

Supplementary Methods

Anti-Nogo-A antibodies

Two monoclonal antibodies (mAbs) against different sites of Nogo-A were used: the mouse **mAb 11C7**^{see 1} was raised against a 18 amino acid peptide of rat Nogo-A (aa623 – 640), close to the most inhibitory region of the Nogo-A protein², which cross-reacts with mouse and monkey Nogo-A. The second antibody used, **mAb hNogo-A** is directed against the Nogo-A specific region of the human Nogo-A sequence. Both antibodies recognize primate Nogo-A monospecifically on Western blots². Both antibodies increase neurite outgrowth in vitro and penetrate deeply the CNS in vivo in monkeys³. The antibodies were purified as IgGs and concentrated to 3.7-10 mg/ml in phosphate buffered saline (PBS).

Control antibodies

Purified IgG of a mouse mAb directed against wheat auxin (AMS Biotechnology, Oxon/UK) was used as control antibody.

Behavioral testing

The experiments were carried out on twelve (3-4 years old) rhesus (*Macaca mulatta*) or cynomolgus (*Macaca fascicularis*) monkeys of either sex (**Supplementary Table 1** online), ranging from 2.5 to 5.5 kg, in accordance to the Guide for the Care and Use of Laboratory Animals (ISBN 0-309-05377-3; 1996) and approved by local (Swiss) veterinary authorities. Monkeys were housed in our animal facilities in rooms of 12 m³, each containing usually 4 monkeys free to move in the room and to interact among each others. In the morning (7 am), before behavioral testing, the animal keeper placed the monkeys in temporary cages for subsequent transfer to the primate chair by the experimenter. The monkeys had free access to water and were not food deprived. The rewards obtained during the behavioral tests represented the first daily access to food. After the tests, the monkeys received additional food (fruits, cereals). To reduce inter-animal variability, "pairs" of two monkeys were formed, placed under the responsibility of the same experimenter, and were subjected simultaneously to the same experimental protocol. Within each "pair" of monkey, one animal was treated with the anti-Nogo-A antibody while the other was treated with the control antibody. However, in most cases, this information was not available to the experimenter ("double-blind" procedure; see **Supplementary Table 1** online). The ID codes refer to individual monkeys, as indicated in **Supplementary Table 1** (online), and comprise for sake of clarity in the text a "C" or a "A" at the fourth digit position, indicating whether the monkey was control-antibody treated or anti-Nogo-A treated, respectively. However, in the course of the experiments, the

animals had different names from which the experimenter could not know which antibody was infused, at least for the “pairs” of monkeys in which the double blind procedure was applied (see **Supplementary Table 1** online). With the goal to reduce variability, part of the data are presented by comparing monkeys within a “pair” (see **Fig. 1a,b,c; Fig. 2c,d; Supplementary Fig. 2a2; Supplementary Fig. 3a,b** online).

The manual dexterity of each hand was assessed in all lesioned monkeys with a finger prehension task (**Fig. 1e**), namely our so-called “**modified Brinkman board**” quantitative test, as described in detail earlier⁴⁻⁶ (see also www.unifr.ch/neuro/rouiller/motorcontcadre.htm). Briefly, tests were done using a Perspex board (10 cm x 20 cm) containing 50 randomly distributed wells, each filled with a food pellet at the beginning of the test. Twenty-five wells were oriented horizontally and twenty-five vertically. The dimension of the wells was 15 mm long, 8 mm wide and 6 mm deep. Retrieval of the food pellets required fractionated finger movements, consisting normally in a dexterous opposition of the index finger and the thumb, corresponding to the so-called “precision grip”. This manual prehension dexterity task was executed daily, alternatively with one or the other hand, 4 to 5 times per week for several months before and after the unilateral cervical cord lesion. A daily behavioral session typically lasted 60 minutes. The performance of each hand was videotaped. The behavioral scores were established by counting the number of wells from which the food pellets were successfully retrieved and brought to the mouth during 30 seconds (**Fig. 1a-c**). After the monkeys reached a stable level of performance (usually after 30-60 days), as indicated by a plateau of behavioral performance, 30-50 daily sessions were considered to establish a stable pre-lesion behavioral score. Functional recovery was expressed quantitatively as the ratio in percent of the plateau post-lesion score to the pre-lesion score.

