In adult macaque monkeys subjected to an incomplete spinal cord injury, numerous large SMI-32 positive fibers were observed into the lesion site.

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INTRODUCTION

Following an incomplete cervical cord hemisection (level C7/C8), corticospinal (CS) fibers of adult macaque monkeys treated with an anti-Nogo-A antibody sprout rostrally and caudally to the lesion site. However, using the anterograde tracer BDA, only few CS axons were found inside the scar tissue (Freund et al., 2006).

As different types of neurons respond differently to injury, the question arises whether some axons, different from CS axons, penetrate and eventually cross the scar tissue.

To investigate this question, we used the SMI-32 antibody seeking after neurofilaments present inside the scar tissue. Furthermore, we investigated whether the presence of growth promoting molecules had an impact on our observations.

MATERIALS & METHODS

Sixteen young adult macaque monkeys (♂) were subjected to an incomplete unilateral section of the spinal cord at the C7/C8 level. The animals were treated with either a control antibody (n=8), or an anti-Nogo-A antibody (n=5), or an anti-Nogo-A antibody combined with BNDF (n=3).

We investigated the number and the cumulative length of SMI-32 (+) fibers present into the lesion site of each groups of animals.

CONCLUSIONS

Conclusion 1
After a spinal cord lesion, the scar tissue can be successfully colonized by certain axons.

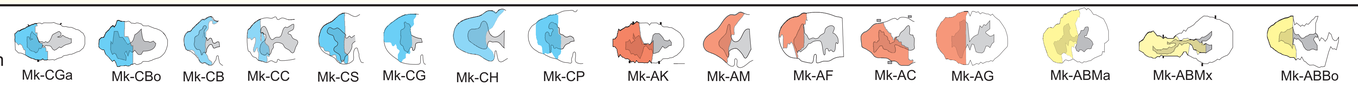
Conclusion 2
Ventral root axons penetrate the lesion, and numerous fibers observed into the scar tissue have a similar size and quality of staining as ventral root axons.
We therefore hypothesize that most of these fibers originate from motoneurons.

Conclusion 3
Neither the anti-Nogo-A antibody alone, nor the combination of this antibody with BDNF influences the number or the length of fibers inside the scar tissue.

RESULTS

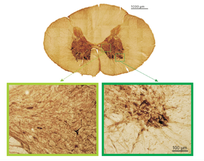
1) Reconstruction and extent of the spinal cord lesion

The extent of the incomplete spinal hemisection varied among animals, ranging between 38 % and 95 %.
Numerous descending & ascending tracts were interrupted ipsilesionally.

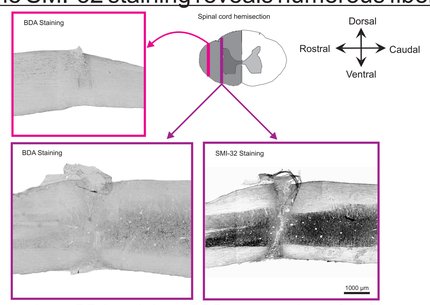


2) Which spinal elements are stained by the SMI-32 staining?

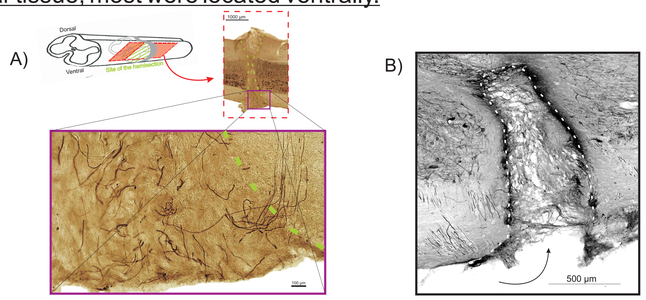
Among spinal neurons, motoneurons were the most heavily SMI-32 stained neurons. Few additional heavily stained neurons were scattered inside the entire gray matter.
In addition to neurons, fibers such as the CS fibers were also distinctly stained.



3) The SMI-32 staining reveals numerous fibers inside the scar tissue; most were located ventrally.

