Modulation of the proteoglycan receptor PTPσ promotes recovery after spinal cord injury

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Contusive spinal cord injury leads to a variety of disabilities owing to limited neuronal regeneration and functional plasticity. It is well established that an upregulation of glial-derived chondroitin sulfate proteoglycans (CSPGs) within the glial scar and perineuronal net creates a barrier to axonal regrowth and sprouting1–5. Protein tyrosine phosphatase σ (PTPσ), along with its sister phosphatase leukocyte common antigen-related (LAR) and the nogo receptors 1 and 3 (NgR), have recently been identified as receptors for the inhibitory glycosylated side chains of CSPGs6–8. Here we find in rats that PTPσ has a critical role in converting growth cones into a dystrophic state by tightly stabilizing them within CSPG-rich substrates. We generated a membrane-permeable peptide mimetic of the PTPσ wedge domain that binds to PTPσ and relieves CSPG-mediated inhibition. Systemic delivery of this peptide over weeks restored substantial serotonergic innervation to the spinal cord below the level of injury and facilitated functional recovery of both locomotor and urinary systems. Our results add a new layer of understanding to the critical role of PTPσ in mediating the growth-inhibited state of neurons due to CSPGs within the injured adult spinal cord.

Our laboratory has developed an in vitro model of the inhibitory extracellular matrix that forms after spinal cord injury (SCI), wherein adult sensory neurons form dystrophic endballs as they attempt to traverse an increasing gradient of CSPG9. Our previous studies focused on dystrophic growth cones stalled within the CSPG gradient that remained active as they recycled membrane13–15. We now report that chronic exposure to CSPG can induce the development of a distinct over-adhered morphology with no forward motility (Fig. 1a–c and Supplementary Videos 1–3). Any newly formed growth cones rapidlyinvolve into large cones. n = 16 laminin, n = 26 CSPG for both treatments. Error bars show standard error of the mean (s.e.m.). ****P < 0.001, F = 19.9, one-way analysis of variance (ANOVA), Tukey’s post hoc test. g–i. Adult sensory neurons expressing a PTPσ–green fluorescent protein (GFP) plasmid. Dashed line indicates CSPG gradient.

Figure 1 | Immobilization of growth cones within gradients of CSPG.

a–c, Time-lapse imaging in which the growth cones of adult sensory neurons are either motile upon a uniform substrate of laminin (a) or stabilizing within the gradient of proteoglycan (b, c). Arrowhead indicates absorbed growth cone. Timestamp indicates time in minutes. Scale bar, 20 μm. See also Supplementary Videos 1–3. d–j, PTPσ staining in motile or stabilized growth cones.
blebs, resulting in a beaded axon with one tip or multiple side branches, all terminating in small punctate adhesive contacts (Fig. 1c and Supplementary Video 3). This morphology is remarkably similar to that described in the early 20th century by Ramon y Cajal in the chronically injured cat spinal cord

Interestingly, although PTPσ is evenly distributed in a punctate pattern within motile axons and growth cones, it becomes concentrated in dystrophic stabilized growth cones (Fig. 1d–i). As PTPσ observed a large concentration of PTPσ in the lesion penumbra after SCI (Extended Data Fig. 1c, d). As PTPσ co-localizes with adhesion plaques and focal adhesions, we hypothesized that it had a critical role in growth cone immobilization and progression into a dystrophic state. Therefore, we sought to target PTPσ to relieve CSPG-mediated inhibition.

Upon analysing the structure of PTPσ and related phosphatases, we identified a highly conserved 24-amino-acid intracellular wedge domain (Fig. 2a and Extended Data Fig. 2a, b). As wedge domains are known to regulate downstream signalling through a variety of mechanisms, we designed intracellular sigma peptide (ISP), a novel peptide-mimetic of the PTPσ wedge with a TAT domain to facilitate membrane penetration (Fig. 2b).

ISP was able to bind to recombinant human PTPσ (Fig. 2c). In rodent brain and spinal cord lysates, ISP pulled down both immature full-length PTPσ and the mature functional complex (Fig. 2d–f)12. In PTPσ-null mice, only a very minor signal was detected, which may reflect non-specific binding to PTPσ, the third LAR family member (Fig. 2d, e)17. No detectable binding was observed between ISP and other CSPG receptors such as LAR and NgRs (Extended Data Fig. 2e, f). Interestingly, a LAR wedge-domain peptide (ILP)14 was also capable of binding PTPσ, but less efficiently than ISP (Fig. 2d, f and Extended Data Fig. 2c, d).