Other behavioral tests were considered to assess further the motor capacity of the monkeys, such as the previously described natural “**reach and grasp drawer**” task⁷⁻¹² (see also www.unifr.ch/neuro/rouiller/motorcontcadre.htm). Originally, this task was developed to study quantitatively bimanual coordination but, in the present case, it was executed only unimanually, testing one hand after the other. In addition to test grasping while the monkey retrieved the pellet from the drawer, the reach and grasp drawer task also allowed assessment of the precise timing for the reaching phase towards the drawer as well as the pulling phase during which the monkey has to produce enough force to open the drawer while holding firmly the drawer's knob. More specifically, in the present report, control-antibody treated- and anti-Nogo-A antibody treated-monkeys will be compared for the reaching and pulling components of the reach and grasp drawer task (**Fig. 2a** and **Supplementary Fig. 2a1,a2** online).

Motor capacity was tested further qualitatively using other tests performed on a weekly basis, at the end of a behavioral session. For instance, “**Ballistic Arm Movements**” (**BAM test**) were

captured on video sequences while the monkey caught, using the two hands or sometimes one hand, pieces of food thrown by the experimenter from a distance of about 1 meter (see also www.unifr.ch/neuro/rouiller/motorcontcadre.htm). In the BAM test, synchrony of the two hands was assessed as well as the pre-shaping ability to open the fingers before retrieving the approaching piece of food (**Fig. 2b** and **Supplementary Fig. 2b** online).

The capacity to grasp with the hindlimb was also tested qualitatively by presenting to the animal a large piece of food accessible only with one or the other foot, before transferring it from the hindlimb to the hand. This "**hindlimb grasp**" test thus allowed assessment of another motor function affected by the cervical lesion, but for which the corresponding motoneurons are located at a much greater distance from the lesion (lumbar cord) than motoneurons involved in manual capacity (**Supplementary Fig. 2c** online; see also www.unifr.ch/neuro/rouiller/motorcontcadre.htm).

Before each daily session, to follow the general health condition of the animal, the body weight was measured daily (except on week-end), in eight of the twelve monkeys involved in the present study (see Supplementary Figure 3f and Supplementary Note). The body weight analysis did not include the four initial monkeys Mk-AF, Mk-AS, Mk-CS and Mk-CC because their body weight was not systematically measured daily but only weekly.

Surgical procedures: partial cervical cord section

Pre-anaesthesia was induced by intramuscular injection of ketamine (Ketalar®; Parke-Davis, 5 mg/kg, i.m.). Atropine was injected i.m. (0.05 mg/kg) to reduce bronchial secretions. Before surgery, the animal was treated with the analgesic Carprofen (Rymadil®, 4 mg/kg, s.c.). An intravenous catheter was placed in the femoral vein for continuous perfusion (0.1 ml/min/kg) with a mixture of 1% propofol (Fresenius®) and a 4% glucose solution (1 volume of Propofol and 2 volumes of glucose solution), inducing a deeper and stable anaesthesia. Methylprednisolone (Solu-Medrol®, Pfizer) was added to the perfusion solution (1 mg/ml). The animal was then placed in a stereotaxic framework, with local anaesthetic put on the ear bars in order to reduce pain possibly originating from the ear canals. During the surgery under aseptic conditions, the following parameters were monitored: heart rate, respiration rate, expired CO₂, arterial O₂ saturation and body temperature. In the initial experiments, an extra intravenous bolus of 0.5 mg of ketamine diluted in saline (0.9%) was added i.v. at potentially more painful steps of the surgical procedure, such as laminectomy. In the other and more recent experiments, ketamine was added to the perfusion solution (65 mg/100 ml) thus administered i.v. continuously throughout the whole surgery. Placed in a ventral decubitus position, the spinal processes from C2 to Th1 were exposed. The paravertebral muscles were retracted and the laminae of the segments C6, C7 and Th1 were dissected. A complete C6 laminectomy and an upper C7 hemi-laminectomy were then performed.

