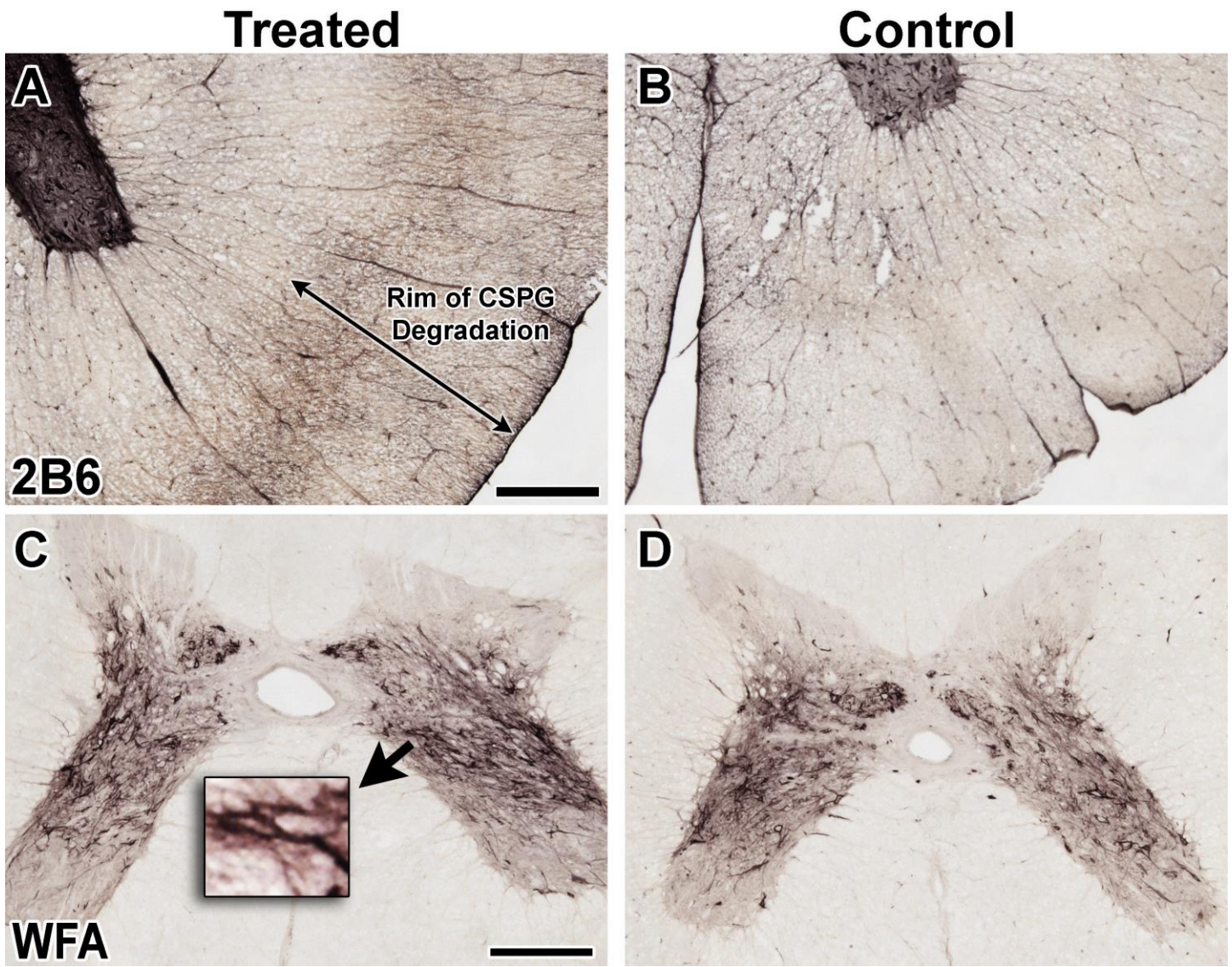


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Chondroitinase improves anatomical and functional outcomes after primate spinal cord injury

