Neurite growth inhibitors restrict plasticity and functional recovery following corticospinal tract lesions

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Anatomical plasticity and functional recovery after lesions of the rodent corticospinal tract (CST) decrease postnatally in parallel with myelin formation. Myelin-associated neurite growth inhibitory proteins prevent regenerative fiber growth, but whether they also prevent reactive sprouting of unlesioned fibers is less clear. Here we show that after unilateral CST lesion in the adult rat brainstem, both intact and lesioned tracts show topographically appropriate sprouting after treatment with a monoclonal antibody that neutralizes these inhibitory proteins. Antibody-treated animals showed full recovery in motor and sensory tests, whereas untreated lesioned rats exhibited persistent severe deficits. Neutralization of myelin-associated neurite growth inhibitors thus restores in adults the structural plasticity and functional recovery normally found only at perinatal ages.

The adult mammalian central nervous system (CNS) has a very limited capacity for functional and anatomical repair after lesions. Myelin and the myelin-associated neurite growth inhibitors NI-35/250 seem to be important for preventing regenerative fiber growth[1]. A monoclonal antibody (IN-1) that neutralizes the inhibitory effect of myelin enhances long-distance regeneration of adult corticospinal axons[2,3] and recovery of locomotor functions[4]. In contrast to the adult CNS, lesions of the perinatal CNS can cause both regeneration of the lesioned fibers and plastic sprouting of unlesioned fibers, which may account for the high degree of functional recovery seen at that age[5–10]. For the rodent corticospinal tract, the capacity for anatomical and functional plasticity declines postnatally[5–7,11] with the onset and progression of myelination[12,13]. If myelin formation in the spinal-cord is experimentally prevented, unilateral section of the CST at the level of the pyramid (a part of the brainstem) in young adult rats causes sprouting of the intact corticospinal tract across the midline of the myelin-free spinal cord into the denervated areas[14].

There are many advantages to using pyramidotomy to investigate structural plasticity. A CST lesion in the brainstem is more likely to spare the other descending and ascending fiber systems than a large spinal cord lesion. The CST runs very superficially in the pyramid with the basilar artery as a landmark between the two CSTs, thus allowing a specific unilateral lesion. Many studies describe lesion-induced sprouting of the CST and other descending tracts in neonatal animals and the absence of sprouting in adults after such a lesion[7,12,15,16]. The normal CST innervation pattern in the spinal cord is very specific and well described[17–21], as are its central projections; for example, the CST projection that originates in the forelimb area of the motor cortex topographically innervates the red nucleus[22,23] and the pontine nuclei[24–27], which lie rostral to the pyramid.

Here we investigated the plastic fiber growth and functional recovery of the mature rodent CST in response to a complete unilateral section at the lower brainstem (pyramidotomy). In one set of experiments, we anterogradely labeled the unlesioned CST and examined its behavior in the cervical spinal cord. In another set of experiments, we labeled the forelimb brainstem projection of the lesioned CST and studied its reaction rostral to the lesion site in the red nucleus and the pontine nuclei, as well as its innervation of the dorsal column nuclei. Our experimental animals were tested for recovery of motor function in a food-pellet reaching task[28], because the integrity of the CST is believed to be necessary for skilled forelimb use. We also used a forelimb footprint analysis to reveal deficits in forelimb placement and rotation. In the sticky-paper test, we assessed the loss and recovery of sensory function. Lesions of the CST led to functional impairment on each of these tasks, and anatomical studies revealed only limited anatomical changes in response to the lesion. In contrast, animals treated with the IN-1 antibody showed full functional recovery on all behavioral assays, and this was accompanied by extensive plastic sprouting of both the lesioned and the contralateral unlesioned CST.

Results

The monoclonal antibody IN-1 was raised against the rat NI-250 myelin protein, a major neurite growth inhibitory component. IN-1 also neutralizes rat NI-35, as well as myelin inhibitory activity of bovine and human spinal cord[29,30]. Unilateral lesions of the CST were performed at the level of the medulla oblongata (see Fig. 4) in adult Lewis rats of either sex at two to three

