Chondroitinase improves anatomical and functional outcomes after primate spinal cord injury

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Inhibitory extracellular matrices form around mature neurons as perineuronal nets containing chondroitin sulfate proteoglycans that limit axonal sprouting after CNS injury. The enzyme chondroitinase (Chase) degrades inhibitory chondroitin sulfate proteoglycans and improves axonal sprouting and functional recovery after spinal cord injury in rodents. We evaluated the effects of Chase in rhesus monkeys that had undergone C7 spinal cord hemisection. Four weeks after hemisection, we administered multiple intraparenchymal Chase injections below the lesion, targeting spinal cord circuits that control hand function. Hand function improved significantly in Chase-treated monkeys relative to vehicle-injected controls. Moreover, Chase significantly increased corticospinal axon growth and the number of synapses formed by corticospinal terminals in gray matter caudal to the lesion. No detrimental effects were detected. This approach appears to merit clinical translation in spinal cord injury.

A number of mechanisms limit the regenerative capacity of the injured adult mammalian CNS, including insufficient trophic support and the presence of inhibitors to axon growth associated with both myelin and the extracellular matrix1,2. Among the extracellular matrix molecules that inhibit axon growth, a major contribution is made by chondroitin sulfate proteoglycans (CSPGs)3,4. The predominant CSPG molecules present in the CNS include neural/glial antigen 2, neurocan and aggrecan3,4. The side chains are the primary determinants of axon inhibition6. As the period of robust developmental plasticity of the nervous system closes, CSPGs form around neurons and synapses in ‘perineuronal nets’ that are postulated to influence the function of the CNS by controlling plasticity and sprouting4. In addition, CSPGs are newly synthesized at sites of CNS injury and directly block axon growth5,6,10–12.

The natural bacterial enzyme chondroitinase (Chase) ABC can degrade the inhibitory carbohydrate side chains on CSPGs4,13,14. Indeed, Chase administration to rats after spinal cord injury (SCI) enhances growth of both corticospinal and sensory axons, and improves functional outcomes4,14–16. These growth-promoting effects of Chase result from the removal of perineuronal nets, which increases sprouting from spared axons below the injury site and formation of new synaptic connections7,18. Alternatively, when a cellular or peripheral nerve bridge is placed into a spinal cord lesion site, Chase can promote axonal regeneration of cut axons into the bridge18,19. Beneficial effects of Chase administration have been reported in rodent models of SCI4,14,15,16,18,19, nigostraiatal injury13 and stroke21–23, and cats with SCI24,25.

New therapies to promote CNS sprouting and regeneration are needed to improve outcomes after human neurotrauma and stroke. Given the replication of Chase efficacy across multiple experimental models by independent laboratories, together with the identification of compelling candidate mechanisms underlying its beneficial effects, we tested Chase therapy in a non-human primate model of SCI as a potential prelude to human clinical trials. Primates, both human and non-human, exhibit potentially important differences from rodents in terms of size, anatomy, systems function and immunology that make predictions of human safety and benefit based solely on rodent studies uncertain, a concern highlighted by numerous failed trials in stroke, SCI and other disorders26–29. First, we employed a porcine model to test methods for Chase administration in the spinal cord of large animals, and found that intrathecal infusions failed to degrade CSPGs in spinal cord gray matter. Accordingly, we moved to intraparenchymal spinal cord injections of Chase; these effectively degraded CSPGs in our rhesus monkey model of C7 spinal cord lateral hemisection30. This experiment hypothesized that degradation of perineuronal nets surrounding neurons in spinal cord gray matter below the lesion would enhance sprouting of spared host axonal systems, including the corticospinal tract (CST), the most important motor system for voluntary movement in primates11,12. Previously, we reported spontaneous sprouting of spared corticospinal axons in monkeys after C7 lesions30, supporting their potential relevance as a target for enhanced growth.
after human SCI, where the majority of lesions spare a superficial rim of white matter including corticospinal axons. Thus, using intraparenchymal injections, we tested Chase administration in rhesus monkeys with hemissections, comparing experimental and control lesion animals.

Results

Route of Chase administration: successful delivery by intraparenchymal but not intrathecal infusion. In a preliminary study, we determined whether intrathecal injections of Chase, a frequent and effective route of administration in rat studies, would effectively diffuse into the spinal cord of a larger animal. We used pigs for this study because their spinal cords measure roughly 8–11 mm in diameter (compared to the rat spinal cord width (3.5 mm)) and the human spinal cord width (12.5 mm)). Seven adult American Yorkshire-Landrace-Duroc pigs received intermittent (every other day for 2 weeks) intrathecal injections of 2 ml of saline \( (n=1) \), 5 U Chase enzyme ml\(^{-1}\) (5 U ml\(^{-1}\), 10 U per dose; \( n=3 \) animals; for Chase, 1 U is the amount that catalyzes the conversion of 1 \( \mu \)mol of chondroitin sulfate A or C per minute at 37°C and pH 8.0), or 25 U Chase enzyme ml\(^{-1}\) (50 U per dose; \( n=3 \) animals) (Chase provided by Acorda Therapeutics). Injections were made at 0.2 ml min\(^{-1}\) via an indwelling catheter into the intrathecal space between vertebrae T11–T13. Pigs were killed 2 d after the last dose of intrathecal Chase. With this infusion method, 2B6 immunohistochemistry revealed degradation of glycosaminoglycan side chains in both low- and high-dose groups in a 1 mm rim of the superficial circumferential thoracic spinal cord white matter (Supplementary Fig. 1a,b). However, perineuronal nets in the spinal cord gray matter were not attenuated, as revealed by Wisteria floribunda agglutinin (WFA) lectin histochemistry (Supplementary Fig. 1c,d). Because a prime target of Chase therapy is removal of perineuronal inhibitory CSPGs in gray matter to enhance plasticity and new synapse formation, we concluded that intrathecal infusions of the current formulation of Chase in the larger spinal cord were not an effective delivery method to enable hypothesis testing in the primate model; thus, in the absence of a more parenchymal-penetrant formulation or method for Chase administration, we moved to intraparenchymal injections in monkeys.

In a preliminary study to test the delivery efficacy of intraparenchymal Chase administration in monkeys, two adult rhesus male monkeys underwent C7 spinal cord hemisection lesions, followed 4 weeks later by intraparenchymal injections of Chase. Four weeks post-lesion is a time point when spontaneous plasticity in primate is occurring. Monkeys received injections of 20 U ml\(^{-1}\) Chase starting at spinal cord level C7 (1 mm caudal to the lesion); 5 \( \mu \)l of Chase were injected at each of 10 sites, spaced 1.5 mm apart in the rostrocaudal axis, on the right side of the spinal cord from C7 to T1. Chase was infused at two dorsoventral depths per injection site, the first targeting the intermediate gray (2.5 mm depth) and the second targeting the ventral gray (3.5 mm depth); 2.5 \( \mu \)l were injected at each depth (infusion rate 0.5 \( \mu \)l min\(^{-1}\) for a total volume of 5 \( \mu \)l per site (Fig. 1). As noted earlier, the primary objective of this injection design is to degrade perineuronal CSPG 'nets' that surround adult neurons in host gray matter below the lesion site, to enhance sprouting from spared host axons that occurs spontaneously after SCI. Animals were transcardially perfused 2 weeks later to assess CSPG degradation by WFA histochemistry. CSPGs were completely degraded in the spinal cord gray matter over a distance of at least 2 mm from each injection site without evident damage to the spinal cord or neuronal loss (Fig. 2 and Supplementary Figs. 2–4). Therefore, we used the same injection design to deliver Chase to the animals in the therapeutic efficacy study. CSPGs were also degraded at the caudal aspect of the C7 spinal cord lesion site (Supplementary Fig. 5).