We next asked whether ISP could release CSPG-mediated axonal inhibition in vitro. ISP treatment allowed adult sensory neurons to extend axons through a CSPG gradient in a dose-dependent manner to the same extent as pre-treatment with chondroitinase ABC (ChABC), which cleaves the glycosylated CSPG side chains and removes the PTPσ ligand (Fig. 2g–i). Time-lapse microscopy revealed that although growth cones treated with ISP were still transiently collapsed by CSPG, they continued to reform growth cones, allowing them to eventually cross the gradient (Fig. 2k and Supplementary Video 4). Additionally, both ISP and ChABC treatments were sufficient to convert already stabilized dystrophic growth cones into a motile state (data not shown). Interestingly, human ISP and the wedge domain peptides of PTPσ and LAR (IDP and ILP) demonstrated some efficacy, suggesting functional redundancy among LAR

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**Figure 2** | **Identification and characterization of ISP.**

**a.** PTPσ structure and wedge domain (red). **b.** Peptide sequences. c–f, Pulldown of human, rat and mouse PTPσ with biotinylated ISP (bISP). Asterisk indicates non-specific recognition of PTPσ. ICD, intracellular domain; D1, PTPσ domain I; D1D2, PTPσ domain I–2; Lad, ladder (kilodaltons); r, recombinant; WT, wild type. Four repetitions. g–i, CSPG gradient crossing assay. Dashed lines indicate CSPG gradient. Scale bar, 50 μm; n > 16 gradients per group. hISP, human ISP; sISP, scrambled ISP; j, ISP treatment on PTPσ-null neurons (n = 12 per group, 3 repetitions). k, Time-lapse imaging of an adult sensory neuron growth cone after 2.5 μM ISP treatment (Supplementary Video 4). Timestamp indicates time in minutes. Scale bar, 20 μm. l, The number of neurons released from a CSPG-rich substrate after agitation (n = 28 vehicle/ILP, n = 16 ISP wells per group). Scale bar, 50 μm. Error bars show s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, one-way ANOVA, Tukey's post hoc test. For additional sample size information, see Methods.

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family wedge domains. The wedge domain of PTPσ (IMP), scrambled ISP, TAT alone, or ISP without TAT were ineffective (Fig. 2h).

Importantly, treatment of adult PTPσ-null sensory neurons with ISP did not further increase crossings (Fig. 2j). In addition, mature astrocytes, which have immunocytochemically undetectable PTPσ, were unable to traverse the CSPG gradient after ISP treatment (Extended Data Fig. 3a). However, PTPσ-expressing satellite glia were induced to cross successfully (Extended Data Fig. 3b). These results suggest specificity of ISP interactions through PTPσ.

ISP concentrations above the optimal dose for gradient crossing greatly reduced neuronal cell attachment, suggesting a reversal of the over-adhered phenotype. ISP treatment decreased interactions between the CSPG-rich substrate and PTPσ, allowing cells to detach in response to agitation (Fig. 2l and Extended Data Fig. 3c, d). This suggests that appropriate levels of adhesion are critical for regeneration across increasing concentrations of innately inhibitory CSPGs.

The extracellular-signal-regulated kinase 1/2 (Erk1/2) cascade regulates a variety of processes, including axonal growth. Erk1/2 phosphorylation was decreased in neuronal cells grown on a CSPG-rich substrate and both ISP and ChABC were able to restore the phosphorylation state of Erk1/2 to levels comparable to that on laminin-only substrates (Extended Data Fig. 4).

TAT-conjugated peptides are known to cross biological membranes, including the blood–brain barrier. One hour after a single subcutaneous injection of fluorescein isothiocyanate (FITC)-conjugated ISP, we were able to visualize ISP in the intact nervous system (Extended Data Fig. 4a). Therefore, we chose a non-invasive systemic treatment paradigm using daily subcutaneous injections, avoiding complications associated with intraparenchymal delivery. Beginning 1 day after contusive SCI, rats were treated with ISP (11 µg per day), ILP (11 µg per day) or vehicle once daily for 7 consecutive weeks (Extended Data Fig. 5c–e).

SCI disrupts connections between the bladder and brainstem micturition control centre, leading to reduced void frequency and an accompanying increase in void volume (Extended Data Fig. 6a, b). Twelve weeks after injury, ISP promoted a significant twofold increase in void frequency versus controls, along with a significant decrease in void volume (Fig. 3a–c and Extended Data Fig. 6c–h). We used urodynamics to assess whether this improvement was a result of physiologically normal bladder activity. Rats urinate by contracting the detrusor muscle, resulting in a rapid increase in bladder pressure while the external urethral sphincter

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**Figure 3 | Functional recovery after ISP treatment.**

a–c. Void frequency and average void volume at 12 weeks after SCI. d–h. Representative urodynamic recordings of detrusor activity (bladder pressure, top trace) and EUS activity (bottom trace, expanded at the points marked with an asterisk).

i. Full trace of EUS activity. Arrows indicate synchronized phasic bursting; arrowhead indicates single burst.

j–k. Locomotor recovery (BBB score) after SCI. l–m. Gridwalk test at 12 weeks after SCI. n. ISP functional recovery Venn diagram (21/26 ISP, 0/21 vehicle, 1/10 ILP). Error bars show s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, repeated measures two-way ANOVA, Tukey’s post-hoc test (BBB); one-way ANOVA, Kruskal–Wallis post-hoc test (gridwalk and micturition). NS, not significant. Black indicates ISP versus control; red indicates ISP versus ILP.
(EUS) bursts phasically, expelling urine (Fig. 3d)²⁵. No vehicle- or ILP-treated animals displayed multiple coordinated bursts of the EUS during a void (Fig. 3c, h). Although the detrusor was still hyperactive, 10 of the 15 analysed ISP-treated animals recovered coordination between bladder contractions and EUS phasic bursting, suggesting reconnection of a functional circuit (Fig. 3f, g)²⁶.

