In adult macaque monkeys a unilateral section of the dorsal funiculus induces a reduction of the number of SMI-32 positive neurons in the ipsilesional external cuneatus nucleus (ECN) that is prevented by anti-Nogo-A antibody treatment

Freund P. (1), Schmidlin E. (1), Bloch J. (2), Mir A. (3), Schwab M. (4), Rouiller E. M. (1) & Wannier T. (1).

(1) Dept Medicine, Uni Fribourg, CH-Fribourg; (2) Dept Neurosurg., Uni Hospital Lausanne, CH-Lausanne; (3) Novartis Inst. Biomed Res, CH-Basel; (4) Dept Neuromorph., Uni & ETHZ, CH-Zürich

12:15 090.10 Poster board D65 - Mon 14/07/2008, 12:15 - Hall 1

Introduction

The ECN is a major relay nucleus that contributes to the integration of sensory inputs from the upper trunk and upper limbs.
Here, we studied in adult macaque monkeys anatomical changes occurring in this dorsal column nucleus after a unilateral partial section of the spinal cord that interrupted part of the dorsal column at low cervical level (C7-C8).
We also investigated whether an anti-Nogo-A antibody treatment aimed at enhancing functional recovery (Freund et al., 2006) also influenced lesion induced changes in the ECN.

Material and methods

Data were obtained from 12 adult macaque monkeys.
Nine animals were subjected to a unilateral section of the cervical spinal cord at the C7/C8 level (Figure 1).
One group of lesioned monkeys (N=5) received a control antibody; a second group (N=4) was treated with an antibody neutralizing Nogo-A. In addition, data from three intact animals were obtained for comparison.
Histological sections (50 μm thick, 250 μm apart) from the brainstem were stained with the SMI-32 antibody which recognizes non-phosphorylated neurofilaments.
The number and size of neurons in the ipsilesional ECN were measured and compared with values obtained from the contralesional ECN.

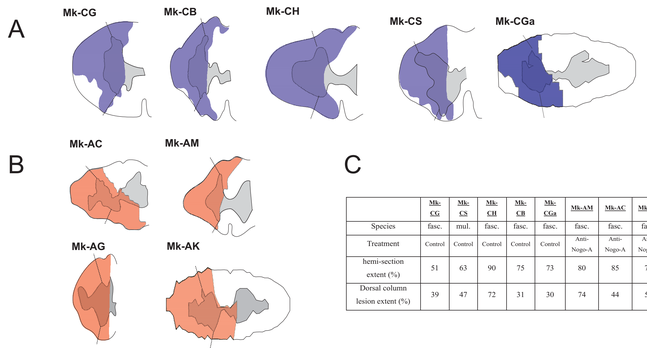
Conclusions

- In control antibody treated lesioned monkeys, the number of SMI-32 positive neurons decreased in the ipsilesional ECN as compared to the contralesional ECN. In contrast, in intact and in anti-Nogo-A antibody treated monkeys, the numbers of SMI-32 positive neurons were comparable in both ECN nuclei.
- The size of SMI-32 positive neurons in the ipsilesional ECN increased in the monkeys with a large lesion extent.
- The anti-Nogo-A antibody treatment did not influence this process.

The data show that the anti-Nogo-A antibody treatment exerts a direct or indirect protective action on deafferented ECN neurons.

Results

1. Reconstruction of the lesion



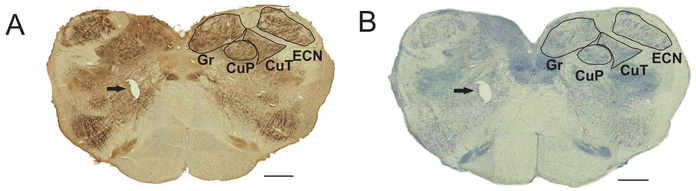
C

	Mk-CG	Mk-CS	Mk-CH	Mk-CB	Mk-CGa	Mk-AM	Mk-AC	Mk-AG	Mk-AK
Species	fasc.	mul.	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.	fasc.
Treatment	Control	Control	Control	Control	Control	Anti-Nogo-A	Anti-Nogo-A	Anti-Nogo-A	Anti-Nogo-A
hemi-section extent (%)	51	63	90	75	73	80	85	78	86
Dorsal column lesion extent (%)	39	47	72	31	30	74	44	56	58

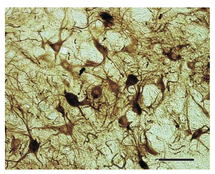
A: control antibody treated monkeys. B: anti-Nogo-A antibody treated monkeys. C: Table indicating the relative extent of the lesion.

