoneuronal axons in the intact white matter. Individual fibers, analyzed either in the intact white matter or in the lesion site (Fig. 2B), exhibited a comparable diameter distribution independently of the
region where the measure was performed, showing that the diameter of motoneuronal axons did not change after they crossed from the intact tissue into the lesion. In the latter, both thick and thin SMI-32 (+) fibers were found, however (Fig. 4A). The thick fibers were usually heavily stained with the SMI-32 antibody (Fig. 4A, white arrowhead) and looked similar to motoneuronal axons in the intact white matter. Other fibers were rather thin and
less densely stained (Fig. 4A, black arrowhead), features that are exceptional for motoneuronal axons inside the intact white matter. For the monkey Mk-ABS, the diameter distribution of the SMI-32 (+) fibers in the scar tissue was compared to that of the motoneuronal axons outside the scar. Both distributions overlapped, but the proportion of small diameter fibers was higher in the lesion site (Fig. 4 B). This difference in fiber distribution was consistently observed in all animals for which diameter measurements were obtained (Fig. 4 C; \( N = 9 \)) and was statistically significant in all cases (Student's \( t \)-test, \( p < 0.01 \)). The data thus suggest that motoneuronal axons are not the only fibers having the capacity to invade the scar tissue.

Do SMI-32 (+) fibers present in the lesion territory respond to the growth-promoting treatments?

To clarify whether the growth of SMI-32 (+) fibers into the lesion territory was affected by the presence of the anti-Nogo-A antibody, combined or not with BDNF, we counted the number of SMI-32 (+) fibers and measured their cumulative length inside the lesion territory. The number of SMI-32 (+) fibers counted in the lesion site varied considerably across the 18 animals (Fig. 5A) and tended to increase in relation to the size of the lesion (Fig. 5B). Comparing the group of animals that received the control antibody to those that received the anti-Nogo-A antibody using a multivariate nonparametric analysis failed to show an influence of the treatment on the growth of the fibers in the scar (\( p > 0.05 \)). The same was observed when comparing the group of animals which received the control antibody to those that received the combination of BDNF and the anti-Nogo-A antibody.

SMI-32 (+) fibers in the lesion territory belong presumably mainly to motoneurons, but since the thinner fibers inside the lesion did not have counterparts inside the motoneuronal axon bundles in the intact white matter, these thin fibers were probably not belonging to motoneurons. By investigating separately the effects of the treatments on the largest and on the thinnest fibers, we tried to assess whether the treatments could have acted differently on different populations of neurons. Considering the distribution of the fibers’ thickness (Fig. 4B, C), we arbitrarily chose the limit of 1.3 \( \mu \text{m} \) to separate the entire population into a group of thin fibers that, presumably, were predominantly not motoneuronal axons and a group of large fibers for which the proportion of motoneuronal axons is large. The percentage of SMI-32 (+) fibers having a diameter of less than 1.3 \( \mu \text{m} \) was then plotted against the extent of the hemi-cord lesion (Fig. 5C). As for the total number of SMI-32 (+) fibers (Fig. 5A), a large variability was observed across animals and, here too, a multivariate nonparametric analysis failed to disclose any effect of the treatments on these thin fibers.

It was also conceivable that the SMI-32 (+) fibers increased in length, while their number remained constant. The length of all fiber segments found in the scar tissue was thus measured and summed into a single cumulated value for each animal. Plotting the
number of SMI-32 (+) fibers found in each animal in relation to their cumulated length revealed a linear relationship (Fig. 5D), which was confirmed by high coefficients of correlation ($r > 0.93$) for each experimental group considered separately, as well as for all animals pooled together. In this situation too, the multivariate nonparametric analysis did not disclose any difference between the groups that could be related to the treatments. Again, this tendency was independent from the diameter of the fibers. Thus, it appears that neither the number nor the length of the SMI-32 (+) fibers observed in the scar tissue was influenced by the anti-Nogo-A antibody alone or in combination with the neurotrophic factor BDNF.

DISCUSSION

We analyzed the lesion territory of 18 adult macaque monkeys in the cervical spinal cord, 3.5–8.5 months after a lateral hemisection lesion. Some of the animals underwent growth and regeneration-promoting treatments, i.e., intrathecal infusion of anti-Nogo-A antibodies (for 1 month after the lesion) with or without BDNF. Here we observed that a sizeable amount of neurites was found in the lesion territory. The bulk of these neurites presumably originated from motoneuronal axons, but it is likely that axons of other origins, though not of corticospinal neurons, also contributed to some extent. The growth of these fibers into the lesion site was affected neither by the presence of the anti-Nogo-A antibody, nor by the presence of a mixture of this antibody with BDNF.