Chase therapeutic efficacy study in non-human primates

A total of 12 adult male rhesus monkeys underwent right-sided C7 complete spinal cord hemisection lesions, which result in persistent deficits in right hand dexterity but retention of bowel/bladder function and locomotion. While this lesion results in extensive impairment in right hand function immediately after the lesion, there is consistent and predictable, but incomplete recovery from 4 to 8 weeks post-lesion, with little further improvement thereafter. Hypothesizing that the period of recovery from 4 to 8 weeks is mediated by spontaneous anatomical plasticity, we injected Chase 4 weeks post-injury to target the enhancement of endogenous plasticity. This delay was also chosen because it is a clinically practicable time to intervene after SCI, when most medical complications in humans have stabilized and patients are better candidates to undergo surgical intervention. Moreover, the extent of functional deficit and probability of further spontaneous clinical recovery can be assessed more reliably in humans 4 weeks post-injury than within days of injury, which are considerations that are helpful in designing a clinical trial.

The primate C7 lesion model was designed to assess potential therapies for improving hand function, since neural circuitry for hand control is located in the C7–T1 spinal levels ranging from 1 to 15 mm below the lesion. These segments received intraparenchymal injections of Chase (20 U ml\(^{-1}\)) 4 weeks after lesions were placed, as indicated earlier (Fig. 1). Four days later, monkeys underwent daily half-hour exposure to a large testing enclosure enriched with numerous objects, perches and food rewards to encourage use of both fore and hind limbs. Monkeys engage in naturalistic and reward-driven behaviors in this environment. Once per week, several functions in the enclosure were evaluated and scored on an ordinal scale by an observer blinded to treatment condition. Some of these functional measures are sensitive to forelimb performance (Supplementary Fig. 6), others to hind limb performance; yet other scores represent composites of overall function (Fig. 3). Separately, monkeys underwent daily exposure to a cage-based Brinkman board task with graded levels of difficulty, requiring retrieval of small food items from baited wells using only the affected right arm (Fig. 3). There were five levels of difficulty on this task, and monkeys were scored weekly on each level. A total of 12 monkeys underwent C7 lesions: 6 received injections...
Chase administration improves functional outcomes

We performed a non-linear principal components analysis (PCA; see Methods) on the ensemble of all behavioral outcome data from the exercise enclosure and the Brinkman board (Fig. 3; n = 6 Chase animals and n = 5 controls). Behavioral measures loaded highly on the first principal component (PC1), which accounted for 68% of the variance in recovery (Supplementary Fig. 8). The resulting weights for each measure were then extracted to create principal component scores for each animal at each time point. PCI scores were then used to test for group differences in performance over time. Overall, PCI showed a significant difference between lesioned controls and Chase-treated animals over time, favoring the Chase-treated group (condition × time, \( F_{(9, 23.87)} = 5.15, P = 0.001 \), Akaike information criterion (AIC) = 94.21, linear mixed model (LMM); Fig. 3a). Lesioned control animals in this study followed a previously reported pattern (stated earlier) of marked impairment in function post-injury, relative to their pre-injury baseline, for the first 4 weeks after C7 hemisection (Fig. 3). This was followed by spontaneous recovery of function (reflected by the PCI scores) over weeks 5–8 (Fig. 3a). The function of lesioned controls did not significantly improve further from weeks 5–8 to weeks 17–20 (main effect of time, \( F_{(3, 12)} = 1.87, P = 0.189, \eta^2_p = 0.319 \), repeated measures analysis of variance (ANOVA); Fig. 3a and Supplementary Video 1). Animals treated with Chase also exhibited marked impairment in hand function over the first 4 weeks post-injury, followed by early spontaneous improvement by weeks 5–8. However, in contrast to the lesioned control group, animals in the Chase treatment group exhibited continued improvement from weeks 5 to 8 to weeks 17–20 (main effect of time, \( F_{(3, 12)} = 7.53, P = 0.003, \eta^2_p = 0.601 \), repeated measures ANOVA; Fig. 3a and Supplementary Video 2). Analysis of performance on individual tasks showed divergence that favored Chase-treated animals on measures sensitive to right hand use (Fig. 3d–i) but not locomotion (Fig. 3j). This observation is consistent with the delivery of Chase to spinal cord segments influencing hand function. In terms of absolute performance levels, monkeys that received Chase exhibited an overall final food object retrieval success rate of 47 ± 12% across all difficulties of the Brinkman board in the final testing period, compared to nearly 100% pre-injury performance, and an inability to retrieve objects in the first 4 weeks after injury. In comparison, lesioned control monkeys recovered to 31 ± 14% final food object retrieval on the Brinkman board, compared to 5 ± 3% performance in the first 4 weeks after injury. This is a 51% difference between groups in the extent of recovery, favoring Chase-treated animals. The difference in absolute performance between groups is significant (\( P = 0.014, \text{LMM, condition} \times \text{time}, F_{(0.7, 12.48)} = 3.26, \text{AIC} = 1461.5 \)).

PCA also generated a second and third principal component that accounted for 10.5 and 5.1% variance, respectively (Supplementary Fig. 8). However, loading patterns for these components did not

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**Fig. 2 | Intraparenchymal Chase injections degrade CSPGs.** a–c. Comparison of Chase-treated (right) and untreated (left) sides of the spinal cord in a rhesus monkey 2 weeks after Chase administration (1 month after lesion). WFA labeling reveals CSPG degradation at the treated side. Boxed regions are shown at higher magnification in b and c. The zone of perineuronal net CSPG degradation is indicated by the dashed outline. d. Tissue section from C7, above the lesion and treated region, has intact CSPGs. e. Series of tissue sections at 2 mm intervals moving caudally from the lesion site reveals effective CSPG degradation from C7 through to T1. The labeling experiment included two animals. Scale bars: a, 500 μm; d,e, 1 mm.

