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Natural and targeted circuit reorganization after spinal cord injury

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A spinal cord injury disrupts communication between the brain and the circuits in the spinal cord that regulate neurological functions. The consequences are permanent paralysis, loss of sensation and debilitating dysautonomia. However, the majority of circuits located above and below the injury remain anatomically intact, and these circuits can reorganize naturally to improve function. In addition, various neuromodulation therapies have tapped into these processes to further augment recovery. Emerging research is illuminating the requirements to reconstitute damaged circuits. Here, we summarize these natural and targeted reorganizations of circuits after a spinal cord injury. We also advocate for new concepts of reorganizing circuits informed by multi-omic single-cell atlases of recovery from injury. These atlases will uncover the molecular logic that governs the selection of 'recovery-organizing' neuronal subpopulations, and are poised to herald a new era in spinal cord medicine.

The circuits that regulate motor and autonomic functions reside in the spinal cord. While these circuits are often regarded as rudimentary components of the CNS under the authoritative control of the brain and brainstem, we are beginning to uncover remarkable sophistication and diversity in the molecular architecture of the neuronal subpopulations that forge these circuits[1,2]. Equally refined is the organization of the brain and brainstem regions that interact with neurons in the spinal cord. Interrogation of neuronal subpopulations based on their identity and/or projection targets have revealed that these regions can no longer be schematized with interacting boxes delivering executive commands to the spinal cord through specialized descending pathways[3]. Instead, each region is composed of specific neuronal subpopulations with distinct receptomes and projectomes that contribute uniquely to the production of behavior[4]. Deciphering how the neuronal subpopulations from the brain, brainstem and spinal cord interact to regulate motor and autonomic functions is a daunting task. Even more intimidating is the attempt to understand how these neuronal subpopulations respond to, and reorganize after, a spinal cord injury (SCI).

Until recently, probing the functional role of neuronal subpopulations has been a laborious endeavor. Our knowledge on the reorganization of circuits after SCI primarily derived from the interrogation of broadly defined pathways and circuits that are known to regulate motor and autonomic functions.

Here, we summarize our knowledge on how the circuits located in the brain, brainstem and spinal cord respond to SCI, and how their reorganization can contribute not only to natural recovery but also to degradation of neurological functions. We focus on motor functions, but also consider autonomic functions when relevant. We discuss how neuromodulation technologies can engage and reorganize the intact neuronal subpopulations above and below an SCI to improve these functions. We then lay out our understanding of the requirements to reconstitute damaged circuits. Finally, we advocate for a shift away from vaguely defined pathways, circuits or regions. We must increase the resolution of our nervous system interrogation by uncovering 'recovery-organizing' neurons—the specific neuronal subpopulations that reorganize anatomically to restore function after SCI—and how

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they can be targeted to further improve spinal cord repair and functional recovery.

Adaptive and maladaptive circuit reorganization

An SCI induces the death of neurons and glia in and around spinal cord lesions and the interruption of axons passing in the vicinity. However, circuits located far from the injury, both above and below the SCI, also undergo profound changes that can contribute to natural recovery[5,6] or instead be detrimental[7,8]. Local growth of residual descending fibers, sprouting of synaptic terminals, adaptations in synaptic transmission and multifaceted changes in molecular properties of neurons have been documented in numerous regions of the brain, brainstem and spinal cord. These adaptive and maladaptive modifications primarily depend on the severity of SCI, as summarized below (Fig. 1).

Reorganization of brain circuits

Neuronal populations embedded in the cerebral cortex are essential for hand dexterity, are the most experimentally accessible neurons, and subserve the motor skills of primate species. Experiments in nonhuman primate (NHP) models have shown that dexterous movements are not possible when corticospinal tract projections are interrupted[9]. Accordingly, the integrity of the corticospinal tract is a key predictor of natural functional recovery after SCI in humans[10].

One of the most striking mechanisms of recovery involves time-dependent, bilateral reorganization of cortical dynamics. This mechanism has been described after lateralized SCI in NHP models[11]. These studies found a bilateral increase in activity of the motor and sensory cortices early after SCI. Once recovery had taken place, however, the increased activity was restricted to the contralesional motor and sensory cortices and to both ventral premotor cortices. Silencing experiments confirmed that ipsilesional cortical regions are necessary for movement execution, but only during the early phase after the SCI[11]. Functional remapping of cortical regions during recovery from SCI in rodents also illustrated the importance of time-dependent changes in cortical dynamics[12,13]. When cortically derived projections in the spinal cord below injury were silenced, the recovery of movement vanished immediately, showing the relevance of this reorganization to the restoration of function after SCI[5,14].