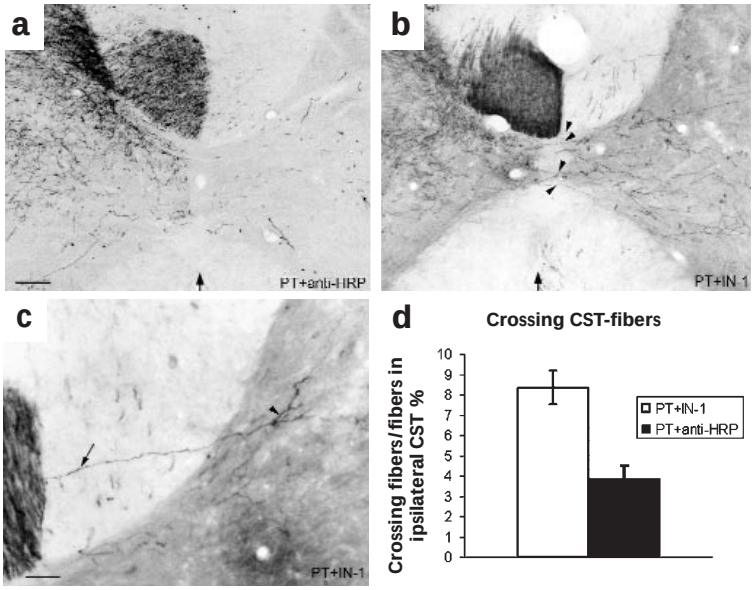

Fig. 1. Treatment with IN-1 increases lesion-induced sprouting of the intact CST, as revealed by the increased number of fibers crossing the spinal cord midline. The sensorimotor cortex of the unlesioned CST was injected with the anterograde tracer biotin dextran amine. **(a, b)** Photomicrographs of cross sections through the cervical spinal cord of pyridotomized animals treated either with control antibody (PT+ anti-HRP; a) or IN-1 (PT+IN-1; b). The position of the midline is indicated by an arrow. In the lesioned group treated with IN-1, many labeled CST axons cross the midline (arrowheads) and branch into denervated regions of the gray matter. In lesioned animals treated with anti-HRP antibodies, only a very small portion of fibers sprout into the denervated half. Note also the few ipsilateral uncrossed CST fibers in the dorsal and ventral funiculi. Scale bar for (a) and (b), 120 µm. **(c)** Occasionally, fibers from the labeled, intact CST cross through the area of the degenerated, contralateral CST (arrow) and branch into the denervated region (arrowhead). Scale bar, 60 µm. **(d)** Fibers crossing the midline were counted blind to treatment, and the counts were averaged per animal (n=10 in each group). Mean values were normalized to the labeled ipsilateral fibers (Methods). The difference is highly significant (p<0.001, Mann-Whitney test). Error bars indicate standard error.

months of age. At the time of surgery, 10^5 hybridoma cells secreting either IN-1 or a control antibody directed against horseradish peroxidase[2] were injected into the cortex contralateral to the lesion. Cyclosporin A (1 mg per 100 g body weight) given postoperatively allowed the growth of small, antibody-secreting aggregates of hybridoma cells. Additional control groups included rats with lesions but no hybridoma cells, and sham-operated rats treated with either anti-HRP or IN-1 hybridomas (antibody-only). For anatomical studies, the sensorimotor cortex on either the intact or the lesioned side was injected on the day of the surgery with the anterograde tracer biotin dextran amine, and the animals were sacrificed by perfusion two weeks later. In one group of experiments, we investigated the effects of lesions and antibody treatment on the intact (contralateral) CST in the cervical spinal cord. In a second group, we examined the projections from the ipsilateral (lesioned) fibers to the contralateral red nucleus and pons. A third group of animals was analyzed behaviorally. In these animals, the dorsal column nuclei of the brainstem were analyzed after the testing period.

Lesion-induced sprouting in the spinal cord

In the rat, the CST decussates almost completely at the pyramidal decussation within the brainstem, and thus most fibers project, via the dorsal funiculus, to the contralateral half of the spinal cord[31]. A small number of fibers does not cross at the pyramidal decussation but instead continues ipsilaterally in the ventral and dorsal funiculi[21,31]. In the control groups, after two weeks we found a small increase in the number of labeled fibers in the denervated spinal cord gray matter contralateral to the lesion (lesion only or antibody only; data not shown). Lesioned animals treated with control antibodies showed a similar sparse sprouting into the denervated areas (Fig. 1a and d). In contrast, lesioned rats treated with IN-1 antibody showed a significant increase in sprouting into the denervated areas (Fig. 1b and d). Fibers grew out of the intact CST running in the dorsal column, crossed the spinal cord midline through the dorsal commissure or through the area of the degenerating CST (Fig. 1c) and extended branches into the denervated dorsal, intermediate and ventral horn (main-

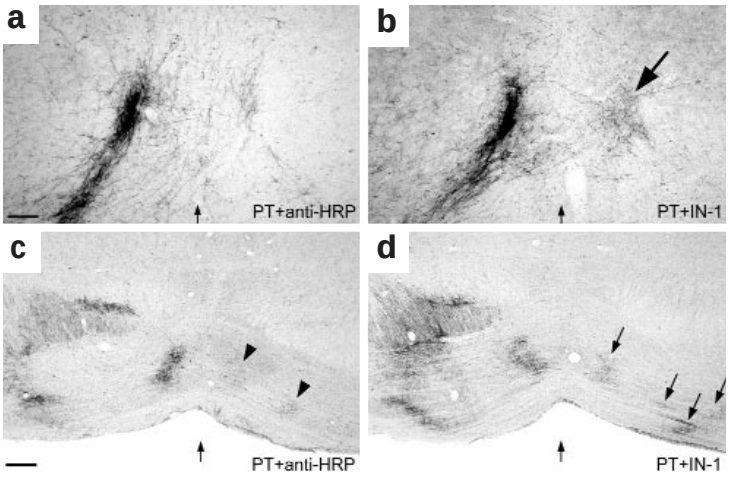

Fig. 2. Corticobulbar axons establish a bilateral projection in the red nucleus and pons after unilateral pyramidotomy and treatment with IN-1. The lesioned tract was traced with biotin dextran amine. **(a)** Photomicrograph of the innervation pattern of the rostral part of the red nucleus of an animal treated with control antibody (anti-HRP). **(b)** Corticorubral projections of an IN-1-treated animal. The extent of the corticorubral fibers terminating in the contralateral red nucleus is enlarged (arrow). Scale bar (a) and (b), 120 µm. **(c)** Typical projection pattern of corticopontine fibers originating from the forelimb motor cortex at the mid-pontine level of an animal treated with control antibody. Note the small contralateral projection (arrowheads). **(d)** Projection fields of corticopontine fibers at the mid-pontine level of an animal treated with IN-1, showing a large increase in contralateral projections and their arborization in the typical forelimb areas (arrows). The position of the midline is indicated by an arrow. Scale bar (c) and (d), 240 µm.