We measured locomotor recovery using the Basso, Beattie and Bresnahan (BBB) scale and a gridwalk test²⁷. After SCI, vehicle- and ILP-treated animals recovered from hindlimb paralysis at day 1 to, on average, occasional weight bearing stepping at week 11 (Fig. 3i). ISP treatment resulted in a significant progressive recovery of locomotion, which began several weeks after injury (Fig. 3i). Thirty per cent of ISP-treated animals (versus zero vehicle/ILP-treated animals) demonstrated, at least, frequent coordinated stepping (BBB ≥ 13), with three rats achieving BBB scores ≥17.5 (Fig. 3j–k). Furthermore, ISP-treated animals made on average 58% fewer foot faults than control rats on the gridwalk test (Fig. 3l, m), suggesting recovery of sensorimotor coordination and balance. Importantly, aside from minor irritation at the injection site after multiple weeks of treatment, ISP did not induce neuropathic pain (Extended Data Fig. 7a, b).

Interestingly, no correlation between ISP-induced recovered behaviours was observed (Extended Data Fig. 7c–e). Using stringent threshold analyses, we determined that 21 of 26 ISP-treated animals recovered at least one behaviour, with 3 animals recovering all three (Fig. 3n). Further analyses demonstrated the degree to which responding animals benefited from treatment (Extended Data Fig. 7f–h). Taken together, this suggests that the re-acquisition of each behaviour is modular and not coincident, and may reflect anatomical differences in the pattern of axon re-innervation.

The behavioural results represent the average of five repetitions, each performed with newly synthesized peptide, blinded behavioural testers and a separate blinded treatment administrator. Although variability existed, ISP increased functional recovery of each behaviour in all cohorts (Extended Data Fig. 8). In a final group of animals, urinary, but not locomotor behaviours, responded to increasing concentrations of ISP, with our maximum (44 µg per day) dose improving urination markedly in all rats (Extended Data Fig. 7g). Therefore, further optimization of the dose or administrative route of ISP may lead to additional functional improvements.

ISP treatment was not neuroprotective, as it did not lead to differences in lesion size or spared white matter (Fig. 4a, b). At the individual animal level, the variability in spared tissue correlated with functional recovery in vehicle-, but not ISP-treated rats (Extended Data Fig. 9). Although regenerative pathways are difficult to examine after con- tusive SCI²⁸, we did not observe any lengthy regenerating biotinylated dextran amine (BDA)-labelled corticospinal tract fibres (data not shown).

We focused further analysis on the serotonergic system (5HT) neurons, which express LAR family receptors and contribute to proper neuromodulatory tone in locomotion and micturition circuitry⁷,²⁶,²⁹,³⁰. Contusive SCI led to a marked decrease in descending 5HT-positive fibres caudal to the lesion (Fig. 4c, e, h). In animals exhibiting functional recovery after ISP treatment, we observed unusually shaped, densely sprouted territories of 5HT fibres below the level of the lesion (Fig. 4d, f, i–n). These patterns corresponded in part with neurofilament staining (Extended Data Fig. 10), but were not present in sections stained for GFAP, ED1 or 4’,6-diamidino-2-phenylindole (DAPI), ensuring that the sharp edges of staining were not due to tissue folds. We speculate that physical constraints of the perineuronal net may confine fibres in these patterns. Their spatial variability, in conjunction with differences in tract sparing from animal to animal after contusion, could partially account for the disparity in behavioural recovery between animals.

Treatment with the 5HT receptor antagonist methysergide at 14 weeks after SCI significantly reduced locomotor and urinary function in ISP-treated, but not vehicle-treated animals (Fig. 4o, p)²⁶,²⁹. This was most

Figure 4 | Anatomical changes after ISP treatment. a, b, Average lesion size (GFAP) and spared white matter (eriochrome cyanine (EC) staining) after SCI. n = 9–10 vehicle, n = 14 ISP. c, d, 5HT intensity in representative longitudinal spinal cord sections. Insets reveal fibre density. Scale bar, 2 mm; inset, 20 µm. e, f, Representative confocal projections of caudal 5HT fibres at L1–L3. Scale bar, 20 µm. g, l, 5HT intensity across lumbar coronal sections. Scale bar, 500 µm. m, n, Average area and percent coverage of 5HT. n = 13 vehicle, n = 18 ISP. P < 0.05, Student’s t-test (two tailed). o–q, Behavioural response to methysergide at 14 weeks after SCI. ‘Responders’ are animals demonstrating functional recovery in each behaviour, n = 8 vehicle, n = 11–12 ISP. Error bars show s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001, two-way repeated measures ANOVA, Tukey’s post-hoc test (BBB); one-way ANOVA, Kruskal–Wallis post hoc test (gridwalk and void frequency). NS, not significant.