Anatomical reorganization of cortically derived projections[15] and ascending pathways[16] parallels these adaptations of cortical dynamics. When the SCI spares a subset of fibers from the corticospinal tract, these fibers undergo directed growth to invade the gray matter territories that have been deprived of supraspinal projections[17,18]. After a lateral SCI, corticospinal tract fibers can branch from the contralesional dorsolateral column, cross the spinal cord midline, and develop dense synaptic projections into the ventral gray matter where they contact neuronal subpopulations producing hand function[19]. Interventions that enhance this growth further augment hand function recovery[20]. Although the mechanisms that trigger this process remain unclear, regression of corticospinal tract neurons to a transcriptional state similar to that of developing neurons appears responsible for enabling this growth[21]. The reorganization of cortically derived projections has been exposed in rodent and NHP models, but evidence suggests that this mechanism also supports recovery in humans[22].

When the SCI interrupts all corticospinal tract projections, the commands from the motor cortex can also be rerouted through alternative pathways with surviving projections below the injury (Fig. 1). For example, the growth of cortically derived projections onto neurons located in the reticular formation restores walking after contusion SCI[5]. Owing to the ubiquitous location of reticulospinal fibers within the rim of spinal cord white matter, a subset of these fibers survives contusion SCI, regardless of the inherently variable topology of damage[5]. Similarly, sprouting of cortically derived projections within the red nucleus enables the motor cortex to convey information to the spinal cord through the rubrospinal tract to regain upper limb function[23–25]. The formation of relays through reticulospinal and rubrospinal neurons underscores the important role of brainstem-derived projections[26] and suggests the existence of recovery-organizing neurons located in distributed regions of the brainstem.

Reorganization of brainstem circuits

Studies have documented the growth of reticulospinal[5], rubrospinal[27] and serotonergic[28] projections within territories located below incomplete SCIs (Fig. 1). This growth often recapitulates the natural innervation pattern of these projections[5,29], suggesting an attempt to reestablish the pre-injury connectivity of these pathways. Electrophysiological, lesion-specific and projection-specific silencing experiments have linked this reorganization with recovery[5,28].

However, the involvement of subcortical structures cannot be restricted to pathways that project directly to the spinal cord. One example is the necessary contribution of the nucleus accumbens to the execution of dexterous hand movements, especially early after SCI[30]. Another unexpected contribution of evolutionary ancient subcortical regions comes from the mesencephalic locomotor region (MLR)[31]. While this region is not necessary to produce walking in the absence of injury[32], neurons distributed within the MLR are capable of recruiting reticulospinal neurons with surviving projections to produce locomotion after SCI[33].

Limiting the survey of recovery-organizing brainstem neurons to regions with residual projections below the SCI also fails to acknowledge the diversity of circuits within and across brainstem regions. For example, the MLR contains distinct subpopulations of glutamatergic neurons that project to specific targets to regulate distinct behaviors[34]. It is also well established that reticulospinal neurons cluster in spatially defined regions based on their contribution to the control of specific limbs[35] or behaviors[36]. Moreover, projection-stratified neuronal subpopulations of the brainstem encode discrete phases of actions, and they may even serve as building blocks for regulating complex movements[37].

This stratification of brainstem neuronal subpopulations into interactive, hierarchically organized layers suggests that multiple regions may interact to pass executive commands downstream. Natural recovery from SCI may involve multifaceted changes in the flow of communication among these subcortical neuronal populations. Concomitant adaptations in the receptome and projectome of these recovery-organizing neuronal subpopulations are likely to take place to support recovery.

These observations reveal that functional recovery after SCI may rely on unexpected circuit reorganization, including evolutionarily conserved mechanisms that are not essential to produce behaviors in the absence of injury, but that become essential to support recovery after SCI. The critical role of mesolimbic and mesencephalic regions provides perfect illustrations of this mechanism, but similar examples have also been described in the spinal cord.

Reorganization of spinal circuits above the injury

The motor cortex is essential for regaining motor functions after SCI, especially in primates. Yet, cortical commands do not have to be transferred directly to the spinal cord below the injury. A remarkable example comes from the ability of propriospinal neurons located in C3–C4 segments to relay cortical signals past an SCI to restore hand dexterity in NHP models[38]. Importantly, these evolutionarily conserved neurons are not critical in the absence of injury, but they become essential for recovery of hand functions when direct corticospinal tract projections are interrupted (Fig. 1).