The graphics are reconstruction of the cervical cord lesion as seen in the frontal plane and reconstructed from parasagittal sections.
Grey area: grey matter. Blue and red areas: lesion extent in control antibody treated animals and anti-Nogo-A antibody treated animals, respectively.
In each reconstruction, straight lines indicate the level of entry or exit of dorsal and ventral rootlets.

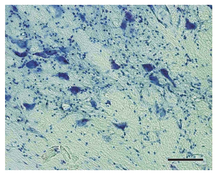
2. ECN and its neurons



Photomicrographs of a coronal section of SMI-32 stained (A) and of an adjacent Nissl (B) stained section.
The black arrows point to a reference mark introduced while sectioning the tissue
Abbreviations: Gr= nucleus gracilis; CuP= nucleus cuneatus, pars rotunda; CuT= nucleus cuneatus, pars triangularis; ECN: external cuneatus nucleus (scale bar: 1 mm)

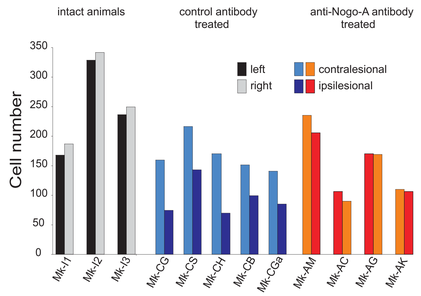


SMI-32 positive ECN neurons show a multipolar shape with several dendritic processes.
Only the neurons with a clear visible nucleus were included in the morphometric analysis (cell count and somatic size). Scale bar: 100μm

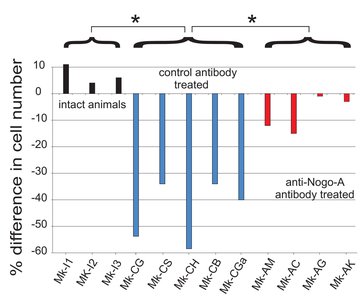


Nissl stained ECN neurons taken from an adjacent section.
Scale bar: 100μm

3.Cell number

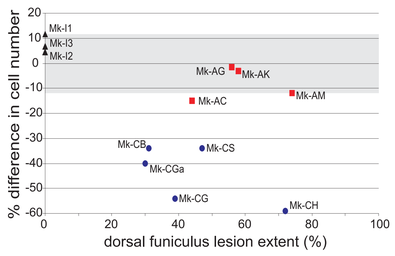


Numbers of SMI-32 positive cells detected in the ECN.
For the intact animals, the left bar depicts data from the left ECN, the right bar for the right ECN. For the lesioned animals, the left bar depicts data from the ECN on the contralesional side, whereas the right bar depicts data from the ECN on the ipsilesional side.
No difference in ECN cell number between the two sides for the intact and for the anti-Nogo-A treated monkeys (pooled data, Wilcoxon signed-rank test, p>0.05). For the five lesioned monkeys treated with the control antibody, less neurons were detected in the ECN on the side of the lesion (p<0.05).

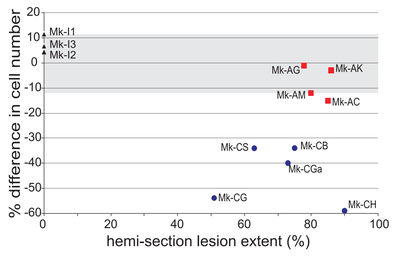


Percentage differences in the number of SMI-32 positive cells between the two sides of the ECN.
The difference among both groups of lesioned animals versus the intact animals was statistically significant (Mann & Whitney, p < 0.05).

