Remodeling of Axonal Connections Contributes to Recovery in an Animal Model of Multiple Sclerosis

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Abstract

In multiple sclerosis (MS), inflammation in the central nervous system (CNS) leads to damage of axons and myelin. Early during the clinical course, patients can compensate this damage, but little is known about the changes that underlie this improvement of neurological function. To study axonal changes that may contribute to recovery, we made use of an animal model of MS, which allows us to target inflammatory lesions to the corticospinal tract (CST), a major descending motor pathway. We demonstrate that axons remodel at multiple levels in response to a single neuroinflammatory lesion as follows: (a) surrounding the lesion, local interneurons show regenerative sprouting; (b) above the lesion, descending CST axons extend new collaterals that establish a “detour” circuit to the lumbar target area, whereas below the lesion, spared CST axons increase their terminal branching; and (c) in the motor cortex, the distribution of projection neurons is remodeled, and new neurons are recruited to the cortical motor pool. Behavioral tests directly show the importance of these changes for recovery. This paper provides evidence for a highly plastic response of the motor system to a single neuroinflammatory lesion. This framework will help to understand the endogenous repair capacity of the CNS and to develop therapeutic strategies to support it.

Key words: demyelinating autoimmune disease • spinal cord • pyramidal tracts • axons • nerve regeneration

Introduction

Inflammatory disorders of the central nervous system (CNS) such as multiple sclerosis (MS) are often accompanied by substantial damage to axons and myelin (1). The extent of axonal damage is of particular importance for the clinical deficit that MS patients accumulate over time (2, 3). However, the clinical outcome will ultimately depend on the balance of damage inflicted on the CNS and the extent of endogenous repair that can counter it. Although axonal destruction in MS has been characterized extensively in recent years, very little is known about how it can be counterbalanced (4, 5). Substantial compensation is evident in the typical clinical course of MS. The relapsing–remitting form of the disease is defined by cycles of newly emerging clinical deficits that resolve within a few days or weeks. The regression of tissue edema and inflammation undoubtedly plays a major role early during remission, but these processes cannot mend structural damage caused by the inflammatory attack. Thus, additional compensatory mechanisms must be considered for complete recovery that often occurs despite substantial structural damage that persists at the site of inflammation.

The neuropathology of MS and its animal model, experimental autoimmune encephalomyelitis (EAE), suggests that limited repair of myelin damage can be achieved (“remyelination”), when oligodendrocytes form new myelin (5, 6). This gives rise to so-called shadow plaques, which are areas of myelin pallor in MS brain tissue. In addition, the redistribution of sodium-channels in demyelinated axons
can help to compensate for myelin loss (7). However, given the close correlation between the extent of axon damage and clinical deficit in MS, one would expect compensation for axon loss to be an even more powerful means of recovery. Surprisingly, little is known about how lost axonal connections are repaired in MS. Early papers have documented changes suggestive of axonal outgrowth in lesions of MS, a finding subsequently confirmed for EAE (8–10). In addition, functional MRI studies provide evidence for cortical adaptation in MS patients (11, 12). The lack of understanding of how recovery from axonal damage occurs in MS is, in part, due to the disseminated nature of MS and EAE. Multiple neuroinflammatory lesions occur in unpredictable locations in these diseases and damage various axonal tracts with high interindividual variability. This makes the analysis of axonal compensation, which occurs equally in a disseminated and variable way, difficult if not impossible.

In the present paper, we have circumvented this problem by using a recently developed “targeted” EAE model in which we can reproducibly induce a single inflammatory, demyelinating lesion in the dorsal column area of the midthoracic spinal cord (13). This area comprises the corticospinal tract (CST), a major descending motor tract that connects cortical layer V neurons with spinal targets and is often affected in MS (14). Targeting lesions to this tract system combined with detection of growth-associated proteins, as well as a variety of anterograde, retrograde, and trans-synaptic tracing techniques allowed us to analyze precisely how axonal compensation is achieved. We demonstrate that a single EAE lesion in the spinal cord induces axonal remodeling on many levels: (a) on the spinal level, in the neuronal circuitry surrounding the lesion, where numerous axons sprout; (b) in descending motor tracts, which create “detour” circuits to circumnavigate the lesion; and (c) at the cortical level, where the map of motor neurons that project to spinal motor targets is remodeled. Finally, behavioral experiments show that at least some of these remodeled connections directly contribute to functional recovery.

In summary, our paper provides insight into the mechanisms of endogenous axonal remodeling in inflammatory CNS diseases. In addition, our results suggest a new strategy for intervention in MS, as they underscore the existence of an endogenous repair program that could be targeted therapeutically.

Materials and Methods

Animals

Adult female Lewis rats (160 and 220 g) were obtained from Harlan. All experiments were approved by the veterinary department of the Canton of Zurich.

Immunogen

An NH₂-terminal fragment of rat myelin oligodendrocyte glycoprotein (rMOG; amino acids 1–125) was expressed in Escherichia coli, purified to homogeneity as described previously (15), dissolved in 6 M urea, and solubilized by dialysis against 20 mM sodium acetate buffer, pH 3.0.

Induction of a Targeted EAE Lesion

A focal EAE lesion was targeted to the CST area in the midthoracic spinal cord by minimal invasive injection of proinflammatory cytokines in MOG immunized animals as described previously (reference 13 and see supplemental information for detailed protocols).

Surgical Procedures

Thoracic Lesion of the CST. A bilateral dorsolateral hemisection of the spinal cord at T8 was performed as described previously (16). The main dorsomedial as well as the minor dorsolateral components of the CST were transected.

Medullar Lesion of the CST. A pyramidotomy was performed at the level of the medulla oblongata using a ventral approach as described previously (17). The entire CST was transected rostral to the decussation on both sides.

 Histopathology

Histopathological evaluation was performed on 3-μm thick sections of spinal cords of animals with targeted EAE lesions at day 7 (n = 4), day 14 (n = 4), and day 28 (n = 4) after lesion induction (see online supplemental material for detailed protocols).

Immunohistochemistry

Cross sections of the spinal cord were cut on a vibratome or cryostat and analyzed using standard immunohistochemistry (see online supplemental material for detailed protocols). The following primary antibodies were used: rabbit anti-c-Jun (1:500; Santa Cruz Biotecntology, Inc.), mouse anti-GAP43 (1:1,000; clone 9-1E12; Chemicon), mouse anti-GFAP (1:200, clone G-A-5, Chemicon), mouse anti-MAP2 (1:100; clone AP20; Chemicon), rabbit antineurofilament (1:100, Chemicon), and mouse ED1 (1:1,000, clone ED1; Serotec).