The same mechanism supports recovery of walking after dorsal or lateral hemisection SCI[13], and even temporally and spatially separated hemisection SCI. In this scenario, all the direct pathways from the brain are interrupted as a result of the two hemisections on opposite sides. Yet, the neurons located between the hemisections can form relays that transfer sufficient information past the injury to restore

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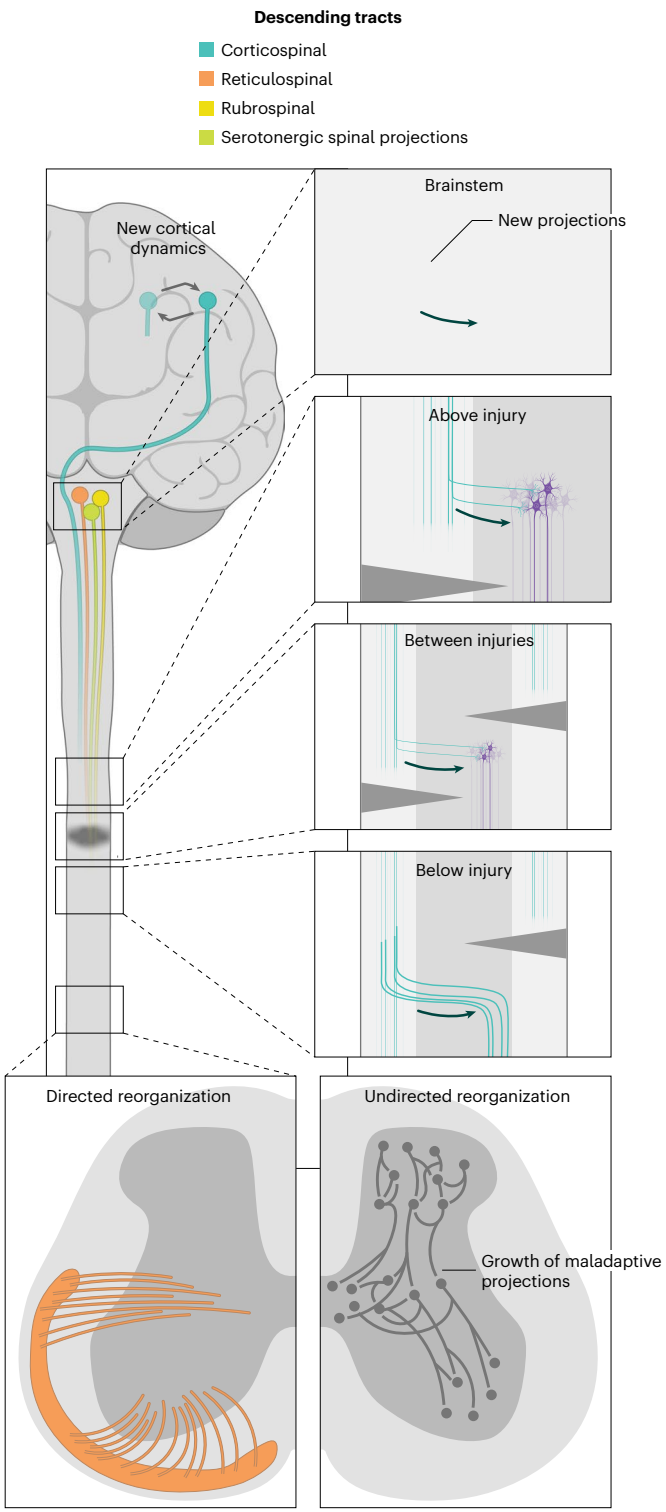

Fig. 1 | Adaptive and maladaptive reorganization of brain, brainstem and spinal circuits following spinal cord injury. An SCI triggers functional and anatomical changes throughout the nervous system. Extensive functional and anatomical reorganization of projections from neurons located in the brain, brainstem and spinal cord above the injury occur naturally after SCI. The topological distribution of these reorganizations is illustrated, including alterations in cortical dynamics and growth of new axonal projections from neurons in the brain, brainstem and spinal cord. The profound changes that these circuits undergo can contribute to natural recovery or instead be detrimental to neurological functions and result in neuropathic pain, autonomic dysreflexia or spasticity. To a large extent, the severity of the SCI determines whether this reorganization is directed or undirected.