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Supplementary Figure 1

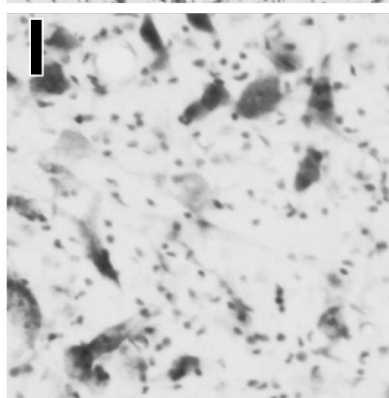
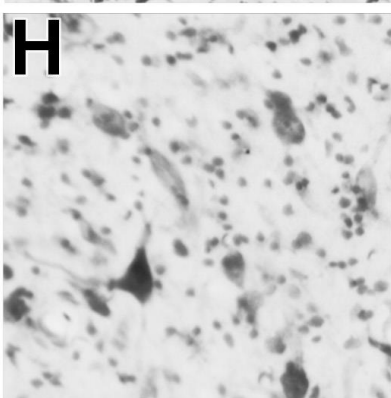
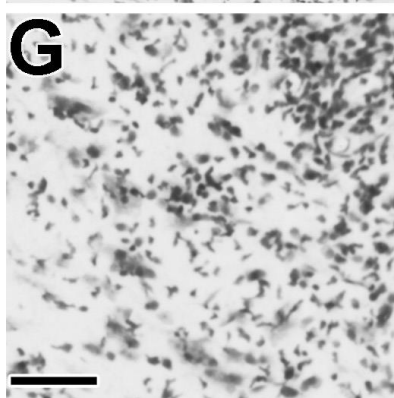
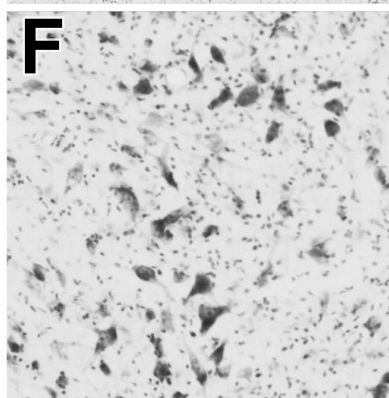
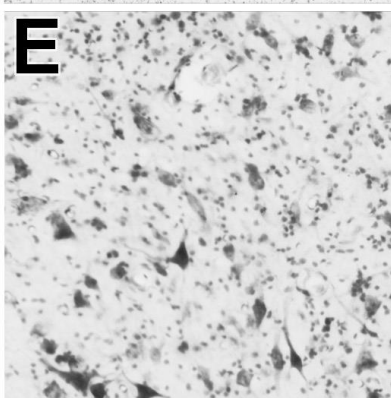
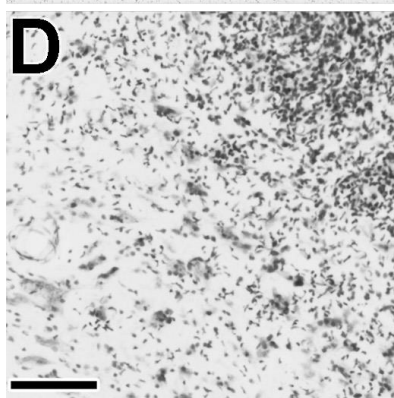
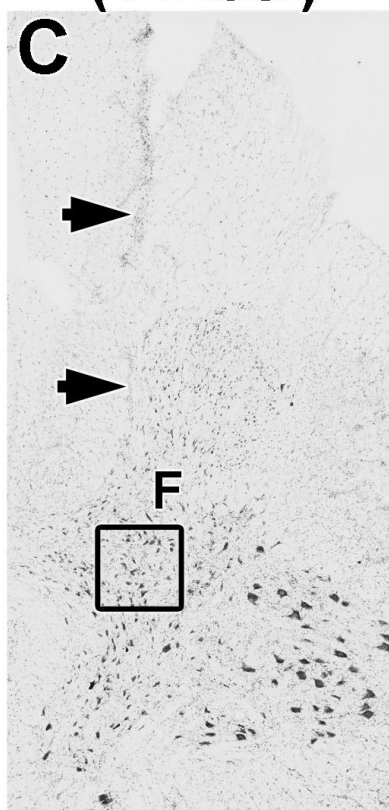
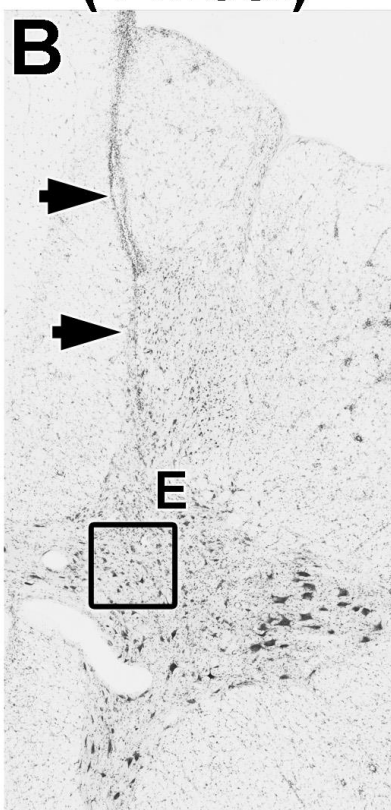
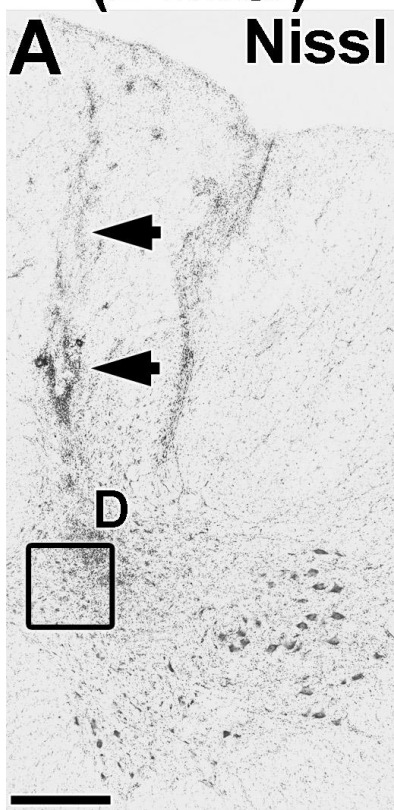
Intrathecal Chase Infusions in the Pig Do Not Degrade Gray Matter Chondroitin Sulfate Proteoglycans

(A) 2B6 labeling of digested stubs of chondroitin sulfate proteoglycans (CSPGs) in transverse spinal cord sections of the porcine spinal cord at C8. Following intrathecal Chase infusions, there is an approximate 1 mm rim of CSPG degradation in the peripheral white matter of Chase-treated subjects. (B) Saline-infused control. (C) Wisteria Floribunda Agglutinin (WFA) labeling of peri-neuronal net CSPGs in gray matter reveals intact peri-neuronal net CSPGs in all Chase-infused subjects, which appear identical to (D) saline-infused controls. Labeling experiments included 6 Chase-infused and 1 saline-infused animals. Scale bars: 500 μ m.