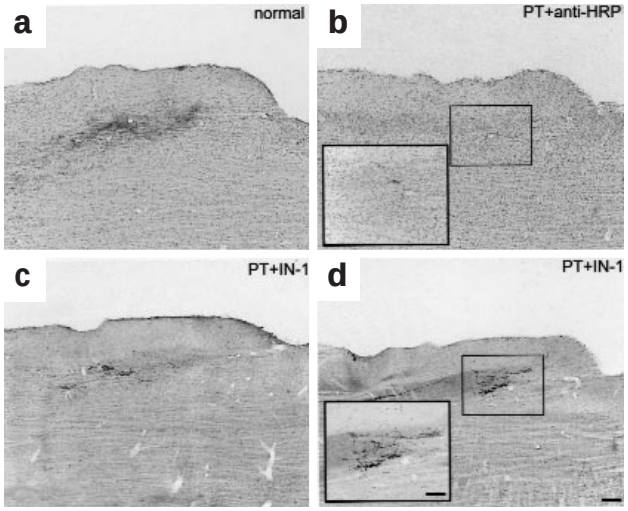

Fig. 3. Treatment with IN-1 induces a partial reinnervation of the dorsal column nuclei after pyramidotomy. All longitudinal sections are taken at the level of the solitary nucleus and counterstained with cresyl violet. **(a)** Photomicrograph of CST innervation of the DCN in a normal animal, traced with biotin dextran amine. **(b)** In lesioned animals treated with control antibodies, only a few fibers re-enter the DCN at 16 weeks postoperatively. Inset, close-up of the CST fibers in the DCN. **(c and d)** Two examples of animals treated with IN-1, 16 weeks after the lesion. Notice the increase in labeling in the DCN. Inset in (d), close-up of the reinnervating fibers and their arborization. Scale bars, 200 µm; inset scale bars, 50 µm.

ly laminae VI and VII). These newly formed collaterals showed bouton-like structures along their length and at their tips (Fig. 1c). In the lesioned group treated with IN-1, the number of fibers crossing the midline was increased more than twofold as compared to animals treated with anti-HRP antibodies (Fig. 1d, $p<0.001$, Mann-Whitney test). Lesioned animals treated with IN-1 were also significantly different from lesion-only and sham-operated, antibody-treated animals (data not shown), whereas there was no significant difference between the lesioned animals treated with anti-HRP antibodies and either of the other control groups. The normalized values shown in Fig. 1d correspond to about 3.2 fibers per section in the IN-1-treated group ($n=10$) and about 1.8 fibers per section in the anti-HRP-treated animals ($n=10$). Pilot experiments using a recombinant, humanized IN-1 Fab fragment infused by osmotic minipumps for two weeks over the thoracic spinal cord showed a similar lesion-induced sprouting[32] (Brösamle *et al.*, *Soc. Neurosci. Abstr.*, 130.2, 1996).

A NEW CONTRALATERAL PROJECTION IN IN-1-TREATED ANIMALS

In the brainstem, we examined the red nucleus and the basilar pontine nuclei. Both structures receive a strong input from the primary motor cortex, i.e. direct corticorubral or corticopontine projections and collaterals of the CST axons[23,27,33]. These nuclei are involved in motor control via the cerebellum. Cortical fibers end mainly in the parvocellular part of the red nucleus; in the basilar pons each cortical area has a distinct termination pattern (Fig. 2c and d, left half). These projections are unilateral, and only a very small number of fibers cross the midline to project to the contralateral nuclei. In lesion-only rats (data not shown) and in lesioned animals treated with control antibody, these so-called corticobulbar projections were almost indistinguishable from normal, except for a slight increase in contralateral terminations in the animals treated with control antibody (Fig. 2a and c). In contrast, in animals treated with IN-1 antibody, the number of fibers crossing the midline was increased, both in the region of the parvocellular red nucleus (Fig. 2b) and in the caudal regions of the pons. In the basilar pontine nuclei, a relatively large increase in the fiber density of the terminal plexus was found on the contralateral side in the group treated with IN-1 antibody (Fig. 2d). Densitometry of these terminal fields showed a contralateral component of 8.3% (± 2.3, $n=5$) in normal animals as compared to 26.3% (± 2.6, $n=5$, $p<0.01$, ANOVA) in the lesioned group treated with IN-1 antibody. These fibers innervated the appropriate contralateral regions (forelimb areas of the pons).

IN-1 INCREASES DORAL COLUMN NUCLEI REINNERVATION

In rodents, CST collaterals projecting to the dorsal column nuclei (DCN; the nucleus cuneatus and nucleus gracilis) originate at the level of the decussation and spread in the rostral direction to innervate the contralateral DCN (Fig. 3a)[34]. Because of this innervation pattern, a lesion of the CST at brainstem level induces a complete denervation of the DCN. Innervation of the DCN was qualitatively compared by labeling the lesioned CST in all animal groups that had undergone behavioral tests. Lesion-only as well as lesioned, anti-HRP-treated animals showed little or no DCN reinnervation (Fig. 3b). In contrast, two thirds of the animals treated with IN-1 had a dense reinnervation of the DCN (Fig. 3c and d). The anatomical results are summarized in Fig. 4.