leg movements[18,39,40]. Again, these neurons are not necessary for walking before the SCI[40], but they become essential after the SCI. Many descending projections from propriospinal relay neurons survive SCIs. However, injury-mediated downregulation of the potassium–chloride cotransporter KCC2 in inhibitory neurons located in the vicinity of the injury leads to excessive inhibition of relay circuits, rendering them nonfunctional[39]. Experimental prevention of KCC2 downregulation maintains the balance between inhibition and excitation, which is sufficient to restore walking[39]. The molecular identities of these relay neurons and the computational principles that enable the transfer of meaningful commands past the SCI remain unknown.

Directed reorganization of spinal circuits below the injury

The interruption of supraspinal projections triggers a reorganization of spinal circuits located below the injury. When residual supraspinal projections are abundant, these surveying fibers extend collaterals onto reorganizing circuits below the injury, which supports functional recovery[5,19,26]. Neurorehabilitation can also direct a reorganization of circuits below the injury that improves recovery, even following complete SCI[41,42]. When deprived of sufficient guidance, however, spinal circuits undergo maladaptive reorganization that provokes clinical syndromes. Below, we summarize our knowledge on directed (adaptive) and undirected (maladaptive) reorganization of spinal circuits below the injury (Fig. 1).

Spinal circuits can produce complex motor behaviors without any contribution from the brain and brainstem[43,44]. Central to this competence is their ability to transform task-specific sensory information into patterns of muscle activity that meet the intrinsic biomechanical constraints and extrinsic environmental demands[45]. When supraspinal projections are compromised, sensory information must thus become the main source of control for movement[45]. Indeed, limb immobilization alters functional recovery after SCI[46]. Instead, neurorehabilitation can direct activity-dependent functional[47] and anatomical[48] reorganization of sensory projections that supports recovery. A logical consequence is the necessity of feedback circuits to execute and regain motor functions after SCI[49], especially those originating from muscle proprioceptive organs[49]. Indeed, the genetic ablation of muscle spindle feedback circuits abolishes the recovery that naturally occurs after incomplete SCI. Concomitantly, brainstem and spinal cord relay neurons fail to respond to injury with the growth of new projections that mediates this natural recovery[6]. These observations help to explain why the outcome of neurorehabilitation depends so singularly on the coincidence between the flow of sensory information and the volitional drive from residual supraspinal projections. However, the nature and topology of the interactions that promote this growth remain unknown. Proprioceptive neurons are thus recovery-organizing cells that steer beneficial reorganization of circuits throughout the nervous system. Moreover, targeting alterations in histone acetylation of proprioceptive neurons after SCI endows their axons with increased regenerative capacities, which can contribute to augmenting recovery[50].

It is difficult to identify neurons below an SCI that are not profoundly perturbed when a severe spinal cord damage occurs. Yet, functional recovery is likely to rely more prominently on the reorganization of specific neuronal subpopulations[45,51]. For example, the recruitment of developmentally defined V2a neurons during the recovery of walking[1,52] and breathing[53] suggests that these cells modify how they interact with the circuits that produce these functions. Neurons located in spinal cord deep layers (dI3) have also been implicated[54].

These observations stress the importance of shifting the focus on clearly defined neuronal subpopulations. Single-cell technologies have exposed a remarkable diversity of neuronal subpopulations in the spinal cord. Original nomenclatures were derived from the known cardinal lineages in the developing and postnatal spinal cord[1,55]. More recently, a top-down approach identified orderly genetic tiers that divide neuronal subpopulations according to their motor–sensory,

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local–long range and excitatory–inhibitory features[2]. We anticipate that this developmentally driven taxonomy will lead to a harmonized catalog of neuronal subpopulations in the developing and adult spinal cord[56] that will guide the rapid identification of recovery-organizing neurons in the spinal cord after SCI.

Undirected reorganization of spinal circuits below the injury

The massive depletion of supraspinal projections induces a collection of maladaptive compensatory responses that are responsible for various clinical syndromes (Fig. 1).