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evident in ISP responders, animals that regained function beyond recovery thresholds. However, behavioural improvements were not fully reverted to vehicle levels and gridwalk scores were only minimally affected (Fig. 4q), suggesting plasticity of other pathways outside the serotonegic system.

While CSPGs have largely been thought to act as repulsive components of the extracellular matrix, our results suggest that regenerating adult growth cones can become permanently immobilized by CSPG gradients. These observations highlight an initial cellular mechanism regulated by PTPσ that leads to the development of axonal dystrophy and the prevention of chronic regeneration and plasticity in vivo. Systemic modulation of PTPσ opens a new therapeutic avenue in non-invasive treatments for enhancing functional recovery after a variety of injuries or diseases in which proteoglycans inhibit the attempt of axons to regenerate or sprout.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 8 July; accepted 16 October 2014. Published online 3 December 2014.

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Supplementary Information is available in the online version of the paper.

Acknowledgements This work was supported by National Institute of Neurological Disorders and Stroke grant NS025713 (J.S.); the Case Western Reserve University Council to Advance Human Health; P. Jing, R. Senior and S. Poon; Unite to Fight Paralysis; The Brumagin Memorial Fund; Spinal Cord Injury Sucks; United Paralysis Council to Advance Human Health; P. Jing, R. Senior and S. Poon; Unite to Fight Paralysis; The Brumagin Memorial Fund; Spinal Cord Injury Sucks; United Paralysis Council to Advance Human Health; P. Jing, R. Senior and S. Poon; Unite to Fight Paralysis; The Brumagin Memorial Fund; Spinal Cord Injury Sucks; United Paralysis Council to Advance Human Health; P. Jing, R. Senior and S. Poon; Unite to Fight Paralysis; The Brumagin Memorial Fund; Spinal Cord Injury Sucks; United Paralysis Council to Advance Human Health; P. Jing, R. Senior and S. Poon; Unite to Fight Paralysis; The Brumagin Memorial Fund; Spinal Cord Injury Sucks; United Paralysis Council to Advance Human Health; P. Jing, R. Senior and S. Poon; Unite to Fight Paralysis; The Brumagin Memorial Fund; Spinal Cord Injury Sucks; United Paralysis Council to Advance Human Health; P. Jing, R. Senior and S. Poon; Unite to Fight Paralysis; The Brumagin Memorial Fund; Spinal Cord Injury Sucks; United Paralysis Council to Advance Human Health; P. Jing, R. Senior and S. Poon; Unite to Fight Paralysis; The Brumagin Memorial Fund; Spinal Cord Injury Sucks; United Paralysis Council to Advance Human Health; P. Jing, R. Senior and S. Poon; Unite to Fight Paralysis; The Brumagin Memorial Fund; Spinal Cord Injury Sucks; United Paralysis Council to Advance Human Health; P. Jing, R. Senior and S. Poon.
Peptide sequences are as follows: ISP, NH2-GRKKRRQRRRCDMAEHMERLKA
Lyophilized peptides were dissolved in sterile water and stored at
6°C for 3 h. Dissociated DRG neurons were plated at a density of 1,000 cells cm⁻².

Dish preparation and time-lapse microscopy. Delta-T-cell culture dishes were prepared as previously described. Culture dishes were rinsed with sterile water, and then coated with poly-L-lysine (0.1 mg ml⁻¹, Sigma-Aldrich) overnight at room temperature, rinsed with sterile water, and allowed to dry. Aggrecan spot gradients were formed by allowing 2 μl of aggrecan solution (2 mg ml⁻¹ in HBSS-CMF, Sigma-Aldrich) to dry onto the culture surface. The surface of the dish was bathed in laminin solution (10 μg ml⁻¹ in HBSS-CMF, Invitrogen) for 3 h at 37°C. The laminin bath was subsequently removed and cells were plated without allowing the surface of the dish to dry. For conditions with laminin alone, the aggrecan spots were excluded from the protocol.

