Published in final edited form as:

Ann Biomed Eng. 2019 March ; 47(3): 744–753. doi:10.1007/s10439-019-02198-0.

Automated Gait Analysis Detects Improvements after Intracellular σ Peptide Administration in a Rat Hemisection Model of Spinal Cord Injury

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Abstract

A promising treatment strategy for spinal cord injury (SCI) is to reduce inhibition from chondroitin sulfate proteoglycans (CSPGs). For example, administering intracellular σ peptide (ISP) can improve the ability of axons to cross inhibitory CSPGs and improve function in rodent models of SCI. To translate such treatments into the clinic, we need robust and sensitive methods for studying rodent models. In this study, we applied a newly developed suite of quantitative gait analysis tools, GAITOR (gait analysis instrumentation and technology optimized for rodents), which consists of an arena and open-source software (AGATHA: automated gait analysis through hues and areas). We showed that GAITOR can be used to detect subtle functional improvements (measured by hindlimb duty factor imbalance) in rats following ISP administration in a T10 hemisection injury model. We demonstrated that SCI-specific parameters (right paw placement accuracy and phase dispersion) can be easily added to GAITOR to track recovery. We confirmed the gait observations via retrograde tracer uptake. We concluded that GAITOR is a powerful tool for measuring recovery after moderate/mild SCI, and could be used to replace expensive/inflexible commercially-available gait analysis techniques.

Keywords

Spinal cord injury; gait analysis; hemisection; intracellular sigma peptide; behavioral analysis

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Conflict of Interest

No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

1 Introduction

Traumatic spinal cord injury (SCI) leaves patients with a permanent impairment, as there is no known clinical method for functionally regenerating spinal cord tissue. Following an initial injury (e.g., a mechanical impact or stab wound), the cellular response is chaotic, and a variety of secondary events occur over different timescales³. Importantly, a permanent barrier scar is formed around the damaged tissue. Scar formation is driven by different cell types, both CNS-intrinsic and extrinsic². Infiltrating cells, such as type A pericytes, locate within the center of the lesion¹⁴. These are surrounded by reactive astrocytes, which deposit inhibitory chondroitin sulfate proteoglycans (CSPGs). Since these reactive astrocytes, and the barrier scar itself, play a beneficial role in the limited recovery that does take place (by compacting the lesion area and supporting the remaining tissue)¹², a potential treatment target is to attack the inhibitory components of the scar while allowing the physical scar matrix to remain in place. One such strategy utilizes intracellular σ peptide (ISP), a membrane-permeable peptide which reduces inhibition from CSPGs by inhibiting receptor PTP σ and has been shown to improve locomotor recovery following contusive SCI⁹. However, its ability to promote recovery from a lateral hemisection SCI (in which half of the spinal cord is cut and removed) is unknown.

One of the primary outcomes in any SCI treatment is to evaluate how it improves locomotion. In rats, which are the predominant laboratory SCI model, it can be difficult to distinguish differences during the later stages of recovery after mild to moderate SCI. Quantitative gait analysis has been commonly employed as an alternative or complimentary technique to standard, observer-scored techniques such as the Basso, Beattie, and Bresnahan (BBB) score¹. Quantitative gait analysis allows researchers to study subtle changes in gait as an animal recovers, which may not be obvious using observer-scored techniques that are conducted in real time. This allows the investigation of how a treatment affects an individual limb (e.g., in a unilateral injury) and/or the isolation of spatial and temporal parameters. Additionally, quantitative analyses remove observer bias from studies that cannot be conducted in a double-blinded manner. However, there are issues with current techniques, as they may be closed source, require manual scoring, or involve a treadmill (which inherently affects locomotion⁴).