**Chase
(2 wks.)**

**Saline
(4 mos.)**

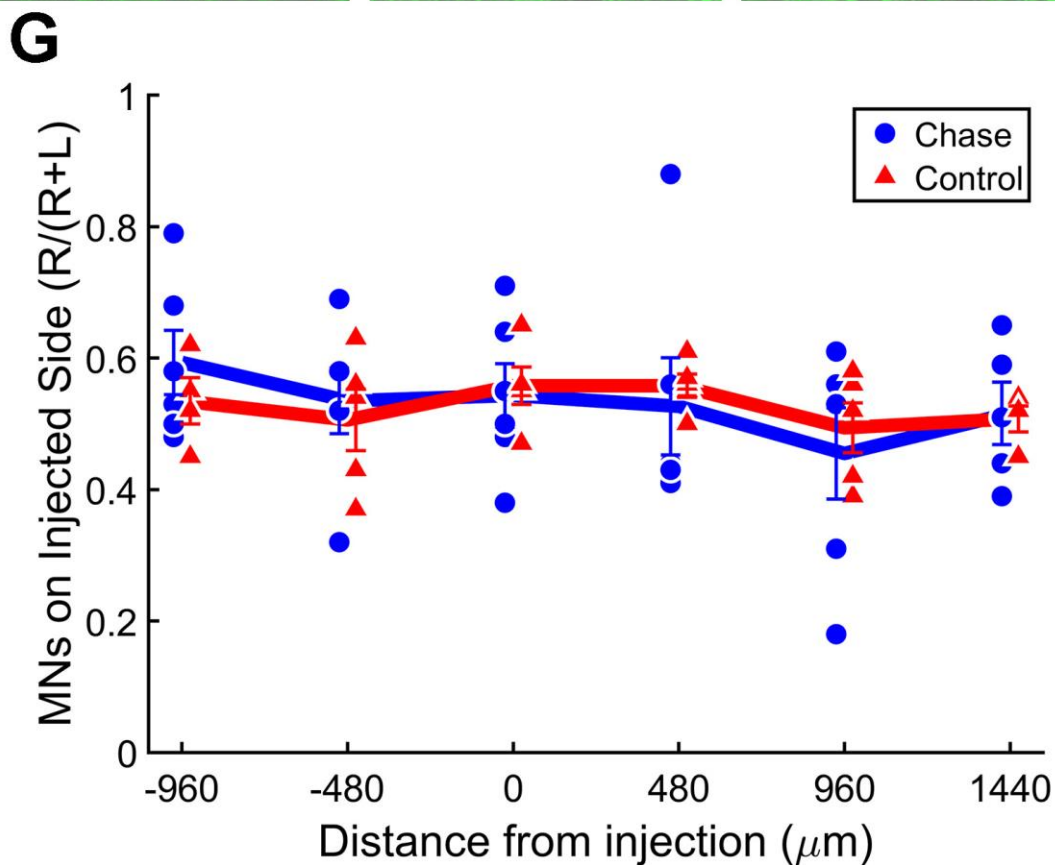
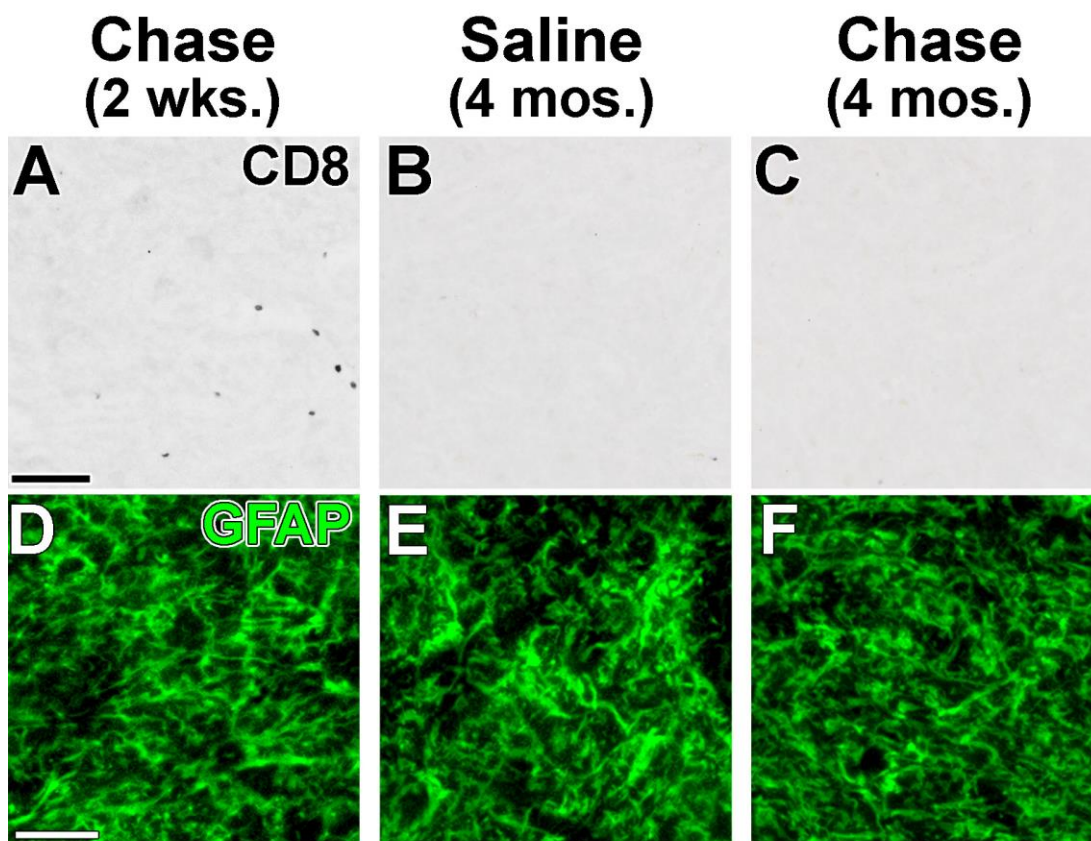
**Chase
(4 mos.)**