Quantitative Analysis of Immunostainings

Expression of c-Jun and GAP43 was quantified on cryostat sections of EAE animals perfused at day 7, day 14, and day 28 (n = 4 for each time point), as well as from untreated control animals (n = 2) and animals receiving a cytokine injection but no immunization (n = 6). For comparison, c-Jun and GAP43 expression were analyzed in animals perfused at day 7, day 14, and day 28 after a traumatic spinal cord lesion (n = 5 for each time point). For analysis, we selected sections with comparable lesions size and counted the total number of c-Jun⁺, GAP43⁺ neurons as well as the number of c-Jun⁺/GAP43⁺ neurons (10–30 sections per animal). For four EAE animals at day 14 after lesion induction, we further reconstructed the localization of c-Jun⁺ and GAP43⁺ neurons on five consecutive sections.

Tracing of Axonal Tract Systems

The hindlimb CST was traced anterogradely and bilaterally with biotinylated dextran amine (BDA) as described previously (18). Contacts between hindlimb CST collaterals and propriospinal neurons (PSNs) were analyzed by anterograde tracing of the CST (BDA) and retrograde tracing of PSNs (fluoroemerald; for detailed tracing protocols, see online supplemental material).

Analysis of CST Damage

The hindlimb CST damage was quantified in targeted EAE animals as described previously (reference 13 and see online supplemental material for detailed protocols).
Analysis of CST Reorganization

We determined the sprouting of the hindlimb CST above and below the level of the EAE lesion on cross sections of the cervical (segments C3–C5, between 40 and 50 sections per animal were evaluated) and lumbar (segments L1–L3, between 20 and 25 sections per tract were evaluated) spinal cord at day 28 as follows. For each side of each section, we determined the number of CST collaterals (the number of fibers emerging from the CST tract and entering the gray matter) and the number of CST branches (the number of fibers crossing grid lines positioned in the gray matter [see Figs. 3 and 4]). To correct for variation in tracing efficiency, we normalized the number of CST collaterals and branches per section to the total number of labeled CST axons on each side (determined as the average of traced fibers on five cross sections between C3 and C5 or L1 and L3). As we detected a substantial contribution of collaterals derived from the minor ventral and lateral components of the CST in the lumbar spinal cord, we determined the number of labeled axons as well as the number of collaterals per lumbar section originating from these CST components and included them in the analysis. For the final analysis, we determined the following indices: the collateral index, as the average number of CST collaterals per section divided by the number of labeled CST fibers and multiplied by 1,000; the branch index, as the average number of CST branches per section divided by the number of labeled CST fibers and multiplied with 1,000; and the ratio of CST branches/collateral as the average number of CST branches per section divided by the average number of CST collaterals per section. We further reconstructed the cervical CST collaterals and branches on 10 consecutive sections derived from comparable levels of the cervical spinal cord in EAE (n = 10) and control animals (n = 4) using a camera lucida. In addition, hindlimb CST collaterals and PSNs were reconstructed on five consecutive sections of the cervical spinal cord in double-traced animals.

Transynaptic Tracing of Axonal Connectivity

To assess changes induced by the EAE lesion in the connectivity between the lumbar spinal cord and the cortex, we used a retrograde transsynaptic tracer, GFP-labeled pseudo-rabies virus Bartha (PRV) as described previously (reference 18 and, for a detailed protocol, see online supplemental material). The number of neurons with PRV immunoreactivity in lamina V was counted throughout the entire cortex and normalized to 100 sections per animal. For all animals, we mapped the localization of PRV+ neurons in lamina V of the cortex in relation to existing maps of the hindlimb and forelimb motor area (19). The following groups of animals were analyzed: animals with a newly transected CST at the

Figure 1. Histopathology of lesion development in the targeted EAE model. (a–c) Spinal cross sections of targeted EAE lesions stained with hematoxylin eosin at days 7 (a), 14 (b), and 28 (c) after lesions induction. (d) Quantification of the area of inflammation based on hematoxylin eosin–stained sections. (e–g) Spinal cross sections of targeted EAE lesions stained with Luxol fast blue at days 7 (e), 14 (f), and 28 (g) after lesion induction. (h) Quantification of the area of demyelination based in Luxol fast blue–stained sections. (i–k) Spinal cross sections of targeted EAE lesions stained by Bielschowsky silver impregnation at days 7 (i), 14 (j), and 28 (k) after lesions induction. (l) Quantification of axon density based on silver impregnated sections. (m–o) Spinal cross sections of targeted EAE lesions immunostained for APP (red, with nuclear counterstain in blue) at days 7 (m), 14 (n), and 28 (o) after lesion induction. (p) Quantification of the behavioral recovery over time in targeted EAE animals (mean ± SEM).
thoracic level (n = 8), animals with an unlesioned CST (n = 6), and animals 4 wk after a thoracic EAE lesion with (n = 8) or without (n = 8) an additional fresh thoracic transection of the CST.

Behavioral Testing

Three behavioral test paradigms were used to assess overall motor performance (standard EAE score; reference 13) as well as CST-related responses (hindlimb placing and proprioceptive aduction; references 18, 20). For a detailed description of the test paradigms, see online supplemental material.

Statistical Analysis

Statistical analysis was performed with Prism software (GraphPad). Differences in the anatomical data were analyzed using either an unpaired Student’s t test or one-way analysis of variance followed by Newman-Keuls Multiple Comparisons tests. Non-parametric behavioral data were analyzed over time with a Friedman test and behavioral scores were compared with a Wilcoxon matched paired test. *, P < 0.05 was considered significant. **, P < 0.01. ***, P < 0.001. Data are presented as mean ± SEM.

Online Supplemental Material

The online supplemental material contains detailed protocols of the staining and tracing techniques used in this paper. It contains comparison of axonal remodeling after inflammatory and traumatic spinal cord lesions. Figure S1 shows comparison of growth-associated gene expression in inflammatory and traumatic spinal cord lesions. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20040452/DC1.