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**Fig. 3 | Functional outcomes.** a. Combining all behavioral data into a PCA demonstrates a significant interaction of Chase treatment with time on PC1 (LMM, \( P = 0.001 \)). The plot shows the change in PC1 scores over time. The shading represents the s.e.m. PC1 explains 68% of the variance in the behavioral data (see Supplementary Fig. 5 for loadings). b,c. Example of food reward retrieval on one of the tasks comprising behavioral testing (Brinkman 5). The arrow indicates the pincer motion used to retrieve the food reward from the well. d–l. Recovery curves for the individual tasks that comprise PC1. These individual task plots show that, although single functional outcome metrics may not be sensitive on their own, combining all metrics in a PCA reveals a robust effect of Chase treatment at the multivariate level. The object manipulation score (d) reflects use of the impaired forelimb to manipulate a large piece of fruit and retrieve food items from inside a Kong brand toy. Brinkman 1 through to Brinkman 5 (e–i) indicates progressively more difficult versions of food retrieval (see Methods; raw Brinkman scores are reported in the text). j. The locomotion score reflects use of the ipsilesional limbs for moving around the exercise enclosure. k. The climbing score reflects use of ipsilesional limbs for vertical climbing in the exercise enclosure. l. The general score is a composite of multiple measures that describe forelimb and hind limb function, as described in Nout et al.42 and Salegio et al.44. Generally, the Chase-treated group exhibited superior recovery on tasks reflecting hand use (the spinal cord region targeted with Chase injections), and no difference in measures that included hind limb function (that is, those not targeted by Chase treatment). n = 6 Chase-treated animals and n = 5 controls. The large data points show the group means, the small data points are the individual animals, and the error bars represent the s.e.m.
indicate clearly definable eigenmodules; thus, scores on these components were not tested.

**Chase administration improves anatomical outcomes**

We hypothesized that, by degrading perineuronal nets in the gray matter surrounding neurons and synapses caudal to the C7 lesion site, Chase administration would increase axonal sprouting and synaptogenesis from spared axonal systems. Previously, we reported that, in rhesus monkeys, spared components of the corticospinal system that decussate across the spinal cord midline undergo spontaneous axonal sprouting after C7 hemisection lesions. In Chase-treated animals, we found greater total length (compared to controls) of corticospinal axons in gray matter caudal to the injury at C8, which is indicative of sprouting of spared corticospinal axons.
that decussate across the spinal cord midline\(^{30,39}\) \((n=6\) Chase-treated animals and \(n=5\) controls; \(F_{1, 30.02} = 4.81, P = 0.036, AIC = 693.89, LMM; \) Fig. 4c\). The same Chase-associated increase in axon density (compared to controls) was observed in CST axons originating in the right motor cortex \((F_{1, 41.15} = 13.15, P = 0.001, AIC = 1239.11, LMM; \) Supplementary Fig. 9). All measures were made in a blinded fashion; analysis of corticospinal axons arising from the left hemisphere was done in one laboratory (M.H.T.), while analysis of axons arising from the right hemisphere was done independently in a second laboratory (J.W.F.).

In addition, Chase treatment was associated with a significant increase, compared to lesioned controls, in the number of corticospinal synapses in gray matter caudal to the injury \((n=6\) Chase-treated animals and \(n=5\) controls; \(F_{1, 38.12} = 12.06, P = 0.001, AIC = 119.18, LMM; \) Fig. 4). CST synaptic connectivity was assessed by quantifying the number of CST terminal boutons that colocalized with synaptophycin immunoreactivity, divided by the sampled volume of gray matter (Fig. 4e).

We also assessed whether serotonergic axons respond to Chase treatment. In a previous study of lesioned animals lacking experimental treatment, serotonergic axons, in contrast to corticospinal axons, did not exhibit detectable sprouting after C7 hemisection lesions\(^{40}\). In the present study, we observed no difference between Chase-treated animals and controls in total serotonergic axon length in motor neuron pools caudal to SCI \((n=6\) Chase-treated animals and \(n=5\) controls; Wald \(\chi^2 = 0.009\), quasi-likelihood under the independence model criterion (QIC) = 4.86, \(P = 0.924\), generalized estimating equation; Supplementary Fig. 10). All anatomical measures were made by observers blinded to treatment group.

Safety
Anatomical analysis of injection sites with Nissl staining revealed no detectable toxicity after intraparenchymal spinal cord injections (Supplementary Fig. 2). We also quantified motor neurons in a series of sections straddling an injection site in each monkey, and found no neuron loss in Chase-injected versus lesioned control monkeys (Supplementary Fig. 3). In addition, we quantified IBA1 immunolabeling in gray matter adjacent to the injection sites to assess microglial responses, and found no difference between Chase-injected, saline-injected and lesion control groups (ANOVA, \(F_{2, 3.79} = 0.11, P = 0.89\); Supplementary Fig. 4). Moreover, cellular-mediated inflammation in gray matter, assessed by labeling for CD8, CD3 and CD45, was extremely mild 2 weeks after Chase injection (Supplementary Fig. 3; CD8 labeling shown). Labeling for CD8, CD3 and CD45 was no longer detectable 4.5 months after injections of either Chase or saline (Supplementary Fig. 3). Systemically, Chase-treated and lesioned control monkeys exhibited no notable differences in weight, activity or post-lesion complications. Although formal pain tests were not employed, monkeys exhibited no behaviors that suggested pain (for example, reduced activity, enhanced startle).

Discussion
Chase treatment showed efficacy on both anatomical and functional outcome measures. On functional assays, effects were only suggested on forelimb measures, which were anatomically targeted by Chase injections into cervical segments mediating hand control, whereas effects were not evident on hind limb measures. Anatomically, Chase treatment significantly increased the length of corticospinal axons in cervical spinal cord segments below the lesion, representing sprouting of axons spared by the lesion, and also significantly increased the number of corticospinal terminals colocalizing with a synaptic marker, indicating a likely increase in the number of corticospinal synapses.

In contrast to studies in rats and cats\(^{41}\), the effects of Chase on serotonergic axons were not detected. Interestingly, serotonergic axons do not detectably sprout following C7 hemisection lesions in rhesus monkeys\(^ {30,39}\). A number of mechanisms could potentially account for this observation, including the possibility that serotonergic axons are highly branched and sustaining collaterals may minimize injury responses in the hemisection model\(^ {40}\).

The overall functional success of food object retrieval on the Brinkman board task, a test of finger use, was 47\(\pm\)12% among Chase-injected animals compared to 31\(\pm\)14% in lesioned controls. Chase-treated animals also showed better recovery than lesioned controls on an object manipulation scale in the testing enclosure (21.25 point improvement versus 12.5 point improvement; Fig. 3d). On anatomical measures, CST axon terminal innervation was 50% denser after Chase treatment (Fig. 4c) and the number of putative synapses by corticospinal axons increased twofold compared to lesioned controls (Fig. 4e). The reticulospinal system was not traced or quantified in this study. It is possible that, like the CST, reticulospinal axons might sprout in response to Chase treatment and mediate functional recovery.

