Restoring Voluntary Control of Locomotion after Paralyzing Spinal Cord Injury

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Half of human spinal cord injuries lead to chronic paralysis. Here, we introduce an electrochemical neuroprosthesis and a robotic postural interface designed to encourage supraspinally mediated movements in rats with paralyzing lesions. Despite the interruption of direct supraspinal pathways, the cortex regained the capacity to transform contextual information into task-specific commands to execute refined locomotion. This recovery relied on the extensive remodeling of cortical projections, including the formation of brainstem and intraspinal relays that restored qualitative control over electrochemically enabled lumbosacral circuitries. Automated treadmill-restricted training, which did not engage cortical neurons, failed to promote translesional plasticity and recovery. By encouraging active participation under functional states, our training paradigm triggered a cortex-dependent recovery that may improve function after similar injuries in humans.

Activity-based interventions exploiting proprioceptive information to enhance spinal motor output during training (1–3) promote plastic changes capable of restoring locomotion after severe though incomplete spinal cord injury (SCI) (3, 4). A recent case study suggests that, in combination with epidural electrical stimulation of lumbosacral segments, activity-based rehabilitation may also restore supraspinally mediated movements after motor complete paraplegia (5). We aimed to design a multisystem neuroprosthetic training program that took full advantage of this concept. We hypothesized that, after the complete interruption of direct supraspinal input, a robotic postural interface encouraging the brain to actively use the paralyzed hindlimbs during electrochemically enabled motor states (6) would reestablish supraspinal control of locomotion by promoting extensive and ubiquitous remodeling of spared neuronal circuitries.

Adult rats received a left lateral over-hemisection at thoracic (T) vertebra T7 and a right lateral hemisection at T10. This SCI interrupts all direct supraspinal pathways (fig. S1, A to C), but leaves half of human spinal cord injuries lead to chronic paralysis. Here, we introduce an electrochemical neuroprosthesis and a robotic postural interface designed to encourage supraspinally mediated movements in rats with paralyzing lesions. Despite the interruption of direct supraspinal pathways, the cortex regained the capacity to transform contextual information into task-specific commands to execute refined locomotion. This recovery relied on the extensive remodeling of cortical projections, including the formation of brainstem and intraspinal relays that restored qualitative control over electrochemically enabled lumbosacral circuitries. Automated treadmill-restricted training, which did not engage cortical neurons, failed to promote translesional plasticity and recovery. By encouraging active participation under functional states, our training paradigm triggered a cortex-dependent recovery that may improve function after similar injuries in humans.

This experimental lesion reproduces key anatomical and functional features of human SCIs, while providing well-controlled conditions to investigate the mechanisms underlying recovery (8). To transform lumbosacral circuits from dormant to highly functional states (9), we applied tonic (40 Hz) epidural electrical stimulation over L2 and S1 spinal segments (6), and systemically administered a tailored cocktail of serotonin receptor agonists (5HT1A/7and 5HT2A/C) and dopamine (D1) receptor agonists (10). By increasing the general level of spinal excitability, this electrochemical spinal neuroprosthesis enables sensory information to become a source of control for stepping (6, 9). This intervention promoted coordinated, although involuntary, bipedal stepping on a treadmill as early as 7 days post injury (Fig. 1C).

These stepping movements are elicited by the moving treadmill belt (6), which suggests that the
rats would not be capable of voluntarily initiating hindlimb locomotion overground. To verify the absence of supraspinal control, we applied the electrochemical neuroprosthesis and positioned the same rats bidirectionally on a robotic posture interface that provided adjustable vertical and lateral trunk support, but did not facilitate locomotion in any direction (Fig. 1B and fig. S2). All the rats ($n = 27$) failed to initiate hindlimb locomotion overground 7 days post injury ($P < 0.001$) (Fig. 1C).

We then designed a multisystem neuroprosthetic training program that encompassed two objectives. First, we aimed to improve the functionality of lumbosacral circuits through treadmill-based training enabled by the electrochemical neuroprosthesis (6). Second, we sought to promote the recovery of supraspinally mediated movements; we exploited the robotic posture interface not only to enable, but also to force, the rats to actively use their paralyzed hindlimbs in order to locomote bidirectionally toward a target.