Results

This work explores how axonal damage is compensated for in an animal model of MS. We approached this problem in a reductionist way, by studying the compensatory response of the motor system to a single, localized neuroinflammatory lesion. A targeted EAE lesion was induced in the dorsal column of the midthoracic spinal cord by minimal invasive injection of proinflammatory cytokines in rats immunized with subthreshold levels of MOG(13). Previously, we had established that these targeted lesions reflect the major pathological hallmarks of MS, including focal inflammatory demyelination and extensive axonal damage (13). We have now characterized the evolution of inflammation, demyelination, and axonal damage in these lesions over time (Fig. 1). After the cytokine injection, a large inflammatory infiltration developed within a few days (Fig. 1 a, D7). However, both the area of inflammation (Fig. 1 d) as well as the density of cell infiltration (day 7: 4229 ± 379; day 14: 5503 ± 848; and day 28: 5428 ± 406 cells/mm²) remained largely unchanged for the remaining experimental period (days 7–28). Parameters of structural damage showed a similar time course. Extensive demyelination occurred up to day 7, but the area of demyelination did not change significantly between days 7 and 28 (Fig. 1, e–h). We did not detect remyelination at any time point. The axon density dropped substantially over the first days of lesion development and again remained unchanged from day 7 up to day 28 (Fig. 1, i–l). In accordance with this finding, acute axonal damage, as assessed by immunohistochemistry for APP, was prominent at an early disease stage (Fig. 1 m, D7) and substantially lower at later stages (Fig. 1, n and o, D14, D28). However, despite the persistent structural damage, the animals showed a nearly complete behavioral recovery over the same time period (Fig. 1 p). These findings point to the existence of compensatory mechanisms, which promote behavioral recovery in the presence of persisting structural deficits. The targeted EAE model now allows us to analyze these compensatory mechanisms on multiple anatomical levels.

Growth-associated Proteins and Neurite Formation in Interneurons near Spinal EAE Lesions. As a first step to understanding how neurons respond to inflammatory axonal damage, we focused our analysis on neurons in the immediate vicinity of the lesion. We asked whether proteins that are part of a growth-associated expression pattern, such as c-Jun and GAP43 (21–23), could be detected in neurons near the lesion and whether their expression correlated with axon outgrowth. We immunostained for c-Jun at various stages after induction of a targeted EAE lesion in the midthoracic spinal cord. Very little c-Jun immunoreactivity was detected in untreated control animals (1.2 ± 0.4 c-Jun+ neurons/section) and in animals receiving a cytokine injection but no immunization (day 7: 1.4 ± 0.2; day 14: 2.4 ± 0.3 c-Jun+ neurons/section). In contrast, in targeted EAE animals, many neurons were immunoreactive for c-Jun with a typical nu-

![Figure 2](https://www.jem.org/cgi/content/full/jem.20040452/DC1)
clear staining pattern (c-Jun⁺; Fig. 2 a and Fig. S1, available at http://www.jem.org/cgi/content/full/jem.20040452/DC1). The number of c-Jun⁺ neurons was strongly increased starting on day 7 (17.5 ± 2.9 c-Jun⁺ neurons/section), peaked at day 14 (23.9 ± 4.5 c-Jun⁺ neurons/section), and declined after. However, persistent expression of c-Jun was still detected up to day 28 (5.1 ± 1 c-Jun⁺ neurons/section). This is in contrast with the comparably short-lasting induction of c-Jun observed after traumatic spinal cord lesion (see online supplemental material and Fig. S1).

Because the persistent expression of c-Jun is associated with axonal outgrowth, in particular if accompanied by other growth-associated proteins such as GAP43 (21, 23), we wondered if this inflammatory damage would induce adaptive changes in descending axons before they entered the lesion (hindlimb CST fibers [percentage of CST damage]). We assessed at day 28 after the induction of a midthoracic EAE lesion. As the main part of the CST in rodents runs in the dorsal column, our targeted lesion transects a variable fraction of all hindlimb CST axons (ranging from 2 to 100% of hindlimb CST fibers [percentage of CST damage]). We wondered if this inflammatory damage would induce adaptive changes in descending axons before they entered the lesion site (i.e., in the cervical spinal cord). In principle,
CST axons could give rise to more collaterals that enter the gray matter, and/or collaterals could show an increased formation of branches (for a definition of collaterals and branches, see Materials and Methods and Fig. 3 a).

In normal animals, very few collaterals and branches emerged from the hindlimb CST in the cervical spinal cord (mean collateral index: 0.38; mean branch index: 0.69; n = 7; Fig. 3 b). In contrast, a nearly 10-fold increased number of hindlimb CST collaterals and branches was detected in the cervical spinal cord of animals with a targeted EAE lesion (mean collateral index: 3.71, P < 0.001 vs. controls; mean branch index 4.1, P < 0.01 vs. controls; n = 29; Fig. 3, c, d, and e). However, the ratio of branches to collaterals was comparable between EAE and control animals (mean ratio of 1.14 CST branches/collateral in EAE animals vs. 1.8 branches/collaterals in control animals; Fig. 3 f). This de novo formation of cervical collaterals is similar to the induction of CST collaterals observed after traumatic spinal cord lesions (see online supplemental material).

To understand whether this reorganization depends on the severity of the lesion, we quantified the extent of CST collateral formation in spinal cords in response to EAE lesions of varying severity. We found that the more severe the lesion is, the more pronounced is the increase in collateral formation (Fig. 3 d).

CST Branches Form below the EAE Lesion. To determine the contribution of spared hindlimb CST fibers to axonal remodeling, we quantified the number of hindlimb collaterals and branches and their ratio in the lumbar spinal cord of animals at day 28 after the induction of a targeted EAE lesion (Fig. 4).

Compared with the CST of control animals (n = 8), the CST of animals with a targeted EAE lesion (n = 22) showed a threefold increase in the number of branches (mean branch index: 97.1 in EAE animals vs. mean branch index: 31.3 in control animals, P < 0.001; Fig. 4, b, c, and e). However, in contrast with the cervical spinal cord, the number of collaterals remained similar between both groups (mean collateral index: 23.4 in EAE animals vs. mean collateral index: 18.8 in control animals, P > 0.05; Fig. 4 d). Accordingly, the branch-to-collateral ratio is increased in EAE animals (mean ratio of 3.43 branches/collateral in EAE animals vs. mean ratio of 1.56 branches/collateral in control animals, P < 0.001; Fig. 4 f). Again, lumbar reorganization was dependent on the number of damaged CST fibers with terminal branching increasing with the amount of CST damage (Fig. 4, e).