In the present study, we applied an open-source, closed-arena gait analysis technique (GAITOR, gait analysis instrumentation and technology optimized for rodents, Figure 1)⁵. GAITOR consists of an arena that allows the collection of high-speed footage of rat locomotion from both sagittal and ventral viewpoints, and a set of software tools to analyze the footage (AGATHA, automated gait analysis through hues and areas⁷). While GAITOR was originally developed for orthopedic injury models, the open-source aspect allowed us to easily calculate additional parameters which are specific for SCI by making simple changes to the AGATHA calculator. We used GAITOR to evaluate recovery from a right T10 hemisection SCI (T10 was chosen due to its position rostral to the motor neuron pools which control rear hindlimb locomotion¹¹, Figure 1E) after ISP administration in rats. In addition to previously calculated parameters (duty factor, step width, stride length), we implemented SCI-specific parameters (paw placement accuracy, phase dispersion⁸). Additionally, we investigated tissue-level recovery via retrograde tracer. Overall, the goal was to determine if

GAITOR could be used to sensitively detect changes in locomotor recovery following treatment for SCI.

2 Materials and Methods

Hemisection Injury Model

All procedures described in this manuscript were conducted under the approval of the University of Akron Institutional Animal Care and Use Committee (IACUC, protocol 16-04-07 LRE) and performed under anesthesia. We performed the hemisection in a manner similar to that which has been described previously¹⁰. Briefly, we made an incision along the midline of female Fisher 344 rats (Envigo, n = 6, 3 total groups: ISP+, ISP–, and naïve). After carefully counting the dorsal processes, we exposed the dorsal surface of the spinal column from approximately T8-T11. We performed a laminectomy on T10, leaving the dura intact. We then performed a right lateral hemisection with a #11 scalpel blade and removed 2mm of the cord, resulting in a moderate injury to the spinal cord. Before continuing, we took care to scrape the bottom of the vertebrae repeatedly to ensure a complete hemisection (i.e., that no neuronal connections remained on the right side of the cord). After homeostasis was achieved, we closed the muscle with 4-0 Vicryl (Ethicon) and the skin with Michel clips. We treated pain with subcutaneous injections of carprofen (5 mg/kg) for 3 days following the injury and expressed bladders as needed until the normal reflex returned. We replaced any animals which did not recover locomotion by week 3 (e.g., dragging limbs, 1 animal from each injury group). We allowed the rats to recover for 13 weeks before perfusion.

ISP Administration

We administered ISP as described by others⁹. Briefly, we prepared a 5 μ M solution of ISP (synthesized by GenScript) in sterile DMSO:saline (1:20) and aliquoted each animal's daily dose (stored at –20 °C). Beginning at 24h post injury and continuing through day 49, we gave each animal a 500 μ L injection of the ISP solution in the subcutaneous tissue near the lesion (within 2 cm of the original incision, on either side of the midline). We conducted the experiment in a double-blinded manner. Vehicle solution (DMSO:saline) was the negative control.

Gait Testing

We allowed rats to explore a specialized gait arena that has been described previously: full dimensions, instructions, and examples can be found in ref⁵. Briefly, the arena consists of a clear acrylic enclosure with a 45° mirror placed below to enable viewing of both the sagittal and ventral planes (Figure 1A). The rats walked freely back and forth across the arena, against a black background, while being recorded at 250 FPS using a high speed camera (MS85K, MegaSpeed Corp). For lighting, we used two 50" DC LED light bars (SuperBrightLEDs) producing $\approx$ 21,400 lumens each: one placed above the arena and one below to illuminate both views. Rats walked unprompted for up to 20 minutes to obtain 6-7 videos per animal. Importantly, we acclimatized the animals to the arena for 2 weeks prior to beginning the study. We rejected videos in which the animal changed speed or stopped before completing three full gait cycles.

Retrograde Tracing

We performed retrograde tracing using FluoroGold (FG, Fluorochrome LLC) as previously described^{10,15}. We dissolved FG in phosphate buffered saline (PBS, 4% w/v) and sterilized the solution via 0.22 μm filtration. We then soaked a small (5×5×2 mm) gelatin pledget (Gelfoam, Pfizer) in the FG solution for >5 min. For tracing animals (we traced half of each group described above, or n=3), 1 week before the study endpoint (week 12), we performed a laminectomy at T12 (2 segments caudal to the lesion), made a small incision in the dura, and placed the pledget on top of the incision, using petroleum jelly to hold it in place. We then closed the surgical site as described above, and allowed the animals to recover for 1 week before perfusion and analysis (see below).