Following deafferentation of a region in the CNS, new synapses derived from unaffected afferent pathways form spontaneously[57]. After SCI, this compensatory homeostatic response[58] contributes to the aberrant sprouting of afferents originating from dorsal root ganglia neurons[7,59], the chaotic growth of new propriospinal connections[60] and the ubiquitous genesis of excitatory synapses below the injury[7,61]. Moreover, the disruption of blood flow below the injury leads to a chronic state of hypoxia owing to paradoxical excess activity of monoamine receptors on pericytes, which impairs neuronal function[62]. Parallel changes in the molecular properties of neurons take place. These include an increase in constitutive activity of monoaminergic receptors[63], serotonin hypersensitivity[64], upregulation of intracellular chloride concentration[65], increased excitability of various classes of neurons[66] and hyperactivity of L-type calcium channels[67].

Unlike the directed reorganization that occurs when sufficient supraspinal projections persist, this undirected formation of circuits after severe SCI leads to clinical syndromes, including abnormal proprioceptive reflex responses[7,68], increased muscle tone[69], spontaneous spasms[7], bladder overactivity[70], neuropathic pain[71] and autonomic dysreflexia[72].

Various types of interneurons and circuits have been implicated in these clinical syndromes[73]. Yet, we surmise that this chaos involves the recruitment of specific neuronal subpopulations whose individual responses are directed toward predictable targets.

Perspectives on natural circuit reorganization

This unstructured registry of pathways, regions and circuits that may contribute to the recovery or deterioration of neurological functions underscores the incompleteness of our knowledge. Moreover, observations that recovery may rely on unexpected mechanisms or regions not critical to neurological functions in the absence of injury leaves the possibility that critically important recovery-organizing neuronal subpopulations may have been overlooked by previous studies that were merely informed by principles identified in uninjured animal models.

The identification of recovery-organizing neuronal subpopulations is contingent on catalogs of genetically accessible neurons. These catalogs are now available for most brain regions[74], but the brainstem and spinal cord have remained comparatively less explored. Consequently, previous studies were forced to rely on developmentally defined neuronal subtypes or broadly defined neuronal subtypes, which may or may not reflect the diversity of neuronal subpopulations in the adult nervous system[1,2,75]. However, current high-throughput technologies are now enabling us to generate spatially resolved catalogs of neuronal subpopulations[52,76], auguring the imminent availability of comprehensive atlases over the entire nervous system. Moreover, the statistical methods to navigate these uncharted territories are expanding quickly[1,52,77].

Understanding the recovery-organizing neuronal subpopulations in the brain, brainstem and spinal cord that contribute to natural recovery of function after SCI is essential, because this knowledge can be harnessed to develop neuromodulation and biological repair therapies that target these neurons to augment recovery, as we discuss below.

Neuromodulation of anatomically intact circuits

Spinal cord damage disrupts the flow of communication between the circuits that regulate motor and autonomic functions, but spares the vast majority of neurons that compose them. Consequently, various neuromodulation strategies based on electricity have tapped into anatomically intact neurons of the brain, brainstem and spinal cord to improve neurological functions after SCI (Fig. 2).