Cervical CST Collaterals Contact PSNs That Relay to the Lumbar Spinal Cord. To investigate the functional role of cervical CST collaterals formed in response to thoracic EAE lesions, we reconstructed the projection pattern of these collaterals in animals with a targeted EAE lesion (n = 10) and control animals (n = 4; Fig. 5). The newly formed collaterals showed a very uniform projection pattern toward the intermediate layers of the spinal cord gray matter (Fig. 5, c and d), suggesting that they may contact a specific target neurons. The most attractive candidate targets in this location are long PSNs, which stand out for several reasons. First, whereas the cell bodies of PSNs are located in laminae V–VIII, their axons travel through a ventral pathway to the lumbar spinal cord (26, 27). This leaves them unaffected by a dorsolateral EAE lesion used in this work. Second, some PSN axons are known to directly contact lumbar motor neurons and, thus, offer an alternate detour circuit to these CST target neurons (26). Third, we have previously found that long PSNs are part of such a CST detour circuit in a dorsal hemisection model of spinal cord injury and that the number of contacts between PSNs and lumbar motor neurons increases in response to complete CST transection (18). To assess whether long PSNs are targeted by CST collaterals, we traced hindlimb CST fibers.
anterogradely and long PSNs retrogradely in animals with a targeted EAE lesion. At 28 d after lesion induction, newly formed CST collaterals form a dense network around the somata of PSNs, and individual CST branches form close appositions with PSNs (Fig. 5, e–n). This suggests that newly formed CST collaterals contact propriospinal interneurons as part of the remodeling process induced by an EAE lesion.

Reorganization of CST Connections Contributes to Functional Recovery. We further analyzed whether these newly formed collaterals can contribute to functional recovery by following the recovery of overall motor function and CST-specific motor responses using specific behavioral tests in animals with targeted EAE lesions (Fig. 6). To show directly that newly formed CST collaterals contribute to functional recovery, the CST was cut above the level where collaterals formed in targeted EAE animals; i.e., at the level of the pyramids in the medulla oblongata, where the CST can be selectively transected without destroying any other spinal motor tracts (“pyramidotomy”; reference 17). If done in animals that previously showed recovery, the drop in motor test performance after pyramidotomy establishes the contribution of the CST to recovery of function (Fig. 6 a). To differentiate recovery induced by the formation of new collaterals from functions dependent on unlesioned CST fibers, we performed the behavioral analysis in animals with both anatomically and functionally nearly complete CST lesions. (n = 8; mean CST damage 99.56%, a range of 98.56–99.97%; complete loss of CST-dependent responses at day 3 after lesion in seven out of eight animals).

At day 3, all targeted EAE animals showed high initial deficits in the overall motor performance (median EAE score: 2.5) as well as a complete loss of CST-related responses (Fig. 6, b and c). The CST-related responses as well as the overall motor function (at day 28: median EAE score: 1.5) slowly recovered over the course of the next 4 wk (Fig. 6, b and c). However, 2 d after pyramidotomy, the animals had lost a substantial part of the previously recovered CST function (Fig. 6, d and e). In contrast, the overall motor performance of these animals, which is insensitive to CST damage, did not change significantly in response to pyramidotomy (P > 0.05). This strongly suggests that recovered CST function is lost as a specific consequence of CST transection by pyramidotomy and not as an unspecific consequence of the surgical procedure. As virtually no CST fibers crossed the EAE lesion in these animals, these results indicate that recovery of CST function in these experiments is at least partially due to reorganization of CST connections between the midthoracic EAE lesion and the pyramidotomy in the medulla oblongata.

Intraspinal Rewiring Is Accompanied by Reorganization of Cortical Motor Representation. Finally, we investigated to what extent the changes of axonal connectivity in the spinal cord are complemented by changes in the cortical motor representation (Fig. 7). For this purpose, we retrogradely traced cortical neurons, which are directly or indirectly connected to motor pools of hindlimb muscles with a transsynaptic tracer, PRV. As we have established previously, PRV reaches the lumbar spinal cord in 2–3 d, the cervical spinal cord in 4–5 d, and the cortex in 6–7 d after PRV injection into hindlimb muscles (18). In normal animals, an average of 304 cortical projection neurons were labeled by PRV per brain; located almost exclusively in or directly adjacent to the hindlimb motor cortex (79% in hindlimb motor cortex; n = 6; Fig. 7, a and e). Control animals with a fresh mechanical transection of the CST showed virtually no labeled cortical projection neurons (average 31 PRV+ neurons per brain; n = 8; Fig. 7, d and e), indicating that the CST is the major descending connection between the lumbar spinal
Axonal Remodeling in EAE

However, targeted EAE animals traced 4 wk after lesion induction showed an average of 174 labeled cortical projection neurons per brain \((n = 8; \text{Fig. 7, b and e})\). This number is similar to the increased number of PRV\(^+\) neurons observed 3 wk after a traumatic spinal cord lesion (see online supplemental material). In EAE animals, these labeled neurons could have been traced both via remaining unlesioned CST fibers and via de novo formed cortico-spinal detour circuits. Two arguments support the conclusion that the labeled cortical projection neurons in EAE animals are at least partly traced through newly formed detour circuits. First, in targeted EAE animals, in which the remaining unlesioned CST fibers were mechanically transected just before PRV injection by a dorsal thoracic hemisection, an average of 105 PRV\(^+\) projection neurons were found per brain \((n = 8; \text{Fig. 6, e})\).

Rewiring of corticospinal connections contributes to functional recovery. (a) Scheme of the pyramidotomy (PTX) experiment. Transection of the CST above the cervical level removes new CST collaterals and permits assessment of their contribution to the recovery of CST function. (b and c) Functional recovery of targeted EAE animals with a complete inflammatory CST lesion from baseline (BL) to day 28 in behavioral test paradigms. CST-related responses such as hindlimb placing (b) and proprioceptive adduction (c) are nearly completely lost at day 3 but afterwards slowly recover up to day 28. (d and e) Comparison of behavioral tests before (corresponds to D28) and 2 d after PTX. Hindlimb placing (d) and proprioceptive adduction (e) lose a significant part of their previous recovery, suggesting the involvement of cervical reorganization in recovery.

Targeted EAE lesions induce a reorganization of the cortical motor representation. (a–d) Position of PRV\(^+\) neurons in hindlimb (blue) and forelimb (red) motor cortex. Neurons outside both areas are depicted in yellow. (a) In controls, numerous cortical projection neurons are almost exclusively found in the hindlimb motor area, which lose their connection to lumbar targets after fresh mechanical transection of the thoracic CST (d). In contrast, PRV\(^+\) neurons in EAE animals after remodeling (b) are located within and outside of the hindlimb motor area (particularly in the forelimb motor cortex). Additional mechanical transection of the thoracic CST does not disconnect all of these neurons (c), suggesting that many use a detour pathway to contact their lumbar targets. (e) Total numbers of PRV\(^+\) cortical neurons (CST-Tr.\(^+\)/EAE\(^+\), presence or absence of mechanical CST lesion; EAE\(^+\)/EAE\(^+\), presence or absence of an EAE lesion). (f) Percentage of PRV\(^+\) cortical neurons located in the forelimb motor area, which is significantly higher in EAE animals (CST Tr.\(^+\)/EAE\(^+\)) compared with control animals (CST Tr.\(^+\)/EAE\(^+\)). This indicates that in animals with a targeted EAE lesion, additional projection neurons have gained access to the lumbar spinal cord. (g) The number of PRV\(^+\) neurons in the hindlimb motor cortex, which retain access to the lumbar spinal cord after mechanical CST transection is significantly higher in EAE animals (CST-Tr.\(^+\)/EAE\(^+\)) than in freshly transected control animals (CST Tr.\(^+\)/EAE\(^+\)). This suggests that some of the original hindlimb CST neurons have regained access to their targets.