We developed the non-human primate model of SCI to enable testing of potential translational therapies, invasive therapies in particular, before human translation. The rhesus monkey represents the most proximate animal model of the human nervous system, and the adult rhesus monkey can exhibit behavioral changes comparable to SCI in humans. Therefore, the developed model should be a useful bridge between preclinical and clinical SCI treatments.
system that is experimentally testable. We have found that rhesus macaques exhibit variation in motivation, task engagement and response to injury that constitutes a source of variability exceeding that encountered in rodent models. This variability may result from the increased complexity of the rhesus brain compared to the rodent brain, and/or the considerable genetic differences in primates that exceed variation found even in outbred rat strains. Consequently, it is probable that this non-human primate model, while less variable than human injuries, incorporates some features of the variability typically encountered in the human clinical setting. Thus, the ability to detect significant differences on several measures following Chase administration, despite intersubject variability in this model, provides support for the initiation of human clinical trials. While the sample size in this study is small, the costs and duration of this work constitute a relative barrier to studying larger numbers of animals. Our use of data-driven multivariate statistical analysis before hypothesis testing allowed us to efficiently test recovery in ensemble across multiple tasks, thereby maximizing information gain with small n, while remaining sensitive to therapeutic effects. Notably, multivariate statistics are not typically employed in clinical trials, which instead generally rely on one or two predefined outcome measures to establish clinical efficacy. Interestingly, had we used classical univariate statistics in the present study, the functional benefit of Chase therapy would likely not have been appreciated. This raises the possibility that a treatment of potential functional benefit might be unappreciated in a clinical trial, despite the presence of a clear biological benefit at the level of axonal growth and synaptogenesis. This finding highlights the great importance of continued consideration and development of multivariate outcome measures in clinical trials, a point that is receiving increasing attention.

There are important limitations to this non-human primate model of SCI. First, the injury itself is a hemisection, rather than a contusion, which is generally considered more representative of human injuries. In fact, we have developed a contusive model of SCI in a non-human primate, which could be employed in future preclinical studies. Second, the C7 level of the hemisection SCI is optimally placed for recovery of hand function. New studies are required to assess the potential benefit to humans with higher cervical injuries or with thoracic injuries, although preceding rat SCI studies have demonstrated the functional benefits of Chase after C4 (refs.14,15) and thoracic SCI. In addition to presenting evidence of efficacy, this study also preliminarily demonstrates that multiple parenchymal injections of Chase appear to be well tolerated. The outcome of the present study, taken together with the beneficial effects observed by many independent research groups in various rodent models of SCI, support the concept of translation of Chase treatment to human clinical trials.

The large-animal porcine model revealed that intrathecal infusions of the native Chase enzyme at even relatively high concentrations did not reach gray matter target regions. Thus, to advance these proof-of-concept studies into the clinically relevant primate model of SCI, we turned to more invasive intraparenchymal injections of Chase. The injections appeared to be well tolerated anatomically based on inflammatory markers and cell counts. Chemical modifications to Chase may enhance its penetrant properties after intrathecal administration, a possibility that merits further study for potential future clinical translation.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41593-019-0424-1.