FUNCTIONAL RECOVERY PARALLELS STRUCTURAL PLASTICITY

The remarkable structural plasticity observed in the rats treated with IN-1 antibody led to the question of whether functional improvements parallel these anatomical changes. Adult Lewis rats were trained daily for two to three weeks in a food-pellet reaching task[28] (testing set-up in Fig. 5a) and then operated (five experimental groups: lesion only, sham operation and anti-HRP, sham operation and IN-1, lesion and anti-HRP, lesion and IN-1). Animals were allowed to recover from the

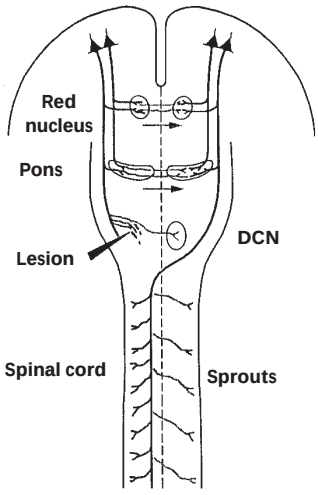

Fig. 4. Schematic representation of the lesion site and the corticospinal projections that were examined in this study. Gray lines represent newly sprouted fibers after the unilateral CST lesion and IN-1 treatment. The arrows indicate the growth direction of the sprouts. The intact CST showed collaterals that recrossed the midline at spinal cord levels to branch into the denervated hemicord. The lesioned tract increased its innervation to the contralateral red nucleus and pontine nuclei and reinnervated the dorsal column nuclei.

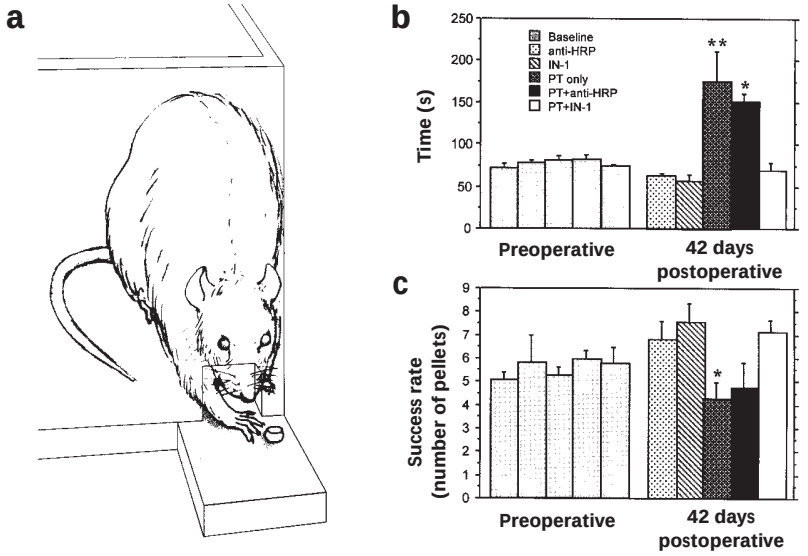

Fig. 5. The monoclonal antibody IN-1 leads to functional recovery in the food-pellet reaching task. **(a)** Testing set-up. **(b)** Time (seconds) needed to grasp 20 stabilized pellets. **(c)** Number of pellets grasped and eaten in the 10-pellet test (success rate), where pellets were presented without stabilization. Preoperative baseline and performance six weeks after operation are shown. Animals treated with IN-1 improve to nearly normal performance levels. Sham-operated, antibody-treated animals (anti-HRP, $n = 4$, or IN-1 only, $n = 4$); pyramidotomy (PT only; $n = 10$); PT+anti-HRP ($n = 10$); PT+IN-1 ($n = 10$). Error bars indicate standard error. Asterisks indicate significance relative to antibody-only animals: * $p<0.05$; ** $p<0.01$, Kruskal-Wallis test.

operation and treatment for two weeks and were then tested daily for the next four weeks. On the first day of postoperative training, most lesioned animals reached with the ipsilateral, unimpaired forelimb. During postoperative training, the animals were forced to switch to the contralateral, impaired forelimb. The animals were required to grasp food pellets from a smooth surface with the impaired forelimb. The first test measured the time required to eat 20 stabilized food pellets (quantitative analysis in Fig. 5b). The preoperative baseline measurement showed that the animals needed about 75 seconds to grasp and eat the 20 pellets. Forty-two days after the operation, lesion-only animals and lesioned animals treated with anti-HRP needed significantly more time, about 150 seconds, as compared to the preoperative baseline or to sham-operated animals. In contrast, sham-operated animals and lesioned animals treated with IN-1 did not differ from the preoperative baseline (75 seconds). In a second task, the success rate to obtain 10 unstabilized pellets from the shelf was recorded (Fig. 5c). Compared to sham-operated animals, which grasped 7 pellets, lesion-only animals did grasp significantly fewer pellets (4.5) on postoperative day 42. The success rate of lesioned animals treated with IN-1 was similar to that of sham-operated animals. An increased success rate was observed in the lesioned animals treated with IN-1 antibody and in sham-operated animals as compared to the preoperative values, which was probably due to the postoperative training (Fig. 5c). In the lesion only and the lesioned, anti-HRP-treated animals, the lower success rate reflects the well known permanent deficits that normally follow CST lesion[28,35].