Figure 6.

Figure 7.
Fig. 7, c and e). This represents a significant increase both in the total number of PRV+ cortical neurons as well as in the number of PRV+ neurons in the original hindlimb motor area compared with freshly lesioned control animals (105 vs. 31; P < 0.01 for the total PRV+ neurons and 69 vs. 18; P < 0.01 for the PRV+ neurons in the hindlimb motor area; Fig. 7, e and g). Second, in contrast with unlesioned control animals, the PRV+ projection neurons in targeted EAE animals are only partially located in the hindlimb motor cortex (44% of PRV+ neurons in hindlimb motor cortex in targeted EAE animals vs. 79% in unlesioned control animals) and partially in other cortical areas, in particular the forelimb motor cortex (37% of PRV+ neurons in forelimb motor cortex in targeted EAE animals vs. 7% in unlesioned control animals; Fig. 7 f).

Discussion

In this work, we investigate how axons remodel in diseases like MS. We show that a single inflammatory lesion in the spinal cord is sufficient to induce changes in axonal connectivity at multiple anatomical levels in the motor system (Fig. 8).

This work also provides a unique opportunity to compare changes induced by neuroinflammation to those found after other forms of lesion (e.g., traumatic spinal cord injury). To investigate the effect of the neuroinflammatory environment on these remodeling processes we have studied axonal sprouting as well as the expression of two proteins, c-Jun and GAP43, that are paradigmatic for factors associated with axonal outgrowth (21–23, 28), in the vicinity of inflammatory EAE lesions as well as traumatic spinal cord lesions.

In the targeted EAE model, local interneurons sprout extensively close to the EAE lesion. Sprouting is paralleled by increased expression of c-Jun and GAP43 in these interneurons. This is in line with previous studies suggesting that successful neuronal outgrowth in the periphery and in the CNS requires activation of many genes, including c-Jun and GAP43 (21–23). Of these, c-Jun is expressed early and appears to indicate a cellular stress response. Current models suggest that c-Jun activation is double edged; it can lead to neuronal apoptosis, if transient and followed by the expression of genes such as JunD, or to axonal growth and regeneration, if sustained and accompanied by the expression of genes such as GAP43 (23). Our results suggest that a large number of spinal interneurons express c-Jun and, thus, mount an initial stress response. We found no evidence of neuronal apoptosis in EAE animals (unpublished data), but a subfraction of c-Jun+ interneurons also expressed GAP43 and started sprouting, primarily close to the lesion in the intermediate layers of the gray matter. This is in line with earlier studies that show that the ability to enter a regeneratory gene expression pattern is limited to subpopulations of neurons in the CNS (29). The extensive local growth response induced in the vicinity of an EAE lesion is in contrast with the comparably minor and short-lasting response induced by traumatic lesions (see online supplemental material). Several properties of the EAE lesion can contribute to this enhanced local growth response. First, it is well established that the infiltrating immune cells themselves can support neuronal outgrowth most likely due to the secretion of soluble growth promoting factors such as neurotrophins (30, 31). The finding that neuronal outgrowth is primarily induced in spinal neurons located in the gray matter immediately adjacent to the inflammatory infiltrate would support this assumption. Second, the extensive demyelination caused by the targeted EAE lesions is expected to lead to a reduced effect of myelin inhibition on neurite outgrowth in and around the lesion area (32, 33). Third, the temporal characteristics of the inflammatory damage are likely to trigger a more prolonged neuronal stress response, which in turn would favor the induction of a neuronal growth program. The distinct temporal profile of prolonged c-Jun and GAP43 expression, which is observed after inflammatory but not after traumatic damage, would support this notion.

On the level of the descending motor tract systems, some of the changes induced by an EAE lesion resemble...
those found after traumatic injury to these axons (i.e., the formation of new cervical CST collaterals (18). However, in contrast with commonly used spinal cord injury models, neuroinflammatory lesions lead only to a partial transection of the axonal tract system and, thus, allow for an additional level of reorganization based on the unlesioned fibers of the tract. We found that remodeling of CST after neuroinflammatory lesions follows two basic principles. First, below the lesion, untransected fibers spared by the EAE lesion increase the number of branches from their collaterals in the lumbar spinal cord. Second, above the lesion, in the cervical cord, CST fibers form new collaterals (that show a relatively normal degree of branching). The finding that the number of these collaterals increases with the extent of CST damage, and that new cervical collaterals are found in animals with complete CST transections, suggests that new collaterals are primarily formed by transected CST axons. These newly formed collaterals contact PSNs in the cervical cord. The formation of collaterals above the lesion provides new access to the cervical spinal cord, but this would be of no functional benefit unless a detour connection to the original target is established. The formation of such a detour connection in targeted EAE animals is suggested by the three following observations. First, cervical CST collaterals form new contacts with PSNs, which provide an intact connection to the lumbar spinal cord. Second, CST neurons can be transsynaptically traced from hindlimb muscles with PRV. Third, recovery is partially lost after pyramidotomy in animals with near complete thoracic CST lesion, showing that transected CST fiber input had previously reestablished a functional connection to the lumbar hindlimb motor pools.