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References
1. Schwab, M. E. Nogo and axon regeneration. Curr. Opin. Neurobiol. 14, 118–124 (2004).
2. Fitch, M. T. & Silver, J. CNS injury, glial scars, and inflammation: inhibitory extracellular matrices and regeneration failure. Exp. Neurol. 209, 294–301 (2008).
3. Kwon, J. C. F., Afshari, F., García-Alias, G. & Fawcett, J. W. Proteoglycans in the central nervous system: plasticity, regeneration and their stimulation with chondroitinase ABC. Restor. Neurol. Neurosci. 26, 131–145 (2008).
4. Tran, A. P., Threen, M. & Silver, J. The biology of regeneration failure and success after spinal cord injury. Physiol. Rev. 98, 881–917 (2018).
5. Dou, C. L. & Levine, J. M. Inhibition of neurite growth by the NG2 chondroitin sulfate proteoglycan. J. Neurosci. 14, 7616–7628 (1994).
6. Levine, J. M. & Nishiyama, A. The NG2 chondroitin sulfate proteoglycan: a multifunctional proteoglycan associated with immature cells. Perspect. Dev. Neurobiol. 3, 245–259 (1994).
7. Jones, L. L., Yamaguchi, Y., Stallcup, W. B. & Tuszynski, M. H. NG2 Is a major chondroitin sulfate proteoglycan produced after spinal cord injury and is expressed by macrophages and oligodendrocyte progenitors. J. Neurosci. 22, 2792–2803 (2002).
8. Grime, B. & Silver, J. A novel DNA enzyme reduces glycosaminoglycan chains in the glial scar and allows microtransplanted dorsal root ganglia axons to regenerate beyond lesions in the spinal cord. J. Neurosci. 24, 1393–1397 (2004).
9. Wang, D. & Fawcett, J. The perineuronal net and the control of CNS plasticity. Cell Tissue Res. 349, 147–160 (2012).
10. Altiyan, H. I., Horn, K. P., Hu, D., Dick, T. E. & Silver, J. Functional regeneration of respiratory pathways after spinal cord injury. Nature 475, 196–200 (2011).
11. Filous, A. R. et al. Entrapment via synaptic-like connections between NG2 proteoglycan+ cells and dystrophic axons in the lesion plays a role in regeneration failure after spinal cord injury. J. Neurosci. 34, 16369–16384 (2014).
12. Lang, B. T. et al. Modulation of the proteoglycan receptor PTPr promotes recovery after spinal cord injury. Nature 518, 404–408 (2015).
13. Moon, L. D., Asher, R. A., Rhodes, K. E. & Fawcett, J. W. Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC. Nat. Neurosci. 4, 465–466 (2001).
14. Bradbury, E. J. et al. Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416, 636–640 (2002).
15. García-Alias, G., Barkhuysen, S., Buckle, M. & Fawcett, J. W. Chondroitinase ABC treatment opens a window of opportunity for task-specific rehabilitation. Nat. Neurosci. 12, 1143–1151 (2009).
16. Caggiano, A. O., Zimber, M. P., Ganguly, A., Blight, A. R. & Gruskin, E. A. Chondroitinase ABCI improves locomotion and bladder function following contusion injury of the rat spinal cord. J. Neurotrauma 22, 226–239 (2005).
17. Galtrey, C. M., Asher, R. A., Nothias, F. & Fawcett, J. W. Promoting plasticity in the spinal cord with chondroitinase improves functional recovery after peripheral nerve repair. Brain 130, 926–939 (2007).
18. Starkey, M. L., Bartus, K., Barritt, A. W. & Bradbury, E. J. Chondroitinase ABC promotes compensatory sprouting of the intact corticospinal tract and recovery of forelimb function following unilateral pyramidalotomy in adult mice. Eur. J. Neurosci. 36, 3665–3678 (2012).
19. Houle, J. D. et al. Combining an autologous peripheral nervous system “bridge” and matrix modification by chondroitinase allows robust, functional regeneration beyond a hemisection lesion of the adult rat spinal cord. J. Neurosci. 26, 7405–7415 (2006).
20. James, N. D. et al. Chondroitinase gene therapy improves upper limb function following cervical contusion injury. Exp. Neurol. 271, 131–135 (2015).
21. Solomon, S., Yip, P. K., Durick, D. A. & Moon, L. D. F. Delayed treatment with chondroitinase ABC promotes sensorimotor recovery and plasticity after stroke in aged rats. Brain 135, 1210–1223 (2012).
22. Hill, J. J., Jin, K., Mao, X. O., Xie, L. & Greenberg, D. A. Intracerebral chondroitinase ABC and heparan sulfate proteoglycan glicipan improve outcome from chronic stroke in rats. Proc. Natl Acad. Sci. USA 109, 9155–9160 (2012).
23. Gherardini, L., Gennaro, M. & Pizzorusso, T. Perilesional treatment with chondroitinase ABC and motor training promote functional recovery after stroke in rats. Eur. J. Neurosci. 25, 202–212 (2015).
24. Tester, N. J. & Howland, D. R. Chondroitinase ABC improves basic and skilled locomotion in spinal cord injured cats. Exp. Neurol. 209, 483–496 (2008).
25. Mondello, S. E., Jefferson, S. C., Tester, N. J. & Howland, D. R. Impact of treatment duration and lesion size on effectiveness of chondroitinase treatment post-SCI. Exp. Neurol. 267, 64–77 (2015).
26. Ginsberg, M. D. Neuroprotection for ischemic stroke: past, present and future. Neuropharmacology 55, 363–389 (2008).
27. Howells, D. W., Sena, E. S. & Macleod, M. R. Bringing rigour to translational medicine. *Nat. Rev. Neurol.* **10**, 37–43 (2014).
28. Schumacher, M., Denier, C., Oudinot, J.-P., Adams, D. & Guennoun, R. Progesterone neuroprotection: the background of clinical trial failure. *J. Steroid Biochem. Mol. Biol.* **160**, 53–66 (2016).
29. Tator, C. H. Review of treatment trials in human spinal cord injury: issues, difficulties, and recommendations. *Neurosurgery* **59**, 957–982 (2006).
30. Rosenzweig, E. S. et al. Extensive spontaneous plasticity of corticospinal projections after primate spinal cord injury. *Nat. Neurosci.* **13**, 1505–1510 (2010).
31. Lawrence, D. G. & Kuypers, H. G. The functional organization of the motor system in the monkey. *I. The effects of bilateral pyramidal lesions. Brain* **91**, 1–14 (1968).
32. Hepp-Reymond, M. C., Trouche, E. & Wiesendanger, M. Effects of unilateral and bilateral pyramidotomy on a conditioned rapid precision grip in monkeys (*Macaca fuscata*). *Exp. Brain Res.* **21**, 519–527 (1974).
33. Bunge, R. P., Puckett, W. R., Becerra, J. L., Marcillo, A. & Quencer, R. M. Observations on the pathology of human spinal cord injury. *A review and classification of 22 new cases with details from a case of chronic cord compression with extensive focal demyelination. Adv. Neurol.* **59**, 75–89 (1993).
34. Kakulas, B. A review of the neuropathology of human spinal cord injury with emphasis on special features. *J. Spinal Cord Med.* **22**, 119–124 (1999).
35. Nourt, Y. S. et al. Animal models of neurologic disorders: a nonhuman primate model of spinal cord injury. *Neurotherapeutics* **9**, 380–392 (2012).
36. Weidner, N., Neer, A., Salimi, N. & Tuszyński, M. H. Spontaneous corticospinal axonal plasticity and functional recovery after adult central nervous system injury. *Proc. Natl Acad. Sci. USA* **98**, 3513–3518 (2001).
37. Brus-Ramer, M., Carmel, J. B., Chakraborty, S. & Martin, J. H. Electrical stimulation of spared corticospinal axons augments connections with ipsilateral spinal motor circuits after injury. *J. Neurosci.* **27**, 13793–13801 (2007).
38. Ghosh, A. et al. Functional and anatomical reorganization of the sensory-motor cortex after incomplete spinal cord injury in adult rats. *J. Neurosci.* **29**, 12210–12219 (2009).
39. Friedli, L. et al. Pronounced species divergence in corticospinal tract reorganization and functional recovery after lateralized spinal cord injury favors primates. *Sci. Transl. Med.* **7**, 302ra134 (2015).
40. Liu, K. et al. PTEN deletion enhances the regenerative ability of adult corticospinal neurons. *Nat. Neurosci.* **13**, 1073–1081 (2010).
41. Lee, J. K. et al. Assessing spinal axon regeneration and sprouting in Nogo-, MAG-, and OMgp-deficient mice. *Neuron* **66**, 663–670 (2010).
42. Nout, Y. S. et al. Methods for functional assessment after C7 spinal cord hemisection in the rhesus monkey. *Neurorehabil. Neural Repair* **26**, 556–569 (2012).
43. Shank, C. D., Walters, B. C. & Hadley, M. N. Management of acute traumatic spinal cord injuries. *Handb. Clin. Neurol.* **140**, 275–298 (2017).
44. Saleglo, E. A. et al. A unilateral cervical spinal cord contusion injury model in non-human primates (*Macaca mulatta*). *J. Neurotrauma* **33**, 439–459 (2016).
45. Rosenzweig, E. S. et al. Extensive spinal decussation and bilateral termination of cervical corticospinal projections in rhesus monkeys. *J. Comp. Neurol.* **513**, 151–163 (2009).
46. Leanza, G., Perez, S., Pellitteri, R., Russo, A. & Stanzani, S. Branching serotonergic and non-serotonergic projections from caudal brainstem to the medial preoptic area and the lumbar spinal cord, in the rat. *Neurosci. Lett.* **200**, 5–8 (1995).
47. Haefeli, J. et al. A data-driven approach for evaluating multi-modal therapy in traumatic brain injury. *Sci. Rep.* **7**, 42474 (2017).
48. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for human use. ICH harmonised tripartite guideline. *Statistical principles for clinical trials. International Conference on Harmonisation E9 Expert Working Group. Stat. Med.* **18**, 1905–1942 (1999).
49. Rasmussen, M. A., Colding-Jørgensen, M., Hansen, L. T. & Bro, R. Multivariate evaluation of pharmacological responses in early clinical trials: a study of rIL-21 in the treatment of patients with metastatic melanoma. *Br. J. Clin. Pharmacol.* **69**, 379–390 (2010).
50. Raghavan, N. et al. The ADAS-Cog revisited: novel composite scales based on ADAS-Cog to improve efficiency in MCI and early AD trials. *Alzheimers Dement.* **9**, S21–S31 (2013).

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Author contributions

E.S.R., E.A.S., J.H.B., J.E.I., A.O.C., A.R.B., J.R.H., L.A.H., J.E.I., A.O.C., A.R.B., Y.S.N.-L., A.R.F., M.S.B., J.C.B. and M.H.T. designed the experiments. E.S.R., A.O.C., A.R.B., Y.S.N.-L., A.R.F., M.S.B., J.C.B. and M.H.T. carried out the experiments. E.S.R., E.A.S., J.J.I., L.W., C.A.W., J.H.B., R.H., J.E.I., J.W.F., A.R.F., M.S.B., J.C.B. and M.H.T. analyzed the data. E.S.R. and M.H.T. wrote the manuscript. E.S.R., E.A.S., J.J.I., L.W., C.A.W., J.H.B., R.H., J.E.I., A.O.C., A.R.B., B.H., J.R.H., L.A.H., Y.S.N.-L., J.W.F., A.R.F., M.S.B., J.C.B. and M.H.T. edited the manuscript.

