Classification: Biological Sciences (Neuroscience)

Serotonergic Modulation of Spinal Circuitry Restores Motor Function after Chronic Spinal Cord Injury

Sarita Walvekar,[1,2] Robert B. Robinson,[1] Hailey M. Chadwick,[1] Rebecca M. Burch,[1] Hanzhang Ding,[1] Steve I. Perlmutter[1] and Samira Moorjani[1,*]

[1]Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195, USA

[2]Present Address: Cleveland Clinic Lerner College of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA

*Corresponding Author: Samira Moorjani, 1705 Northeast Pacific Street, Health Sciences Building G-424, Box 357290, Seattle, WA 98195, USA; Phone: (206) 221-5338; E-mail: moorjani@u.washington.edu.

Author Contributions: S.I.P. and S.M. conceived the original idea. S.W., R.B.R., H.M.C., S.I.P. and S.M. contributed to research design. R.B.R. and H.M.C. performed all the surgeries. S.W., H.M.C., R.M.B. and H.D. carried out experiments. S.M. performed all the analyses. S.W. and S.M. wrote the paper.

Competing Interest Statement: The authors declare no conflict of interest.

Keywords: Spinal Cord Injury; Paralysis; Serotonin; Neuromodulation; Rehabilitation; Motor Recovery

This file includes the following:

Main Text

Figures 1 – 6

Note: A Preprint of this manuscript has been deposited on *bioRxiv* 510858 (CC-BY-NC-ND 4.0 International license).

Abstract

Electrical stimulation of the nervous system, in conjunction with use-dependent rehabilitation, has been employed for treatment of paralysis following spinal cord injury. However, the therapeutic effects almost always rely on the sustained activation of muscles or neurons, making the benefits largely contingent on continued delivery of stimulation. In the present study, we describe a neuromodulatory intervention that combined intraspinal delivery of serotonergic agonists with physical rehabilitation to restore motor function after a chronic moderate-to-severe cervical contusion (that produces impairments in forelimb movements and dexterity). We show that targeted delivery of quipazine, a broad-spectrum serotonergic-receptor agonist, increased the effectiveness of use-dependent rehabilitation—which is the current gold standard of treatment—leading to substantially improved motor-recovery outcomes. Injured rats in our study received physical rehabilitation combined with intraspinal delivery of quipazine/vehicle. All rats that received quipazine recovered 100% of their pre-injury reach ability and showed substantial improvements in dexterity, which led to sizable gains in motor performance and reduced variability during post-therapy follow-up. In comparison, physical rehabilitation alone provided significantly reduced benefits, which were more variable across animals. This difference may be attributed to the beneficial effect of quipazine on motor recovery after a severe spinal cord injury. While therapeutic benefits in skilled reach and grasp movements were comparable between the two intervention groups in moderately-injured animals, quipazine significantly augmented motor gains after a severe injury. Remarkably, quipazine-mediated behavioral improvements persisted for weeks after termination of neuromodulator delivery, alluding to modification of spared adult spinal circuitry that drives lasting motor recovery.

Significance Statement

Existing therapeutic approaches employed for treatment of paralysis, after a spinal cord injury, produce temporary reanimation through acute modification of the underlying circuitry that is damaged by the neurological trauma. We describe a neuromodulatory intervention that relies on intraspinal delivery of serotonergic agonists to promote motor recovery after chronic cervical spinal cord injury that compromises forelimb movements and dexterity. Our results show that targeted delivery of serotonergic agonists increased the effectiveness of use-dependent rehabilitation, which is the current gold standard of treatment, leading to substantially improved motor-recovery outcomes. Remarkably, therapeutic gains produced by our intervention persisted for weeks after termination of neuromodulator delivery—a finding that is both unique and clinically relevant—signaling plasticity induction in chronically-damaged adult spinal circuitry.

Introduction

An estimated 27 million people worldwide live with spinal cord injury (SCI; 1), which often results from trauma suffered during motor-vehicle accidents, falls, or sporting and recreational injuries (2). Among developed regions, annual incidence of traumatic SCI varies from 39 cases per million of the population in North America to 15 – 16 cases per million in Western Europe and Australia (3). SCI can lead to motor deficits in the upper and lower extremities as well as sexual, bowel and bladder dysfunction, depending on the location and severity of the injury (2, 4). Motor deficits after SCI range from weakness in the limbs, abnormal muscle tone and posture, disruption in movement synergies, spasticity, and loss of balance and coordination to complete paralysis (5, 6). Secondary health complications often develop over time due to low levels or the complete lack of movement, further compromising the quality of life and patient autonomy (7). Most spontaneous recovery is limited to the first few months after the injury (2, 4). Additionally, a limited number of therapies have been developed for acute SCI (8), but they offer vastly diminished to no benefits to patients with chronic injuries, creating a need for interventions that target chronic motor damage.

Due to the limited intrinsic capacity of the adult central nervous system for remyelination and regrowth of damaged axons, existing approaches to motor recovery after chronic injuries seek to leverage spared circuitry for reinforcing functionally-relevant neuronal firing patterns through targeted physical rehabilitation (9). Use-dependent movement interventions, such as robot-assisted body-weight-supported treadmill training (10-12) and constraint-induced therapy (13-15), have been widely explored as treatment options in this effort, but they typically—and at best—lead to marginal recovery of motor function. Greater success has been achieved through hybrid approaches that combine use-dependent rehabilitation with epidural electrical stimulation (16-27). However, the therapeutic gains almost always rely on the continued delivery of stimulation. In a small number of studies, residual effects were observed (16, 20), but such benefits were appreciably smaller compared to those obtained in the presence of stimulation. This lack of endurance in (the extent of) therapeutic benefits has created a need for development of alternate strategies that produce more lasting changes through plasticity induction in damaged circuitry. Towards this effort, we describe a neuromodulatory intervention that relies on intraspinal delivery of serotonergic agonists below the lesion to restore motor function after chronic SCI.

Serotonin (or 5-hydroxytryptamine; 5-HT) is an important neuromodulator in the spinal cord (28). Serotonergic regulation of spinal-neuron excitability is critical for normal sensorimotor integration and movement production (29). The activation of serotonergic receptors promotes a general increase in motoneuron excitability through amplification of synaptic inputs, depolarization, and increase in firing rates (30). The excitatory effects of serotonin on motoneurons depend on the identity, location and level of receptor activation (30-32). Nonetheless, serotonergic modulation of spinal circuitry provides a potential therapeutic avenue for motor recovery by reinstating the (disrupted) excitation–inhibition balance after SCI (33, 34).

Previous studies have shown that serotonergic therapies can promote motor gains after SCI (35-43). Serotonin-receptor agonists were able to induce stepping and rhythmogenesis in spinalized rats after thoracic injuries through activation of spinal locomotor networks (35-40, 43). While serotonergic agonists have been extensively examined after thoracic SCI (35-40, 43), administration of 5-hydroxytryptophan, which is a 5-HT precursor, also evoked activity in phrenic motoneurons following a C1 – C2 transection (41). Additionally, delivery of serotonergic agonists produced recovery of skilled

reach and grasp movements after a C4 injury (42). However, therapeutic benefits did not persist after withdrawal of the neuromodulators in these studies.

We describe a hybrid intervention that combined intraspinal delivery of quipazine, a non-specific 5-HT_2 receptor agonist (36, 38, 44), with targeted physical rehabilitation to promote motor recovery in an adult rodent contusion model of chronic cervical SCI that predominantly damages the corticospinal tract (critical for control of voluntary forelimb movements and dexterity; 45). Injured rats that received the hybrid intervention showed remarkable improvements in forelimb reach-and-grasp motor performance compared to rats that received physical rehabilitation alone. Importantly, therapeutic benefits in our study outlasted neuromodulator delivery, signaling modification of the chronically-damaged spinal circuitry that drives lasting improvements in motor function.

Results

Experimental design. Experiments were conducted in 20 adult female Long-Evans rats. Rats were trained on a standard forelimb reach-and-grasp behavior (46), using operant-conditioning protocols (47), for food-pellet rewards. Briefly, animals were placed inside clear plexiglass arenas and trained to reach out through a slit in the arena, grasp a food pellet placed on a block outside the arena with their dominant forepaw, and bring the pellet through the slit back into the arena and then to their mouth.

Rats were assigned a reach-and-grasp performance score of 1 (or 100%) for successful completion of all the steps in the forelimb-motor sequence. If the rat was unable to touch the pellet, due to inaccuracy in aim or the forelimb not extended far enough, it was counted as a "miss" and was assigned a score of 0. Partial scores were assigned for completion of some—but not all—steps in the motor sequence. To assign partial scores, the motor task was divided into 'reach' and 'grasp,' with successful completion of the individual components leading to a score of 0.50 (on each component and a combined score of 1). Scoring on reach was binary with rats receiving either 0 or 0.50, whereas scoring of grasp occurred on a quaternary scale (with rats receiving 0, 0.17, 0.33 or 0.50). For example, if the rat dropped the food pellet inside the arena while bringing it to the mouth, it received a reach score of 0.50 and a grasp score of 0.33 (bringing the combined score to 0.83). If the pellet, however, was dropped outside the arena, the rat received a reach score of 0.50 and a (lower) grasp score of 0.17 (bringing the combined score to 0.67). Lastly, if the rat was only able to touch the pellet (but not grasp it), it received a reach score of 0.50 and a grasp score of 0 (making the combined score 0.50). Additional details of the forelimb-motor task and the scoring system can be found in Materials and Methods and Table S1.

Behavioral training on the forelimb-motor task occurred 10X/week, with the sessions divided across the five weekdays. Each behavioral session, in turn, consisted of 20 trials, which were preceded by a short warm-up. When rats had attained an average weekly reach-and-grasp performance of 80% or higher on the forelimb-motor task (which typically took 1 – 4 weeks), they received a unilateral (incomplete) moderate-to-severe C4 – C5 spinal contusion injury on the dominant forelimb side (48). This type of injury produces marked deficits in extension of the elbow, wrist and digits, consequently, resulting in impaired performance on the previously-mastered skilled reach-and-grasp behavior (49). During week 4 after SCI, rats were implanted with a biocompatible plastic cannula caudal to the lesion in the C7 spinal segment. The cannula was coupled upstream to a pre-programmed battery-operated microinfusion pump (50), which was implanted subcutaneously. Seven weeks after SCI, a pre-therapy assessment on the same task was performed to quantify the functional deficits produced by the cervical cord injury. Therapy was initiated during week 8 after injury, which represents a common time point for a chronic SCI model in rats (51, 52), and was administered during weeks 8 – 13. Therapy consisted of targeted physical rehabilitation combined with delivery of quipazine or vehicle. Physical rehabilitation consisted of ten sessions per week of retraining of the impaired forelimb on the pellet-retrieval task and occurred during periods of ongoing neuromodulator/vehicle delivery. To eliminate biases in scoring, all retraining was performed by researchers blinded to the therapy of the rats. Quipazine/vehicle was delivered intraspinally below the lesion for approximately 4.5 hours/day for a total of six weeks. After completion of therapy, rats continued to be tested in the forelimb-motor task for an additional four weeks (in the presence of saline) to assess retention of motor gains in the absence of the neuromodulator. A terminal surgery was performed after completion of post-therapy follow-up to assess the integrity and location of the cannula. A detailed experimental timeline is shown in Figure 1A.