Supplementary Figure 2

Parenchymal Integrity at Chase Injection Sites - Nissl

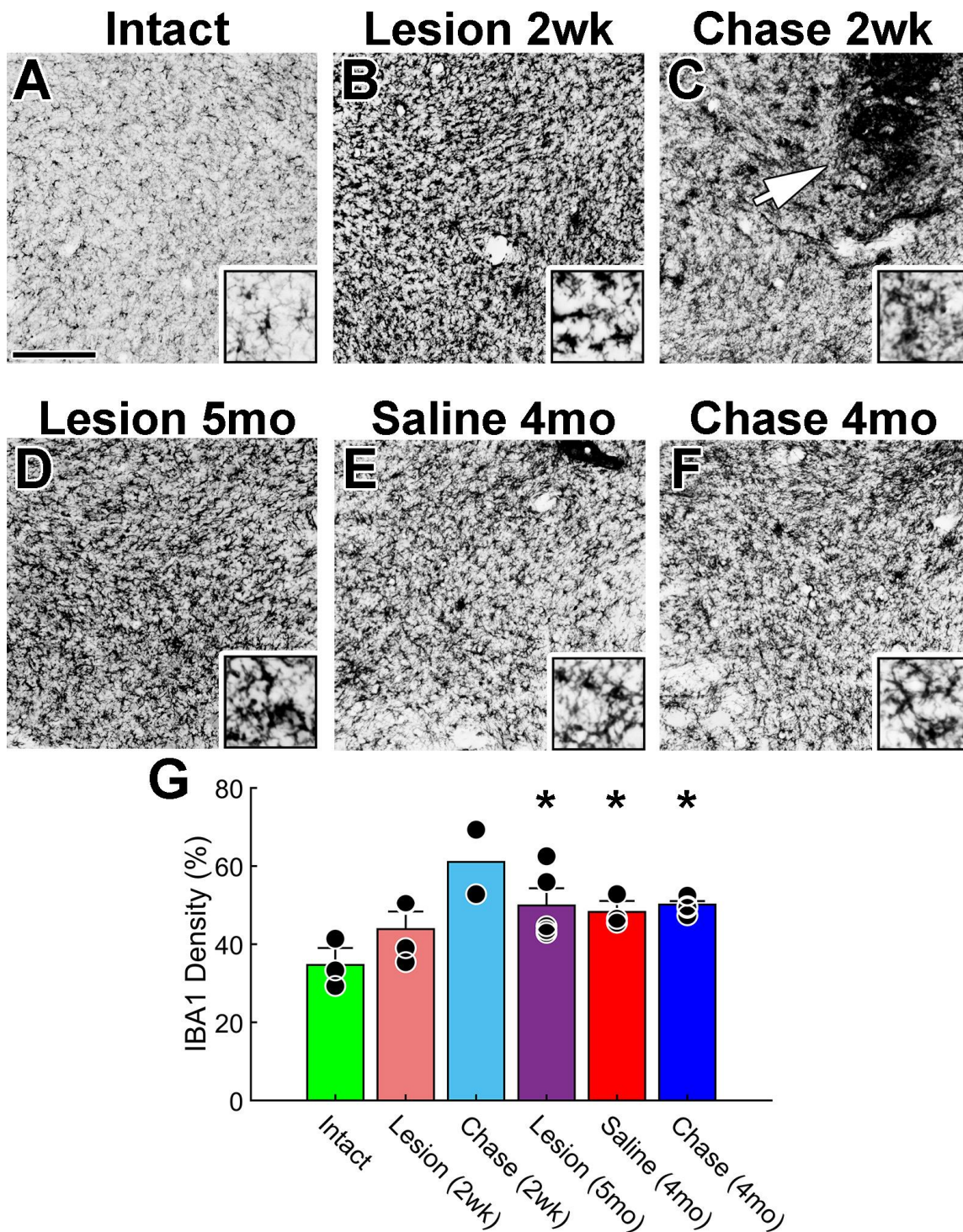
Intraparenchymal injections of Chase in the monkey spinal cord do not cause extensive damage or inflammation. Images of transverse sections very near injection sites at C8-T1. Left column of images is from a short-term Chase subject (2 wks post injection, 6 wks post SCI); middle column is from a saline-injected subject (4 mos post injection, 5 mos post SCI); right column is from a Chase-injected subject (4 mos post injection, 5 mos post SCI). **(A)** Labeling for Nissl substance reveals mild hypercellularity along the injection track (**arrowheads**) 2 weeks after Chase injection. **(B-C)** By 4 months after injections, there are no evident differences in Nissl stains comparing saline and Chase injection sites. **(D-F)** Higher magnifications of sections in **A-C**. **(G-I)** Further magnification of panels D-F. Labeling experiments included 2 short-term Chase subjects, 4 saline-injected subjects, and 6 Chase-injected subjects. Scale bars: A-C, 500 μm ; D-F, 100 μm ; G-I, 50 μm .



Supplementary Figure 3

Parenchymal Integrity at Chase Injection Sites – CD8, GFAP, Motor Neurons

Intraparenchymal injections of Chase in the monkey spinal cord do not cause extensive damage or inflammation. Images of transverse sections midway between injection sites at C8-T1. **(A-C)** CD8 labeling in gray matter reveals rare T-cells 2 weeks after Chase injection; these cells are not detected 4 months after injection. CD3 and CD45 labeling showed the same pattern (data not shown). **(D-F)** Labeling for GFAP (astrocytes) in gray matter shows no qualitative differences between short-term Chase, long-term saline, and long-term Chase. Immunolabeling experiments included 2 short-term Chase subjects, 4 saline-injected subjects, and 6 Chase-injected subjects. **(G)** Injections of either Chase or saline resulted in no loss of spinal motor neurons (MNs) on injected vs. uninjected sides of the spinal cord (expected proportion is 0.5). Moreover, there was no drop in MN numbers at the actual injection site, nor when comparing motor neuron numbers in Chase vs. control animals (Chase proportion: 0.53 ± 0.04 ; Control proportion: 0.51 ± 0.01 ; ANOVA: $F_{(1, 0.00055)} = 0.11$, $P=0.75$). $N=6$ Chase and $N=5$ controls. Lines show group means, bars show SEM, data points are individual tissue sections, 5-6 total sections per subject (up to one section per x-axis position). Scale bars: 100 μm .