Competing interests

J.E.I., A.O.C. and A.R.B. are employees of Acorda Therapeutics; J.W.F. and M.H.T. were members of the Acorda scientific advisory board at the time that these studies were performed. All other authors declare no competing interests.

Additional information

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Sulfate A or C per minute at 37 °C and pH 8.0. The low dose was comparable to rat Molex) were attached to a NanoFil 100 µm outside diameter; 300 nl of the anterograde neuronal tracer BDA (10,000 MW , 5% in PBS overnight and then transferred to 20% glycerol/2% DMSO in PBS (pH 7.4) for 24 h. Tissue was transferred to 10% glycerol/2% dimethylsulfoxide (DMSO) in PBS overnight and then transferred to 20% glycerol/2% DMSO in PBS (pH 7.4) and was stored refrigerated (2–8 °C). Tissue was sent to UCSD and sectioned in the transverse plane at 60–80 µm and was coverslipped with Mowiol mounting medium (http://cshprotocols.cshlp.org/content/2006/1/pdb.re10255). All antibodies were used previously in monkeys.

Light-level labeling. For WFA and BDA detection, sections were washed in TBS and then quenched in 0.6% H2O2 in 50% methanol in TBS for 30 min. Sections were then washed with TBS and incubated overnight at 4 °C in WFA-biotin (1:1,000; reduced form; Sigma-Aldrich) or Vectastain Elite ABC solution (Vector Laboratories) at 0.25% Triton X-100 in TBS, washed with TBS, then incubated with Vectastain Elite ABC solution for 1 h. All sections were then washed again and developed with 3,3′-diaminobenzidine (DAB) and NiCl2. Sections were mounted on gelatin-subbed glass slides, dehydrated and coverslipped with DPX mounting medium (Sigma-Aldrich).

For A488, CD8, CD3 and CD45 detection, sections were washed in TBS and then quenched in 0.6% H2O2 in 100% methanol in TBS for 30 min. Sections were then washed with TBS, blocked for 1 h in 5% horse serum (Lampire Biological Laboratories) and 0.25% Triton X-100 in TBS (TBBS++) and incubated at 4 °C in TBBS++ with rabbit anti-A488 antibody (1:5,000; Thermo Fisher Scientific, #A11094, Lot #1314344) over 2 nights (~42 h), or mouse anti-CD8 antibody (1:250; BD Pharmingen #555364, Lot #36720, Clone RPA-T8), mouse anti-CD3 antibody (1:250; BD Pharmingen #555480, Lot #50013, Clone HIT3A) or mouse anti-CD45 antibody (1:500, BD Pharmingen #555337, Lot #M067088, Clone HI103A) over 2 nights (~42 h). Sections were then washed again and incubated with 1 µg/ml of a fluorophore-conjugated secondary antibody (ABC; 1:500; Thermo Fisher Scientific, #A11094, Lot #1314344) over 2 nights (~42 h). Sections were washed again and developed with DAB and NiCl2. Sections were mounted on gelatin-subbed glass slides, dehydrated and coverslipped with DPX mounting medium (Sigma-Aldrich).

For 2B6 detection, porcine tissue sections were washed in TBS and then quenched in 0.6% H2O2 in 100% methanol in TBS for 30 min. Sections were then washed with TBS and incubated overnight at 4 °C in WFA-biotin (1:1,000; reduced form; Sigma-Aldrich) or Vectastain Elite ABC solution (Vector Laboratories) at 0.25% Triton X-100 in TBS, washed with TBS, then incubated with Vectastain Elite ABC solution for 1 h. All sections were then washed again and developed with 3,3′-diaminobenzidine (DAB) and NiCl2. Sections were mounted on gelatin-subbed glass slides, dehydrated and coverslipped with DPX mounting medium (Sigma-Aldrich).

For A488, CD8, CD3 and CD45 detection, sections were washed in TBS and then quenched in 0.6% H2O2 in 100% methanol in TBS for 30 min. Sections were then washed with TBS, blocked for 1 h in 5% horse serum (Lampire Biological Laboratories) and 0.25% Triton X-100 in TBS (TBBS++) and incubated at 4 °C in TBBS++ with rabbit anti-A488 antibody (1:5,000; Thermo Fisher Scientific, #A11094, Lot #1314344) over 2 nights (~42 h), or mouse anti-CD8 antibody (1:250; BD Pharmingen #555364, Lot #36720, Clone RPA-T8), mouse anti-CD3 antibody (1:250; BD Pharmingen #555337, Lot #M067088, Clone HI103A) over 2 nights (~42 h). Sections were then washed again and incubated with 1 µg/ml of a fluorophore-conjugated secondary antibody (ABC; 1:500; Thermo Fisher Scientific, #A11094, Lot #1314344) over 2 nights (~42 h). Sections were washed again and developed with DAB and NiCl2. Sections were mounted on gelatin-subbed glass slides, dehydrated and coverslipped with DPX mounting medium (Sigma-Aldrich).

For 2B6 detection, porcine tissue sections were washed in TBS and then quenched in 0.6% H2O2 in 100% methanol in TBS for 30 min. Sections were then washed with TBS, blocked for 1 h in 5% horse serum (Lampire Biological Laboratories) and 0.25% Triton X-100 in TBS (TBBS++) and incubated at 4 °C in TBBS++ with rabbit anti-A488 antibody (1:5,000; Thermo Fisher Scientific, #A11094, Lot #1314344) over 2 nights (~42 h), or mouse anti-CD8 antibody (1:250; BD Pharmingen #555364, Lot #36720, Clone RPA-T8), mouse anti-CD3 antibody (1:250; BD Pharmingen #555337, Lot #M067088, Clone HI103A) over 2 nights (~42 h). Sections were then washed again and incubated with 1 µg/ml of a fluorophore-conjugated secondary antibody (ABC; 1:500; Thermo Fisher Scientific, #A11094, Lot #1314344) over 2 nights (~42 h). Sections were washed again and developed with DAB and NiCl2. Sections were mounted on gelatin-subbed glass slides, dehydrated and coverslipped with DPX mounting medium (Sigma-Aldrich).
Nissl substance was labeled in sections from a 1:12 series covering spinal cord segments C3, C7, C8, T1 and T2. Sections were washed in TBS, fixed in buffered 4% PFA for 1 h at room temperature, mounted on gelatin-subbed glass slides and dried overnight. Sections were then dehydrated in a 1:1 mixture of chloroform and ethanol, rehydrated, placed briefly in 0.25% thionin, dehydrated, cleared and coverslipped.

Quantification of axons, synapses, motor neurons and microglia. Axon density was quantified with Image v.1.41c (NIH) and a custom-written script (J.L.), derived from previous methods (E.S.R.)30; 200× magnification of the entire spinal cord were obtained and loaded into ImageJ. All images were corrected for brightness and contrast equally to reduce background noise. For CST axons, regions of interest (ROIs) were drawn around the right gray matter. For 5-HT-labeled raphespinal axons, ROIs were drawn around the right and left motor pools. The image was auto-thresholded, the detected fiber profiles were skeletonized (so that sum of pixels=total axon length) and measurements for each ROI were recorded in the right gray matter.