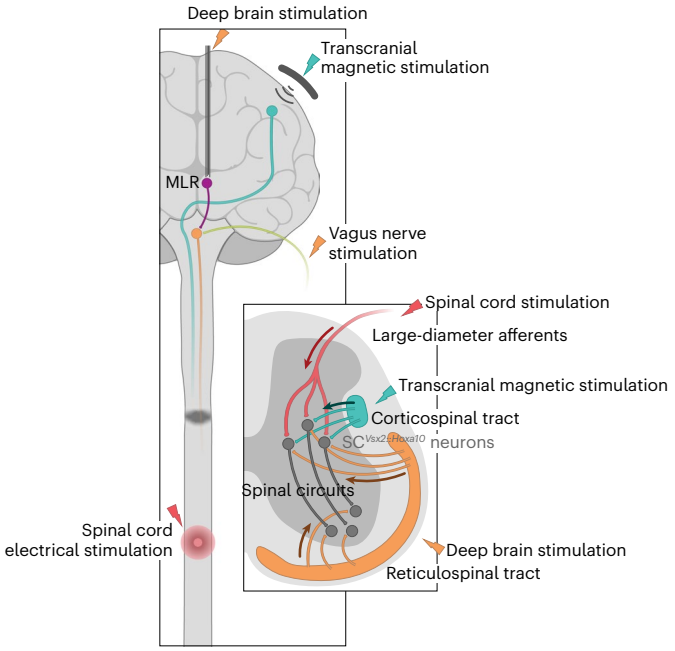

Fig. 2 | Neuromodulation of anatomically intact circuits. Neuromodulation strategies, which are primarily based on electrical stimulation methods, tap into anatomically intact circuits of the brain, brainstem and spinal cord to improve neurological functions immediately and/or promote long-term recovery of neurological functions after SCI. When the SCI is incomplete, electrical stimulation is delivered in the brain or brainstem to reactivate circuits in the spinal cord or to reinforce the strength of connections between the brain and spinal cord. When an SCI is severe, the circuits in the spinal cord lack the source of modulation and excitation that they require to be functional. Electrical spinal cord stimulation, in particular EES, can reactivate these circuits through the modulation of large-diameter afferents. When combined with neurorehabilitation, the stimulation directs the growth of residual projections from the brain and brainstem (arrows) to $SC^{Vsx2::Hoxa10}$ neurons that are activated by the stimulation[52]. This reorganization promotes recovery of volitional movement even when the stimulation is turned off.

Neuromodulation of cortical circuits

Targeting cortical circuits provides broad access to a multitude of circuits located downstream. A perfect example of this hierarchical organization is illustrated in the immediate recovery of walking when the motor cortex is stimulated after a lateral hemisection[78]. Despite the interruption of corticospinal tract projections on one side, adjusting the amplitude of intracortical closed-loop stimulation patterns was sufficient to control the amplitude of stepping movements from the paralyzed leg on the denervated side[78]. The same observations were obtained during graded optogenetic activation of cortical projection neurons after contusion SCI[5]. Because this injury abolished all corticospinal projections, the optogenetically evoked signals were relayed through glutamatergic reticulospinal neurons that retained projections below the SCI. These results indicate that activation of cortical circuits propagates commands that cascade downstream through brainstem neurons with spared yet functionally silent projections below the SCI.

Activity-dependent stimulation protocols can also strengthen the residual connections between the brain and spinal cord (Fig. 2). The molecular mechanisms remain unclear, but it is now established that the repeated activation of the motor cortex with noninvasive or invasive electrical stimulation methods promotes activity-dependent

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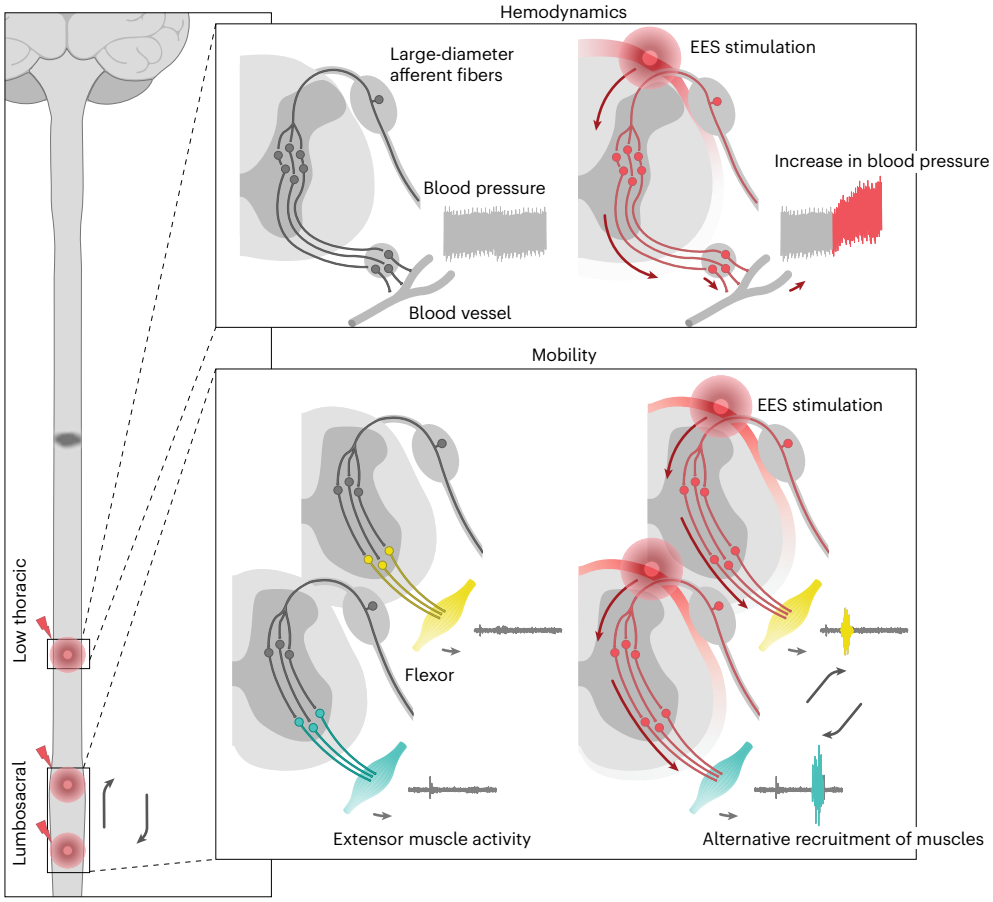