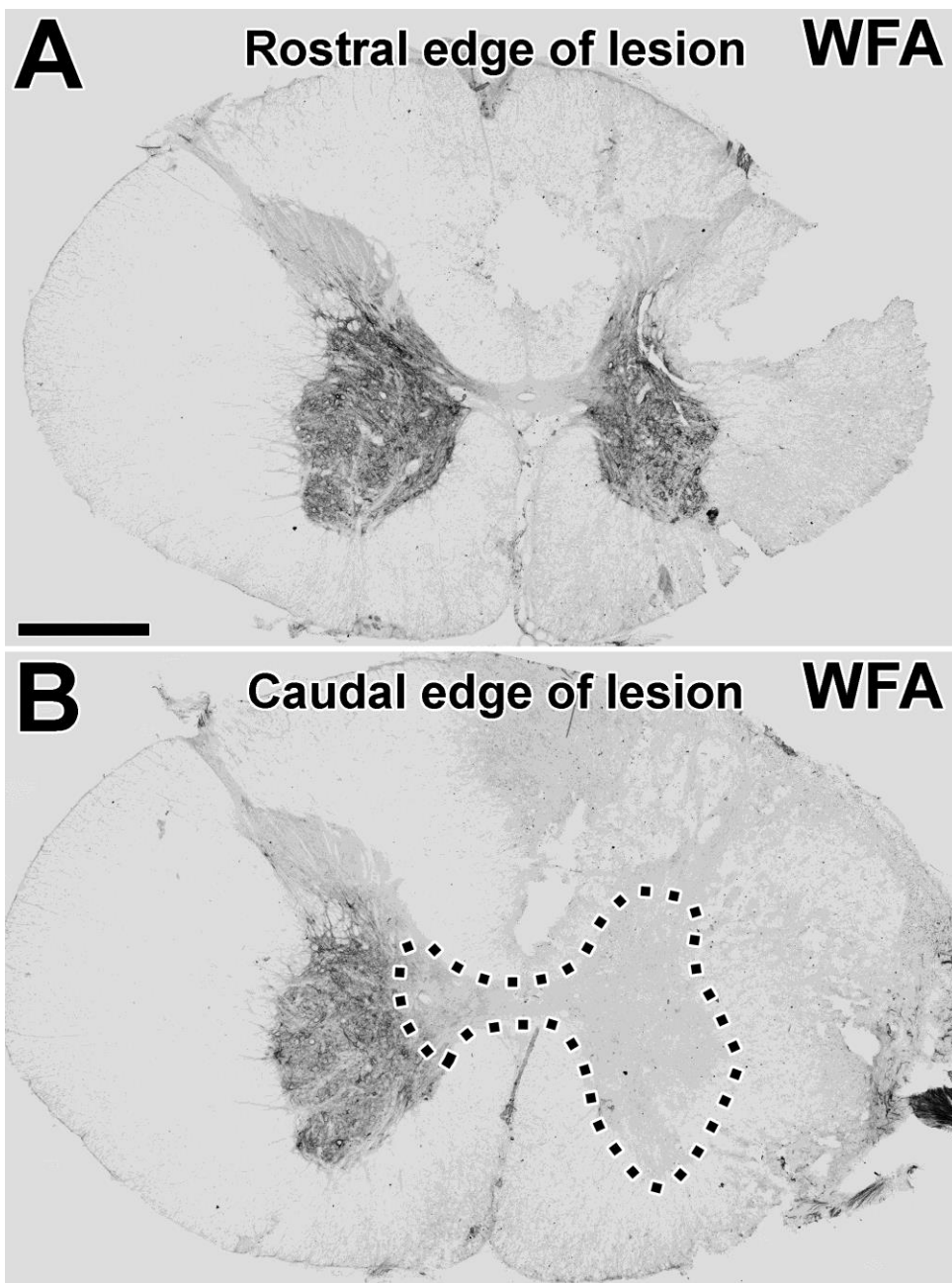


Supplementary Figure 4

Temporary Microglial Activation at Chase Injection Sites

Images of transverse sections immediately adjacent to injection sites (or similar regions in C8-T1 spinal cord for uninjected subjects), labeled for IBA1 (microglia) at the indicated time points. There are significant differences in density of IBA1 labeling across groups (Overall ANOVA: $F_{(5, 197.30)} = 4.43$, $P = 0.009$). Analyses include sections from animals involved in a previous study ([30]; N=3 Intact

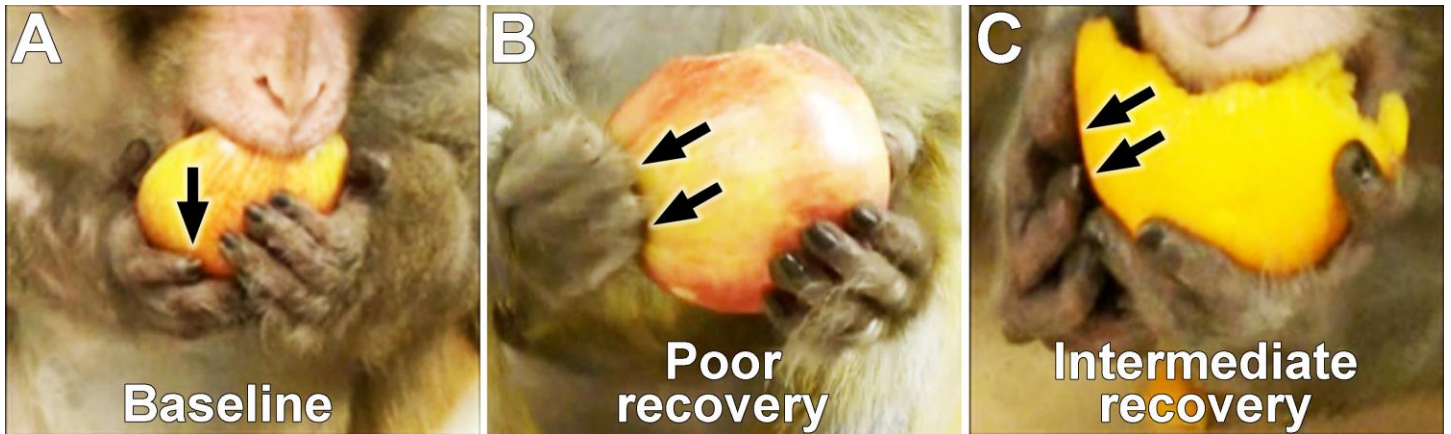
subjects, N=4 Lesion 2wk subjects, N=3 Lesion 5mo subjects). Main panels show example actual images used for quantification; insets are 3x zooms. Images shown are from subjects whose results were closest to their group's mean value. Quantifications in panel G. **(A)** Intact subjects show the baseline level of microglial labeling. **(B)** Two weeks after lesion, a trend toward increased microglial labeling is observed compared to baseline (N=4 subjects, ANOVA: $F_{(1, 144.57)} = 2.79$, $P = 0.16$, compared to N=3 Intact subjects). **(C)** Two weeks after Chase injections (six weeks after lesion), increased IBA1 labeling is evident immediately adjacent to the injection site (**arrow**) (N=2 subjects). **(D-F)** At longer time points after lesions, increased IBA1 labeling remains evident and statistically significant compared to intact baseline (Lesion 5mo, N=5 subjects, ANOVA: $F_{(1, 435.10)} = 6.74$, $P = 0.04$; Saline 4mo, N=3 subjects, ANOVA: $F_{(1, 277.44)} = 10.34$, $P = 0.03$; Chase 4mo, N=6 subjects, ANOVA: $F_{(1, 479.47)} = 35.19$, $P = 0.0006$; all compared to N=3 Intact subjects). However, there are no significant differences among animals with lesions alone, Saline injections or Chase injections (Lesion 5mo vs. Saline 4mo, vs. Chase 4mo, ANOVA: $F_{(2, 3.78)} = 0.11$, $P=0.89$). **(G)** Bar graphs show means, data points are individual subjects, error bars represent SEM, asterisks indicate statistical difference from intact baseline, statistical results described above. Scale bar: 200 μm .