Note that rats in both intervention groups received retraining on the reach-and-grasp pellet-retrieval task during weeks 7 – 17. Retraining on pellet retrieval served as both targeted physical rehabilitation and a measure of behavioral recovery promoted by our interventions.

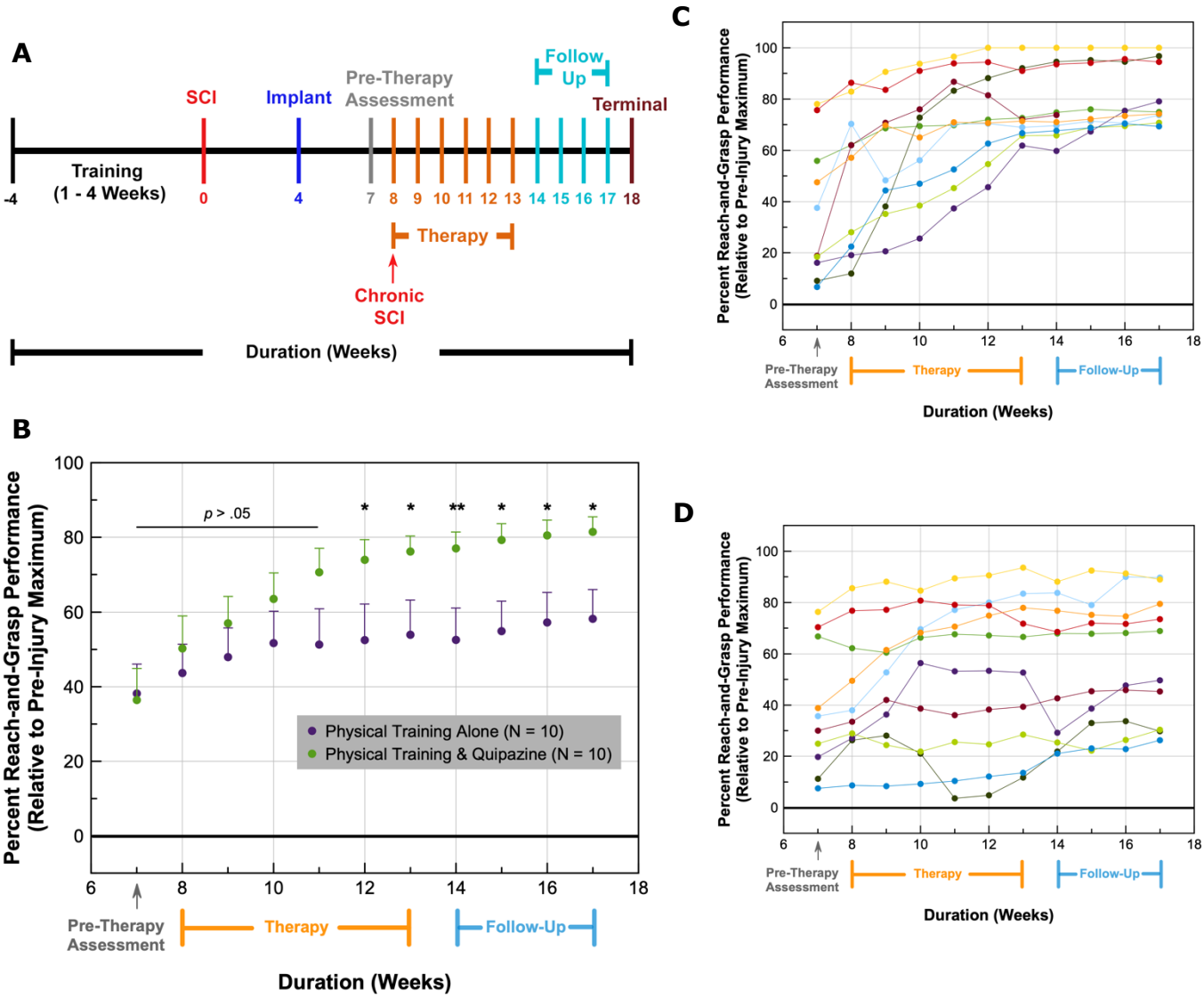

Figure 1. Intraspinal quipazine enhances motor recovery after chronic SCI. (A) Experimental timeline. (B) The reach-and-grasp score of injured rats, as a percentage of their individual pre-SCI maximum, was used to quantify the forelimb-motor performance before, (at several time points) during and after therapy. Data points represent weekly averages of all the rats in their respective intervention groups. 'N' indicates the total number of rats in each group. Error bars represent standard error of the mean. Only positive error bars are shown in the plot to reduce clutter. *p* values were obtained using paired-samples *t* tests. $*p \leq .05$; $**p \leq .01$. (C, D) Individual reach-and-grasp performance profiles of rats, whose means are shown in (B), that received quipazine (C) or vehicle (D) during therapy. Motor-recovery profiles of the rats are color-coded in the plots to reflect impairment-matching between the two groups.

Intraspinal quipazine improves motor-recovery outcomes after chronic SCI. Chronically injured rats, after a moderate-to-severe SCI, received physical rehabilitation combined with intraspinal delivery of (20 µg/week) quipazine or vehicle. Depending on their

pre-therapy (or week 7) reach-and-grasp scores, impairment-matched animals were randomly assigned to the two intervention groups. Impairment matching was used to account for the variability in motor impairment across animals arising from the large range of injury severity tested in our study (see Materials and Methods and Table S2 for additional details). Paired-samples *t* tests were employed for statistical comparisons between impairment-matched (case–control) groups (53, 54). Figure 1B shows the average weekly reach-and-grasp performance of rats in the two groups during the pre-therapy, therapy and follow-up periods. The reach-and-grasp performance of the injured rats is reported as a percentage of their individual pre-SCI maximum scores.

Injured rats that received physical training combined with intraspinal delivery of quipazine showed greater improvement in their forelimb-motor performance compared to rats that received physical training alone (Figure 1B). During the pre-therapy assessment, the reach-and-grasp performance of rats in the two groups was indistinguishable from each other ($t(9) = -0.845$, $p > .05$, paired-samples *t* test). Starting with the first week of the intervention, rats that received physical training and quipazine showed greater improvement in their performance scores relative to those that received physical training alone, and this difference in motor performance widened as therapy progressed. The motor-performance scores of rats in the two groups, however, were not significantly different from each other during the first four weeks of the intervention (*i.e.,* weeks 8 – 11). Beginning with the fifth week of the intervention (or week 12 after SCI), rats that received quipazine performed significantly better than those that received physical training alone. Importantly, therapeutic gains were maintained during the follow-up period (*i.e.,* during weeks 14 – 17) in the absence of quipazine. During the four-week follow-up period, the average forelimb-skilled performance of rats that received the combined intervention was 79% ± 4%, while rats that received physical training alone recovered 56% ± 8% of their pre-injury ability. The above changes, as well as those in the following sections, are reported as mean ± standard error (unless otherwise indicated).

Figures 1C and 1D show the individual motor-recovery profiles of rats that received intraspinal quipazine and vehicle, respectively. Motor profiles of rats that started at similar functional injury levels are represented by the same color in the two plots to illustrate differences in intervention-mediated recovery between them (*e.g.,* yellow curves depict the profile of the least injured rat in the two groups, while the darker-blue curves represent the most severely injured rat). Because rats in the two groups were impairment-matched, and they spanned a range of impairments, there was a high—but comparable—variability in motor performance within the two groups during the pre-therapy week (36% ± 9% and 38% ± 8% for rats in the quipazine and vehicle groups, respectively). Variability in motor performance gradually decreased as therapy progressed in rats that received the combined intervention, with variability at its lowest during the follow-up period (79% ± 4%; Figure 1C). In contrast, variability in the physical-training-alone group continued to remain high during therapy and post-therapy periods, with an average of 56% ± 8% during (post-therapy) follow-up (Figure 1D). Notably, in the combined-intervention group, rats with higher injury severity (or lower reach-and-grasp scores) achieved larger therapeutic gains that resulted in follow-up scores that were closer to the moderately-injured rats in the group, which ultimately reduced the overall variability over time (Figure 1C). This decreasing trend in performance variability was not seen in rats that received physical training alone (Figure 1D).

Quipazine-mediated gains persist beyond the intervention. We assessed the persistence of quipazine-mediated benefits by tracking the motor performance of injured rats for four additional weeks, after the end of therapy, in the absence of quipazine (Figures

1A, 1B and 1C). During the four-week follow-up period, rats received the same amount of physical training (10X/week), in the presence of saline, which also served as a measure of the behavioral recovery promoted by our intervention. A comparison of motor-performance scores during the follow-up period (*i.e.,* weeks 14 – 17) found statistically significant differences across the four weeks ($\chi^2(3, N = 9) = 15.835$, $p = .001$, Friedman test; N, sample size). Note that one of the rats was excluded from the statistical analysis due to listwise deletion from some missing post-therapy scores, reducing the sample size to nine. Next, we compared performance scores between the last week of therapy (*i.e.,* week 13) and the first week of post-therapy follow-up (*i.e.,* week 14) and found that there were no significant differences in the scores between the two weeks ($t(9) = 1.764$, $p > .05$, paired-samples *t* test; Figure 2). In contrast, significant increases were observed during weeks 15 – 17 compared to scores obtained during week 13 (Figure 2). Both paired-samples *t* test and Wilcoxon signed-rank test were used for these analyses, depending on the distribution of the data. While the differences in scores were significant during weeks 15 – 17, the size of the effect ranged from very small to small (0.19 – 0.37), and this small increase may have been promoted by the continued physical training during the follow-up period. Details of effect-size calculations and thresholds used for qualitative interpretation of the magnitude of observed effects can be found in Materials and Methods and Table S3.