Motor function was also assessed with a rope-climbing task. Foot slips on the impaired side were counted while rats were climbing up a rope (Fig. 6a), and the number of slips per step was calculated (Fig. 6b). This test was done exclusively with female rats (see Methods). At 42 days after surgery, lesion-only and lesioned, anti-HRP-treated animals made a mean of 4.8 or 4.5 slips respectively per 10 steps (Fig. 6b), as compared to 2.5 slips per 10 steps in the sham-operated groups. Lesioned animals treated with IN-1 antibody performed as well as sham-operated animals, which were significantly better than the lesioned control groups. Compared to the preoperative performance, all animals made more footslips, which might be due to the increased body weight.

Recovery of sensory function was assessed using the sticky paper test, known to be particularly sensitive to somatosensory deficits[49]. At 42 days postoperatively, the time the rats needed to remove adhesive tapes from the palm of the forepaws was recorded for the unimpaired and the impaired side (Fig. 6c). Sham-operated animals removed the paper within 15 seconds from either forelimb. After CST lesions, animals needed 30 seconds to remove the paper from the unimpaired side (Fig. 6d). Removal of the paper sticking to the impaired forepaw was significantly prolonged to a mean of 110 seconds ($p<0.05$ for lesion only, Scheffe test). When initiated, the movements leading to paper removal were fast, showing that the motor component of this behavior was not visibly affected. Lesioned animals treated with IN-1 showed no statistical difference from preoperative values or sham-operated rats. The lesioned animals treated with IN-1 removed the papers from both the impaired and unimpaired sides within the same time (20 seconds; Fig. 6d). Interestingly, re-innervation of the DCN correlated with fast removal of the sticky paper of the impaired forepaw in the lesioned animals treated with IN-1.

We also used a forelimb footprint analysis to examine forelimb placement and rotation. Forelimb exorotation, i.e. an outward rotation of the impaired side, was significantly increased at 42 days postoperatively in the lesion-only group and lesioned animals treated with anti-HRP (27 and 25 degrees, respectively, compared to 20 degrees in sham-operated animals and lesioned animals treated with IN-1 antibody; Fig. 7). Base of support, stride length and toe spreading did not differ between the experimental groups.

Discussion

This study shows that the monoclonal antibody IN-1, which neutralizes the inhibitory properties of myelin, increases lesion-induced structural plasticity in adult rats after unilateral lesion of the corticospinal tract, resulting in a bilateral innervation of the cervical spinal cord, red nucleus and pons. Interestingly, the innervation of the dorsal column nuclei that was lost due to the lesion was also re-established in animals treated with IN-1. Moreover, IN-1 treatment resulted in the functional recovery of precise forelimb movements and sensory function.

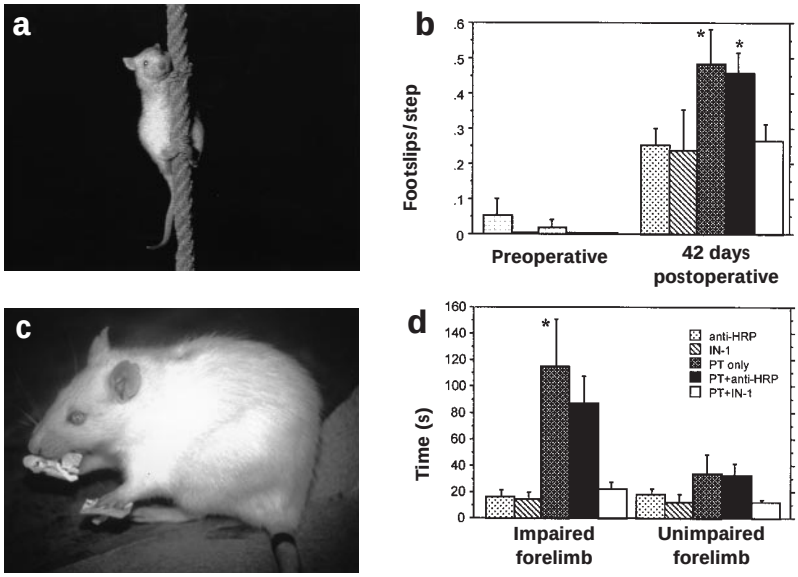

Fig. 6. Treatment with IN-1 improves performance in the sticky-paper and rope-climbing tests. **(a)** The rope-climbing test. Performance was measured as the rat climbed a vertical rope. **(b)** The number of foot slips per step during rope climbing was measured preoperatively (baseline) and 42 days after the lesion for female animals. This test showed improved climbing ability in IN-1-treated animals as compared to lesioned control groups. Anti-HRP, sham-operated with anti-HRP treatment (n = 2); IN-1, sham-operated with IN-1 treatment (n = 3); PT only, lesion only (n = 4); PT+anti-HRP, lesion with control antibody treatment (n = 5); PT+IN-1, lesion with IN-1 treatment (n = 8). Error bars indicate standard error. *p<0.05, Kruskal-Wallis test. **(c)** Sticky-paper test. The time to remove self-adhesive paper from the palm of the forelimb was measured. **(d)** Time (seconds) to remove sticky paper was recorded for all experimental groups on day 42 after lesion for the contralateral (impaired) and ipsilateral (unimpaired) side. Lesioned animals treated with IN-1 removed the paper as rapidly as sham-operated animals. Anti-HRP, sham-operated with anti-HRP treatment (n = 4); IN-1, sham-operated with IN-1 treatment (n = 4); PT only, lesion only (n = 10); PT+anti-HRP, lesion with control antibody treatment (n = 10); PT+IN-1, lesion with IN-1 treatment (n = 10). Error bars indicate standard error. *p<0.05, Scheffe test.