Automated Gait Analysis

A detailed overview of how AGATHA works, including a comparison with manual digitization, can be found in ref⁷. In brief: first, the user selects filtering parameters that enable AGATHA to filter the sagittal view to contain only the rat, and the ventral view to contain only the paws (Figure 1B). AGATHA then locates the row of pixels which represents the floor of the arena in the sagittal view. Next, AGATHA excludes any regions where the nose or tail are contacting the floor. AGATHA determines the earliest (foot-strike) and last (toe-off) frames in which a paw is in contact with the ground. These foot-strike/toe-off events are plotted in a foot-strike toe-off (FSTO) diagram, which shows each step as a rectangle, each foot-strike event as a green circle, and each toe-off event as a red circle (Figure 1D). From this information, AGATHA calculates temporal parameters (Table 1 shows a list of parameters used in this study: for additional parameters that AGATHA can calculate, as well as an explanation of what each parameter means, see^{7,6}). For the spatial parameters, AGATHA starts by estimating the foot-strike location from the sagittal view. From this approximate location, the centroid of each foot-strike is determined in the ventral view. High-resolution images of each foot-strike are then overlaid onto one figure, showing each footfall as the rat moves across the arena (Figure 1C). The centroid is used to calculate spatial parameters (Table 1). Finally, the centroid of the entire rat is used to determine the overall velocity of the rat during each video.

Tissue Processing

After week 13, we perfused the rats with PBS, followed by 4% paraformaldehyde (PFA) in PBS. We then carefully explanted the entire spinal cord and placed it in 4% PFA for 4h at 4 °C. We rinsed each cord once and stored them in 0.2 M phosphate buffer overnight at 4 °C. Finally, we placed each cord in 30% sucrose for 3 days at 4 °C. We cut the cords into ≈ 2 cm blocks. Then, we embedded blocks containing the lesion and a segment rostral to the lesion (for analysis of retrograde tracer) in OCT Compound (Tissue-Tek) and stored them at -80 °C until cryosectioning (Leica CM 1850). We collected 25 μm longitudinal (for analyzing the lesion area) and cross (for tracer analysis) sections.

Immunohistological Analysis

We stained the longitudinal sections with rabbit anti-Tau (Abcam ab32057, 1:250 dilution) or mouse anti-glial fibrillary acidic protein (GFAP, Millipore-Sigma MAB360, 1:500

dilution). For secondary antibodies, we used either donkey anti-rabbit 488 (Invitrogen A21206, 1:400 dilution) or goat anti-mouse 546 (Invitrogen A11030, 1:400 dilution). We conducted the staining as described before¹⁰. Briefly, we permeabilized with 0.1% Triton X-100, blocked with 10% fetal bovine serum (FBS), incubated with 1° antibodies overnight at 4 °C, washed 3x with PBS, incubated with 2° antibodies at RT for 1h, washed 3x with PBS, stained for nuclei with Hoechst 33342 (10×10^{-6} M) at RT for 7 m, and finally washed 3x with PBS before mounting using Cytoseal XYL (ThermoFisher). We imaged the slides on an Olympus IX81 fluorescent microscope using the same acquisition settings for all sections. We minimally processed each image using MetaMorph (Molecular Devices) to correct background fluorescence and prepared them for publication (cropping/resizing) using Photoshop (Adobe).