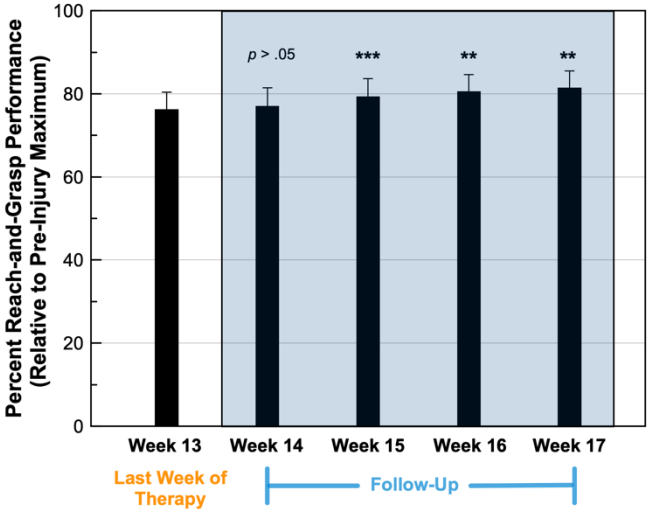

Figure 2. Intraspinal quipazine produces lasting motor recovery after chronic SCI. Bar plot shows the average reach-and-grasp performance during the last week of therapy and four weeks of post-therapy follow-up (shown in the blue-shaded region) for animals that received physical training combined with intraspinal delivery of quipazine (see Figures 1B and 1C). Error bars represent standard error of the mean. *p* values and asterisks indicate statistical comparisons between the performance of rats during the corresponding follow-up week and week 13. *p* values were obtained using paired-samples *t* test and Wilcoxon signed-rank test. ***$p \leq .001$; **$p \leq .01$.

Intraspinal quipazine substantially augments motor gains after severe traumatic SCI. Figure 3A shows the average therapeutic gains (= mean post-therapy performance – pre-therapy performance) produced by our interventions. The mean post-therapy performance was calculated by averaging the reach-and-grasp scores obtained during weeks 14 – 17. Therapeutic gains were 2.4-fold larger with quipazine (43% ± 7%) compared to physical training alone (18% ± 5%). We used a paired-samples *t* test to compare performance gains between impairment-matched rats, which revealed a significant difference between the two groups ($t(9) = 2.925$, $p = .02$; effect size = 1.57, very large

effect).

We also plotted therapeutic gains as a function of the injury severity (quantified by the pre-therapy/week 7 performance scores) for individual rats (Figure 3A, inset). Next, we (arbitrarily) classified the rats according to their injury severity: severely-injured rats, whose functional injury level (or pre-therapy performance) was less than or equal to 30% (shown in the pink-shaded region), and moderately-injured rats whose pre-therapy performance was greater than 30% (shown in the unshaded region in the inset). The average functional injury level in the moderately-injured cohort was 58% and 59% for rats that received vehicle and quipazine, respectively. We found that there were no significant differences in intervention-mediated reach-and-grasp performance gains in the moderately-injured animals ($t(4) = 0.413$, $p > .05$, paired-samples t test; Figure 3B, unshaded region). Delivery of quipazine, therefore, produced no additional benefit after moderate SCI. On the other hand, the average functional injury level in the severely-injured cohort was 19% and 14% for rats that received vehicle and quipazine, respectively. In stark contrast, the average reach-and-grasp performance gain was 4.3-fold larger in severely-injured rats that received physical training and quipazine compared to those that received physical training alone ($t(4) = 8.066$, $p = .001$, paired-samples t test; effect size = 6.05, very large effect; Figure 3B, pink-shaded region). These results indicate that intraspinal quipazine significantly augmented forelimb-motor gains after severe cervical SCI.

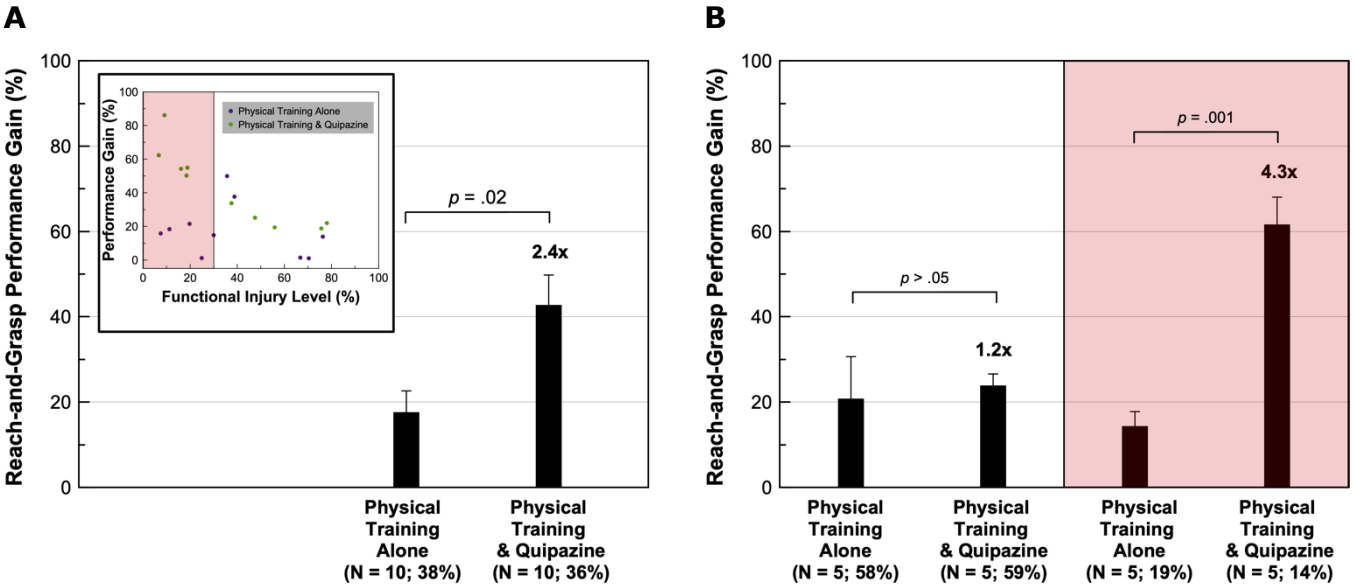

Figure 3. Intraspinal quipazine augments motor gains after severe SCI. (A) Bar plot shows the average reach-and-grasp performance gains for animals that received physical training and intraspinal delivery of vehicle/quipazine. Inset shows the performance gains as a function of injury severity for individual rats in the two intervention groups. (B) Bar plots show intervention-mediated performance gains after a moderate *vs.* severe SCI. Severely-injured animals are shown in the pink-shaded regions, while moderately-injured rats are shown in the unshaded region of the plots in inset and (B). Numbers (in bold) above the bars quantify the relative performance of rats that received the combined intervention over the physical-training-alone group. Error bars represent standard error of the mean. p values were obtained using paired-samples t tests. Number of rats included in each group (indicated by 'N'), along with the average injury severity of the group, are reported in parentheses below the bars.

Intraspinal quipazine enhances recovery of skilled reach and grasp movements after severe cervical SCI. The forelimb-motor behavior—that served as targeted physical

rehabilitation—consists of two functional movements that are differentially affected after a cervical SCI: reach and grasp (49, 55, 56). To determine the effect of quipazine on the recovery of these movements, we compared intervention-mediated changes in the reach and grasp motor performance, assessed individually, using the data from injured rats reported in Figure 1B.

Figure 4A shows the average weekly reach performance of injured rats as a percentage of their individual pre-SCI maximum scores. The pre-therapy scores of the two groups were similar (57% ± 10% and 53% ± 11% for rats in the vehicle and quipazine groups, respectively). Starting with the first week of the intervention, rats that received physical training and quipazine showed greater improvement in their reach performance relative to those that received physical training alone, and this difference continued to widen as therapy progressed. Importantly, performance variability decreased over time in the combined intervention group, with all rats recovering 100% of their pre-injury reach ability at the end of the post-therapy follow-up. In comparison, recovery of reach in the impairment-matched rats that received physical training alone ranged from 46% to 100%.

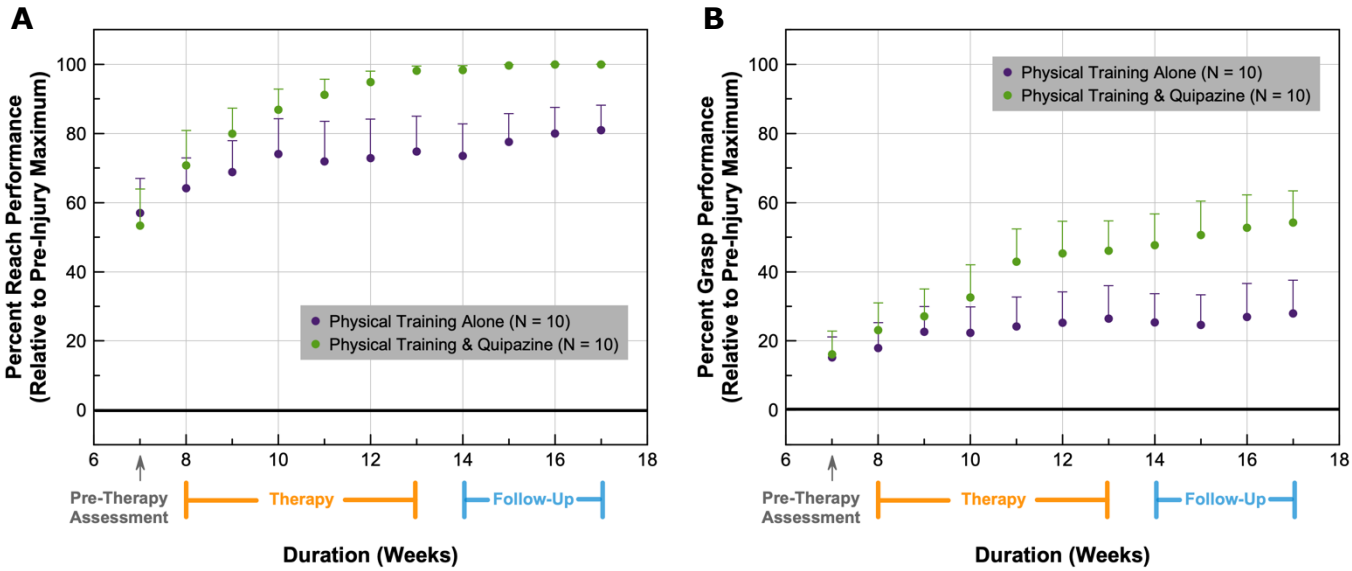

Figure 4. Intraspinal quipazine improves recovery of skilled reach and grasp movements after chronic cervical SCI. (A, B) The average weekly reach (A) and grasp (B) performance of rats, reported as a percentage of their individual pre-SCI maximum scores, is plotted for the pre-therapy, therapy and follow-up periods. Rats received physical training and intraspinal delivery of vehicle/quipazine during the six-week therapy period. Error bars represent standard error of the mean.