The potential of these detour pathways is underlined by transsynaptic tracing experiments. We show that a substantial fraction of cortical neurons in hindlimb motor cortex have regained access to lumbar motor pools after only 4 wk. This timeframe coincides with the formation of CST collaterals and the recovery of CST function (18). Many neurons that can be traced from the hindlimb muscles are found in the original hindlimb motor cortex and must have been connected after the lesion. The mechanical transection of preserved CST fibers demonstrates that a substantial part of these hindlimb motor neurons were reconnected to their target area using remodeled intraspinal pathways. Furthermore, the distribution of PRV neurons in cortex after a targeted EAE lesion indicates that the additional recruitment of cortical neurons contributes to cortical reorganization. Cortical neurons located outside the primary hindlimb motor cortex (e.g., in the forelimb motor cortex) can be traced from the hindlimb after remodeling. It is plausible that anatomical changes in cortical projection observed by PRV tracing could account for some adaptive changes in cortical activation patterns as occur in response to various types of CNS lesions in animals and humans (34, 35). For example, it has been shown previously that hand movements activate a larger area in functional magnetic resonance images in MS patients, likely representing an attempt to functionally compensate the impaired hand function (11, 12). This provides a remarkable parallel to our work, where animals with impaired hindlimb function recruit new cortical projection neurons outside the hindlimb motor area, which in turn should result in a larger cortical area activated during hindlimb movements. Interestingly, the adaptive changes of the cortical motor representation in response to targeted EAE lesions are highly reminiscent of those induced by traumatic spinal cord lesions (18). This suggests that on the cortical level a default program of reorganization is implemented after injury, which depends on the extent of injury, but not on the type of injury. This is in contrast with the local reorganization on the spinal level, which is influenced not only by the extent of injury but also by its type.

From this work and other studies (18, 36), a common theme arises: structural reconnection and functional recovery can be achieved as long as sufficient “reserve capacity” for remodeling is present in the CNS, where true long-distance regeneration does not occur. Our work provides insight into the anatomical basis of this reserve capacity. Although spinal interneurons can sprout neurites, descending motor fibers can grow collaterals, and cortical projections can be reorganized, the success of all this critically depends on the presence of preserved detour pathways. Only as long as sufficient numbers of intact axonal pathways exist that allow damaged pathways to reestablish connections with reasonable probability and efficiency, recovery will be possible. The concept of a limited reserve capacity of axonal pathways in the CNS is particularly informative when explored in the context of MS (37). Early in the disease, deficits can often be readily compensated despite the fact that they are accompanied by substantial axonal damage. We speculate that during this stage the reserve capacity of the CNS is still largely intact. Thus, damage to axonal tract systems can be compensated for by both the remaining unlesioned tract fibers as well as by the formation of detour circuits. Although this leads to complete or partial recovery, it also increases the vulnerability of axonal systems as they depend on the patency of increasingly widespread detour pathways that are at high risk of being destroyed during subsequent inflammatory episodes. In parallel, each cycle of transection and detour formation decreases the remaining reserve of parallel pathways that can substitute for lost axonal connections. Therefore, compensation of clinical deficits is limited as this reserve becomes depleted. As the disease progresses, clinical deficits begin to remain, as new lesions become more and more likely to “hit” an axonal pathway that has exhausted its reserve, and at some point starts to progress steadily (as almost all axonal pathways have reached this point of no return). Once the reserve capacity is exhausted, new axonal damage is directly translated into clinical deficit giving rise to a progressive disease course.

This concept has far-reaching implications for the therapy of MS. First, our results clearly emphasize the importance of preventing axonal damage throughout the disease course of MS. This becomes even more pertinent in later stages of the disease: first, to preserve the reserve capacity
for remodeling and, second, because the CNS will become more vulnerable to subsequent axonal damage after it underwent several cycles of damage and remodeling. As long as a sufficient number of connections to the target area exist, endogenous processes of axonal rewiring can be implemented and compensate arising functional deficits. Aggressive attempts to limit axonal damage even late during the relapsing-remitting stage, may prevent or at least delay the transition of the disease to the progressive stage. Second, therapeutic strategies that foster endogenous reorganization (e.g., by including unlesioned tract systems in the rewiring process) may provide additional benefits by enlarging the endogenous reserve capacity. This could be achieved in several complementary ways. Physical therapy can support remodeling processes, as has been demonstrated in numerous studies in humans and animals (34, 38). Growth-promoting therapies might help reestablish axonal connections, either by promoting sprouting or by disinhibiting endogenous growth restriction (33, 39, 40).

In summary, our work underscores the compensatory potential of the adult CNS, and raises the hope that the presence of powerful rewiring machinery in the CNS can ultimately be therapeutically harnessed to benefit patients with MS.

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Material and Methods

Induction of a “Targeted” EAE Lesion
For the induction of a targeted EAE lesion, rats were injected s.c. at the base of the tail with a total volume of 100 μl of rMOG (5–25 μg diluted in saline) in IFA (Sigma-Aldrich). This immunization protocol alone rarely induces disseminated disease; the few animals in which dissemination occurred were excluded from analysis. 18–22 d later, 150 U of recombinant rat IFN-γ 150 (PeproTech), 250 ng of recombinant rat TNF-α (R&D Systems), and Monastral blue (to visualize the injection tract; Sigma-Aldrich) were injected into the spinal cord through a dorsal laminectomy at thoracic level 8 (T8) using a minimally invasive stereotactic technique (2 μl volume over a 5-min period; CST coordinates: 0.7-mm depth in midline) as described previously (1).

Histopathology
Animals were perfused transcardially with 4% paraformaldehyde after survival times of 7–28 d. Spinal cords were dissected and embedded in paraffin, and 3-μm thick sections were cut. To assess inflammation, sections were stained with hematoxylin–eosin, and the surface area of inflammatory infiltration (expressed as mm²) was measured on the level of the lesion epicenter. The density of inflammatory infiltrates within a lesion was measured in an area of 300–500 μm² and expressed as cells/mm². Demyelination and remyelination were detected by Luxol-fast blue (LFB) staining and by immunostaining for myelin basic protein (MBP, A0623; DakoCytomation) and cyclic nucleotide 3’-phosphodiesterase (CNP, SM91; Sternberger Monoclonals). The extent of demyelination was quantified by measuring the surface area of complete myelin loss (expressed as mm²) at the level of the lesion epicenter. Bielschowsky silver impregnation was used to determine the extent of axonal damage, whereas immunostaining for amyloid precursor protein (APP, clone 22C11; Chemicon) was used as a marker of acute axonal damage. Relative axonal density was measured at the level of the lesion epicenter using a stereological grid as described previously (2). The density of CST fibers in animals with targeted EAE (n = 12) was expressed as the percentage of axonal density in control animals (n = 4). Avidin–biotin technique with 3,3’-diaminobenzidine (for MBP, CNP) or alkaline phosphatase–anti-alkaline phosphatase (for APP) was used for the visualization of bound primary antibodies.

Immunohistochemistry

Tissue Preparation. After transcardial perfusion with 4% paraformaldehyde (PFA; Sigma-Aldrich), the brain and spinal cord of the animals were dissected and postfixed for 24 h in PFA. Tissue for cryostat sectioning was transferred to a phosphate-buffered solution containing 30% sucrose (Sigma-Aldrich) and incubated for several days before embedding. Tissue for vibratome sectioning was transferred to a Tris or phosphate-buffered solution before embedding.