Retrograde Tracing Analysis

To analyze tracer intensity, we imaged cross sections of the cord at 2.5 cm rostral to the lesion site. We carefully collected the cross-sections sequentially, to ensure that we knew the location (i.e., in multiples of 25 μ m from the lesion site) of each section. We directly imaged mounted (Cytoseal) sections (no additional staining) using an Olympus IX81. To evaluate tracer intensity, we analyzed 10 sequential sections per animal (comprising a total distance of 250 μ m) in the following manner: first, we collected images using identical acquisition settings (exposure time, contrast, etc.). We then applied an identical threshold to each image and measured the total number of bright pixels (i.e., above the threshold) in a consistently-sized region of interest on each side (left and right) of the cord. Finally, we took the ratio of right/left to control for overall uptake of tracer between animals. This analysis was conducted in MetaMorph.

Experimental Design and Data Processing

Only one treatment factor was analyzed in this study: ISP (yes or no). We also included a comparison to naïve rats at each timepoint, for 3 groups total ($n = 6$). We collected gait data beginning at 3 weeks post-injury (this was the time by which all rats were consistently stepping) and continuing until week 13 (for a total of 11 timepoints). Prior to analyzing the gait parameters, we plotted the naïve rat data for each parameter vs. velocity (i.e., all total data points over 11 weeks). For any parameter which was found to be velocity-dependent, we calculated velocity-weighted residuals at each timepoint⁶. These are expressed in the legend for each figure (e.g., “stride length residual”).

Statistical Analyses

For duty factor, paw placement accuracy, step width, and stride length, our hypothesis was that ISP administration would affect the mean value of each parameter after 13 weeks. To test this hypothesis, we used an ANOVA with Tukey’s *post-hoc* on the week 13 data with the week 3 data (e.g., starting values) as a covariate. This allowed us to take into account any differences in starting values between groups. For phase dispersion, our hypothesis was that ISP would affect the spread of values (consistency, as measured by standard deviation) after 13 weeks. To test this, we performed an ANOVA on the mean standard deviation for each rat⁸. Lastly, for the tracing data, our hypothesis was that the ISP administration would affect the mean value of the tracer intensity. To test this, we used Student’s t-test.

When an ANOVA was performed, groups not sharing a letter were found to be significantly different from each other. Descending letters (beginning with A) indicate smaller means. For Student's t-test, the p-value is shown on each graph. For all statistical comparisons, we considered $p < 0.05$ to be significant.

3 Results

Duty Factor Imbalance and Right Paw Placement Accuracy Improve after ISP Administration

Duty factor imbalance measures the degree to which an animal favors one hindlimb over another (i.e., an antalgic or limping gait). Based on our methodology, a number greater than 0 indicates that the animal is spending more time standing on the right hindlimb, and less than 0 indicates more time standing on the left hindlimb. Over the course of 13 weeks, rats that were administered ISP improved their imbalances, steadily moving closer to 0 (Figure 2A), while rats administered a vehicle control saw essentially no change in duty factor imbalance. A statistical comparison of the week 13 values, using the week 3 values as covariates, indicated that the ISP+ group bridges the gap between the ISP- and naïve groups. No imbalance between right and left forelimbs was observed (Figure 2B), demonstrating that our T10 hemisection did not cause limping in the forelimbs. Likewise, we saw similar statistical patterns in right paw placement accuracy. During healthy locomotion, rats will place ipsilateral fore/hind limbs in the same place (see Figure 1C for an example). However, after receiving a hemisection, they lose this ability and will place their limbs much farther apart (Figure 2C), but only on the injured side. Paw placement accuracy measures the distance between the centroid of each paw, relative to that of a healthy animal (a value of 0 is healthy, while > 0 indicates they are being placed farther apart and < 0 closer together). The animals which were administered ISP improved their placement accuracy over time, while untreated animals did not. The same statistical comparison as in duty factor imbalance yields similar results — the ISP+ animals bridge the gap between ISP- and naïve. No change in accuracy on the left side of the animals was observed (Figure 2D), demonstrating that our T10 hemisection only caused a deficit in motor control on the injured side.