Prior to time-lapse imaging, adult neurons were incubated at 37°C for 4–6 days in Neurobasal-A media with either vehicle (water) or 25 μM peptide. Neurobasal-A media with HEPEs (50 μM, Sigma-Aldrich) and either vehicle or 25 μM peptide was added to the cultures before imaging in a heated stage apparatus. Time-lapse images were acquired every 30 s for at least 1.5 h with a Zeiss Axiovert 405M microscope using a heated ×100 oil-immersion objective. Growth cones that extended normal to the spot rim were chosen for analysis. We tracked and charted the behaviour of growth cones in our in vitro assay with Metamorph software. n = 19 laminin (5 μg ml⁻¹) and aggrecan (25 μg ml⁻¹) bath

PTPα concentration quantification. High-magnification images of PTPα expression (R&D Systems, 1:100) in growth cones, either motif on uniform substrates of laminin (5 μg ml⁻¹) or dystrophic within the CSPG gradient were analysed with ImageJ. Both the growth cone and axon were manually traced and the mean pixel intensity was calculated. All images were taken using identical settings.

CSPG gradient crossing assay. CSPG gradients were prepared as described previously. Glass coverslips (1.6 mm, Fisher Scientific) coated with poly-L-lysine and nitrocellulose were spotted with a 2 μl solution of aggrecan (0.7 mg ml⁻¹) and laminin (5 μg ml⁻¹) in HBSS-CMF (4 spots per coverslip). After the spots were allowed to dry, the coverslips were incubated with laminin (5 μg ml⁻¹) in HBSS-CMF at 37°C for 3 h. Dissociated DRG neurons were plated at a density of 1,000 cells cm⁻² in Neurobasal-A supplemented with B27, Glutamax and penicillin/streptomycin and incubated for 5 days at 37°C.

For peptide experiments, appropriate concentrations of peptide were added to the media at the time of plating. For ChABC experiments, 0.1 μM ChABC (Seikagaku) was added to coverslips for 2 h after the laminin bath before cell plating.

At 5 days, cultures were fixed in 4% paraformaldehyde in PBS for 30 min. After several rinses in PBS, the coverslips were incubated in blocking solution (5% normal goat serum, 0.1% BSA, and with or without 0.1% Triton X-100 in PBS) for 1 h at room temperature and then incubated overnight at 4°C in primary antibody. Anti-IIIl-tubulin (1:500; Sigma-Aldrich), anti-CSPG (1:100; Sigma-Aldrich), anti-PTPα (1:100, R&D systems), anti-GFP (1:500, Invitrogen), anti-sI00 (1:1,000, Sigma-Aldrich), anti-GFAP (1:500, DAKO), anti-NgR (1:100, Millipore) and anti-LAR (1:100, Santa Cruz) were used as primary antibodies.

Coverslips were rinsed several times in PBS and then incubated in the appropriate secondary antibody (Molecular Probes) overnight at 4°C. Coverslips were rinsed with PBS again, and mounted on glass slides in Citifluor (Ted Pella) mounting medium. Specimens were examined using a Leitz Orthoplan 2 fluorescence microscope.

Wedge identification. The intracellular domains of rat PTPα (Protein Data Bank (PDB) accession 2HFT) and human LAR (PDB accession 1RPM) were visualized using UCSF Chimera 1.6. The wedge domains of human, rat and mouse PTPα and PTP6 were identified by BLAST alignment with the known wedge domain of LAR.

Peptide preparation. Peptides were synthesized commercially with C-terminal amidation (GenScript), and purity was assessed >98% by mass spectrometry. Lyophilized peptides were dissolved in sterile water and stored at -80°C until use. Peptide sequences are as follows: ISP, NH2-GRKKRRQRRRCDMAEHMERLKA

SDS–PAGE and western blot. Thirty microtubes of the pulldown material with 4× Laemmli Buffer was boiled for 10 min at 100°C and loaded into 7.5% TBI Mini-Protean Gels (Bio-Rad 456-1029) with Bio-Rad Precision Plus Protein Standard (Bio-Rad 161-0374). The gel was run for about 1.5 h at 100 V in 1× Tris Glycine SDS Buffer. Gels were stained with Sypro Ruby Red (Sigma-Aldrich) to visualize proteins within the gel. Transfer occurred overnight at 15 V using a PVDF membrane. After transfer, bands at 2 h in 5% milk power, 0.1% Tween-20 before overnight incubation with an antibody against GST (Cell Signaling), PTPα ICD (1:100, Abnova), PTPα ECD (1:1,000, Abcam), anti-NgR (1:500, Millipore) or anti-LAR (1:1,000, R&D Systems). We washed five times for 5 min in 1× PBS-0.1% Tween-20 before blocking overnight with a horseradish peroxidase (HRP)-conjugated secondary (1:1,000). The blot was developed using a chemiluminescence substrate (Thermo Sci) after five times five 1× PBS-0.1% Tween-20 washes. All experiments were repeated >3 times.