In comparison to deficits in reach, grasping ability was more impaired after the cervical contusion SCI with rats starting at appreciably lower pre-therapy (grasp) baselines: 15% ± 6% and 16% ± 7% for rats in the vehicle and quipazine groups, respectively (Figure 4B). Additionally, even though trends in motor performance were similar (*cf.* Figures 4A and 4B), gains were smaller, with rats recovering 54% of their pre-injury grasp ability at the end of the follow-up period in the presence of quipazine and only 28% with physical training alone. Finally, in contrast to changes in reach performance, variability in recovery of grasp continued to remain high during the therapy and follow-up periods and was comparable between the two intervention groups.

Figure 5 shows the average therapeutic gains in reach and grasp produced by our interventions. The average gain in reaching ability was 2.2-fold larger in injured rats that

received physical training and quipazine compared to those that received physical training alone ($t(9) = 2.642$, $p = .03$, paired-samples t test; effect size = 0.96, large effect). Quipazine-induced gains in grasp were even larger with injured rats recovering 3.1-fold more grasp compared to those that received physical training alone ($t(9) = 2.622$, $p = .03$, paired-samples t test; effect size = 1.07, large effect). These results indicate that intraspinal quipazine significantly improved recovery of skilled reach and grasp movements after chronic cervical SCI.

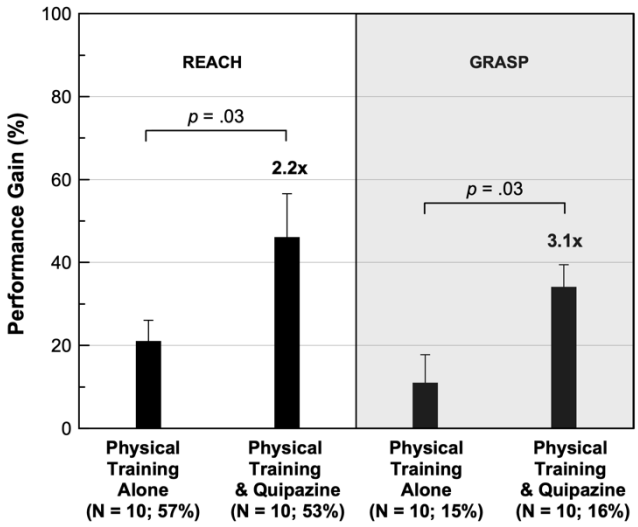

Figure 5. Intraspinal quipazine promotes greater recovery of skilled movements after chronic SCI. The reach (left unshaded panel) and grasp (right gray panel) performance gains are plotted for injured rats that received physical training and intraspinal delivery of vehicle/quipazine. Numbers (in bold) above the bars quantify the performance of rats that received the combined intervention relative to those that received physical training alone. Error bars represent standard error of the mean. p values were obtained using paired-samples t tests. Number of rats included in each group (indicated by 'N'), along with the average injury severity (or the pre-therapy reach/grasp baseline) of the group, are reported in parentheses below the bars.

Finally, we plotted the performance gains in reach and grasp as a function of injury severity, using the injury classification developed in Figure 3A (inset), to characterize the effect of our interventions after a moderate *vs.* severe SCI. Using paired-samples t tests, we found that there was no significant difference in the reach or grasp gains produced by our interventions in the moderately-injured animals ($t(4) = -0.320$, $p > .05$ and $t(4) = 0.784$, $p > .05$ for gains in reach and grasp, respectively; Figure 6A). In striking contrast, delivery of quipazine, caudal to the lesion, produced substantially larger improvements in both the reach and grasp ability after severe cervical SCI (Figure 6B). Severely-injured rats that received quipazine showed a 3.1-fold larger gain in their reach ability relative to the physical-training-alone group ($t(4) = 8.139$, $p = .001$, paired-samples t test; effect size = 4.01, very large effect). Quipazine-mediated gains in grasp after severe SCI were also larger ($t(4) = 3.523$, $p = .02$, paired-samples t test; effect size = 10.89, very large effect). Individual gains in grasp were less than 3% in the absence of quipazine after severe SCI. In the presence of quipazine, the average gain in grasp in the severely-injured animals was 36%. These results demonstrate the effectiveness of intraspinal quipazine in promoting recovery of skilled reach and grasp movements after severe cervical SCI.

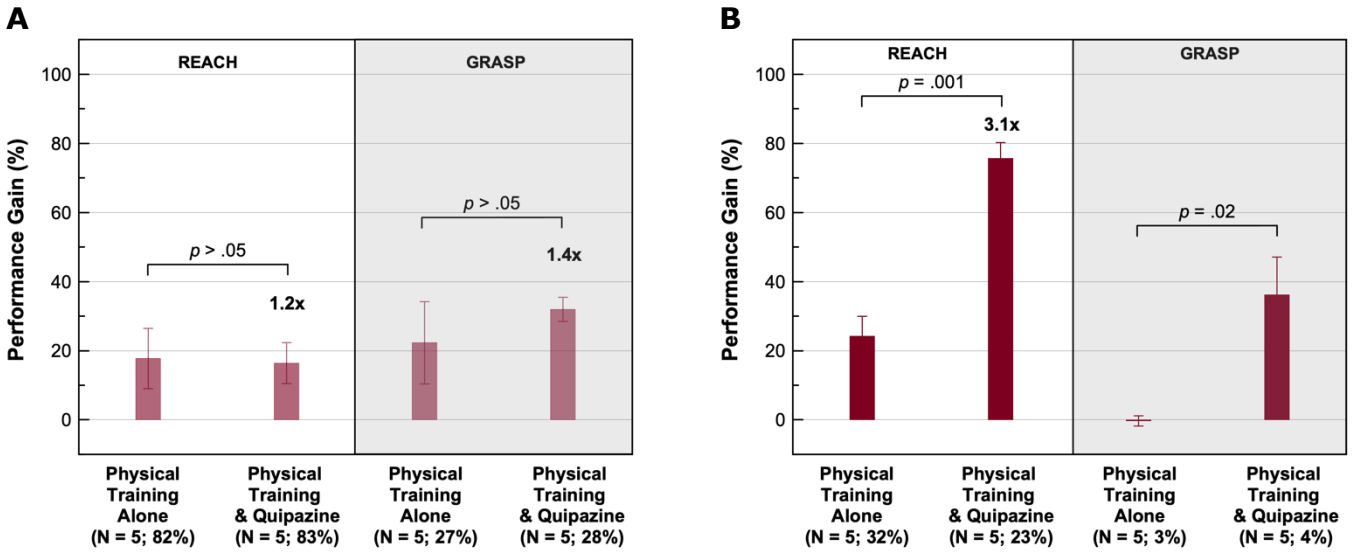

Figure 6. Intervention-mediated forelimb-performance gains after a moderate *vs.* severe SCI. (A, B) The reach (left panel) and grasp (right panel) performance gains after a moderate (A) *vs.* severe (B) SCI are plotted for rats that received physical training and intraspinal delivery of vehicle/quipazine. Numbers (in bold) above the bars quantify the performance of rats that received the combined intervention relative to the physical-training-alone group. Error bars represent standard error of the mean. *p* values were obtained using paired-samples *t* tests. Number of rats included in each group (indicated by 'N'), along with the average injury severity (or the pre-therapy reach/grasp baseline) of the group, are reported in parentheses below the bars. Note that relative gains are not reported for grasp in the severely-injured cohort (B, right panel) due to the lack of gains in the physical-training-alone group (which produced an inflated ratio when comparing gains between the two interventions).

Discussion

This study describes a neuromodulatory intervention that relies on intraspinal delivery of serotonergic agonists to promote forelimb-motor recovery after chronic moderate-to-severe cervical SCI. Chronic motor deficits are one of the most debilitating symptoms experienced by individuals with SCI, and restoration of upper-extremity function has been identified as an important recovery priority (57). Impairments in upper-extremity function can compound difficulties in other areas—such as bowel and bladder management, both of which have also been identified as recovery priorities (57)—to further reduce independence and quality of life. Physical rehabilitation and use-dependent movement interventions are currently the gold standard of treatment after SCI, but they offer limited benefits, especially after chronic trauma (10-15). Our results demonstrate that targeted delivery of quipazine, a non-specific 5-HT_2 agonist (36, 38, 44), caudal to the lesion substantially increased the effectiveness of physical rehabilitation in promoting forelimb-motor recovery in a rodent contusion model of chronic cervical SCI. Rats in our study received physical rehabilitation combined with intraspinal delivery of quipazine/vehicle. All rats that received quipazine recovered 100% of their pre-injury reach ability and showed substantial improvements in grasp, which led to sizable gains in forelimb-motor performance and reduced post-therapy performance variability in this intervention group. In comparison, physical rehabilitation alone provided significantly reduced benefits, which were also more variable across animals. This difference may be attributed to the beneficial effect of quipazine on motor recovery after severe SCI. While recovery of skilled reach and grasp movements was comparable between the two interventions in moderately-injured animals, quipazine significantly augmented forelimb-motor gains after severe SCI. Gains in reach were substantially smaller in the absence of quipazine after severe SCI, and physical training alone was completely ineffective in the rescue of skilled grasping movements in severely-injured animals. Notably, our experiments were conducted in an adult chronic model of cervical SCI. Our data, therefore, highlight an important role for serotonin in restoration of motor function after a severe and chronic injury of the spinal cord. Remarkably, quipazine-mediated improvements in forelimb function outlasted neuromodulator delivery, suggesting that our intervention had modified the underlying spared, but malfunctional, circuitry to drive lasting motor recovery (in contrast to the temporary reanimation of spinal circuitry produced by existing epidural-stimulation approaches).

Potential mechanisms underlying quipazine-mediated motor recovery after chronic severe SCI. The dendrites of motoneurons have voltage-dependent channels that can generate strong sodium and calcium persistent inward currents (PICs), which result in the characteristic sustained motor-unit firing pattern. The amplitude of the inward current is proportional to the level of neuromodulatory input from the brainstem, and serotonin plays an important role in the modulation of PICs (58). SCI results in a loss (or reduction) of brainstem-derived serotonin. In the days following SCI (*i.e.,* during the acute phase of the injury), motoneurons have small PICs and reduced excitability, and spinal reflexes are weak or absent, which leads to impairments in motor function and paralysis (33, 58).