IN-1 ENHANCES PLASTICITY OF CORTICOFUGAL PATHWAYS

The monoclonal antibody IN-1 recognizes a high-molecular-weight novel protein (NI-250), with very potent neurite growth inhibitory activity in rat, bovine and human CNS myelin[29,36]. Our results show that substantial structural plasticity (fiber growth from intact and lesioned axons; Fig. 4) can occur in the adult CNS if myelin-associated neurite growth inhibitory activity is neutralized. This fiber growth is very precise and topographically specific in both the spinal cord and the brainstem. Fibers sprouted from the unlesioned CST and crossed the spinal cord midline to arborize in the denervated half of the spinal cord. Bouton-like structures on the new collaterals suggest the presence of synaptic terminals. Thus, although the denervated half of the spinal cord has lost its normal cortical input following the degeneration of the ipsilateral CST, the observed structural plasticity may allow it to be controlled by the intact contralateral CST, and hence by the other cortical hemisphere. Some regeneration of the lesioned CST axons across or around the lesion site does occur in rats treated with IN-1, but it is quantitatively weak, possibly due to the axons' difficulty in navigating through the pyramidal decussation (O.R., unpublished observations). Our findings agree with earlier observations after neonatal lesions where a similar 'aberrant ipsilateral pathway' by recrossing of CST fibers at spinal cord levels was described[7,12,31]. We also observed sprouting of corticobulbar axons, which originate from the lesioned CST rostral to the lesion site. These sprouts, which innervate the contralateral red nucleus and pons, might have similar consequences as the sprouting found in the spinal cord. By enhancing the contralateral projection to these nuclei, the cortical hemisphere that has lost its access to the spinal cord might (for example, via the cerebellum) obtain an indirect influence on its former target regions. Interestingly, similar projections develop following unilateral lesions of one sensorimotor cortex in neonatal animals: the intact cortex sends bilateral projections to the red nucleus[37,38] and the basilar pontine nuclei[37,39]. Thus, neutralization of the inhibitory properties of myelin results in compensatory structural plasticity that is normally only found after neonatal lesions.

UNDERLYING MECHANISMS

In the spinal cord, sprouting occurred into a denervated area. Denervation may induce an upregulation of neurotrophic factors and chemoattractants[40], which may guide the newly growing collaterals across the midline and to appropriate target regions. The nature of these factors in the spinal cord remains to be determined. A different mechanism would presumably be required to explain the sprouting that occurred in the con-

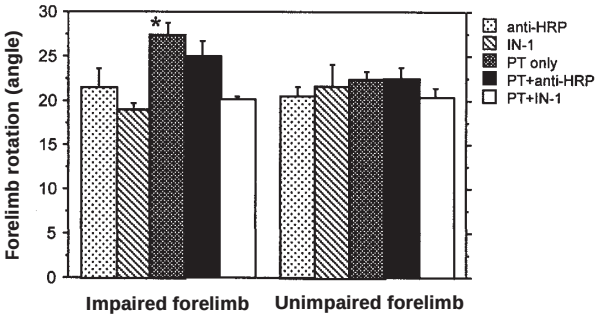

Fig. 7. Footprint analysis: angle of forelimb rotation. Compared to preoperative values, the forelimb rotation of the impaired side was increased at 42 days after lesion in the lesion-only and the lesioned, anti-HRP-treated animals. The lesioned, IN-1-treated animals showed no significant change in forelimb rotation. Sham-operated, antibody-treated animals (anti HRP, n = 4 or IN-1 only, n = 4); pyramidotomy (PT only; n = 10); PT+anti-HRP (n = 10); PT+IN-1 (n = 10). Error bars indicate standard error. Asterisks indicate significance relative to antibody-only animals: *p<0.05; **p<0.01; Kruskal-Wallis test.

tralateral pons and red nucleus, which are rostral to the lesion site and are not themselves denervated by the lesion. Possibly this form of plasticity might be induced by factors expressed as a consequence of the functional imbalance of the system, for instance by neurotrophic factors that are regulated by neuronal activity[41]. The anatomical specificity of the sprouting suggests that mechanisms for target recognition are present, or can be re-expressed, in the adult lesioned CNS.