Fig. 3 | Mechanisms through which epidural electrical stimulation restores hemodynamics and mobility. Evidence suggests that EES elicits electrical currents that do not modulate neurons directly, but instead flow around the spinal cord within the cerebrospinal fluid where they depolarize large-diameter afferent fibers. Owing to their low impedance and heavy myelin contents, these fibers are prone to depolarization, especially where they enter the spinal cord through the dorsal root entry zones. There is evidence that alternative stimulation methods act through the same mechanisms. The recruitment of large-diameter afferent fibers activates visceral and somatic motor neurons both directly and indirectly, and helps to transform spinal circuits from a dormant to a highly excitable state. Because flexor and extensor motor pools are located in different spinal segments, targeting the dorsal roots that project to the segments wherein these motor pools reside enables the modulation of specific muscles with a timing that reproduces the natural activation of these muscles.

growth of corticospinal tract projections[79]. Pairing motor cortex stimulation with precisely timed recruitment of peripheral nerves or even intraspinal stimulation further enhances transmission along residual neural pathways[80]. The resulting spike-timing-dependent reorganization of circuits has mediated lasting improvement of motor functions and reduced spasticity in animal models[81] and people with SCI[82].

Neuromodulation of cortical circuits can also be indirect. For example, vagus nerve stimulation activates diverse nuclei throughout the brainstem, provoking a release of acetylcholine and norepinephrine throughout the brain that predisposes vicariously distributed circuits to increase their responses to neurorehabilitation[83] (Fig. 2). As with most neuromodulation therapies, the timing of the stimulation must coincide with relevant motor events to maximize recovery[84]. When delivered after cervical SCI, closed-loop vagus nerve stimulation promoted the growth of neuronal projections involved in the control of hand musculature[84]. Improvement of manual dexterity paralleled this anatomical reorganization, but whether and how specific neuronal populations contributed to this recovery remains unknown.

Neuromodulation of brainstem circuits

The majority of SCIs spare bridges of white matter within which mixed populations of brainstem-derived projections reside. When few projections are spared, they fail to mediate volitional muscular contractions. This understanding led to neuromodulation therapies that aim to recruit these spared projections to elicit movement. For example, the delivery of deep brain stimulation within the MLR activates reticulospinal neurons, which improved walking after SCI[33] (Fig. 2). These results are comparable to those observed during motor cortex stimulation, suggesting that the brain fails to engage the entire population of reticulospinal neurons with spared projections below an SCI.

The relevance of this ancestral locomotor system remains unclear in humans. Moreover, this region is difficult to target surgically because the MLR is primarily defined functionally within the scattered topology of the pedunculopontine nucleus. Accordingly, deep brain stimulation of this region to improve gait after Parkinson's disease led to variable outcomes[85]. Recent studies have segregated the MLR into anatomically distinct nuclei with defined populations of projection-stratified neurons that regulate specific behaviors[36]. These studies open the possibility that more refined targets could be identified, or even upstream regions with more circumscribed anatomical topology.

Neuromodulation of spinal circuits

Ultimately, spinal circuits generate the muscular contractions that regulate motor actions. Therefore, it is logical that tapping into these circuits results in the generation of organized muscular activity. Experiments from the past century documented the initiation of walking when the isolated spinal cords of cats and dogs were stimulated electrically[86]. The same observations were made in humans during the 1980s[87].

It is now well established that delivering electrical stimulation to the lumbar spinal cord using epidural[88,89], intraspinal[90] or transcutaneous[91] methods can elicit rhythmic leg movements, weight-bearing standing, and even independent stepping in animal models[18] and humans with SCI[88,89,92] (Fig. 2). Pharmacological neuromodulation based on monoaminergic agents can also reactivate the spinal cord below an SCI[93], and acts synergistically with electrical neuromodulation therapies to promote movement[45]. When a sufficient number of supraspinal projections is spared, these therapies even enable these otherwise silent residual projections to engage spinal circuits[5]. This mechanism, which was unmasked in preclinical models[5], has restored volitional control over the activity of paralyzed muscles in humans[88,89].