Analysis of the Coexpression of c-Jun with Cell-type Markers and GAP43. Coexpression of c-Jun with cell-type markers and GAP43 was analyzed by double immunofluorescence in thoracic spinal cords of untreated control animals and animals receiving a cytokine injection but no immunization as well as EAE animals and animals with traumatic spinal cord lesions at various time points after disease induction as follows. Cross-sections (20–30 μm) of the thoracic spinal cord were cut on a cryostat, blocked, and incubated overnight at 4°C with the primary antibodies before detection with fluorochrome-coupled secondary antibodies (Jackson ImmunoResearch Laboratories). The following primary antibodies were used: polyclonal rabbit anti-c-Jun antibody (1:250, Santa Cruz Biotechnology, Inc.), mouse anti-GAP43 (1:1,000, clone 9-1E12; Chemicon), mouse anti-GFAP (1:200, clone G-A-5; Chemicon), mouse anti–MAP2 antibody (1:100, clone AP20; Chemicon), rabbit anti-neurofilament (1:100; Chemicon), and mouse ED1 (1:1,000, clone ED1; Serotec). For confocal microscopy, 100-μm-thick vibratome sections were stained with a similar protocol, except for longer incubation periods with the primary (2 d at room temperature) and secondary antibodies (24 h at room temperature) and the addition of Neurotrace Far red (1:200; Molecular Probes) to the solution containing the secondary antibodies. The sections were mounted in Vectashield (Vector Laboratories) and imaged with an Olympus FV 500 or Biorad MRC 1024 confocal microscope. All confocal stacks are shown as maximum intensity projections unless noted otherwise.

Tracing of Axonal Tract Systems

Tracing of Hindlimb CST. The hindlimb corticospinal tract was traced anterogradely and bilaterally as follows. The area of the hindlimb motor cortex was identified by measuring positions on the skull relative to bregma. Injections of a 10% solution of biotinylated dextran amine (BDA 10,000; Molecular Probes) in 0.01 M of phosphate buffer, pH 7.4, were performed with a thin glass capillary into the left and right motor cortices (coordinates: 2-mm posterior to bregma, 2-mm lateral to bregma, and 1.5-mm depth). After 14 d, the animals were perfused transcardially with 4% PFA and the spinal cords were dissected, embedded, and cut on a vibratome. Semi–free floating cross-sections (50 μm) of the spinal cord were developed using avidin-peroxidase (ABC Elite; Vector Laboratories) and DAB (Sigma-Aldrich) enhanced with 0.4% ammonium nickel sulfate (Sigma-Aldrich) as described previously (3). Sections were air dried, counterstained with cresyl violet, and coverslipped with Eukitt (Kindler). Micrographs were taken on an Axioplan with an Axiocam camera (Carl Zeiss MicroImaging). To capture sprouts running through multiple focal plains, several frames were taken and combined by extracting in focus information using Adobe Photoshop.

Double Tracing of Hindlimb CST and Propriospinal Neurons Contacts between collaterals of the hindlimb CST and propriospinal neurons were analyzed in the cervical spinal cord by anterograde tracing of the hindlimb CST and retrograde tracing of long propriospinal neurons (PSNs). The hindlimb CST was traced using BDA 10,000 as described before. At the same time, PSNs
were labeled retrogradely as previously established (4). In brief, 1 μl of fluoroemerald (10% in 0.01 M phosphate buffer; Molecular Probes) was pressure-injected with a Hamilton syringe into both sides of the spinal cord at thoracic level T12. 2 wk after the injections, the animals were transcardially perfused with 4% PFA, and the spinal cords were dissected. Sections of animals labeled with BDA (hindlimb CST) and fluoroemerald (PSNs) were cut on a vibratome, and BDA detection was performed as described before, resulting in a violet–black reaction product for the CST. Fluoroemerald was detected using an antifluorescein antibody (1: 500; Molecular Probes) and subsequently visualized using DAB without ammonium nickel sulfate resulting in a brown reaction product.

Analysis of Corticospinal Tract Damage
For anatomical experiments, hindlimb CST damage was quantified in animals with a BDA-traced CST at day 28. For each animal, the number of CST fibers was determined on cross-sections above (cervical spinal cord) and below (lumbar spinal cord) the level of the EAE lesion separately for the right and left tract. Five counted sections were averaged and the decrease in labeled CST fibers between the cervical and lumbar level was determined. This value was corrected for the decrease of labeled CST fibers between the respective levels in healthy control animals and expressed as a percentage of CST reduction for each CST.

For behavioral experiments, we quantified the CST damage using Bielschowsky silver impregnation on 5-μm-thick consecutive cross-sections of paraffin-embedded thoracic spinal cord spanning the EAE lesion and the corresponding cervical spinal cord. The total number of CST fibers was determined for the cervical spinal cord and the epicenter of the EAE lesion. To obtain the final CST damage the reduction of CST fibers between cervical spinal cord and EAE epicenter was calculated and corrected as described before.

Trans-synaptic Tracing of Axonal Connectivity
To assess changes induced by the EAE lesion in the connectivity between the lumbar spinal cord and the cortex, we used a retrograde trans-synaptic tracer, a GFP-labeled pseudo–rabies virus Bartha (PRV; references 4–6). Viral cDNA was transfected into rabbit kidney cells (RK13) and the virus expanded. 30 μl of virus solution (titer = 108 PFU/ml) was injected into the hindlimb muscles (gastrocnemius lateralis and tibialis cranialis) of animals, which were killed 6–7 d after virus injection. After transcardial perfusion with 4% PFA, the spinal cords and cortices were dissected. To analyze the distribution of the PRV tracer in the cortex, we cut consecutive sagittal sections (50 μm) of the entire cortex. The PRV tracer was visualized using a rabbit anti-PRV antibody (1:10,000; Affinity BioReagents, Inc.) and DAB/ammonium nickel sulfate detection.

Behavioral Testing

EAE Score. For evaluation of overall motor performance, the rats were examined with a standard EAE scoring scale (1, 7, 8) as follows: grade 0, no clinical disease; grade 0.5, partial tail weakness or slight loss of muscle tone; grade 1, tail weakness; grade 1.5, slightly clumsy gait; grade 2.0, hindlimb paresis; grade 2.5, marked hindlimb paresis (partial dragging); grade 3.0, hindlimb paralysis; grade 3.5, hindlimb paralysis and forelimb paresis; grade 4.0, complete paralysis (tetraplegy); and grade 5, moribund. Because of the thoracic localization of the targeted EAE lesions, animals usually scored in between grades 0 and 3 on this scale.