FUNCTIONAL RECOVERY

Skilled forelimb use is strongly and permanently impaired after unilateral lesion of the CST at the level of the brainstem in adult rodents[6,42], and there is only limited spontaneous recovery. The very high degree of functional recovery of forelimb reaching observed here in the rats treated with IN-1, like the anatomical plasticity, is normally observed only after lesions at perinatal ages[28,43]. It remains to be determined to what extent this functional recovery is due to the newly grown CST collaterals in the spinal cord or to the new bilateral corticobulbar projection. Under the growth-permissive conditions established by IN-1, other tracts such as the rubrospinal tract may also react by increased and adaptive plasticity. Plasticity may also occur in sensory systems (see below) and in the cortex. To confirm that the observed functional recovery is mediated by structural plasticity and not by regeneration of the lesioned CST fibers, we introduced a second lesion rostral to the first one, thus cutting the CST again, including the regenerating fibers. This procedure did not abolish the functional recovery in the food-pellet reaching task of the IN-1-treated animals (W.J.Z., in preparation). Forelimb footprints showed an increased exorotation on the impaired side, an effect that was also reversed in the rats treated with IN-1.

The dorsal column nuclei, the most important relay station of the ascending somatosensory system, are densely innervated by the CST. This input was lost following the lesion and partially re-established by sprouts from the lesioned axons in the IN-1-treated rats. After IN-1 treatment, the sticky-paper test revealed a high degree of sensory recovery. Our data thus support former suggestions that the CST modulates sensory information[44] and imply that reinnervation of the DCN might influence the sensory recovery after such lesions. The increased reinnervation of the DCN might at least partially account for the sensory recovery in the sticky-paper and reaching tests.

Neutralization of myelin-associated neurite growth inhibitors with IN-1 induced a very high degree of structural plasticity and functional recovery in adult rats. In humans, the outcome of CNS injury depends strongly on the age at which the injury was sustained; affected patients show a much better motor performance when the lesion occurs very early in life[8,10,45]. Human spinal cord contains neurite growth inhibitors (IN-1 antigens) with biochemical properties very similar to those of rat or bovine myelin[29]. Therefore, the present results suggest a new possibility for therapeutic approaches after CNS lesions or stroke, for which tools like recombinant and humanized IN-1 Fab fragments are now becoming available[32] (Brösamle *et al.*, *Soc. Neurosci. Abstr.*, 130.2, 1996).

Methods

PYRAMIDOTOMY. Unilateral lesions of the CST were performed in two- to three-month-old Lewis rats. Rats were anesthetized by an intraperitoneal injection of Hypnorm (0.3 mg/kg body weight, i.p.; Janssen, Buckinghamshire, England) and Dormicum (0.6 mg/kg body weight, i.p.; Roche, Basel, Switzerland). The medullary pyramids were exposed by a ventral approach through an opening of the occipital bone as described[16]. The left CST was transected rostral to the decussation using a fine tungsten needle, with the basilar artery serving as a landmark for the midline. At the time of the surgery, hybridoma cells secreting either monoclonal antibody IN-1 (n = 25) or anti-HRP as control antibody[2] (n = 25) were injected into the cortex or the hippocampal region contralateral to the lesion. 6 µl of cell suspension, containing 10^5 cells, was injected. Cyclosporin A (10 mg/kg body weight, i.p.; Sandimmun, Novartis, Basel, Switzerland) was given daily during the first eight days postoperatively to allow the transplants to grow. To prevent infections Co-trimoxazol (0.83 ml/kg, i.p.; Bactrim, Roche, Basel, Switzerland) was given with cyclosporin A.

TRACING. In one group of animals, the sensorimotor cortex of the unlesioned CST was pressure injected at the day of operation with the anterograde tracer biotin dextran amine (10% BDA in 0.1M phosphate buffer; Molecular Probes, Eugene; 2.5 µl into three to four injection sites). To examine the topography of the corticorubral and corticopontine projections in the second group of animals, we wanted to trace exclusively the forelimb area of the motor cortex, but without allowing the tracer to diffuse too far, so we chose a iontophoretic injection. The ipsilateral motor cortex was mapped by intracortical microstimulation and five points of the caudal forelimb motor area were iontophoretically injected with the anterograde tracer BDA (7 s pulse, 14 s pause, 5 µA, duration 15 min). The animals that underwent intracortical microstimulation were pretreated with atropine (0.025 mg, i.p., Sintetica S.A., Mendrisio, Switzerland) and anesthetized with ketamine (100 mg/kg body weight; i.p., Ketalar, Parke-Davis, New Jersey). After a survival time of two weeks, the corticospinal projections of the non-lesioned CST and the corticorubral and corticopontine projections of the lesioned CST were examined. After the behavioral tests, the dorsal column nuclei were investigated after tracing of the ipsilateral motor cortex using BDA (pressure injection).