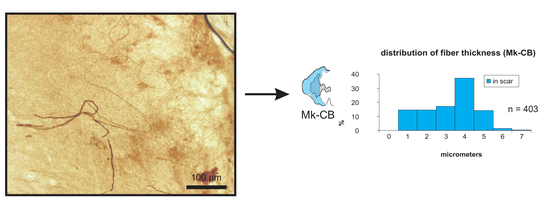


Compared to a BDA staining of CS axons, where few fibers attempt to penetrate the scar tissue, the SMI-32 staining reveals numerous positive fibers in the ventral part of the lesioned tissue.

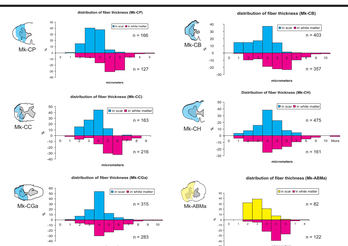


Numerous SMI-32 (+) fibers invade the scar tissue (A,B). They are irregularly orientated and some cross the lesion (B). Some of those axons, have a segment inside the intact white matter, where they are included in bundles otherwise forming ventral roots (A).
This demonstrates that some of these fibers belong to motoneurons.

4) The scar tissue contains SMI-32 (+) fibers of different thickness.



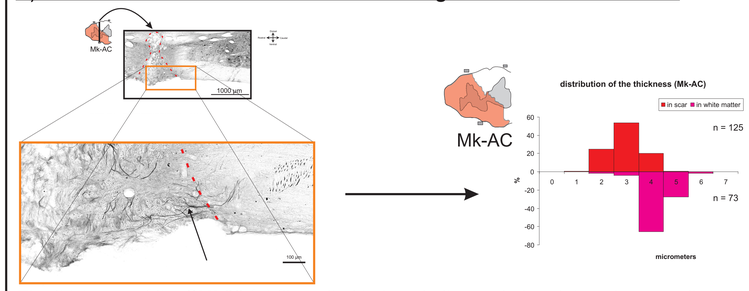
The thickness of SMI-32 (+) fibers observed into the lesion site ranges from thin (< 2 µm) to large fibers (> 6 µm).



The distribution of SMI-32 (+) fibers width in the scar and in the ventral white matter overlap, but differ. This indicates that fibers from neurons other than motoneurons, invade also the scar.

In oder to evaluate whether different types of axons responded diffrently to the presence of growth promoting molecules, we decided to split arbitrarily these fibers populations into two groups:
- a "thick fibers" group (∅ > 3.1 µm), presumably containing a high proportion of motoneurons axons.
-a "thin fibers" group (∅ < 3.1 µm), presumably containing a large proportion of non-motoneuron axons.

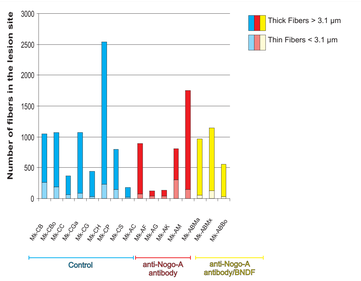
5) Some of the "thin" fibers are not coming from the ventral roots.



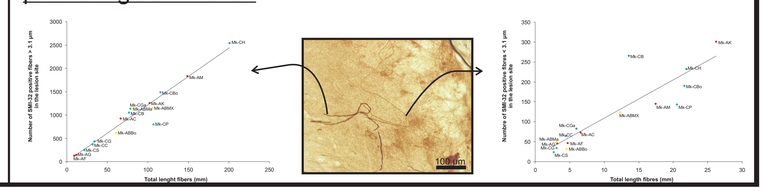
The thickness of the SMI-32 (+) fibers that belong to the ventral root do not vary as they penetrate into the scar and the fibers width distribution is different in most animal when comparing the scar and the white matter.This suggests that some of "thin" fibers do not originate from ventral roots.

6) The number of SMI-32 (+) fibers present into the scar is independent of the treatment

Both treatments did not promote a better colonization into the scar tissue of the two types of width fibers.



7) The relationship between the number and the cumulated fiber length of SMI-32 (+) fibers is linear and not affected by both growth promoting treatments.



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