Supplementary Figure 5

Chase Injections Degrade Chondroitin Sulfate Proteoglycans up to the Caudal Lesion Border

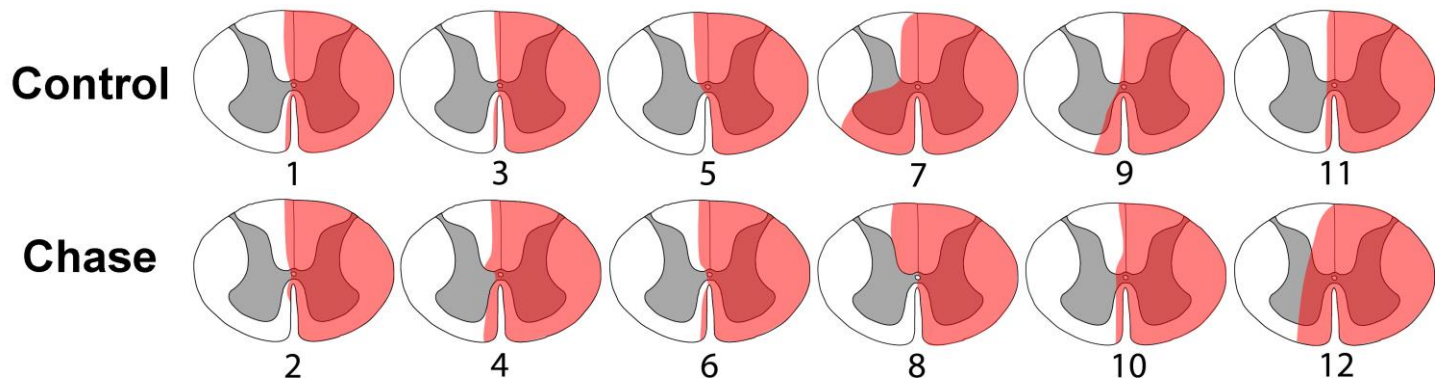
(A) 2 wk after Chase administration (1 mo after lesion), WFA labeling reveals that CSPGs are intact at the rostral end of the lesion site. (B) In contrast, CSPG degradation is evident up to the caudal lesion border. However, because no “bridge” was placed in the lesion site to allow the growth of axons through the lesion, this CSPG degradation likely had no impact on experimental outcomes. Approximate zone of peri-neuronal net CSPG degradation indicated by **dashed lines**. Labeling experiment included 6 control subjects, and 6 Chase-injected subjects. Scale bar: 1 mm.



Supplementary Figure 6

Object Manipulation

(A) Intact subject manipulating an apple bimanually. Note palmar contact of right hand (arrow) with object, digit extension and thumb apposition, all features of normal object manipulation. (B) Manipulation by a lesioned subject early after injury. Note the flexed digits of the impaired right hand (arrows). (C) Partial recovery of the impaired right hand during object manipulation. The hand is more normally positioned and less tightly flexed, but the dorsal aspect of the digits are still in contact with the fruit, and the thumb remains close to digit two (arrows). The score for object manipulations is based on these features in combination with evaluations of movement of the wrist and fingers during manipulation, and the presence of pincer grasp [42,44].



Supplementary Figure 7

Lesion Reconstructions

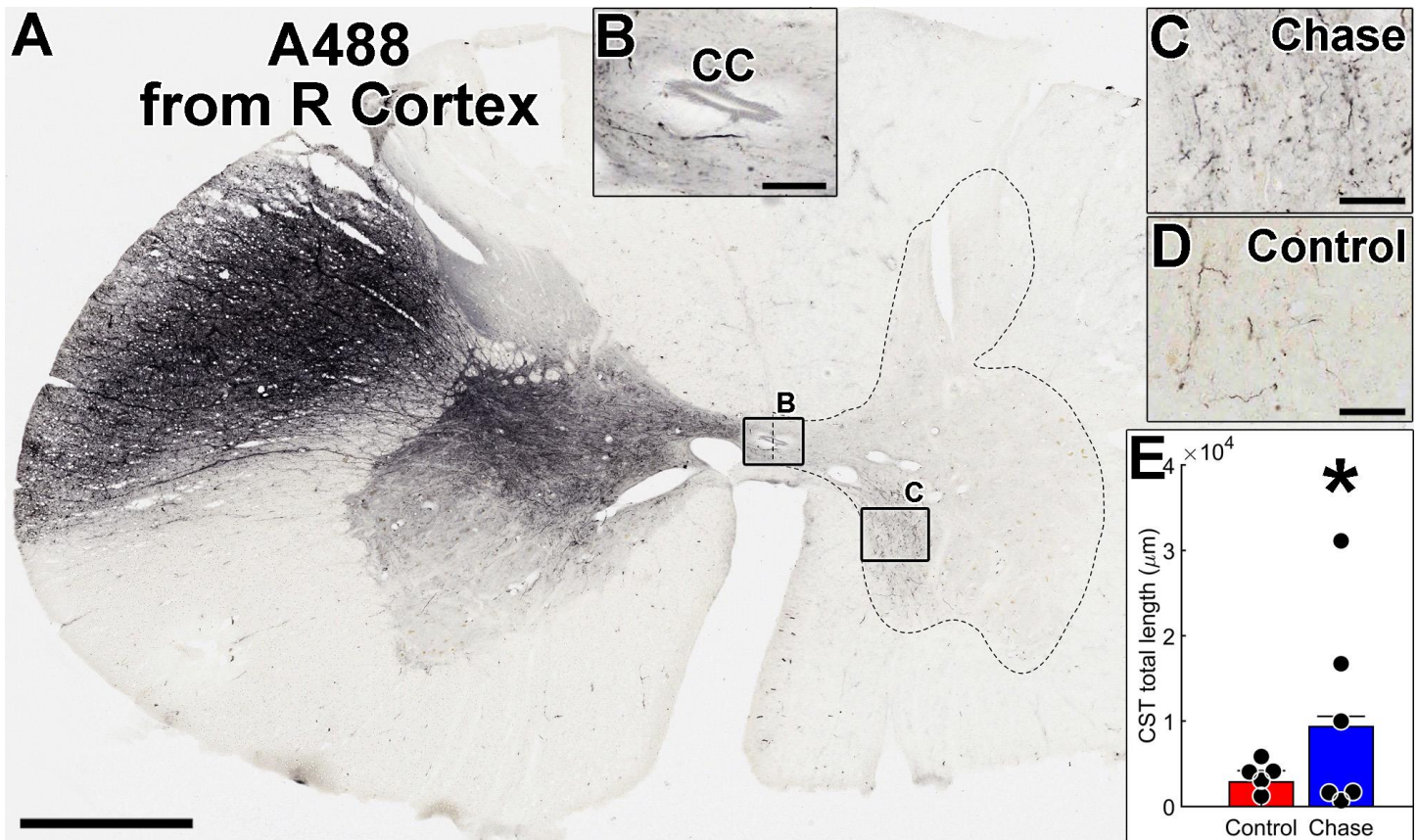
Extent of lesion is shown in red shading. Control subject #7 was considered an 'overlesion' and was excluded from behavioral analyses (but not CST anatomical analysis, as the left dorsolateral tract, which carries nearly all of the spared CST axons, was unaffected by the overlesion). Control subject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