Hindlimb Step Width and Stride Length are Unaffected by T10 Hemisection

Interestingly, neither hindlimb step width (perpendicular to the direction of travel) nor hindlimb stride length (along the direction of travel) were affected by our injury model (Figure 3). There is a slight increase in step width for both injured groups at week 3, but this was not statistically significant, and furthermore, both groups recovered at largely the same rate. Stride length was not affected at any timepoints. This highlights the specificity of a T10 hemisection for only the right hindlimb — the animals can compensate for the limping and loss of accuracy (Figure 2) such an injury causes and not have it affect the width or length of their steps.

Phase Dispersion Consistency is Improved after ISP Administration

Phase dispersion (PD) measures the degree to which paw placement is coordinated in time^{8,13}. Healthy rats place diagonal (e.g., right forelimb and left hindlimb) paws at roughly

the same time, which corresponds to a phase dispersion of 0 (measured as a percentage of the total step cycle time). When the corresponding hindpaw is placed after the forepaw, this results in a positive PD, whereas placement of the hindpaw before the forepaw results in a negative PD. By calculating the mean standard deviation for each rat at starting and ending time-points (i.e., weeks 3 and 13), we can obtain a quantitative measurement of PD consistency (Figure 4)⁸. A comparison of mean standard deviations for right hindpaw-left forepaw pairs across groups indicated that ISP treatment improved PD consistency (lower mean standard deviation) compared with the ISP- group (Figure 4A). Unlike duty factor imbalance or paw placement accuracy, ISP+ was significantly different from both groups (naïve was the lowest and ISP- the highest). Left hindpaw — right forepaw PD was not affected by our injury model (Figure 4B), again highlighting the specificity of a T10 right hemisection for right hindlimb function only.

Improvements in Gait Parameters are Confirmed by Retrograde Tracing

At week 12, we administered FluoroGold (FG, a retrograde tracer) at T12 (2 segments caudal to the lesion) to measure uptake of the tracer across the injury site (Figure 5). The animals were allowed to recover for 1 week before sacrifice and analysis of tracer intensity at week 13. After explanting the spinal cords, we collected transverse sections rostral to the lesion. A comparison of intensity 1 cm rostral to the lesion (normalized to the uninjured side) indicated that the ISP+ animals transported significantly more tracer across the lesion than did the ISP- animals (Figure 5C), although both groups transported less tracer across the injured side than the non-injured (relative intensities < 1). This was confirmed via Student's t-test. Tracer uptake is another way of measuring function, as the tracer will only be transported rostrally by functional neurons — thus, more functional neurons equates to a greater tracer intensity. The results from our tissue-level functional test match those from the locomotor functional tests: ISP improves function on the injured side, but not to the level of a healthy animal (which would have a normalized tracer intensity of 1).

Tau and GFAP Expression at the Lesion Show Minor Improvements

Lastly, to briefly assess whether ISP affected axonal growth into the lesion from the rostral and caudal stumps, we stained for the axonal marker Tau (Figure S1). In the animals treated with ISP, we observed more Tau expression both within and immediately surrounding the lesion area, and it appears that the edges have been filled in with Tau+ axons. Of note are the intensity (brighter staining around the lesion) and appearance (evenly distributed around the lesion border) of the Tau stain, which may indicate sprouting of axons from the rostral and caudal stumps into the lesion. This phenomenon is not observed in the ISP- animals. We also stained for GFAP around the lesion area (Figure S2). The purpose of ISP is not to reduce glial reactivity, but to reduce the effect of the inhibitory CSPGs. Expectedly, intensity and localization around the lesion edges, a hallmark of astrocyte reactivity, was similar between groups (Figure S2B vs. D).

4 Discussion

The overall purpose of this study was to implement GAITOR for evaluating locomotor changes after SCI, and see if it could track changes following ISP administration in a lateral

hemisection. We opted not to compare GAITOR with BBB score, as BBB was developed for bilateral injury models, so it has limitations when applied to a right lateral hemisection. Additionally, we wanted to keep the focus of the manuscript on introducing a new technique, so we instead confirmed locomotor improvements via tracing. Our purpose was not to supplant BBB or other functional tests (gridwalk, etc.) but to introduce a new, complementary technique.