CSPG signalling

Plating SH-SY5Y cells on laminin and CSPG substrates. The SH-SY5Y neuroblastoma cell line (ATCC) was grown in Hyclone Dulbecco’s modified Eagle’s high-glucose media (GE Healthcare Life Sciences, SH-30081.02) supplemented with 4 mM-glutamine, 1 mM sodium pyruvate, 1% penicillin/streptomycin/neomycin (PSN) and 10% heat-inactivated fetal bovine serum (Invitrogen). SH-SY5Y cells were plated at an initial density of 12,000 cells cm⁻² onto tissue culture surfaces for
Dorsal column crush injury. Cells were harvested from culture plates 4 days after cell plating and homogenized in RIPA buffer (Thermo Fisher) containing SigmaFast Protease Inhibitor (Sigma). A total of 30–50 μg protein was loaded into the gel and then transferred to a nitrocellulose membrane (Bio-Rad). The membranes were then blocked in 5% non-fat milk in Tween Tris buffered saline (TBST) for 1 h and incubated overnight at 4 °C with P-p44/42 MAPK (Cell Signaling, Rabbit 1:500) diluted in the blocking solution. The membranes were washed and incubated with HRP-conjugated goat anti-rabbit antibodies (1:4,000, Biorad). Membranes were then incubated in ECL plus immunoblotting detection reagents (Thermo Scientific Pierce) according to the manufacturer’s specifications. Blots were then stripped of their primary and secondary antibodies for 30 min in 0.2 M NaOH and re-probed with primary antibody pp44/42 MAPK (Erk1/2) (Cell Signaling, Rabbit 1:1,000) overnight followed by incubation with secondary antibody and ECL.

Quantification. Immunoreactive bands were quantified using AlphaEaseFC (FluoroChem, 8900). The ratio of phosphorylated (p)Erk1/2 to total (t)Erk1/2 for each condition was calculated. Data are reported as means ± s.e.m., and P < 0.05 was considered significant. Statistical analyses of intensity measurements were tested by one-way ANOVA comparing conditions followed by post-hoc pairwise multiple-comparison testing by the Holm–Sidak method.

Animals and contusive SCI

Animals. Adult female Sprague–Dawley rats (225–250 g) were obtained from Harlan. All procedures were approved by the Institutional Animal Care and Use Committee. PTPre-null mice were provided by M. Tremblay. All PTPre-null experiments were performed in collaboration with Y. Shen.

Contusive SCI. Briefly, adult female Sprague–Dawley rats (230–250 g) were obtained from Harlan and acclimated to the animal resource centre, behaviour analysis chambers and handlers. Rats were injected intraperitoneally with ketamine (60 mg kg⁻¹) and xylazine (10 mg kg⁻¹). The musculature was cut from T7–T9 and the dorsal surface of T8 was exposed by laminectomy. The vertebral column was stabilized by clamping the T7 and T9 vertebral bodies with forceps fixed to the base of an Infinite Horizon Impact Device. The animals were situated on the platform, and the 2.5 mm stainless steel impactor tip was positioned over the mid-point of T8 and impacted with 250 kdyn force. The overlying musculature was closed using sutures, the skin was closed using wound clips and the animals were treated with Marcaine at the incision site. The force/displacement graph was used to monitor impact consistency and any closed using wound clips and the animals were treated with Marcaine at the incision site. The force/displacement graph was used to monitor impact consistency and any.
respectively, and signal was sampled at a rate of 10 kHz (Power 1401, Spike2: Cambridge Electronic Design). Continuous cymotystograms (CMGs) were collected using constant infusion (6 ml h⁻¹) of room temperature saline (Aladin-1000 single syringe infusion pump; World Precision Instruments) through the catheter into the bladder to elicit repetitive voids. The bladder pressure was recorded via the same catheter used for saline infusion, using a pressure transducer (Grass Technologies) connected to the recording system and sampled at a frequency of 2 kHz. Animals that received methysergide did not receive urodynamic analysis. n = 11 vehicle, 15 ISP, 6 ILP.

**Immunocytochemistry and tracing**

**Perfusion and sectioning.** To obtain spinal cord sections, rats were transcardially perfused with ice-cold 4% paraformaldehyde in PBS, and the spinal cords were dissected out. After the tissue was postfixed in 4% paraformaldehyde overnight at 4°C and cryoprotected with 30% sucrose, spinal cords were frozen in OCT mounting media and sectioned on a Hacker cryostat at a thickness of 20 μm.

**Immunocytochemistry.** Mounted sections were washed three times with PBS followed by blocking in 5% normal goat serum (NGS) and 0.1% bovine serum albumin (BSA) in PBS. 0.1% Triton X-100 was added to the blocking buffer depending on the antigen used. After blocking, sections were incubated in primary antibody diluted in blocking buffer overnight at 4°C. Primary antibodies used were mouse anti-NeuN (1:100, Chemicon), anti-GFAP (1:1,000, Dako), mouse anti-ED1 (Chemicon, 1:100) rabbit anti-5-HT (1:500, Immunostar), and anti-neurofilament (1:500, Sigma-Aldrich). For in vivo PTPζ staining, sagittal 20 μm sections encompassing regions both rostral and caudal to the lesion were probed with anti-PTPζ antibody (1:500, Abnova). The next day, the sections were washed extensively with PBS and incubated in the appropriate secondary antibody or avidin substrate conjugated to AlexaFluor 488, 594 or 633 (1:500, Molecular Probes) overnight. After extensive washing, the sections were stained with DAPI (1:1,500 in PBS, Sigma-Aldrich), washed, coverslipped and viewed with a confocal microscope (Zeiss, Germany). Pixel intensity was measured on images taken on a standard fluorescent microscope (Leica) with a uniform exposure setting and analysed using ImageJ.