		PC1	PC2	PC3
%Variance Explained		67.73	10.38	5.13
Open Field	General Locomotion	0.459	0.516	0.574
	Climbing	0.584	0.401	0.555
	Object Manipulation	0.764	0.244	0.216
	Overall	0.650	0.492	0.558
Brinkman Board	sloped_1	0.901	0.106	0.109
	sloped_2	0.924	0.104	0.073
	sloped timetoclear	-0.613	0.553	-0.381
	non_sloped_1	0.612	0.089	-0.066
	non_sloped_2	0.942	0.102	0.022
	non_sloped timetoclear	-0.470	0.619	-0.228
	four_config_1	0.915	0.078	-0.041
	four_config_2	0.926	0.085	-0.053
	four_config_3	0.929	0.070	-0.010
	four_config_4	0.944	0.085	0.011
	four_config_timetoclear	-0.532	0.731	-0.264
	six_config_1	0.896	-0.008	-0.163
	six_config_2	0.931	0.083	-0.109
	six_config_3	0.944	0.049	-0.125
	six_config_4	0.934	0.011	-0.121
	six_config_5	0.938	0.024	-0.139
	six_config_6	0.930	0.012	-0.071
	six_config_timetoclear	-0.470	0.803	-0.172
	nine_config_1	0.888	-0.050	-0.138
	nine_config_2	0.918	0.014	-0.192
	nine_config_3	0.926	0.039	-0.127
	nine_config_4	0.909	-0.024	-0.164
	nine_config_5	0.843	-0.092	-0.147
	nine_config_6	0.932	0.011	-0.175
	nine_config_7	0.922	0.056	-0.111
	nine_config_8	0.882	-0.031	-0.114
	nine_config_9	0.886	-0.006	-0.101
	nine_config_timetoclear	-0.396	0.794	-0.206

Supplementary Figure 8

Behavioral Task Loadings on Principal Components Analysis

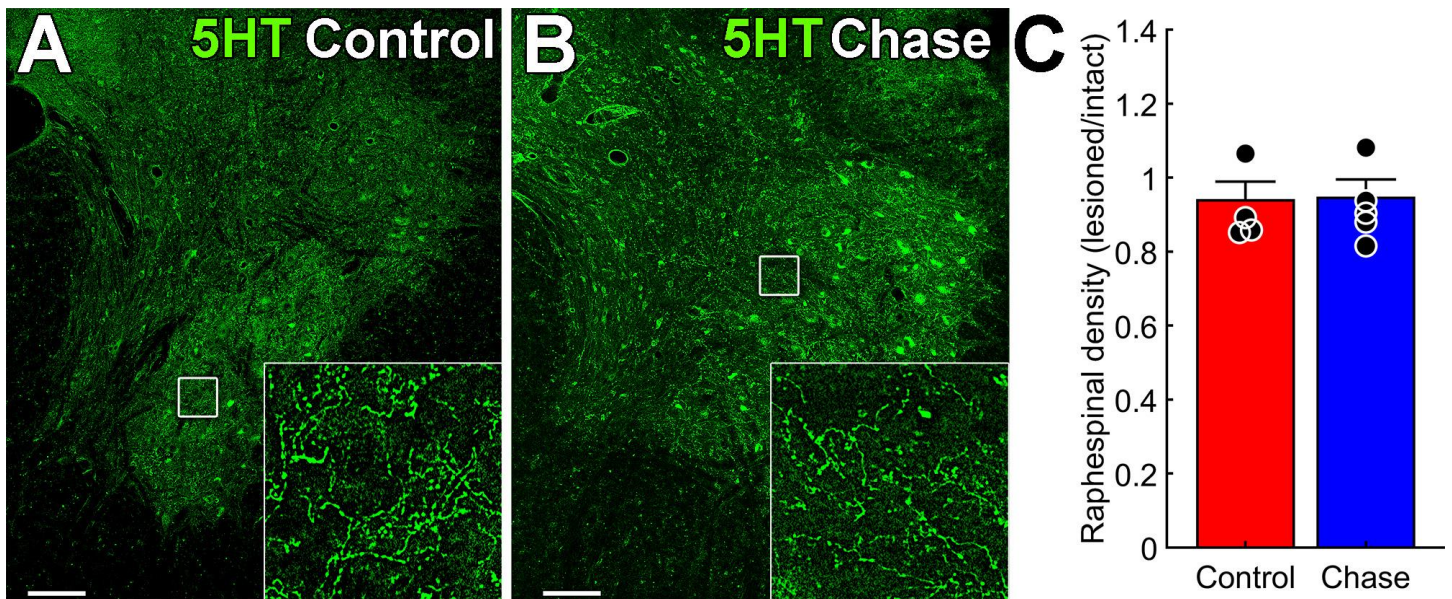
Non-linear principal components analysis of all behavioral tasks (open-field tasks and Brinkman board) across all time points revealed strong loadings of all behavioral components on PC1, which accounted for 67.7% of the variance. For PC1, positive loadings (in red) indicate increased behavioral score, and negative loadings (in blue) are found on Brinkman board clearance time measurements (where lower values indicate improved performance). The loading pattern for PC1 indicates that this component broadly reflects improved behavioral performance. Two more principal components were extracted, with much lower percent variance accounted for (10.4% for PC2 and 5.1% for PC3). The loading patterns for these components did not indicate clearly definable eigenmodules, and scores on these components were therefore not tested. N = 11 subjects, with results confirmed through 1000 iterations of bootstrap subsampling at 60%.