This study highlights the power of GAITOR for studying locomotor changes in rodents as they recover from SCI, especially in the later stages or after mild injuries, when BBB score may plateau. Here, we have used a rat injury model, but the same system (with minor changes) can be used to study mice as well. The open-source nature of GAITOR, combined with the ability to observe high-resolution images of the paws during the entire stance phase, is particularly useful in SCI research. In this study, we have added 2 parameters to AGATHA that are specific to SCI (paw placement accuracy and phase dispersion), but more could be easily studied. For example, AGATHA could be used to study the magnitude and speed of paw rotation during stance phase (e.g., if the animal places the paw parallel and then rotates it prior to toe-off). The ability to implement new parameters is of particular interest to SCI, which often has to rely on closed-source gait analysis techniques (e.g., DigiGait, Treadscan) that may not offer as much adaptability to various injury models. Additionally, such commercially available techniques are expensive and typically require the use of a treadmill, which itself alters locomotion (rather than a closed arena, which does not)⁴.

It is also important to discuss some drawbacks to GAITOR. First, the animals must be consistently walking — if the animal is dragging limbs (e.g., in response to severe contusion), it cannot be studied. Additionally, it requires some knowledge of computer programming to fully harness. Online resources do exist⁵, and support is steadily growing, but the “do-it-yourself” nature of GAITOR (which is also arguably its greatest strength) will likely require collaboration for some neuroscientists to use effectively.

Our results are similar to those published by Lang et al., describing the benefits of ISP in a bilateral contusion model⁹. They found that ISP administration improved function in a contusion model, as measured by BBB score, over a period of 11 weeks post-injury. Here, we build upon that study and show that ISP improves function in a lateral thoracic hemisection model as well. Importantly, we also show that GAITOR can be used to sensitively track such changes. We also show that GAITOR can be used to study a more severe hemisection, where an entire piece of the spinal cord is removed. Typically, groups that are studying hemisection injuries do not remove a piece of the cord, but in the case of studying a biomaterial scaffold/bridge, some of the cord must be removed in order to implant the device¹⁰. In this study, we demonstrate that GAITOR can be used to study animals with such a defect, laying the groundwork for future studies involving biomaterial scaffolds. We also show that GAITOR can be used by other groups studying injury models of similar severity (i.e., where the animals are at least stepping), or expanded to injury models involving different deficits simply by adding new parameters or changing existing ones.

In general, the results from the tissue-level analyses match those from the gait analyses. ISP administration improves outcomes over a negative control, but treated animals fail to recover to their normal state. Some of the statistical tests indicated that the ISP+ group essentially bridged the gap between ISP– and naïve, being statistically different from neither after 13 weeks (see Figure 2). However, other tests indicated that that ISP+ animals occupy a distinct middle ground between the two (see Figure 4). It is important to also look at the actual trends, and observe that, while the ISP+ animals might be statistically similar to the naïve group, they do not achieve the same actual values. We can also see that there is no bridging of neurites across the entire gap (see Figure S1), so true regeneration was not achieved. This highlights the need for a biomaterial scaffold in conjunction with ISP administration to provide a way to bridge the gap in patients who experience removal or degeneration of a portion of their spinal cord.

In conclusion, we have shown that GAITOR detects improvements in rat locomotor recovery following ISP administration to block glial scar inhibition and that these are confirmed by increased tracer uptake across the lesion. We added SCI-specific parameters to AGATHA, which we also show are specific and track recovery alongside more general parameters. Finally, we discussed how GAITOR can be used to study any SCI model where the animals are stepping, providing a useful, quantitative technique that can be used alongside existing behavioral analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Dr. Hossein Tavana (UA) for the use of his Leica Cryostat and the faculty/staff at the Ohio State University Spinal Cord Injury Training Program (sponsored by the Craig H. Neilsen Foundation) for their valuable input and advice. This work was supported by the National Institute of Neurological Disorders and Stroke (grant 1R21NS096571-01).