Rats ($n = 10$) were trained daily for 30 min with a combination of both paradigms, starting 7 to 8 days post injury (fig. S3). The first, effortful voluntary steps emerged after 2 to 3 weeks of training ($P < 0.01$) (Fig. 1D). As voluntary movements recovered, we gradually increased the relative duration of overground training (fig. S3B). Five to 6 weeks post injury, all the rats (fig. S4) were capable of initiating and sustaining full weight-bearing bipedal locomotion for extended periods of time, but only during electrochemically enabled motor states (Fig. 1, C and D, fig. S1D, and movie S1). Kinematic analyses (fig. S5) revealed that overground-trained rats deployed a similar control strategy as intact animals to produce locomotion (Fig. 1, A and C, and fig. S5). To measure recovery, we adapted the clinically standardized 6-min walk test (11) to bidirectionally stepping rats. Overground-trained animals with a paralyzing SCI covered distances as long as 21 m in 3 min (Fig. 1D).

We next tested whether treadmill-restricted step training under electrochemically enabled states would also promote the recovery of voluntary locomotion ($n = 7$ rats). This automated step training failed to reestablish overground locomotion despite repeated testing during 4 to 8 sessions 9 weeks post injury ($P < 0.001$) (Fig. 1, C and D, and movie S1). Moreover, treadmill-trained rats were not capable of sustaining robotically initiated locomotion overground (fig. S6).

To further enhance supraspinal contribution, we introduced stairs and obstacles; two conditions requiring voluntarily mediated gait tuning (12). After 2 to 3 additional weeks, overground-trained rats were capable of bidirectionally sprinting up stairs and avoiding obstacles (Fig. 1C, fig. S7, and movie S1). To accomplish these paradigms, the animals displayed a range of task-specific adjustments of hindlimb movements (fig. S7).

Anatomical examinations highlighted an extensive remodeling of supraspinal and intraspinal projections in rats that regained voluntary locomotion. We first conducted retrograde tract tracing from lumbar (L) vertebrae L1/L2 locomotor centers (Fig. 2A). We found a significant increase ($P < 0.05$) (Fig. 2, B and C) in the number of labeled neurons in intermediate and ventral laminae of T8/T9 segments in both overground-trained and treadmill-trained rats compared with nontrained animals. Analysis of the activity-dependent marker, c-fos, after continuous overground locomotion confirmed that the labeled neurons were active during walking (Fig. 2F). The number of c-fos$^{\text{ON}}$ nuclei in the regions rich in neurons retrogradely labeled from L1/L2 locomotor centers was larger in overground-trained rats compared with all the other groups ($P < 0.01$) (Fig. 2D and E). Thoracic neurons may thus play a pivotal role in restoring voluntary locomotion (8, 13, 14). To address this hypothesis, we ablated T8/T9 neurons by infusing the axon-sparing excitotoxin N-methyl-D-aspartic acid (NMDA) (8) (Fig. 2G and fig. S8). Infusion of NMDA abolished the regained voluntary locomotion ($P < 0.01$) (Fig. 2H and movie S2), despite uncompromised functionality of lumbosacral circuits (fig. S8). Likewise, overground-trained rats lost voluntary control of locomotion after the complete interruption of supraspinal input to T8/T9 neurons ($P < 0.01$) (Fig. 2, G and H).

We labeled projections from the left hindlimb motor cortex with injections of biotinylated dextran amine (BDA) (Fig. 3A). The bilateral interruption of the dorsal column at the T7 over-hemisection spared a few (1 to 2%) (15) corticospinal tract (CST) axons in the right dorsolateral funiculus (fig. S9E). Consequently, nontrained rats showed sparse CST labeling in T8/T9 segments (Fig. 3, B and C, and fig. S9E). Treadmill-restricted training did not promote significant changes in the density of thoracic CST projections (Fig. 3, B and C, and fig. S9E). In contrast, we found a reconstitution of $45 \pm 7\%$ of prelesion bilateral fiber density in overground-trained rats (Fig. 3, B to D). These CST axons exclusively branched from the right dorsolateral funiculus (Fig. 3D), and they profusely innervated the right and, more unexpectedly, the left gray matter of T8/T9 segments (fig. S9F) (16). We detected multiple CST fibers extending from the gray matter at the T7 lesion site into the right dorsolateral funiculus (Fig. 3, E and F). These ectopic fibers, suggestive of regenerative sprouting (17), led to a near twofold increase in the CST axon density of the T8/T9 dorsolateral funiculus ($P < 0.01$) (fig. S9G). Thoracic CST fibers bypassed the T7 over-hemisection through the right dorsolateral funiculus, branched into the gray matter, and recrossed the midline (Fig. 3E). These fibers developed large axonal structures with boutonlike swellings suggestive of sprouting in terminal arbors (fig. S9F).