The ligamentum flavum was removed. The dura mater was exposed and incised longitudinally. Under the microscope, the dorsal root entry zones were easily identified. A unilateral incomplete section of the cervical cord at the C7/C8 border was performed using the dorsal root entry zone as the most medial landmark. From this target, a surgical blade (no 11, Paragon®) was inserted 4 mm in depth perpendicularly to the spinal cord, and the section was prolonged laterally to completely cut the dorsolateral funiculus. From previously available anatomical material, the rostro-caudal level where the dorsal rootlets entering respectively the 7th and the 8th cervical spinal segments meet was determined, corresponding to the rostral zone of the spinal portion covered by the 6th cervical lamina. The aimed lesion was located caudal with respect to the pool of biceps motoneurons but rostral to the pool of triceps, forearm and hand muscle motoneurons¹³ (see also **Supplementary Fig. 1a** online). The muscles and the skin were sutured. The animal usually recovered from anesthesia 15-30 minutes after interruption of the perfusion with propofol and was treated post-operatively with an antibiotic (Ampiciline 10%, 30 mg/kg, s.c.). Additional doses of Carprofen were given daily during one week (pills of Rymadil mixed with food). After the spinal lesion, the animal was kept alone in a separate cage for a couple of days, to allow better conditions for recovery than the usual group housing with other monkeys.

Antibody treatments

The treatment with control (six monkeys) or anti-Nogo-A (six monkeys) antibodies lasted during 4 weeks post-lesion delivered from an osmotic pump (Alzet®, 2ML2), placed in the back of the animal, using a small silastic tube positioned intrathecally 3-5 mm above the cervical lesion. The pump was implanted within a few minutes after the lesion of the cervical cord. The pump had a volume of 2 ml, allowing treatment during 2 weeks, after which it was replaced under anaesthesia by a second pump for another 2 weeks treatment. In five monkeys (pilot animals), the experimenters knew which one of the two antibodies was contained in the pumps (**Supplementary Table 1** online). For the seven other monkeys, the antibody contained in the pump was blind for the experimenters (**Supplementary Table 1** online) until the end of the experiments (sacrifice of the animal and reconstruction of the cervical lesion). After four weeks of treatment, the pump and the silastic tube were removed. When removed, each pump was checked for the volume left in order to ensure that the antibody was indeed delivered.

Post-lesion behavioral, electrophysiological and pharmacological investigations

The manual dexterity assessment using the "modified Brinkman board" task was pursued along a period of two to three months post-lesion, in order to reach a plateau (**Fig. 1a-c**). Then an amount of 30-50 daily sessions were considered to establish the "post-lesion" behavioral score,

corresponding to the so-called “functional recovery” expressed in percent of the pre-lesion behavioral score. In three monkeys (“Mk-CC”, “Mk-CS” and “Mk-AF”), extensive intracortical microstimulation (ICMS) sessions were conducted in the motor cortex bilaterally in order to study the post-lesional plasticity of the motor map both in the contralesional⁴ and ipsilesional¹⁴ hemispheres. In the same three monkeys, the post-lesional ICMS sessions were followed by reversible inactivation sessions by infusing the GABA agonist muscimol in the contralesional or ipsilesional hand representation area in M1^{4,14}.

Tracing experiments and histological assessment of the lesion

After completion of the post-lesion behavioral and electrophysiological (ICMS) sessions, the anterograde tracer “Biotinylated Dextran Amine” (BDA, Molecular Probe®) was injected in the contralesional hemisphere. In the three monkeys subjected to electrophysiological mapping, the chronic chamber allowed positioning of Hamilton syringes containing BDA at sites in the M1 hand area identified based on the pre- and post-lesion ICMS data. In order to obtain an uptake of BDA also from more proximal CS neurons, BDA was also injected at ICMS sites corresponding to forearm territories (wrist, elbow, shoulder) as well as more medially in M1 leg territories. In the other monkeys, using stereotaxic landmarks, a craniotomy was performed under propofol anesthesia to expose the central and arcuate sulci contralesionally. Injections of BDA were performed in M1, i.e. in the rostral bank of the central sulcus, following the central sulcus going from lateral (hand representation) to medial (leg representation). A second series of BDA injections were performed more rostrally with respect to the central sulcus, also going from lateral to medial, but at a depth aiming for the part of M1 located on the surface of the cerebral cortex. The parameters of BDA injections are given for each monkey in **Supplementary Table 1** (online). In the first two monkeys (Mk-CC, Mk-CS), the survival time after BDA injection was set to three weeks, as used before in intact monkeys¹⁵. However, the cervical lesion substantially slowed down the anterograde axonal transport of BDA and therefore the tracer did not reach the cervical segments. These animals were not considered further for the anatomical analysis (**Supplementary Table 1** online). In contrast, a much longer survival time (60 days) allowed transport of BDA up to thoracic level in the other ten monkeys (**Supplementary Table 1** online). The animals were finally sacrificed under deep, lethal anaesthesia (90 mg of sodium pentobarbital/kg body weight) by transcardiac perfusion with 0.9% saline (400 ml) and continued with fixative (3 liter of 4% phosphate buffered paraformaldehyde in 0.1 M phosphate buffer, pH=7.6). Perfusion was continued with solutions (2 liters each) of the same fixative containing increasing concentrations of sucrose (10, 20 and 30%). The brain and spinal cord were dissected and placed in a 30% solution of sucrose (in phosphate buffer) for cryoprotection during seven days. Frozen sections (50