**White matter analysis.** Spared white matter analysis was conducted by EC staining as previously published. Briefly, room temperature 20 μm sections were placed in fresh acetic for 10 min, removed and allowed to dry for 30 min. Sections were stained with freshly filtered EC solution (Sigma-Aldrich) for 30 min and washed in running tap water for 5 min. The stain was differentiated in 5% ferric ammonium sulphate (Sigma-Aldrich) for 15 min and again washed with running tap water for 5 min. The differentiation was completed with borax-ferricyanide solution (Sigma-Aldrich) for 10 min, briefly washed with running tap water and allowed to dry. Slides were dehydrated in 70%, 95% and 100% ethanol for 2 min each followed by xylene for 2 min. Slides were crosslinked with a VectaMount Permanent Mounting Medium (Vector Laboratories). n = 10 vehicle, 14 ISP.

**Lesion volume.** Spinal cord sections stained with GFAP were analysed for lesion volume. After staining, sections were digitalized with a Leica SCN 400 Slide Scanner. Fifteen 20 μm coronal sections (one of every ten serial sections was stained, 200 μm between sections) both rostral and caudal of lesion epicentre were analysed for a total of 6 mm of spinal tissue. Sections were traced in ImageJ for volume calculation. n = 9 vehicle, 14 ISP.

**SHT analyses.** Coronal sections of lumbar spinal cord were analysed for SHT intensity. The staining and imaging settings were uniform for all images. High exposure settings were used to maximize the signal to noise ratio, allowing for large patterns of SHT expression to be visualized. ImageJ threshold analysis was used to eliminate all background from each section, leaving only the patterns of SHT expression. The area of innervation and per cent coverage of the grey matter was identified by analysing all particles in the grey matter at threshold intensity. Eight 20 μm sections, (corresponding to 1.6 mm of lumbar tissue) were analysed in ImageJ and averaged, with the highest and lowest intensity removed. n = 13 vehicle, 18 ISP.

**Tracing.** Cortical spinal tract labelling was performed as published previously. Ten per cent BDA (Molecular Probes) was injected into 16 locations in the rat motor cortex at 12 weeks after injury. Animals were killed 2 weeks after labelling. BDA labelling was visualized using an avidin–biotin peroxidase incubation followed by diaminobenzidine and H2O2 (Vector Labs).

**Statistical analysis.** All statistical analyses were performed using Graphpad Prism. All results are presented as mean ± s.e.m. Sample sizes were initially determined using statistical software to calculate the minimum total required number of animals or assays. All reported groups are above the minimum calculated sample size.

**In vitro.** For PTPζ intensity measurements, gradient crossings and adhesion assays, all statistical analysis was performed with one-way ANOVA between all groups in each individual experiment.

**In vivo.** For void frequency, void volume and gridwalk data sets, D’Agostino–Pearson and Shapiro–Wilk tests were first performed to determine if any individual data set abided to a normal, Gaussian distribution. Naïve, vehicle control and ILP all passed the normality test, while ISP failed in all. Next, we performed ROUT analysis to identify outliers. No values were excluded from analysis, although a single ILP void frequency data point was identified as an outlier. Statistical analysis on these behaviours was performed with a one-way ANOVA and post-hoc Kruskal–Wallis test. For BBB, we performed a repeated measures two-way ANOVA to compare vehicle, ILP and ISP. No normality tests were performed because BBB is a nonlinear scale. Post-hoc analysis at individual time point was performed with Tukey’s test.

After methysergide treatment, BBB analysis was performed with repeated measures two-way ANOVA. For both gridwalk and void frequency, we compared pre-methysergide to post-methysergide treatment with a two-way ANOVA. The analysis between responding and non-responding animals was performed separately.

**Anatomy.** SHT area and per cent grey matter coverage, the average value over the eight lumbar sections of ISP and vehicle control were compared with a Student’s t-test.

**Regression analysis.** Regression analysis was performed by creation of a matrix of behavioural and anatomical scores for each animal. A separate matrix was created for vehicle and ISP treatment. Regression values (r) were identified by comparing two individual variables. The Pearson or Spearman coefficients were identified for parametric and non-parametric data sets, respectively.