Supplementary Figure 9

Chase Also Increases Density of Axons Originating in Right Motor Cortex

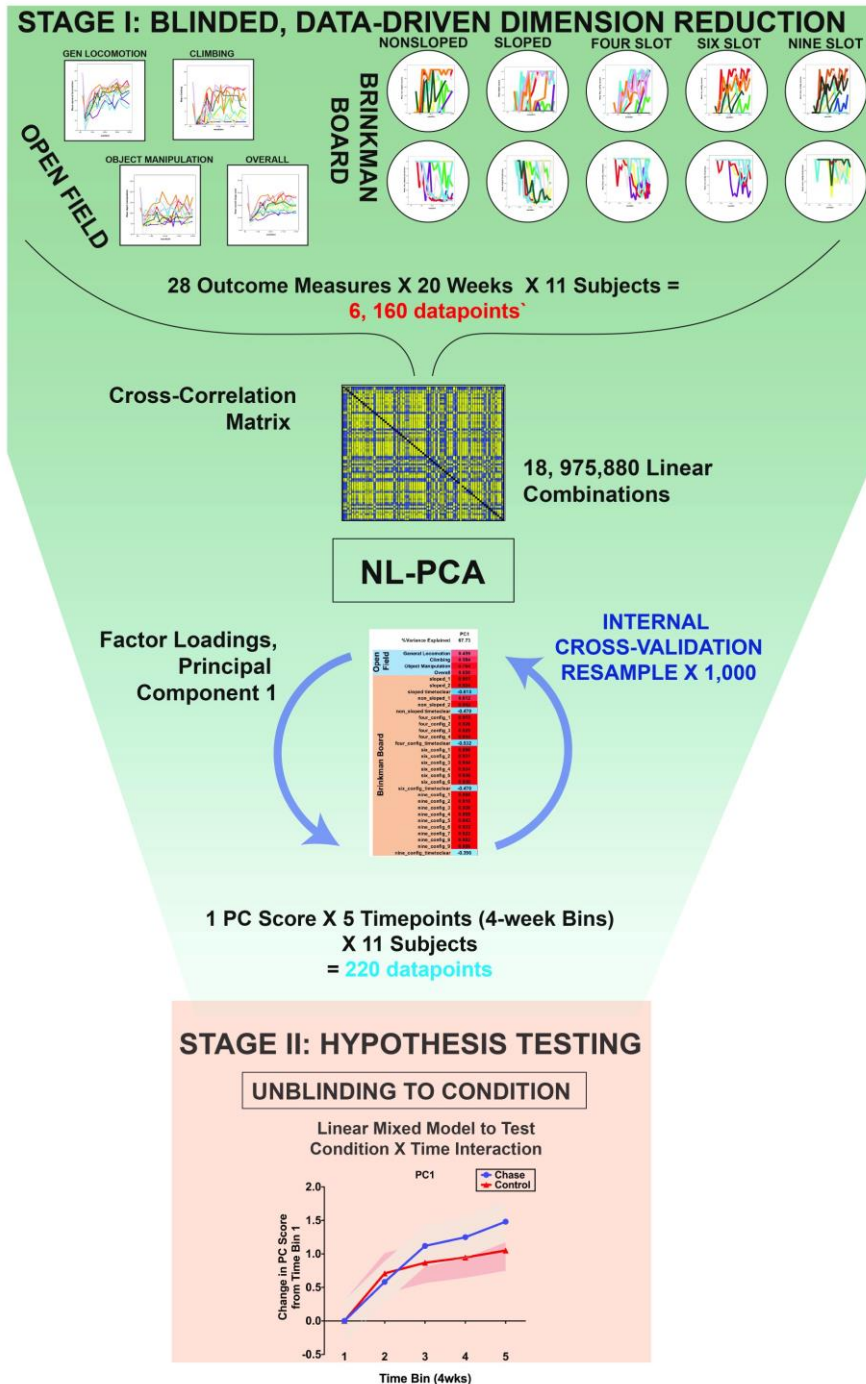
(A) A488-traced CST axons originating in the right motor cortex; 90% of these axons cross midline at the medullary pyramids and descend in the intact left hemicord, hence the greater density of descending axons compared to Fig 4. (B) Some of the axons cross midline near the central canal (CC) and (C) terminate primarily in the ventromedial gray matter. (D) Ventromedial gray matter from control subject. (E) Quantification shows that, just as with axons originating in the left motor cortex, Chase increases the density of these CST axons caudal to the spinal cord hemisection (total length in lesion-side GM per 40 μm section; $P=0.001$, linear mixed model). Immunolabeling and quantification included $N=6$ Chase and $N=5$ controls. Bars represent means, data points are individual subjects, error bars represent linear mixed model SEM. Scale bars: A, 1 mm; B-D, 100 μm .



Supplementary Figure 10

No Chase Effect on Raphespinal Density

Serotonin labeling (5HT) in lesion-side gray matter in transverse sections at C8 in (A) control and (B) Chase-treated subjects. Higher magnification shown in insets. (C) Quantification reveals no differences between treated and control subjects in raphespinal density in motor neuron pools ($P=0.924$, generalized estimating equation). $N=5$ Chase and $N=4$ controls. Bars represent means, data points are individual subjects, error bars represent generalized estimating equation SEM. Scale bars: 200 μm .



Supplementary Figure 11

Non-linear Principal Components Analysis Workflow

Analysis was divided into two distinct stages. In Stage I, we compiled the full behavioral data outcome matrix (open-field measures and Brinkman board measures), and created a cross-correlation matrix of data from from all animals at each timepoint, regardless of condition. Non-linear principal component analysis then determined the extent to which each outcome measure correlated with the

variance explained by the principal component (PC Loadings). The PC loadings were then examined and cross-validated to confirm the resulting PC scores serve as legitimate multidimensional outcome metrics. A PC score was then generated for each animal at each timepoint. In Stage II, we performed a directed, single hypothesis test (linear mixed model), unblinded to the effect of Chase on PC1 score.