References

1. Basso DM, Beattie MS, and Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma* 12:1–21, 1995. [PubMed: 7783230]
2. Burda JE and Sofroniew MV. Reactive gliosis and the multicellular response to CNS damage and disease. *Neuron* 81:229–48, 2014. [PubMed: 24462092]
3. Ham TR and Leipzig ND. Biomaterial strategies for limiting the impact of secondary events following spinal cord injury. *Biomed Mater* 13:024105, 2018. [PubMed: 29155409]
4. Jacobs BY, Kloefkorn HE, and Allen KD. Gait analysis methods for rodent models of osteoarthritis. *Curr Pain Headache Rep* 18:456, 2014. [PubMed: 25160712]
5. Jacobs BY, Lakes EH, Reiter AJ, Lake SP, Ham TR, Leipzig ND, Porvasnik SL, Schmidt CE, Wachs RA, and Allen KD. The open source gaitor suite for rodent gait analysis. *Scientific Reports* 8:9797, 2018. [PubMed: 29955094]
6. Kloefkorn HE, Jacobs BY, Loye AM, and Allen KD. Spatiotemporal gait compensations following medial collateral ligament and medial meniscus injury in the rat: correlating gait patterns to joint damage. *Arthritis Res Ther* 17:287, 2015. [PubMed: 26462474]

7. Kloefer HE, Pettengill TR, Turner SM, Streeter KA, Gonzalez-Rothi EJ, Fuller DD, and Allen KD. Automated gait analysis through hues and areas (agatha): A method to characterize the spatiotemporal pattern of rat gait. *Ann Biomed Eng* 45:711–25, 2016. [PubMed: 27554674]
8. Kloos AD, Fisher LC, Detloff MR, Hassenzahl DL, and Basso DM. Step-wise motor and all-or-none sensory recovery is associated with nonlinear sparing after incremental spinal cord injury in rats. *Exp Neurol* 191:251–65, 2005. [PubMed: 15649480]
9. Lang BT, Cregg JM, DePaul MA, Tran AP, Xu K, Dyck SM, Madalena KM, Brown BP, Weng Y-L, Li S, Karimi-Abdolrezaee S, Busch SA, Shen Y, and Silver J. Modulation of the proteoglycan receptor ptpsigma promotes recovery after spinal cord injury. *Nature* 518:404, 2014. [PubMed: 25470046]
10. Li H, Ham TR, Neill N, Farrag M, Mohrman AE, Koenig AM, and Leipzig ND. A hydrogel bridge incorporating immobilized growth factors and neural stem/progenitor cells to treat spinal cord injury. *Advanced Healthcare Materials* 5:802–812, 2016. [PubMed: 26913590]
11. Mohan R, Tosolini AP, and Morris R. Segmental distribution of the motor neuron columns that supply the rat hindlimb: A muscle/motor neuron tract-tracing analysis targeting the motor end plates. *Neuroscience* 307:98–108, 2015. [PubMed: 26304758]
12. Okada S, Hara M, Kobayakawa K, Matsumoto Y, and Nakashima Y. Astrocyte reactivity and astrogliosis after spinal cord injury. *Neurosci Res* 126:39–43, 2018. [PubMed: 29054466]
13. Redondo-Castro E, Torres-Espin A, Garcia-Alias G, and Navarro X. Quantitative assessment of locomotion and interlimb coordination in rats after different spinal cord injuries. *J Neurosci Methods* 213:165–78, 2013. [PubMed: 23291085]
14. Sabelstrom H, Stenudd M, and Frisen J. Neural stem cells in the adult spinal cord. *Exp Neurol* 260:44–9, 2014. [PubMed: 23376590]
15. Tsai EC, van Bendegem RL, Hwang SW, and Tator CH. A novel method for simultaneous anterograde and retrograde labeling of spinal cord motor tracts in the same animal. *J Histochem Cytochem* 49:1111–22, 2001. [PubMed: 11511680]