4.The preservation of ECN neurons in anti-Nogo-A treated monkeys is not explained by a small lesion size

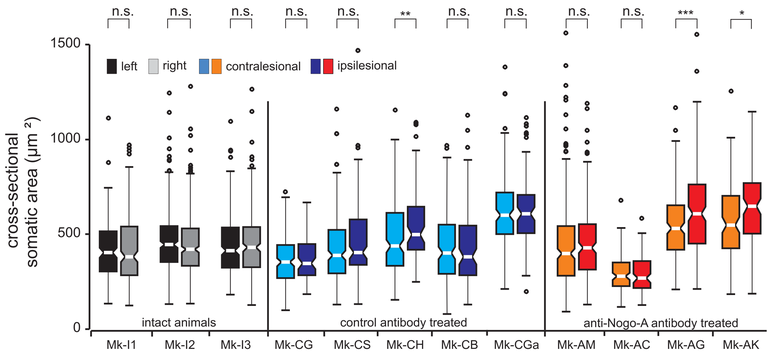


Plot of the reduction of SMI-32 positive cells against the relative extent of the dorsal column lesion.
The lesion extent tends to be larger for the anti-Nogo-A treated animals than for the group of control antibody treated animals.
Black triangles: intact animals; blue circles: control antibody treated animals; red squares: anti-Nogo-A antibody treated animals.
The grey zone delimits the maximal variability of ECN neuron numbers observed in the intact animals.



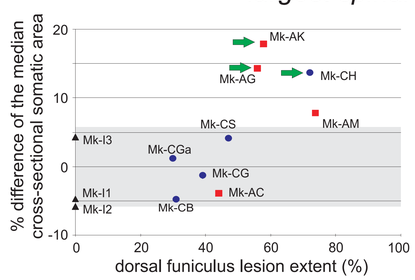
Plot of the reduction of SMI-32 positive cells against the relative extent of the hemi-cord lesion.
The lesion extent tends to be larger for the anti-Nogo-A treated animals than for the group of control antibody treated animals.
Black triangles: intact animals; blue circles: control antibody treated animals; red squares: anti-Nogo-A antibody treated animals.
The grey zone delimits the maximal variability of ECN neuron numbers observed in the intact animals.

5.Cell size

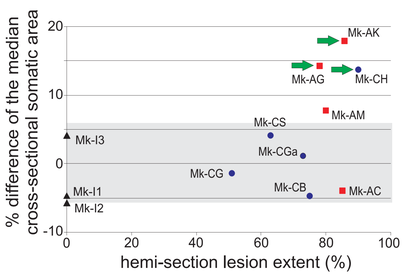


Box and whisker plots indicating the distribution of somatic cross-sectional areas of SMI-32 positive ECN neurons.
For each lesioned animal data from the contralesional and from the ipsilesional ECN are shown respectively on the left and on the right.
In the box and whisker plots, the horizontal white line in the box corresponds to the median value, whereas the top and bottom of the box are for the 75 and 25 percentile values, respectively. The top and bottom extremities of the whiskers are delimited by the lower and highest values at 1.5 interquartile interval from the first and third quartile, respectively. The soma area of the SMI-32 positive neurons in both ECN nuclei was comparable for all intact animals and for four of the five control antibody treated animals. In two of the four anti-Nogo-A treated animals, the size of the SMI-32 positive neurons was significantly larger in the ipsilesional side. (Mann and Whitney test, ***: p<0.001, **: p<0.01; *: p< 0.05)

6. A cell size increase is observed in animals with the largest spinal lesion extent



Percentage difference between the median values of somatic cross-sectional areas in both ECN plotted the relative extent of the dorsal funiculi versus the lesion extent.
The variability amongst values in lesioned animals appears independent of the antibody treatment. Only four animals were subjected to a dorsal column lesion exceeding 45%. Of these, three animals (green arrows) presented sizable soma shrinkage of the SMI-32 positive neurons in the ipsilesional ECN.



Percentage difference between the median values of somatic cross-sectional areas in both ECN plotted the relative extent of the hemi-section versus the lesion extent.
Here too, the variability in lesioned animals appears independent of the antibody treatment. Green arrows point to the three animals which presented sizable soma shrinkage of the SMI-32 positive neurons in the ipsilesional ECN.

Supported by Swiss National Science Foundation (NCCR) and Novartis Pharma.