Over time, as the injury enters the chronic phase, motoneurons compensate for the loss of serotonergic input and degeneration of descending (serotonergic) neurons through an overexpression of 5-HT_2 receptors—primarily 5-HT_{2A} (59-61) and 5-HT_{2C} (33, 62) subtypes—caudal to the lesion. Moreover, these receptors switch to a constitutive phenotype (33, 63), which restores the PICs and, in turn, motoneuron excitability to enable sustained muscle contractions (in the continued absence of brainstem-derived serotonin)

that, ultimately, lead to some spontaneous recovery of motor function. However, in the absence of normal inhibitory input, especially from spinal interneurons that are also regulated by descending tracts (64, 65), these self-sustained depolarizations can produce involuntary muscle spasms or spasticity (33, 58), which is a debilitating symptom that affects 65 – 78% of individuals with chronic SCI (5), further contributing to the range of motor dysfunction.

Other studies have reported that low levels of serotonin (2 – 12%), derived in part from spinal serotonergic neurons associated with the autonomic system, persist in the spinal cord below a chronic transection (28, 66, 67). This combined with the enhanced sensitivity of motoneurons to residual monoamines after a chronic injury (51, 52, 68, 69) have also been postulated to be critical to the redevelopment of PICs. Bennett and coworkers found that very low levels (0.5 – 3%) of serotonin were sufficient to facilitate spinal reflexes after a chronic SCI relative to the dose required after an acute injury of the cord (51, 52, 69).

Our study found that intraspinal delivery of quipazine, caudal to the lesion, provided no additional benefit over physical rehabilitation alone after a moderate SCI. Considering that we implemented a chronic model of incomplete cervical SCI, it is expected that there will be some brainstem-derived serotonin caudal to the lesion. It is, therefore, not surprising that the residual serotonin—derived from both spared descending pathways and autonomic sources—combined with the supersensitivity of motoneurons after a chronic injury (as discussed above) led to no additional benefits from our serotonergic intervention after a moderate SCI. In contrast, delivery of quipazine significantly improved motor-recovery outcomes after a severe SCI in chronically-injured animals, perhaps due to a greater loss of brainstem-derived serotonin, which would be expected after a severe injury. Since motoneuron function and normal movement production require both excitatory and inhibitory control (58), and serotonergic modulation is expected to increase motoneuron excitability (70), we can only speculate that the incomplete nature of our cervical injury spared enough inhibitory circuits, and descending tracts that regulate them (64, 65), to allow for restoration of forelimb movements without occurrence of appreciable levels of spasticity. While spasticity was not quantitatively assessed in our study, the large extent of motor recovery observed in the severely-injured animals that received quipazine provides support for the above argument.

Lastly, several studies have found that monoamines, primarily serotonin and norepinephrine, increase the sensitivity of motoneurons to both excitation and inhibition (71-73). Furthermore, a wide variety of spinal interneurons are strongly influenced by serotonergic inputs to the cord (74-77) through activation of 5-HT_{1B} and 5-HT_{1D} receptors (77, 78). Therefore, delivery of a non-specific serotonergic agonist, such as quipazine, may also have modulated the inhibitory input (in addition to enhancing motoneuron excitability; 70) to promote motor recovery after a severe and chronic injury of the spinal cord. Larger-scale investigations, incorporating *in vivo* electrophysiology and genetic strategies (such as transgenic animals and viral vectors), are needed to elucidate how (and which) specific subsets of spinal neurons and serotonergic receptors contribute to the motor recovery mediated by delivery of quipazine caudal to the lesion after chronic SCI.

Therapeutic benefits outlast neuromodulator delivery. Our results show that delivery of quipazine, caudal to the lesion, substantially improved motor function after chronic severe SCI. Remarkably, quipazine-mediated benefits persisted for at least four weeks after the end of therapy (in the absence of quipazine; Figure 2). The maintenance of quipazine-driven recovery for weeks beyond the intervention is both a unique and clinically-relevant finding. It distinguishes our intervention from existing epidural-stimulation approaches (16-27) whose benefits are largely contingent on the continued delivery of therapy.

Serotonergic agonists have been used extensively to promote motor recovery after SCI (35-43). However, only transient benefits were observed in these studies. In one study by Antri and coworkers, daily injections of quipazine and 8-OHDPAT (which is an agonist for 5-HT_{1A} and 5-HT_7 receptors) for one month, after a T8 – T9 SCI, led to long-term improvements in locomotion that lasted for up to 46 days after the end of neuromodulator administration (79). While the longevity of motor gains is remarkable, serotonergic therapy was initiated the day after SCI. Furthermore, serotonergic interventions were delivered during the acute or subacute phase of the injury in all the above studies (35-43, 79). However, the majority of SCI patients have chronic injuries. Our study, in which quipazine delivery was initiated eight weeks after SCI (which represents a common time point for a chronic SCI model in rats; 51, 52), therefore, provides critical evidence for the important role of serotonin in repair after a chronic and severe contusion of the cervical cord. The substantial and lasting recovery promoted by our intervention further underscores its clinical relevance for motor restoration after chronic damage.

Lastly, a previous study from our laboratory, by McPherson *et al*., used targeted activity-dependent spinal stimulation (TADSS) to strengthen spared motor pathways after an incomplete SCI (80). Delivery of intraspinal TADSS, triggered by residual electromyographic (EMG) activity in the impaired forelimb, produced lasting motor recovery in a rodent contusion model of chronic cervical SCI (similar to the one used in the current study). The average pre-therapy baseline across the different intervention groups in the McPherson *et al*. study was 13% ± 2%, relative to pre-SCI levels, with no between-group differences in performance before the start of treatment. Forelimb-motor performance was calculated using a binary measure that relied only on the number of successes (which is different from the metrics employed in the current study). Using the McPherson-*et*-*al*. metric (for direct comparison), we found that rats in our (current) study were more injured averaging at 4.0% ± 1.9% when all rats were included (N = 20) and 0.2% ± 0.2% in the severely-injured cohort (N = 10) with no significant difference between animals in the two intervention groups before the start of therapy ($Z = -0.943$, $p > .05$, Wilcoxon signed-rank test). Moreover, animals with a pre-intervention score of zero did not benefit from TADSS (unless they achieved an average of at least one success in either of the first two weeks of the intervention), perhaps due to the lack of enough spared pathways necessary for EMG-triggered stimulation. In contrast, six out of the ten animals that received quipazine had a pre-intervention score of zero, and they all showed substantial and lasting improvement in motor function. These comparisons highlight a critical role for serotonin in long-term motor restoration after chronic severe SCI that is perhaps less reliant on spared pathways and could involve axonal sprouting and formation of new synaptic connections (81, 82).

Conclusions. Physical rehabilitation and use-dependent movement interventions have been widely explored as treatment options after SCI (9-15), but they are only marginally effective after severe and chronic neurological trauma. Our results demonstrate that intraspinal delivery of quipazine (which is a non-specific serotonergic-receptor agonist; 36, 38, 44), caudal to the lesion, can significantly improve motor-recovery outcomes promoted by physical rehabilitation after a chronic and severe contusion of the spinal cord. Notably, delivery of quipazine resulted in substantial behavioral restoration that persisted for weeks after the end of therapy (in the absence of quipazine). The ability of our intervention to induce sustained benefits underscores its clinical relevance and signals a serotonin-driven reorganization of chronically-damaged adult spinal circuitry. Subsequent studies will explore less-invasive intrathecal or epidural routes of neuromodulator administration. Finally, our experiments provide new insights into serotonergic modulation of spinal circuitry, which will inform future investigations and therapies targeting chronic SCI.

Materials and Methods

All animal-handling, behavioral training and surgical procedures were approved by the University of Washington's Institutional Animal Care and Use Committee, and they conformed to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (83).

Subjects. Experiments were performed with 20 adult female Long-Evans rats (procured from Charles River Laboratories). A weight cut-off of 250 g (84), which corresponds to an age cut-off of approximately 90 days (85), was imposed (before the SCI) to be considered an adult animal. Pre-injury weights of rats in the two intervention groups ranged between 266 – 354 g.

Behavioral training. After allowing several days for acclimation, rats were trained to perform a skilled forelimb reach-and-grasp behavior (46) during their dark (*i.e.,* active) cycle, using operant-conditioning protocols (47) for food-pellet (Bio-Serv, F0299) reward. To initiate training, animals were placed in a custom-designed clear plexiglass arena that contained two vertical slits near the lower-front corners. A food pellet was inserted inside a small dimple in a (custom-made) plastic block that was placed outside the arena in front of one of the vertical slits. A gap between the arena and the plastic block ensured that the food pellet would fall and land out of reach when not firmly grasped by the rats. After identification of the dominant forelimb, rats were trained to reach out through the slit, grasp the food pellet placed on the block with their dominant forepaw, and bring the pellet through the slit into the arena and to their mouth. The width and position of the slit were chosen to ensure that rats could reach the pellets only with one forelimb. After SCI, this design discouraged reaching with the contralesional forelimb, thus focusing retraining on the impaired limb.

Behavioral scoring. Each behavioral session consisted of 20 pellet-retrieval trials, which were preceded by a short unscored warm-up of five trials. Performance was quantified by the cumulative total of the outcome of individual trials in the session. There were five possible outcomes for a pellet-retrieval attempt: [1] the rat retrieved the food pellet and brought it inside the arena to its mouth with the dominant forelimb (which was counted as a success), [2] the rat retrieved the food pellet, but the pellet dropped inside the arena on the way to its mouth (considered a drop-inside), [3] the rat retrieved the food pellet, but the pellet dropped outside the arena (scored as a drop-outside), [4] the rat extended the dominant forelimb far enough to touch the food pellet, but no grasp was involved (counted as a touch), or [5] the rat made no contact with the pellet (which was considered a miss). These outcomes (success, drop-inside, drop-outside, touch, miss) were quantified based on the level of reach and grasp ability involved in the effort. The rat was assigned a reach score of 0.50 for success, drop-inside, drop-outside and touch. These different outcomes received the same reach score due to the similar level of forelimb extension involved in each of the outcomes. In contrast, the rat received a grasp score of 0.50, 0.33 and 0.17 for success, drop-inside and drop-outside, respectively. A touch received a grasp score of 0 (since the rat did not lift the pellet out of the dimple in the block). These scores were graded based on the grasping ability involved in each outcome. Trials that resulted in a miss received 0 for both reach and grasp. Individual reach and grasp scores were summed up to generate the combined reach-and-grasp score per trial. This scoring scheme is summarized in Table S1. Next, scores from the 20 individual trials were added to compile behavioral scores for the session. Ten sessions of forelimb-motor training occurred per week, with the sessions

divided across the five weekdays. Daily sessions were at least 30 minutes apart to prevent confounds due to fatigue. Scores from the ten sessions were averaged and expressed as a percentage of the 20 trials to compile weekly reach, grasp and reach-and-grasp performances. Training was complete when rats attained a weekly reach-and-grasp performance score of at least 80% (which took 1 – 4 weeks).