TISSUE HANDING. After a survival time of 14 days, the animals were killed by an overdose of pentobarbital (450 mg/kg body weight; Nembutal, Abbott Laboratories, Cham, Switzerland) and perfused transcardially with Ringer's solution containing 0.25% $NaNO_2$ and 100,000 units/l heparin, followed by 4% paraformaldehyde, 5% sucrose in 0.1 M phosphate buffer (PB) at pH 7.4. The brain and spinal cord were dissected, postfixed for 2 hours at 4°C and stored in a 30% sucrose solution for 36 hours at 4°C for cryoprotection. The spinal cord or the brainstem were embedded in a gelatin-chicken albumin solution polymerized with 25% glutaraldehyde, covered with Tissue Tek and frozen by immersion in isopentane at -40°C. Cross sections of 50 µm were cut on a cryostat. The BDA was detected by immunohistochemistry as described[46]. Briefly, sections were collected in cold 0.1 M PB, rinsed 3 x 30 min in TBS-X (50 mM TRIS; 0.9% NaCl; 0.5% Triton X-100; pH 8.0) and incubated overnight with an avidin-biotin-peroxidase complex (Vectastain ABC Elite Kit, Vector Burlingame, CA, 1:100 in TBS-X) at room temperature. After 3 x 30 min washing in TBS-X, the sections were rinsed with 50 mM TRIS-HCl (pH 8.0) and preincubated in 0.4% nickel ammonium sulfate in 50 mM TRIS-HCl for 10 min. Sections were further preincubated for 10 min in the nickel ammonium sulfate solution to which 0.015% of 3,3' diaminobenzidine tetrahydrochloride (DAB; Sigma) was added, and finally reacted in a nickel ammonium/DAB mixture containing 0.004% H_2O_2. After 10-30 min, the reaction was stopped with 50 mM TRIS-HCl, and the sections were rinsed 3 x 10 min in 50 mM TRIS-HCl. Sections were mounted on slides, dehydrated and embedded in Eukitt (Kindler, Germany). Sections of the brainstem were counterstained with cresyl violet.

MEASUREMENT OF CST SPROUTING. Fibers crossing the midline were counted blind to treatment at an Olympus microscope. Fibers were counted at a 200x magnification in six to eight sections per animal, and the counts were averaged per animal. To compensate for tracing efficiency in individual animals (due to variances in tracer uptake and transport), we counted labeled ipsilateral fibers (ventromedial and dorsoventral funiculus) and calculated the ratio to crossing fibers for each individual animal. (The proportion of the main, crossed CST in

relation to the ipsilateral CST fibers is very stable and was determined in normal animals, $n = 4$). Statistical analysis was performed using the Mann-Whitney test.

FOOD-PELLET REACHING TASK. Tests were performed as described[47] in a transparent Plexiglas box (30 x 36 x 30 cm) with a rectangular opening (1.5 x 3 cm) in the front wall adjacent to the left side wall (Fig. 3a). The floor was constructed as a mesh that ensured that dropped food pellets were lost to the rats. A smooth Plexiglas shelf was attached to the wall underneath the rectangular opening. Small food pellets (dustless precision pellets, 45 mg, Bioserv, Frenchtown, NJ) were placed one after the other onto the shelf. A plastic bar between the shelf and the opening prevented scooping of pellets. By the placement of the opening and position of the pellets at a distance of 1.5 cm from the opening, animals were biased to use the impaired forelimb. After weight reduction to about 95% of their initial weight, animals underwent a training phase and five days preoperative baseline measurements. Postoperatively, the animals were trained daily, starting two weeks and ending six weeks after the lesion. Statistical significance was tested using the Kruskal-Wallis test. In both pre- and postoperative sessions, the time to obtain 20 stabilized pellets was measured, starting from when they first touched a pellet. In the 10-pellet test, the animals received ten pellets without stabilization by placing them one after the other onto the shelf, which required the rats to reach accurately and carefully. The number of pellets grasped and eaten (success rate) was recorded in each session. If animals used the ipsilateral limb for reaching or did not start to reach at all, a maximum time of five minutes was given before the session was ended.

ROPE CLIMBING. To examine grip strength, the rope climbing test was performed. Animals had to climb a 160 cm vertical rope to reach a platform[48]; the number of footslips of the affected side (fore- and hindlimb) was counted and reported as total number of footslips per total number of steps. As error rates increase with body weight, the pre- and 42-days-postoperative values are given only for females ($n = 22$).

STICKY-PAPER TEST. Self-adhesive labels (1.3 x 2.6 cm) were placed onto the palm of the forepaws as described[49]. The time the rats needed to remove the paper was recorded for each side. This test was originally developed to assess somatosensory asymmetry and sensory function after sensorimotor cortex lesions.

FOOTPRINT ANALYSIS. Footprint analysis was modified from ref. 50. The forepaws were inked and footprints were made on paper covering a 7-cm-wide, 50-cm-long runway that forced the rats to walk in line in a given direction. A series of at least ten sequential steps, recorded in two trials, was used to determine mean values of limb rotation, stride length, base of support and toe spread. Limb rotation was estimated by the angle formed by the intersection of the line through the print of the third digit and the print representing the metatarsophalangeal joint with the line through the metatarsophalangeal print parallel to the walking direction. Stride length was measured between two consecutive prints on each side. The base of support was determined by measuring the core-to-core distance of the print representing the planar cushion underlying the metatarsophalangeal joint. Toe spread was measured as the distance between toe one and toe four.

STATISTICAL ANALYSIS OF BEHAVIORAL DATA. Analysis of behavioral data was performed with StatView 4.53 statistical package (Abacus Concepts, Inc., Berkeley, CA). For parametric data (time measurements, sticky paper test and footprint analysis), the Scheffe test was used. For non-parametric data (success rates), the Kruskal-Wallis test or the Wilcoxon signed rank test was used. A p-value less than 0.05 per number of samples was chosen as significance level. All data are presented with standard error. As there were no differences among sham-operated rats, anti-HRP-treated rats and sham-operated rats treated with IN-1, they were combined into one group (antibody only) to increase the power of the statistical analysis.

All protocols are approved by the cantonal veterinary department of Zürich.

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