μm thick) of the brain were cut in the frontal plane and collected in eight series, whereas frozen sections (50 μm thick) of the cervical cord (approximately segments C6-T3) were cut in the paralongitudinal plane and collected in 3 series for later histological processing. Upper cervical segments and lower thoracic spinal segments were cut in the frontal plane at 50 μm thick and sections were also collected in three series. One series of spinal sections were revealed for BDA staining, as described in detail in previous reports^{15,16}. The second series of spinal cord sections were immunohistochemically processed to visualize the corticospinal axons using the marker SMI-32, as recently reported¹⁷, a series of sections also used to reconstruct the location and extent of the cervical lesion, as described in detail earlier^{4,17}.

As other descending motor tracts (e.g. rubrospinal, reticulospinal) may also contribute to recovery, the overall extent of the lesion was quantitatively assessed by the percentage of hemi-cord lesioned. In other words, the extent of the cervical lesion was expressed quantitatively in percent, given by the ratio of the reconstructed lesion area (as shown by the red or blue area in **Supplementary Fig. 1b** online) to the total area of the corresponding hemi-cervical cord. The extent of the cervical lesion ranged across monkeys from 38% to 90% of the spinal hemi-cord (**Supplementary Table 1** online). The rostrocaudal extent of the cervical lesion ranged from 488 to 1367 μm (see also **Supplementary Table 1** online). The third series of sections were processed to visualize another anterograde tracer (Dextran-Fluorescein) injected in the ipsilesional hemisphere, but these data will not be presented here.

Measurement of CS axonal arborization

On every paralongitudinal section processed for BDA (150 μm interval), the presence of BDA labelled CS axonal arbors was investigated caudal to the lesion at 200x magnification on a light microscope (Olympus®). Using *neurolucida*® software, each BDA-labelled axonal segment was traced and the location of boutons *terminaux* and *en passant* were registered. The same software allowed counts of axonal swellings and the cumulated length of axonal arbors labeled with BDA caudal to the lesion. Boutons *en-passant* were defined as a swelling with a diameter twice the diameter of the axonal branch before and after the swelling itself. For each monkey, the contour of the lesion (vertical arrow in **Fig. 2c-d** and **Supplementary Fig. 3a-b** online) and the labeled CS axonal segments were plotted on a drawing of superimposed reconstructions of paralongitudinal sections of the cervical-thoracic cord (**Fig. 2c-d** and **Supplementary Fig. 3a-b** online). Rostral to the lesion, the densely packed line segments represent the BDA-labeled CS tract above the lesion and interrupted by the transection of the dorsolateral funiculus (**Fig. 2c-d** and **Supplementary Fig. 3a-b** online). Some of the transected CS axons exhibited a retraction from the rostral limit of the lesion (data not presented here).

Out of the ten monkeys injected with BDA and for which a survival time was long enough (**Supplementary Table 1** online), the seven monkeys conducted according to the “double-blind” procedure were selected for the quantitative analysis of BDA labeling caudal to the lesion. The expected number of CS axonal arbors and swellings labeled with BDA depends on the size of the BDA injection in M1, on the efficiency of tracer uptake and axonal transport. In order to normalize the data across monkeys, the number of BDA-labeled CS axons was counted rostral to the lesion, at C5 level on a frontal section. Then the cumulated length of CS axonal arbors caudal to the lesion was divided by the total number of labeled CS axons at C5 level (**Fig. 2e and g**). A similar normalization was performed for the number of CS axonal swellings (**Fig. 2f-g**).

The statistical comparisons between the two groups of monkeys (anti-Nogo-A treated versus control-antibody treated) regarding functional recovery and CS axonal sprouting were conducted using the non parametric Mann and Whitney test.

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