Spinal contusion. After rats attained a reach-and-grasp performance score of at least 80% on the forelimb-motor task, they received a unilateral moderate-to-severe dorsal contusion injury at the border of C4 – C5 spinal segments on their dominant forelimb side (80), using an Ohio State impact device (48). Briefly, rats were anesthetized with a mixture of ketamine (70 mg/kg) and xylazine (10 mg/kg), administered intraperitoneally, and received local intradermal anesthetics (1% lidocaine and 0.25% bupivacaine) along the incision line and subcutaneous antibiotics (enrofloxacin, 0.70 mg/kg) pre-operatively. In an aseptic procedure, the dorsal neck musculature over the cervical spinal column was retracted, and a hemilaminectomy was performed on the C4 vertebrae on the dominant forelimb side. Clamps of the Ohio State device were then placed on the lateral processes of C3 and C5 segments to fix the spinal column into a suspended position. Next, the tip of the impactor probe was aligned at the dorsal root entry zone of the exposed cord, lowered until probe sensors detected contact, and then triggered to rapidly displace the cord (for 14 milliseconds) to simulate an impact injury. Injury displacements of the animals in our study ranged between 0.61 – 0.71 mm. After the contusion, the rat was removed from the clamps, and the musculature and skin were sutured closed (with 4-0 polyglycolic acid and 5-0 nylon suture, respectively; AD Surgical). Rats recovered with fluid and heat support. Injured animals received post-operative drugs (1 mg/kg subcutaneous sustained-release buprenorphine and 0.20 mg/mL enrofloxacin in their water) and manual bladder expressions (two times per day) until voiding reflexes returned, usually within 1 – 4 days.

All animals exhibited near-complete paralysis of the ipsilesional forelimb in the first 1 – 2 days after SCI. Pronounced ipsilesional forelimb deficits persisted chronically and were characterized by a flexion bias at the elbow, wrist and digits, and minimal ability to volitionally extend the limb. Paw placement during exploratory rearing behavior was generally asymmetrical and dominated by the contralesional forelimb, which was also dominant during weight-bearing, grooming and locomotion functions. Additionally, movements of the impaired forelimb were generally slower and lacked precise coordination. In the severely-injured animals, spontaneous recovery was negligible, and near-complete paralysis of the ipsilesional forelimb remained present at the start of the intervention.

Intraspinal cannula and subcutaneous pump implants. Four weeks after SCI, rats were implanted with a custom-designed cannula whose distal tip terminated in the ipsilesional C7 spinal segment (caudal to the injury site; see experimental timeline in Figure 1A). The cannula assembly consisted of a 3-mm piece of 0.13-mm inner-diameter polyurethane spinal catheter (Instech Laboratories, BTPU-010) of which 2 mm were inserted into (180 mm of) an intermediate-sized silicone tubing (whose inner diameter was 0.30 mm; DuPont, 508-001). The overlap junction was secured with 4-0 silk suture (AD Surgical) and medical-grade liquid silicone adhesive (Factor II). Next, a custom-cut polyamide disc was placed at the junction to act as a (subdural) depth stop for limiting the penetration depth to 1 mm. The intermediate tubing served to interface the spinal catheter with the larger-diameter outlet tubing from a peristaltic pump (Alzet, iPrecio SMP-200; 50), which was implanted subcutaneously in the same surgery or in a separate procedure (as detailed below) and was used for controlled delivery of fluids into the cord.

Rats were anesthetized using inhaled isoflurane (2 – 3% in oxygen), placed on a

heating pad, and administered subcutaneous antibiotics (enrofloxacin, 0.70 mg/kg), corticosteroids (dexamethasone, 0.50 mg/kg), analgesics (meloxicam, 1 mg/kg) and lactated Ringer's solution (for fluid support) pre-operatively. Surgical plane was verified *via* toe pinch and ocular reflex, and vitals were monitored continuously throughout the surgery using a SomnoSuite surgical monitoring system (Kent Scientific). Under aseptic conditions, an incision was made along the dorsal midline above C7, the muscle was separated and retracted, and the spinal column was exposed. A full C7 and a partial-to-full C6 hemilaminectomy was performed on the ipsilesional vertebrae. The cannula was flushed with 70% isopropanol and sterile saline, then primed with saline, and tied shut at the proximal (or the silicone) end using 4-0 silk suture. Next, the dura mater over the C7 dorsal root entry zone was incised and the polyurethane end of the cannula was inserted 1 mm into the cord, as set by the depth stop. The dura mater was then closed atop the depth stop and around the cannula with 7-0 silk suture (AD Surgical) to anchor it in place. The remaining length of the cannula extended dorsally through the muscle (which was closed with 4-0 polyglycolic acid suture) and was coiled inside a subdermal pocket.

During the cannula-implant surgery or in a separate brief procedure (that occurred at the end of the pre-intervention week and before the start of therapy), a second incision was made several centimeters rostral to the cannula-implant site and a subdermal pocket was created *via* blunt dissection for placing a battery-operated microinfusion pump (Alzet, iPrecio SMP-200; 50) whose delivery profile was programmed before implantation. The pump-outlet tubing was trimmed to 20 mm, and the reservoir and outlet tubing were primed with saline. Next, the cannula was tunneled subdermally to the pump pocket, 5 mm of its proximal length (at the silicone end) were snipped, and it was inserted inside the pump-outlet tubing. The junction was secured using 4-0 silk suture and liquid silicone (World Precision Instruments, Kwik-Sil). Note that an additional small incision was made above the cannula-implant site to expose the proximal (or silicone) end of the cannula when the pump was implanted in a separate procedure. Lastly, the pump was anchored inside the subdermal pocket using silicone-coated 4-0 silk suture, and the incision sites were sutured closed (with 5-0 nylon). Rats recovered on a heating pad and were administered analgesics (1 mg/kg subcutaneous sustained-release buprenorphine) and antibiotics (0.20 mg/mL enrofloxacin in their water). Animals were allowed two weeks of post-operative recovery before assessment of their injury severity.

Impairment matching and group assignments. Saline was delivered during two weeks of post-operative recovery, which was followed by pre-therapy motor assessment on the previously-mastered pellet-retrieval task (46) during week 7 (also in the presence of saline; see experimental timeline in Figure 1A) to quantify the functional deficits produced by the cervical contusion. To account for the variability in motor impairment across animals arising from the large range of injury severity tested in our study, we used an impairment-matched design, which involved classifying rats into different injury-severity levels based on their pre-therapy reach-and-grasp performance scores. Within a given injury-severity level, rats were then randomly assigned to one of the two intervention groups. Table S2 details this classification, showing the minimum, maximum and average pre-therapy motor performances of rats tested at each injury-severity level.

To assess the effect of therapy on forelimb-motor recovery as a function of the severity of the cervical injury, rats with a pre-therapy reach-and-grasp motor performance score of less than or equal to 30% were ranked severely injured (Table S2, injury-severity levels I_4 and I_5) and rats with a pre-therapy performance of greater than 30% were classified as moderately injured (Table S2, injury-severity levels I_1 – I_3) in our study.

Therapy and post-therapy follow-up. Before the start of therapy, animals were briefly sedated using inhaled isoflurane, and the fluid in the pump reservoir was exchanged from saline to quipazine (Sigma-Aldrich, Q1004), *via* a percutaneously-accessible septum, in rats that received the combined intervention. To maintain uniformity of procedures across all animals, a saline-to-saline exchange was performed in the control animals.

Therapy, consisting of physical rehabilitation and neuromodulator/vehicle delivery, was administered during weeks 8 – 13 after SCI (see experimental timeline in Figure 1A). The 8-week delay between injury and start-of-therapy was chosen to allow the contusion to translate to a chronic SCI model (51, 52). Quipazine/vehicle was delivered intraspinally below the lesion for approximately 4.5 hours per day (between 11:00 – 15:24) for six weeks. Pumps were pre-programmed (before implantation) to gradually increase the flow rate from 0.5 – 4.0 μL/hour during the therapy window, with the flow rate doubled every 30 minutes and then maintained at 4.0 μL/hour. A gradual delivery profile was chosen due to its association with more durable effects, such as sustained activation of receptors and downstream signaling pathways, compared to fast chemical application (86). Pumps stayed off during the remaining hours of the day (*i.e.,* outside the therapy window). Rats received 20 μg/week of quipazine. The therapy duration of six weeks was chosen due to the plateau in gains observed at this time point (Figure 1B). Physical rehabilitation consisted of ten sessions per week of retraining of the impaired forelimb on the pellet-retrieval task, divided between the five weekdays, and occurred during periods of ongoing neuromodulator/vehicle delivery. Each physical-training session, in turn, consisted of 20 pellet-retrieval trials, preceded by a short warm-up (as described above in this section).

After completion of therapy, a reverse fluid exchange from quipazine to saline was performed under brief isoflurane anesthesia in rats in the combined intervention group, while a (second) saline-to-saline exchange was performed in the control animals. Rats continued to be tested in the pellet-retrieval task for an additional four weeks (in the presence of saline; weeks 14 – 17; see experimental timeline in Figure 1A) to assess if neuromodulator-mediated gains persisted in the absence of quipazine.

Pumps were refilled with quipazine/saline as needed during therapy and follow-up periods. Reservoir-volume checks on the pumps were performed during all exchanges/refills to confirm fluid delivery and rule out any clogging issues. Clogging was observed in two rats in the control group who did not receive the entire volume of saline. Due to the long-term nature of the experiments and their assignment within the control group, these rats were included in the study.

All rats in both intervention groups received the same amount of retraining on pellet retrieval during (per week) pre-therapy assessment, therapy and follow-up periods. The pellet-retrieval task served as both a form of use-dependent physical training and a means to quantify changes in forelimb-motor performance. To eliminate biases in behavioral scoring, all retraining was performed by researchers blinded to the group assignments of the rats. Additionally, these individuals were not involved in data analyses or interpretation. Moreover, surgeries and procedures were similar in all animals, and personnel involved in surgeries and procedures, related to fluid exchanges and reservoir refills, did not train animals.