![Image](https://www.sciencemag.org/content/336/6082/1183/F2.large.jpg)
Collectively, these analyses demonstrate that automated treadmill-restricted training failed to mediate anatomical changes in descending pathways, whereas active training under highly functional states promoted multilevel plasticity in cortex- and brainstem-derived axonal systems.

Contrary to primates, the rodent motor cortex is not essential to produce locomotion (20). Consequently, we sought to demonstrate that training-induced remodeling of motor cortex projections did contribute to controlling voluntary locomotion. First, we stimulated epidural electrodes over the left motor cortex to verify that the reorganization of neuronal pathways reestablished connectivity across the lesion. Before the SCI, applying a train of low intensity (0.7 to 1.5 mA) electrical stimuli evoked large responses in the left tibialis anterior (TA) muscle (Fig. 4A). The SCI permanently abolished these responses in nontrained rats (P < 0.01) (Fig. 4A). In contrast, overground-trained rats regained responses below the lesion, averaging about 10% of their prelesion amplitude (P < 0.001) (Fig. 4B). These responses were delayed by 12 ± 3 ms (P < 0.01) (Fig. 4A), which suggests that a larger number of synaptic relays was necessary to convey the supraspinal volley to hindlimb motor pools. The amplitude of responses substantially increased during electrochemically enabled motor states (P < 0.01) (Fig. 4, A and B), which indicated enhanced transmission of the supraspinal command (14).

Second, we implanted a microwire array in the vicinity of CST neurons projecting to T8/T9 segments (Fig. 4C) and recorded neuronal modulations during voluntary locomotion in overground-trained rats (n = 3). We found a variety of neurons (n = 17/24 neurons) whose modulation patterns significantly (P < 0.05) (Fig. 4D) correlated with gait initiation, sustained locomotion, and corrective movements (fig. S12 and movie S3). A substantial number of motor cortex neurons (36%) exhibited a sharp increase in firing rate before any overt movement or locomotor-related muscle activity had occurred (Fig. 4E). Instead, the firing rate of motor cortex neurons significantly decreased during involuntarily locomotion compared with quiet standing (P < 0.05) (fig. S13, A to C). Third, we inactivated the left motor cortex with a microinjection of the γ-aminobutyric acid (GABA) agonist muscimol (Fig. 4F). Muscimol immediately suppressed voluntary hindlimb locomotion (P < 0.01) (Fig. 4G and movie S3), despite uncompromised functionality of lumbosacral circuits (fig. S14).

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Confocal microscopy confirmed that thoracic CST fibers bore synaptic elements because they colocalized with synaptophysin (Fig. 3G). These fibers established contacts with relay neurons retrogradely labeled from L1/L2 locomotor centers (Fig. 3G).

Remodeling of motor cortex axonal projections was not restricted to the spared tissue bridge. Quantification of CST fibers at T4/T5, above the injury, revealed a significant bilateral increase of axon density in overground-trained compared with nontrained, treadmill-trained, and intact rats (P < 0.01) (fig. S9, A to D). We found a near fourfold increase in the density of cortical projections in various brainstem motor areas (Fig. 3H and fig. S10), including the left and right vestibular nuclei (P < 0.01), the entire reticular formation (P < 0.001), and parapyramidal regions (P < 0.01). These areas contain reticulospinal neurons and spatially projecting serotonergic neurons (fig. S10C) that both contribute to initiating and sustaining locomotion (18, 19). Descending 5HT fibers might thus reorganize with training. We found a nearly complete, lamina-specific restoration of T8/T9 serotonergic innervation in overground-trained rats, which contrasted with the depletion of 5HT fibers in nontrained and treadmill-trained animals (P < 0.05) (fig. S11).

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Thus far, functional restoration after SCI has been interpreted as the need to promote long-distance regeneration of severed fibers to their
original targets (21, 22). Undoubtedly, neuroregeneration will be essential following near-complete SCI. However, a more immediate approach might capitalize on the remarkable capacity of spared neuronal systems to reorganize through use-dependent mechanisms (3, 5, 23). Here, we established training conditions that not only enabled but also forced the brain to construct a multiplicity of de novo brainstem and intraspinal relays to regain quantitative and qualitative access to electrochemically enabled lumbosacral circuits. There is growing evidence that active training with appropriate sensory cues is markedly superior to passive, robot-guided rehabilitation to improve stepping capacities in humans (3, 5, 23–26). Likewise, automated treadmill-restricted training, which did not engage cortical neurons, promoted sublesional plasticity, but failed to promote remodeling of descending pathways. Treadmill-trained rats did not regain supraspinally mediated locomotion. Instead, our new training paradigm encouraged active neuroregen-

**References and Notes**


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