Figure 1:

Overview of GAITOR and injury model. GAITOR is an open-source suite of hardware and software tools for preclinical gait analysis. High-speed footage of rats walking in an arena is collected, including sagittal and ventral views. AGATHA (one software component of GAITOR) examines each video frame-by-frame to extract relevant gait parameters. For detailed information about GAITOR, see^{7,5}. A: An example frame from a GAITOR video. B: AGATHA filters the rat from the background in the sagittal view and paws from the background in the ventral view. These are then binarized and the sagittal view is used to determine which paws are in contact with the ground in each frame (red arrows). C: Detailed images showing each paw placement (in space) for each video are then generated. Notice how the right paws are placed farther apart on average when compared to the left (orange box). D: Plots showing foot strike (when the paw contacts the ground, green circle on top of each box) and toe-off (when the paw leaves the ground, pink circles on bottom) are also generated. Forepaws are blue, while hindpaws are red. The y-axis is descending frame number (so the first frame is at the top) and the x-axis is the horizontal pixels of each frame. The right hindlimb (steps 2, 6, 10, 14, and 18) can be seen to contact the ground for less than the left (steps 4, 8, 12, 16) and at a delay (red box). Inset shows an example step in greater detail. E: We performed a T10 hemisection, which disrupts the motor neuron tracts responsible for right hindlimb movement.

Figure 2:

ISP improves duty factor imbalance and right hindpaw placement accuracy, while forelimb duty factor imbalance and left hindpaw placement accuracy are unaffected by a T10 right lateral hemisection. A: Hindlimb duty factor imbalance (right duty factor - left duty factor) improves in ISP treated animals over 13 weeks. B: Forelimb duty factor imbalance is unaffected by our injury model. C: Right paw placement accuracy (linear distance between ipsilateral hindlimb/forelimb paw centroids) also improves over 13 weeks. D: Our injury model did not affect paw placement accuracy on the left side. For all graphs, data are shown as mean $\pm$ standard error, $n = 6$. Letters denote significance as determined by ANOVA (W3 values as covariates) on the week 13 means with Tukey's *post-hoc* ($p < 0.05$).

Figure 3: Neither hindlimb step width (A) nor stride length (B) are affected by our injury model. For all graphs, data are shown as mean $\pm$ standard error, n = 6.

Figure 4:

ISP treatment improves coordination between right hindpaw - left forepaw pairs as shown by phase dispersion, while left hindpaw - right forepaw pair coordination is unaffected by our injury model. A: Both of the injured groups showed a much greater spread of PD values for right hindpaw - left forepaw pairs at week 3 when compared to the naïve group. However, the ISP+ group recovered some of the spread by week 13 (i.e., lower mean standard deviation) when compared to the ISP- group. Neither group recovered to the same level as naïve. B: All three of the groups had the same PD distributions for left hindpaw - right forepaw pairs, indicating that our injury model does not affect coordination for this pairing.

For all graphs, data are shown as histograms normalized to total step count, $n = 6$. #, ##, and ### denote significance in descending order of mean standard deviation as determined by ANOVA (W3 values as covariates) on the week 13 mean standard deviations with Tukey's *post-hoc* ($p < 0.05$).

Figure 5:

Retrograde tracer uptake confirms gait analysis results as determined by the ratio of tracer intensity on the right side (injured) to that on the left (uninjured). A: ISP+ animals showed more tracer on the right side (relative to the left) than did ISP- groups (B). Quantification of tracer intensity shows the same results, and a comparison of the means confirms that there is a statistical difference between the two groups. Data are shown as mean $\pm$ standard deviation, $n = 3$. * indicates significance as determined by Student's t-test ($p < 0.05$).

Table 1:

List of gait parameters used in this study.

Temporal Parameters	Spatial Parameters
Duty Factor	Paw Placement Accuracy
Phase Dispersion	Step Width
	Stride Length

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