Synapse counts (colocalization of BDA and synaptophysin) were performed with 60× confocal image stacks loaded into ImageJ. Three non-overlapping image stacks were obtained in the right intermediate zone of each of four tissue sections per animal. The experimenter selects one slice and sampled the entire image stack for clearly colocalized BDA and synaptophysin in bouton-like swellings connected to BDA-labeled axons. The number of putative synapses was divided by the volume of the image stack (approximately 900,000 μm³) and converted to synapses per mm². Spinal motor neurons were counted in Nissl-labeled sections at sequential rostrocaudal distances from a sample injection site on both injected and uninjected sides of the spinal cord. The number of motor neurons on the injected side was divided by the sum of the number of motor neurons on the injected and uninjected sides, such that the expected proportion was 0.5; 5–6 total sections were counted per animal (up to 1 section per per axon position).

Microglial density was quantified using IBA1 labeling. IBA1 pixel density was calculated using the ImageJ auto-thresholding function on images of the intermediate zone of the gray matter in tissue sections immediately adjacent to the injection sites (or similar regions in the C8–T1 spinal cord for uninjected animals). Tissue sections from animals in the present study were immunolabeled for IBA1 concurrently with sections from animals involved in a previous study30. n = 3 intact animals, n = 4 animals 2 weeks after C7 hemisection SCI and n = 3 animals 5 months after C7 hemisection SCI.

Experimenter were blind to group membership during all quantitative analyses.

Functional testing. Functional outcomes were assessed in an open field paradigm using an ordinal scale, as described in detail in Nout et al.31 and Salegio et al.18. In the open field testing paradigm, monkeys were placed at various levels, climb along the enclosure walls and manipulate objects containing small food rewards using dexterous hand movements. Use of each hind limb and forelimb on the tasks was rated by an observer over the 30 min observation period; video recordings allow reassessments as necessary. A total of 72 points on the scale are possible. We also rated monkeys on a 22-point forelimb subcorticomedullary extrapyramidal quotient, 16 points on arm, hand and digit use during object manipulation1818. Monkeys underwent pre-lesion baseline training, followed by three half-hour exposures per week to the enclosure to encourage activity and limb use. Performance was videotaped and rated once weekly. We compared the level of functional performance of lesioned control monkeys (n = 5) to Chase-treated monkeys (n = 6).

The ability of animals to perform fine motor functions with the affected hand were also assessed using a modified, cage-mounted Brinkman board, which required use of the affected (right) hand to retrieve small food items. This custom-made Plexiglas box is hung at the front of each animal’s cage. The left hand were also assessed using a modified, cage-mounted Brinkman board, the time they took to clear the board and whether they used a pincer grasp. All functional outcomes were measured and recorded by observers blinded to treatment group.

Statistical analysis. For statistical analysis of functional data, we employed an a priori established, blinded, data-driven statistical workflow (Supplementary Fig. 11 (ref. 33)). Diagnostic analyses of raw ordinal data revealed non-normal and non-parametric features across measures; thus, non-parametric and non-linear statistical analyses were applied throughout. Analysis was divided into two distinct stages. First, we compiled the full behavioral data outcome matrix (animals×tests×time points) and performed non-linear PCA on the whole matrix from all animals (both treated and untreated). The principal component loadings were then examined and cross-validated to confirm that the resulting principal component scores served as legitimate multidimensional outcome metrics33. This was done in a computationally unsupervised manner by applied biostatisticians (J.R.H., A.R.B.) blind to experimental condition. Second, we performed a directed single hypothesis test (LMM; Fig. 3a), unblinded to the effect of Chase on PCI. This staged statistical analysis approach brings together machine learning-based pattern detection (stage 1), with hypothesis testing (stage 2) to maximize outcome information without relying on multiple hypothesis testing, thereby limiting the false discovery rate. Technically, the non-linear PCA approach (stage 1) reduces the large amount of behavioral data (open field and each level of Brinkman board for each animal at each time point) into an overall integrated behavioral score, while taking into account the level of measurement of each measure (ordinal, continuous, etc.) and a wide range of possible distributions, through optimal scaling transformations and pattern detection using the alternating least squares algorithm34. The resulting principal components are orthogonal and, by definition, normally distributed, with the first principal component accounting for the most variance. The principal component loading reflects the correlation between each measure and the non-linear principal component. The squared factor loading for each of these measures indicates the percentage of variance in a principal component that is explained by that measure. A bootstrapping procedure was then used for internal validation of the loading pattern. The bootstrapping procedure was a non-parametric approach that resampled the dataset 1,000 times, with each iteration producing loadings from a slight variation of the original dataset. This step was followed by a pattern-matching algorithm to test the similarity between the simulation and bootstrapped loading patterns. After choosing the optimal component pattern stability, principal component loading weights were applied to derive a principal score for each animal at each individual time point. In stage 2, we applied an LMM to assess the impact of Chase on principal component scores over time for each group, using a diagonal covariance matrix structure with each individual animal as a random factor, thereby standardizing scores for each animal according to the baseline and bootstrapped loading patterns. After choosing the optimal component pattern, principal component loading weights were applied to derive a principal score for each animal at each individual time point.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability
The custom-written ImageJ scripts are available from the corresponding author upon reasonable request.

References
51. García- Alias, G. et al. Therapeutic time window for the application of chondroitinase ABC after spinal cord injury. Exp. Neurol. 210, 331–338 (2008).
52. Lin, R., Swok, J. C. F., Crespo, D. & Fawcett, J. W. Chondroitinase ABC has a long-lasting effect on chondroitin sulphate glycosaminoglycan content in the injured rat brain. J. Neurochem. 104, 400–408 (2008).
53. Hu, H. Z., Granger, N., Pai, S. B., Bellamkonda, R. V . & Jeffery, N. D. Therapeutic efficacy of microtube-inserted chondroitinase ABC in a canine clinical model of spinal cord injury. Brain 141, 1017–1027 (2018).
54. Garcia Alías, G. et al. Single, high-dose intraspinal injection of chondroitinase reduces glycosaminoglycans in injured spinal cord and promotes corticospinal axonal regrowth after hemisection but not contusion. J. Neurotrauma 25, 334–349 (2008).
55. Jefferson, S. C., Tester, N. J. & Howland, D. R. Chondroitinase ABC promotes recovery of adaptive limb movements and enhances axonal growth caudal to a spinal hemisection. J. Neurosci. 31, 5710–5720 (2011).

56. Steinmetz, M. P. et al. Chronic enhancement of the intrinsic growth capacity of sensory neurons combined with the degradation of inhibitory proteoglycans allows functional regeneration of sensory axons through the dorsal root entry zone in the mammalian spinal cord. J. Neurosci. 25, 8066–8076 (2005).

57. Rosenzweig, E. S. et al. Restorative effects of human neural stem cell grafts on the primate spinal cord. Nat. Med. 24, 484–490 (2018).