Terminal surgery. After completion of post-therapy follow-up, a terminal surgery was performed in all rats from both intervention groups, during week 18 (see experimental timeline in Figure 1A), in which we ensured that pumps were still operational at the end of the study. We also verified the integrity of fluidic junctions and flow through the entire line. Next, we checked the location of the distal tip and entrance angle of the cannula to confirm that fluid was delivered in the spinal cord (*vs.* in the spinal cavity or epidurally) caudal to

the lesion. Lastly, we looked for signs of infection in the spinal cord and muscle layers. No infection was seen in any of the rats in the study.

Behavioral analyses. Forelimb-motor performance on the pellet-retrieval task was the primary outcome measure employed in our study to assess both the functional deficits produced by the cervical-contusion injury and intervention-mediated changes in motor function. We quantified the reach and grasp motor performance, individually and together, after SCI relative to pre-injury performance levels. The weekly motor performance during the pre-therapy, therapy and follow-up periods was expressed as a percentage of the rat's individual pre-SCI maximum, which was the maximum weekly score obtained during behavioral training. Additional details can be found in SI Materials and Methods. Note that, while the ability to reach could be assessed independently, the assessment of grasp was dependent on the rat accurately extending its forelimb outside the arena far enough to reach the food pellet, implying that only pellet-retrieval trials in which rats achieved a touch, drop-outside, drop-inside or success could be used for evaluating grasp. While this could potentially result in an underestimation of grasp ability (due to its dependence on reach), there were only two (out of 40) sessions during the 4-week post-therapy follow-up in one control rat alone (out of the 20 rats in our study across the two intervention groups) in which the rat was unable to reach the pellet and all trials resulted in misses. These two sessions were, therefore, excluded from the grasp analysis.

We also calculated the therapeutic gains, which was the difference between the (mean) post-therapy and pre-therapy motor performances, produced by our interventions. The post-therapy performance was calculated by averaging scores obtained during weeks 14 – 17.

Statistics. Motor-performance scores and therapeutic gains are reported as mean ± standard error (unless otherwise stated). Paired-samples *t* tests were employed for comparisons of performance scores/gains between the two impairment-matched intervention groups (53, 54) and across (two) time points (such as in Figure 2). Before performing paired *t* tests, we verified that the differences between the paired data followed a normal distribution. Normality was assessed using the Shapiro-Wilk test. Wilcoxon signed-rank test, which is the non-parametric equivalent of the paired-samples *t* test, was used when the data were not normally distributed. We also used the Friedman test, which is the non-parametric equivalent of repeated-measures analysis of variance, to compare data when more than two time points were involved. All statistical analyses were performed in IBM SPSS Statistics (version 27). Significance for all statistics was set at the .05 level.

In addition to assessing statistical significance, we quantified the magnitude of the effect using the "effect size" metric, which is the standardized difference between the means of two groups (87). Additional details of effect-size calculations can be found in SI Materials and Methods. Effect-size descriptors developed by Cohen (87) and extended by Rosenthal (88), which are summarized in Table S3, were used for qualitative interpretation of the magnitude of observed effects.

Acknowledgements

We thank Gawin Mai, Pierce Lovinger, Juliana Baranov and Amulya Jolepalem for behavioral training of rats and assistance with handling and care of the animals. This work was supported by grants from the National Institutes of Health (NS099872), the Craig H. Neilsen Foundation (384977), the Paralyzed Veterans of America (3082) and the University of Washington Royalty Research Fund (A166877). Additionally, the study was supported by an Early Investigator Catalyst Award from the Institute of Translational Health Sciences at the University of Washington.

References

1. James SL*, et al.* (2019) Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 18(1):56-87.
2. Ahuja CS*, et al.* (2017) Traumatic spinal cord injury. *Nat. Rev. Dis. Primers* 3:17018.
3. Cripps RA*, et al.* (2011) A global map for traumatic spinal cord injury epidemiology: towards a living data repository for injury prevention. *Spinal Cord* 49(4):493-501.
4. Alizadeh A, Dyck SM & Karimi-Abdolrezaee S (2019) Traumatic spinal cord injury: An overview of pathophysiology, models and acute injury mechanisms. *Front. Neurol.* 10:282.
5. Adams MM & Hicks AL (2005) Spasticity after spinal cord injury. *Spinal Cord* 43(10):577-586.
6. Thomas CK, Zaidner EY, Calancie B, Broton JG & Bigland-Ritchie BR (1997) Muscle weakness, paralysis, and atrophy after human cervical spinal cord injury. *Exp. Neurol.* 148(2):414-423.
7. Johnson RL, Gerhart KA, McCray J, Menconi JC & Whiteneck GG (1998) Secondary conditions following spinal cord injury in a population-based sample. *Spinal Cord* 36(1):45-50.
8. Rowland JW, Hawryluk GWJ, Kwon B & Fehlings MG (2008) Current status of acute spinal cord injury pathophysiology and emerging therapies: promise on the horizon. *Neurosurg. Focus* 25(5):E2.
9. Roy RR, Harkema SJ & Edgerton VR (2012) Basic concepts of activity-based interventions for improved recovery of motor function after spinal cord injury. *Arch. Phys. Med. Rehabil.* 93(9):1487-1497.
10. Hornby TG, Zemon DH & Campbell D (2005) Robotic-assisted, body-weight-supported treadmill training in individuals following motor incomplete spinal cord injury. *Phys. Ther.* 85(1):52-66.
11. Lam T, Pauhl K, Krassioukov A & Eng JJ (2011) Using robot-applied resistance to augment body-weight-supported treadmill training in an individual with incomplete spinal cord injury. *Phys. Ther.* 91(1):143-151.
12. Hicks AL*, et al.* (2005) Long-term body-weight-supported treadmill training and subsequent follow-up in persons with chronic SCI: effects on functional walking ability and measures of subjective well-being. *Spinal Cord* 43(5):291-298.
13. Taub E, Crago JE & Uswatte G (1998) Constraint-induced movement therapy: A new approach to treatment in physical rehabilitation. *Rehabil. Psychol.* 43(2):152-170.
14. Antoniadou E*, et al.* (2022) Use of the modified constraint induced movement therapy protocol to improve function of the predominant arm on a patient with incomplete spinal cord injury. *Am. J. Phys. Med. Rehabil.* 101(4):e62-e64.
15. Taub E, Uswatte G & Elbert T (2002) New treatments in neurorehabiliation founded on basic research. *Nat. Rev. Neurosci.* 3(3):228-236.
16. Wagner FB*, et al.* (2018) Targeted neurotechnology restores walking in humans with spinal cord injury. *Nature* 563(7729):65-71.
17. Wenger N*, et al.* (2014) Closed-loop neuromodulation of spinal sensorimotor circuits controls refined locomotion after complete spinal cord injury. *Sci. Transl. Med.* 6(255):255ra133.
18. Harkema S*, et al.* (2011) Effect of epidural stimulation of the lumbosacral spinal cord on voluntary movement, standing, and assisted stepping after motor complete paraplegia: a case study. *Lancet* 377(9781):1938–1947.
19. Rowald A*, et al.* (2022) Activity-dependent spinal cord neuromodulation rapidly

restores trunk and leg motor functions after complete paralysis. *Nat. Med.* 28(2):260-271.
20. Angeli CA, Edgerton VR, Gerasimenko YP & Harkema SJ (2014) Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans. *Brain* 137(5):1394-1409.
21. Ichiyama RM*, et al.* (2008) Step training reinforces specific spinal locomotor circuitry in adult spinal rats. *J. Neurosci.* 28(29):7370-7375.
22. Courtine G*, et al.* (2009) Transformation of nonfunctional spinal circuits into functional states after the loss of brain input. *Nat. Neurosci.* 12(10):1333-1342.
23. Wenger N*, et al.* (2016) Spatiotemporal neuromodulation therapies engaging muscle synergies improve motor control after spinal cord injury. *Nat. Med.* 22(2):138-145.
24. van den Brand R*, et al.* (2012) Restoring voluntary control of locomotion after paralyzing spinal cord injury. *Science* 336(6085):1182-1185.
25. Asboth L*, et al.* (2018) Cortico–reticulo–spinal circuit reorganization enables functional recovery after severe spinal cord contusion. *Nat. Neurosci.* 21(4):576-588.
26. Gill ML*, et al.* (2018) Neuromodulation of lumbosacral spinal networks enables independent stepping after complete paraplegia. *Nat. Med.* 24(11):1677-1682.
27. Angeli CA*, et al.* (2018) Recovery of over-ground walking after chronic motor complete spinal cord injury. *N. Engl. J. Med.* 379(13):1244-1250.
28. Shapiro S (1997) Neurotransmission by neurons that use serotonin, noradrenaline, glutamate, glycine, and γ-aminobutyric acid in the normal and injured spinal cord. *Neurosurgery* 40(1):168-177.
29. Schmidt BJ & Jordan LM (2000) The role of serotonin in reflex modulation and locomotor rhythm production in the mammalian spinal cord. *Brain Res. Bull.* 53(5):689-710.
30. Perrier J-F, Rasmussen HB, Christensen RK & Petersen AV (2013) Modulation of the intrinsic properties of motoneurons by serotonin. *Curr. Pharm. Des.* 19(24):4371-4384.
31. Heckman CJ, Mottram C, Quinlan K, Theiss R & Schuster J (2009) Motoneuron excitability: The importance of neuromodulatory inputs. *Clin. Neurophysiol.* 120(12):2040-2054.
32. Perrier J-F & Cotel F (2015) Serotonergic modulation of spinal motor control. *Curr. Opin. Neurobiol.* 33:1-7.
33. Murray KC*, et al.* (2010) Recovery of motoneuron and locomotor function after spinal cord injury depends on constitutive activity in 5-HT_{2C} receptors. *Nat. Med.* 16(6):694-700.
34. Perrin FE & Noristani HN (2019) Serotonergic mechanisms in spinal cord injury. *Exp. Neurol.* 318:174-191.
35. Ganzer PD, Beringer CR, Shumsky JS, Nwaobasi C & Moxon KA (2018) Serotonin receptor and dendritic plasticity in the spinal cord mediated by chronic serotonergic pharmacotherapy combined with exercise following complete SCI in the adult rat. *Exp. Neurol.* 304:132-142.
36. Antri M, Orsal D & Barthe J-Y (2002) Locomotor recovery in the chronic spinal rat: effects of long-term treatment with a 5-HT_2 agonist. *Eur. J. Neurosci.* 16(3):467-476.
37. Dugan EA & Shumsky JS (2015) A combination therapy of neural and glial restricted precursor cells and chronic quipazine treatment paired with passive cycling promotes quipazine-induced stepping in adult spinalized rats. *J. Spinal Cord Med.* 38(6):792-804.
38. Gerasimenko YP*, et al.* (2007) Epidural spinal cord stimulation plus quipazine administration enable stepping in complete spinal adult rats. *J. Neurophysiol.*