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Extended Data Figure 1 | CSPG receptors LAR and NgR in vitro and PTPσ in vivo. **a**, LAR expression in motile (left, on laminin) and immobilized (right, within CSPG gradient) growth cones. **b**, Nogo receptors in a soma and axons. Scale bars, 20 μm. **c, d**, PTPσ expression in the spinal cord 14 days after dorsal column crush injury. The arrowhead pointing upwards represents a labelled structure with dystrophic morphology. Scale bar, 50 μm.
Extended Data Figure 2 | LAR structure, sequence alignment and pulldown analysis. a, BLAST alignment of the known sequences of mouse, rat and human LAR, PTPø, PTPδ and PTPμ. The wedge domain of each protein is aligned within the box. b, The tandem intracellular phosphatase domains of human LAR with the previously characterized wedge domain (red)\textsuperscript{14}. c, d, Pulldown of recombinant PTPø with biotinylated (b)ISP or ILP. e, f, Eluted lysate after pulldown was probed with antibodies against either LAR or pan-NgRs. Input is 10% lysate control.
Extended Data Figure 3 | Astrocyte and satellite cell response to ISP and the adhesion assay. a, Purified GFAP-positive mature astrocytes (green) did not respond to ISP. b, S100-positive satellite glia were able to cross the gradient of CSPG after ISP treatment. Scale bar, 50 μm. c, d, Response of neurons and axons upon a CSPG-rich substrate to agitation after ISP treatment. Scale bar, 50 μm.
Extended Data Figure 4 | CSPG and ISP regulation of Erk1/2. Western blot analysis revealed a significant decrease in the phosphorylation ratio of Erk1/2 in SH-SY5Y cells plated on laminin (2 μg ml$^{-1}$) plus CSPGs (15 μg ml$^{-1}$) compared with laminin-only substrates, which was reversed by either pre-treatment with ChABC (0.1 U ml$^{-1}$) or 4 days of ISP treatment (2.5 μM). pErk1/2, phosphorylated Erk1/2; tErk1/2, total Erk1/2. Data normalized to laminin control. $N = 4$ independent experiments. *$P < 0.05$ versus all other conditions, one-way ANOVA, Tukey’s post-hoc test.
Extended Data Figure 5 | FITC-ISP in vivo and infinite horizon impaction.

**a**, **b**, Spinal cord 1 h after subcutaneous injections of FITC-ISP or vehicle. Scale bar, 100 μM.

**c**, Experimental design and timeline for in vivo experiment.

**d**, **e**, The force and impaction velocity of all animals that received an infinite horizon contusive injury. All impactions are within 10% from the target force of 250 kdyn, with an average force of 258.2 for both ISP and vehicle.
Extended Data Figure 6 | Metabolic cage analysis at 6 weeks after injury.

a, b, Void frequency and average void volume at 6 weeks after injury. n = 11 naive, 21 vehicle, 10 ILP and 26 ISP. c–h, Representative smoothed metabolic cage traces of a normal animal (c) and five treated animals (vehicle (d), ILP (e) and ISP (f, h)) 12 weeks after injury. Void volume is plotted (in ml) as a function of time. Scale bar, 4 h.
Extended Data Figure 7 | Additional behavioural data and analyses.

a, b, Average response to thermal (Hargrave’s test) and mechanical (Von Frey test) stimuli at 12 weeks after injury (n = 11 naive, 21 vehicle, 10 ILP and 26 ISP for thermal, n = 10 vehicle, 4 ILP and 10 ISP for mechanical).

c–e, Correlations between recovery of each individual motor behaviour in the ISP treatment group.
f–h, For each behaviour, the ISP-treated animals that recovered to two standard deviations relative to vehicle mean were separated and plotted as ‘responders’ while those that did not were plotted as ‘non-responders’. The n for the responding and non-responding group for each behaviour is listed on the graph.
i, In vivo ISP dose–response plot in a single cohort of animals. A dose-dependent increase occurred in void frequency at 12 weeks after SCI.
Extended Data Figure 8 | Five repetitions of in vivo experiments. a–c, The individual results of five repeats of in vivo experiments are plotted as individual cohorts of animals for BBB, gridwalk and void frequency. *P < 0.05, **P < 0.01, repeated measures two-way ANOVA, Tukey’s post-hoc test (BBB); one-way ANOVA, Kruskal–Wallis post-hoc test (gridwalk and void frequency). Black indicates ISP versus control; red indicates ISP versus ILP. n for each condition is listed.
Extended Data Figure 9 | Correlation between spared tissue and behavioural recovery. a–l, Spared tissue volume (as measured by GFAP-positive tissue) or area of spared white matter at the epicentre (as measured by eriochrome cyanine staining) were plotted against behavioural scores for vehicle- (a–f) and ISP-treated (g–l) animals. Pearson’s correlation coefficient (r value) is reported for each comparison. Only animals whose spinal cord was processed and cut coronally were included in the analysis.
Extended Data Figure 10 | Neurofilament staining at lumbar levels.
a–f, Representative caudal neurofilament expression in lumbar spinal cord. Responders are ISP animals that demonstrated functional improvement (see Fig. 3n). All images were taken using identical settings. Scale bar, 500 μm.