Hindlimb Placing Test. For the evaluation of CST-dependent motor responses, the rats were examined with the hindlimb placing test as described previously (4, 9, 10). In brief, each hindlimb was tested independently for placing in response to tactile/propioreceptive stimulation applied to the top of each hindfoot. Each test was repeated for each hindlimb up to six times or until three correct responses were obtained. The number of completed placing responses (maximum, 3) for each hindlimb was noted, and the results for both hindlimbs were added to obtain a hindlimb placing score (minimum, 0; maximum, 6).

Proprioceptive Adduction Test. For further evaluation of CST-dependent motor responses, the proprioceptive adduction of the rats hindlimbs was examined as described previously (9, 10). In brief, rats were placed along the edge of an elevated table, and a paw was gently pulled down and away from the table edge. Upon sudden release, the immediate retrieval of the paw indicates a positive adduction response. The number of positive adduction responses (maximum, 3) for each hindlimb was noted, and the results for both hindlimbs were added to obtain a proprioceptive adduction score (minimum 0, maximum 6).

Results

Reorganization of the Spinal Circuitry after Inflammatory or Traumatic Spinal Cord Lesions. A comparison of the reorganization of the local spinal circuitry was made by studying the neuronal expression of the growth-associated proteins c-Jun and GAP43 over time in parallel in animals with either inflammatory or traumatic lesions of the dorsal midthoracic spinal cord. As detailed in the Results section, animals with an inflammatory spinal cord lesion showed a profound and prolonged increase of c-Jun expression starting from day 7, reaching a maximum at day 14, and lasting up to day 28 (see Results and Fig. S1 e). In contrast, traumatic spinal cord lesions of comparable size led only to a short-lasting increase of c-Jun expression. Although elevated numbers of c-Jun+ neurons were present at day 7 (12.6 ± 1.8 c-Jun+ neurons/section), their number declined rapidly up to day 14 (4.9 ± 0.9 c-Jun+ neurons/section) and reached baseline levels at day 28 (1.1 ± 0.1 c-Jun+ neurons/section; Fig. S1 e). The expression of GAP43 showed similar differences between traumatic and inflammatory spinal cord lesions. Although targeted EAE lesions led to a robust induction of GAP43 expression that peaked at day 14 (see Results and Fig. S1 f), GAP43+ expression was only induced transiently in a small number of neurons after traumatic spinal cord injury (day 7: 1.6 ± 0.5; day 14: 0.7 ± 0.4; day 28: 0.1 ± 0.04 GAP43+ neurons/section; Fig. S1 f). In line with these findings, only few GAP43+ sprouts were detected in the vicinity of traumatic spinal cord lesions. These data indicate that, compared with traumatic lesions, inflammatory lesions lead to a more pronounced and long-lasting growth response of local neurons and, thereby, presumably to a more extensive plastic reorganization of the local spinal circuitry.
we analyzed the reorganization of corticospinal projections by comparing the formation of cervical CST collaterals induced by either inflammatory or traumatic lesions of the dorsal mid-thoracic spinal cord. for this purpose, we determined the number of collaterals/main tract in EAE animals using the same evaluation method used previously after spinal cord injury (4).

Control unlesioned animals showed only few CST collaterals in both EAE (0.39 ± 0.12 collaterals/main tract) and spinal cord injury studies (0.5 ± 0.13 collaterals/main tract; reference 4). the number of cervical CST collaterals was significantly increased in response to thoracic EAE lesion (2.55 ± 0.35 collaterals/main tract). the extent of this increase was in a comparable range to the one observed after a traumatic spinal cord lesion (2.4 ± 0.61 collaterals/main tract at 3 wk after injury; reference 4). if the extent of CST damage in the EAE animals was taken into consideration, those with a severe CST damage (61–100%) had even a slightly higher number of CST collaterals (3.52 ± 0.46 collaterals/main tract) than the animals with a traumatic and, thus, complete CST lesion. alternately, animals with mild (0–30%) or medium (31–60%) CST damage displayed a comparably lower number of collaterals (0.78 ± 0.08 and 1.72 ± 0.19 collaterals/main tract for animals with mild and medium CST damage). overall, these data indicate that the CST reorganization above the level of the lesion evolves similarly in animals with either inflammatory or traumatic spinal cord lesions. this conclusion is further supported by the analysis of the projection pattern of the CST collaterals (see Results and Fig. 5), which indicates that cervical propriospinal interneurons are used as relay station after inflammatory lesions just as described after traumatic spinal cord lesions (4). however, in contrast with the anatomically complete traumatic lesions of...
the CST, inflammatory lesions usually lead only to a partial transection of the CST. In targeted EAE animals, this allows for an additional reorganization of CST connections achieved by branching of unlesioned CST fibers below the level of the lesion (see Results and Fig. 4).

Reorganization of Cortical Maps after Inflammatory or Traumatic Spinal Cord Lesions. We compared the reorganization of cortical maps between animals with either inflammatory or traumatic (4) lesions of the spinal cord by analyzing the number and distribution of cortical projection neurons labeled trans-synaptically from hindlimb muscles by PRV. Unlesioned animals were used as positive controls in both studies and displayed a high number of retrogradely labeled PRV+ cortical neurons due to the intact corticospinal connection (304 ± 88 PRV+ neurons per brain in the EAE study; 420 ± 112 PRV+ neurons per brain in the spinal cord injury study; reference 4). In contrast, very few PRV+ cortical neurons were detected in freshly lesioned animals, which served as negative controls in both studies (31 ± 7 PRV+ neurons per brain in the EAE study; 33 ± 12 PRV+ neurons per brain in the spinal cord injury study; reference 4). The number of PRV+ neurons was increased to a similar extend in both animals 3 wk after a traumatic spinal cord lesion (150 ± 21 PRV+ neurons per brain; reference 4) and 4 wk after an inflammatory spinal cord lesion (174 ± 39 PRV+ neurons per brain). Consistent with these findings, the distribution of PRV+ neurons in the cortex was altered after both inflammatory and traumatic lesions. Although the vast majority of PRV+ cortical neurons are located in the hindlimb motor area in unlesioned animals, a substantial fraction of PRV+ neurons are located outside the hindlimb motor cortex, in particular in the forelimb motor cortex, in animals after inflammatory (37 ± 7% located in the forelimb motor area) and traumatic (16 ± 6% located in the forelimb motor area; reference 4) lesions of the spinal cord. Together, these data indicate that similar adaptive changes in the cortical motor representation follow injuries of the spinal cord irrespective of the pathomechanism of injury.

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