58. Linting, M., Meulman, J. J., Groenen, P. J. F. & van der Kooij, A. J. Nonlinear principal components analysis: introduction and application. Psychol. Methods 12, 336–358 (2007).

59. Lever, J., Krzywinski, M. & Altman, N. Principal component analysis. Nat. Methods 14, 641–642 (2017).

60. Ferguson, A. R. et al. Derivation of Multivariate Syndromic Outcome Metrics for Consistent Testing across Multiple Models of Cervical Spinal Cord Injury in Rats. PLoS One 8, e59712 (2013).

61. Gifi, A. Nonlinear Multivariate Analysis. (Wiley, 1990).

62. Cnaan, A., Laird, N. M. & Slasor, P. Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. Stat. Med. 16, 2349–2380 (1997).
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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection  No software was used for data collection.

Data analysis  NIH ImageJ was used for image analysis as described in Methods. Adobe Photoshop CSS was used for image brightness and contrast adjustment. SPSS v.25 was used for statistical analyses as described in Methods.

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- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Field-specific reporting

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- Life sciences
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Life sciences study design

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Sample size

Rhesus monkeys are a critical model for human spinal cord injury, but represent an especially precious resource. We have chosen the minimum number of subjects possible for study that can yield statistically valid and meaningful data. POWER ANALYSIS: With N=6 per group, a 30% effect size with 15% standard deviation (estimates consistent with our previous work in lesioned and intact CST; Rosenzweig et al., 2010) is detectable at 89% power (http://www.quantitativeskills.com/sisa/calculations/power.htm).

Data exclusions

Control subject #7 was considered an ‘overlesion’ and was excluded from behavioral analyses (but not CST anatomical analysis, as the left dorsolateral tract, which carries nearly all of the spared CST axons, was unaffected by the overlesion). Control subject #3 did not receive cortical tracer due to weight loss, and was therefore excluded from CST density analyses.

Replication

Full replication was not attempted in this study. However, partial replication was accomplished by enlisting the collaboration of the Fawcett lab in Cambridge, UK. Analysis of corticospinal axons arising from the left hemisphere was done in one laboratory (MHT), while analysis of axons arising from the right hemisphere was done independently in a second laboratory (JWF). The similar effects of Chase on the separate axonal systems supports the replicability of the anatomical results.

Randomization

Subjects entered the experiment in pairs. One subject of each pair was assigned by a single experimenter (ESR) to the Chase-treated group according to post-lesion performance on functional tests such that pre-treatment functional deficits were equivalent across groups.

Blinding

A group membership list was maintained by a single experimenter (ESR), who did not participate directly in functional data collection or anatomical data analysis. Unblinding occurred after analyses were complete.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒  | Antibodies            |
| ☐  | Eukaryotic cell lines |
| ☒  | Palaeontology         |
| ☐  | Animals and other organisms |
| ☒  | Human research participants |
| ☐  | Clinical data         |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒  | ChIP-seq              |
| ☒  | Flow cytometry        |
| ☒  | MRI-based neuroimaging |

Antibodies

| Antibodies used |
|-----------------|
| Serotonin (goat, Immunostar #20079, Lot #1530001, 1:5000) |
| Synaptophysin (mouse, Sigma S5768, Lot #123K4879, Clone SVP38, 1:300) |
| IBA1 (rabbit, Wako 019-19741, Lot #WDJ3047, 1:1500) |
| GFAP (chicken, Encor Bio #CPCA-GFAP, isotype 2, Lot #6701-2, 1:1500) |
| A488 (rabbit, ThermoFisher A11094, Lot #1314344, 1:5000) |
| 286 (mouse, Seikagaku 270432, Lot #06902, Clone 2-8-6, now available from Amsbio 270432-CS, 1:2000) |
| CD8 (mouse, BD Pharmingen #555364, Lot #36720, Clone RPA-T8, 1:500) |
| CD3 (mouse, BD Pharmingen #555337, Lot #M067088, Clone HIT3A, 1:500) |
| CD45 (mouse, BD Pharmingen #555480, Lot #50013, Clone Hi30, 1:500) |
| IL-6Ralpha (goat, R & D Systems AF-227-NA, Lot # unknown, 1:500) |

Validation

| Validation |
|------------|
| Serotonin (goat, Immunostar #20079): Manufacturer’s website lists reactivity in monkey, use in immunohistochemistry in >50 references. |
| Synaptophysin (mouse, Sigma S5768): Manufacturer’s website lists reactivity in human and rodent (suggesting reactivity in monkey), use in immunohistochemistry in >50 references. |
| IBA1 (rabbit, Wako 019-19741): Manufacturer’s website lists reactivity in human and rodent (suggesting reactivity in monkey), use in immunohistochemistry in >10 references. |
| GFAP (chicken, Encor Bio #CPCA-GFAP): Manufacturer’s website lists reactivity in human and rodent (suggesting reactivity in monkey), use in immunohistochemistry in 4 references, including the original 1972 paper (Bignami et al., Brain Res). |
| A488 (rabbit, ThermoFisher A11094): Manufacturer’s website lists reactivity in monkey, use in immunohistochemistry in 6 references. |
| 286 (mouse, Seikagaku 270432, now available from Amsbio 270432-CS): Manufacturer’s website lists reactivity in all animal species, use in immunohistochemistry in 5 references. |
| CD8 (mouse, BD Pharmingen #555364): Manufacturer’s website lists reactivity in monkey, use in immunohistochemistry. |
CD3 (mouse, BD Pharamingen #555337): Manufacturer’s website lists reactivity in human (suggesting reactivity in monkey).
CD45 (mouse, BD Pharamingen #555480): Manufacturer’s website lists reactivity in human (suggesting reactivity in monkey), use in immunohistochemistry.
IL-6R alpha (goat, R & D Systems #AF-227-NA): Manufacturer’s website lists reactivity in human (suggesting reactivity in monkey).

### Animals and other organisms

**Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research**

| Category                        | Information                                                                 |
|---------------------------------|-----------------------------------------------------------------------------|
| Laboratory animals              | We studied a total of 14 male rhesus macaques (Macaca mulatta), aged 6–10 years, that had not been used in any previous experiments. We studied 7 male American Yorkshire-Landrace-Duroc pigs, aged 4-6 months, that had not been used in any previous experiments. |
| Wild animals                    | This study did not involve wild animals.                                    |
| Field-collected samples         | This study did not involve field-collected samples.                         |
| Ethics oversight                | All surgical and experimental procedures adhered to the principles outlined by American Association for the Accreditation of Laboratory Animal Care. Porcine subjects were housed and tested at MPI, Inc., which is USDA registered and compliant with the Animal Welfare Act (AWA), and has Assurance of Compliance with the Public Health Service Policy for the Humane Care and Use of Laboratory Animals (PHS/OLAW Assurance). Non-human primates were housed and surgeries performed at the California National Primate Research Center (CNPRC, Davis, CA); all primate procedures were approved by the CNPRC Institutional Animal Care and Use Committee (IACUC). |

Note that full information on the approval of the study protocol must also be provided in the manuscript.