98(5):2525-2536.
39. Ichiyama RM*, et al.* (2008) Dose dependence of the 5-HT agonist quipazine in facilitating spinal stepping in the rat with epidural stimulation. *Neurosci. Lett.* 438(3):281-285.
40. Ung R-V*, et al.* (2008) Role of spinal 5-HT_2 receptor subtypes in quipazine-induced hindlimb movements after a low-thoracic spinal cord transection. *Eur. J. Neurosci.* 28(11):2231-2242.
41. Mitchell GS*, et al.* (1992) 5-Hydroxytryptophan (5-HTP) augments spontaneous and evoked phrenic motoneuron discharge in spinalized rats. *Neurosci. Lett.* 141(1):75-78.
42. Jin B*, et al.* (2021) Serotonergic facilitation of forelimb functional recovery in rats with cervical spinal cord injury. *Neurotherapeutics* 18(2):1226-1243.
43. Fong AJ*, et al.* (2005) Spinal cord-transected mice learn to step in response to quipazine treatment and robotic training. *J. Neurosci.* 25(50):11738-11747.
44. Guertin PA (2004) Role of NMDA receptor activation in serotonin agonist-induced air-stepping in paraplegic mice. *Spinal Cord* 42(3):185-190.
45. Cohen O, Sherman E, Zinger N, Perlmutter S & Prut Y (2010) Getting ready to move: transmitted information in the corticospinal pathway during preparation for movement. *Curr. Opin. Neurobiol.* 20(6):696-703.
46. Whishaw IQ & Pellis SM (1990) The structure of skilled forelimb reaching in the rat: a proximally driven movement with a single distal rotatory component. *Behav. Brain Res.* 41(1):49-59.
47. Staddon JE & Cerutti DT (2003) Operant conditioning. *Annu. Rev. Psychol.* 54(1):115-144.
48. Stokes BT, Noyes DH & Behrmann DL (1992) An electromechanical spinal injury technique with dynamic sensitivity. *J. Neurotrauma* 9(3):187-195.
49. Schrimsher GW & Reier PJ (1992) Forelimb motor performance following cervical spinal cord contusion injury in the rat. *Exp. Neurol.* 117(3):287-298.
50. Tan T, Watts SW & Davis RP (2011) Drug delivery: enabling technology for drug discovery and development. iPRECIO® Micro Infusion Pump: programmable, refillable, and implantable. *Front. Pharmacol.* 2:44.
51. Harvey PJ, Li X, Li Y & Bennett DJ (2006) 5-HT_2 receptor activation facilitates a persistent sodium current and repetitive firing in spinal motoneurons of rats with and without chronic spinal cord injury. *J. Neurophysiol.* 96(3):1158-1170.
52. Li Y, Harvey PJ, Li X & Bennett DJ (2004) Spastic long-lasting reflexes of the chronic spinal rat studied in vitro. *J. Neurophysiol.* 91(5):2236-2246.
53. Niven DJ, Berthiaume LR, Fick GH & Laupland KB (2012) Matched case-control studies: a review of reported statistical methodology. *Clin. Epidemiol.* 4:99-110.
54. Breslow NE & Day NE (1980) *Statistical Methods in Cancer Research Volume 1: The Analysis of Case-Control Studies* (International Agency for Research on Cancer, Lyon, France).
55. McKenna JE & Whishaw IQ (1999) Complete compensation in skilled reaching success with associated impairments in limb synergies, after dorsal column lesion in the rat. *J. Neurosci.* 19(5):1885-1894.
56. Whishaw IQ, Pellis SM, Gorny B, Kolb B & Tetzlaff W (1993) Proximal and distal impairments in rat forelimb use in reaching follow unilateral pyramidal tract lesions. *Behav. Brain Res.* 56(1):59-76.
57. Simpson LA, Eng JJ, Hsieh JT, Wolfe DL & Spinal Cord Injury Rehabilitation Evidence (SCIRE) Research Team (2012) The health and life priorities of individuals with spinal cord injury: a systematic review. *J. Neurotrauma* 29(8):1548-1555.

58. Heckman CJ, Gorassini MA & Bennett DJ (2005) Persistent inward currents in motoneuron dendrites: implications for motor output. *Muscle Nerve* 31(2):135-156.
59. Kong X-Y, Wienecke J, Hultborn H & Zhang M (2010) Robust upregulation of serotonin 2A receptors after chronic spinal transection of rats: An immunohistochemical study. *Brain Res.* 1320:60-68.
60. Kong X-Y, Wienecke J, Chen M, Hultborn H & Zhang M (2011) The time course of serotonin 2A receptor expression after spinal transection of rats: an immunohistochemical study. *Neuroscience* 177:114-126.
61. Navarrett S, Collier L, Cardozo C & Dracheva S (2012) Alterations of serotonin 2C and 2A receptors in response to T10 spinal cord transection in rats. *Neurosci. Lett.* 506(1):74-78.
62. Ren L-Q*, et al.* (2013) The time course of serotonin 2C receptor expression after spinal transection of rats: An immunohistochemical study. *Neuroscience* 236:31-46.
63. Fouad K*, et al.* (2010) Locomotion after spinal cord injury depends on constitutive activity in serotonin receptors. *J. Neurophysiol.* 104(6):2975-2984.
64. Matsuyama K*, et al.* (2004) Locomotor role of the corticoreticular–reticulospinal–spinal interneuronal system. *Prog. Brain Res.* 143:239-249.
65. Riddle CN & Baker SN (2010) Convergence of pyramidal and medial brain stem descending pathways onto macaque cervical spinal interneurons. *J. Neurophysiol.* 103(5):2821-2832.
66. Newton BW & Hamill RW (1988) The morphology and distribution of rat serotoninergic intraspinal neurons: An immunohistochemical study. *Brain Res. Bull.* 20(3):349-360.
67. Hadjiconstantinou M, Panula P, Lackovic Z & Neff NH (1984) Spinal cord serotonin: A biochemical and immunohistochemical study following transection. *Brain Res.* 322(2):245-254.
68. Barbeau H, Filion M & Bedard P (1981) Effects of agonists and antagonists of serotonin on spontaneous hindlimb EMG activity in chronic spinal rats. *Neuropharmacology* 20(2):99-107.
69. Li X, Murray K, Harvey PJ, Ballou EW & Bennett DJ (2007) Serotonin facilitates a persistent calcium current in motoneurons of rats with and without chronic spinal cord injury. *J. Neurophysiol.* 97(2):1236-1246.
70. Heckman CJ, Lee RH & Brownstone RM (2003) Hyperexcitable dendrites in motoneurons and their neuromodulatory control during motor behavior. *Trends Neurosci.* 26(12):688-695.
71. Perrier J-F & Hounsgaard J (2003) 5-HT_2 receptors promote plateau potentials in turtle spinal motoneurons by facilitating an L-type calcium current. *J. Neurophysiol.* 89(2):954-959.
72. Hultborn H, Denton ME, Wienecke J & Nielsen JB (2003) Variable amplification of synaptic input to cat spinal motoneurones by dendritic persistent inward current. *J. Physiol.* 552(3):945-952.
73. Kuo JJ, Lee RH, Johnson MD, Heckman HM & Heckman CJ (2003) Active dendritic integration of inhibitory synaptic inputs in vivo. *J. Neurophysiol.* 90(6):3617-3624.
74. Jankowska E (1992) Interneuronal relay in spinal pathways from proprioceptors. *Prog. Neurobiol.* 38(4):335-378.
75. Hammar I & Jankowska E (2003) Modulatory effects of α1-, α2-, and β-receptor agonists on feline spinal interneurons with monosynaptic input from group I muscle afferents. *J. Neurosci.* 23(1):332-338.
76. Hammar I, Chojnicka B & Jankowska E (2002) Modulation of responses of feline ventral spinocerebellar tract neurons by monoamines. *J. Comp. Neurol.* 443(3):298-309.

77. Bras H, Jankowska E, Noga B & Skoog B (1990) Comparison of effects of various types of NA and 5-HT agonists on transmission from group II muscle afferents in the cat. *Eur. J. Neurosci.* 2(12):1029-1039.
78. Miller JF, Paul KD, Rymer WZ & Heckman CJ (1995) 5-HT1B/1D agonist CGS-12066B attenuates clasp knife reflex in the cat. *J. Neurophysiol.* 74(1):453-456.
79. Antri M, Barthe J-Y, Mouffle C & Orsal D (2005) Long-lasting recovery of locomotor function in chronic spinal rat following chronic combined pharmacological stimulation of serotonergic receptors with 8-OHDPAT and quipazine. *Neurosci. Lett.* 384(1-2):162-167.
80. McPherson JG, Miller RR & Perlmutter SI (2015) Targeted, activity-dependent spinal stimulation produces long-lasting motor recovery in chronic cervical spinal cord injury. *Proc. Natl. Acad. Sci. USA* 112(39):12193-12198.
81. Bareyre FM*, et al.* (2004) The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. *Nat. Neurosci.* 7(3):269-277.
82. Fouad K, Pedersen V, Schwab ME & Brösamle C (2001) Cervical sprouting of corticospinal fibers after thoracic spinal cord injury accompanies shifts in evoked motor responses. *Curr. Biol.* 11(22):1766-1770.
83. National Research Council (2011) *Guide for the Care and Use of Laboratory Animals* (National Academies Press, Washington, DC) 8 Ed.
84. Sengupta P (2013) The laboratory rat: relating its age with human's. *Int. J. Prev. Med.* 4(6):624-630.
85. Plotsky PM*, et al.* (2005) Long-term consequences of neonatal rearing on central corticotropin-releasing factor systems in adult male rat offspring. *Neuropsychopharmacology* 30(12):2192-2204.
86. Ji Y*, et al.* (2010) Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. *Nat. Neurosci.* 13(3):302-309.
87. Cohen J (1988) *Statistical Power Analysis for the Behavioral Sciences* (Lawrence Erlbaum, Hillsdale, NJ) 2 Ed.
88. Rosenthal JA (1996) Qualitative descriptors of strength of association and effect size. *J. Soc. Serv. Res.* 21(4):37-59.