SMI-32-stained elements inside the spinal cord

The scar tissue formed after a spinal cord lesion is known to hinder the growth of nerve fibers into the lesion (Ramon y Cajal, 1928; Brown and MacCouch, 1947; Clemente, 1955; Reier et al., 1983; Stichel and Muller, 1998; Fawcett and Asher, 1999; Shearer and Fawcett, 2001; Silver and Miller, 2004; Fawcett, 2006). In the present study, 129–2539 SMI-32 (+) fibers were found within the lesion site of all the animals. Indirect evidence suggests that a large proportion of these fibers are motoneuronal axons. First, in spinal cord sections, strongly stained SMI-32 (+) neurons were, for a majority, motoneurons; none of the SMI-32 (+)-descending or -ascending axons present in the white matter showed such a darkly stained appearance. Second, many of the strongly SMI-32 (+) fibers in the lesion exhibited a large diameter, characteristic of motoneurons’ axons. Third, motoneuronal axons

![Fig. 5.](http://doc.rero.ch)
Comparisons between the SMI-32 (+) neurons and those expressing choline acetyltransferase revealed that 82–100% of the positive neurons located in the ventral horn of rats and primates (marmoset and rhesus monkeys) were double-stained. However, the SMI-32 staining is not specific for motoneurons. It also stains, though often less densely, neurons outside the ventral horn, such as for instance neurons in the Clark’s columns or in the intermediate cell column (Carriedo et al., 1996; Tsang et al., 2000). Our observations are in accordance with those reports. As shown in Figs. 1 and 2, the majority of the darkly SMI-32-stained neurons was located in the motoneuronal pools of the ventral horn. Other strongly SMI-32-stained neurons were detected in positions corresponding to Rexed laminae V–VIII, but they were clearly less numerous.

In the cat, intraspinal sprouting of motoneuron axons was observed after root avulsions and spinal cord injuries (Linda et al., 1985, 1992; Havton and Kellerth, 1987). Regeneration included the production of axon-like processes from dendrites and that were called “dendraxons” (Linda et al., 1985, 1992; Havton and Kellerth, 1987). These axons were myelinated, oriented toward the ventral root fascicles and kept a constant diameter. Although the existence of dendraxons has, at least to the best of our knowledge, never been reported in the primate, we cannot exclude that some of the SMI-32 (+) fibers observed here in the lesion site are “dendraxons”.

**CS fibers fail to grow inside scar tissue**

In a previous report on macaques that had been treated with either a control antibody or the anti-Nogo-A antibody during 1 month after injury, CS fibers were only exceptionally found to penetrate into the lesion site (Freund et al., 2007). This observation is confirmed here in four additional animals receiving the control antibody and in two animals treated with the anti-Nogo-A antibody. We also show that, in five animals that received a combination of the anti-Nogo-A antibody with BDNF, the number of fibers’ numbers in the lesion territory were not different from those found in the non-treated monkeys. In rodents, the absence of axotomized CS fibers in the lesion has repeatedly been observed (Schnell and Schwab, 1990; Bregman et al., 1995; Inman and Steward, 2003) and in primates (Fouad et al., 2004; Freund et al., 2006). In these species, while a treatment neutralizing Nogo-A often leads to enhanced sprouting and regeneration of axotomized CS fibers, the fibers grow around the lesion instead of through the lesion. Interestingly, spontaneous invasion of the lesion site occurs by dorsal root afferents like those containing the calcitonin gene-related peptide (CGRP) or Substance P, and by serotonergic-descending fibers which were reported to cross the inhibitory boundary formed by reactive astrocytes and to penetrate deeply into the scar (De Castro et al., 2005).

In addition, neurites containing glycine and GABA were also found within spinal scars (Brook et al., 1998). Recently, it has been shown that the axons of adult cat’s spinal commissural interneurons can spontaneously grow through a local midline spinal section in spite of the presence of CSPGs in the lesion site and that these axons formed functional synaptic connections with appropriate neural targets (Fenrich and Rose, 2009). It thus appears that the mammalian spinal scar tissue does not constitute an environment hostile to regeneration for all fiber types. While this is firmly established for rodents and cats (Matthews et al., 1979; Linda et al., 1985, 1992; Havton and Kellerth, 1987; Wallace et al., 1987; Wang et al., 1996; Brook et al., 1998; Fenrich and Rose, 2009, 2011), it has, to our knowledge, never been reported for adult macaque monkeys. Our data show that SMI-32 (+) fibers may even be attracted by the scar tissue, to invade the lesion site.

**Possible impact of the invasion of motoneuronal neurites in the scar**

Compared to the 400,000 axons in the corticospinal tract of macaque monkeys, the relatively important number of fibers observed here in the lesion may have a positive impact on supraspinal control of movement and consequently on the functional recovery. Nevertheless, their chaotic distribution in the spinal cord could impair efficient CS neurites to grow in the scar tissue and rewire the motor centers to the motoneurons.

Confronting the present morphological data with the percentage of functional recovery (Table 1), there was no relationship between number of fibers in the lesion territory or their cumulative lengths and the functional recovery.

**Anti-Nogo-A antibody treatment alone or combined with BDNF does not induce detectable changes in the number or growth of the SMI-32 (+) fibers in the lesion territory**

The SMI-32 (+) fibers encountered in the spinal lesion in the present investigation demonstrate that this tissue is growth permissive for some types of neuronal fibers. Nogo-A is not expressed in the scar tissue of rodents, but it was of interest to ascertain whether or not an antibody treatment neutralizing Nogo-A or an additional delivery of BDNF would enhance the growth of SMI-32 (+) fibers inside the lesion territory. However, we did not detect any growth-promoting effect of these treatments on the SMI-32 (+) fibers into the lesion site. Finally, independent of the treatments and the thickness of the fibers, a great variability of the number and length of the fibers in the scar was observed across all animals; local properties of the scar and its interface with the surrounding gray and white matter tissue may account for this variability, but the underlying molecular mechanisms remained to be analyzed.

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