These observations motivated studies that dissected the mechanisms of epidural electrical stimulation (EES). It was found that EES elicits electrical currents that do not modulate neurons directly, but instead flow within the cerebrospinal fluid where they depolarize large-diameter afferent fibers[94]. Owing to their relatively low impedance, these fibers are prone to depolarization, especially where they enter the spinal cord through the dorsal root entry zones[94,95] (Fig. 3). Alternative stimulation methods are likely to act through the same mechanisms[96]. The recruitment of large-diameter afferent fibers activates motor neurons both directly and indirectly, and contributes to the transformation of spinal circuits from a dormant to an excitable state[95,97].

These experiments revealed that the biophysical properties of the spinal cord operate as functional filters that direct unspecific electrical currents toward specific neuronal subpopulations. EES leverages large-diameter afferent fibers as a gateway to modulate motor neurons. Since large-diameter afferent fibers primarily modulate motor neurons located in the spinal segment innervated by the root wherein these afferents reside[89,98], targeting individual dorsal roots enables the modulation of distinct motor pool ensembles[89,98,99]. This biological principle led to stimulation strategies[98–101] that target the individual dorsal root entry zones with a temporal structure aiming to reproduce the natural spatiotemporal activation pattern of motor neurons[102] (Fig. 3). This principle enables the configuration of activity-dependent biomimetic stimulation programs to support standing, walking, biking, swimming and even trunk movements in people with paralysis[103].

While the lumbar spinal cord has been the primary focus of studies on EES, experiments in NHP models[104] and two humans with tetraplegia[105] suggested that targeting the cervical dorsal root entry zones also improves hand dexterity. However, complex hand gestures may rely on more complex stimulation programs that are controlled directly by the brain. Brain-controlled regulation of EES can be achieved using brain–computer interface technologies[99].

Neurorehabilitation supported by EES improves functional recovery[89]. Individuals with chronic SCI have regained volitional control over previously paralyzed muscles, and some could even walk naturally without any stimulation. This recovery involved an unexpected reduction in the neural activity in the lumbar spinal cord during walking, suggesting that specific neuronal subpopulations are selected during neurorehabilitation. Indeed, interrogation of a single-cell atlas of recovery in mice revealed that excitatory interneurons expressing *Vsx2* are selected during neurorehabilitation supported by EES[52]. These cells are nested within the intermediate lamina of the spinal cord, where they receive projections from large-diameter afferents and brainstem neurons with surviving projections[5,18]. Neurorehabilitation reinforces these projection patterns, enabling these recovery-organizing neurons to transform information from brainstem locomotor regions and large-diameter afferents into commands that are broadcasted to the ventrally located neurons to produce movement (Fig. 3).

The understanding that EES utilizes large-diameter afferents as gateways to circuits in the spinal cord opened up the possibility of developing neuromodulation therapies that improve other neurological functions, especially autonomic functions, which are ranked among the top priorities for people with SCI (Box 1).

BOX 1

Neurotechnologies to improve autonomic functions

Preliminary studies in feline models and humans have shown that EES applied over the sacral spinal cord could modulate spinal circuits involved in the regulation of bladder and bowel functions[134].

Even more conclusive were studies targeting hemodynamic instability[135,136]. People with cervical and upper thoracic SCI experience frequent drops in blood pressure that not only affect their quality of life but also lead to life-threatening conditions. These hemodynamic events, which are referred to as orthostatic hypotension, are due to the interruption of descending signals from brainstem vasomotor regulatory circuits[136]. Consequently, the sympathetic circuits in the spinal cord no longer respond to orthostatic challenges.

Circuits embedded in the low thoracic spinal cord are enriched in sympathetic preganglionic neurons[136]. Engaging these neurons recruits ganglionic neurons, which release norepinephrine to activate alpha$_1$ receptors on splanchnic blood vessels and thus provoke arterial vasoconstriction[136]. Large-diameter afferent fibers recruited with EES can activate sympathetic preganglionic neurons through an intermediary glutamatergic interneuron[136] (Fig. 3). This mechanism enables the precise regulation of sympathetic circuits. Closed-loop control of EES targeting these circuits maintained blood pressure stability during transient, varying, and sustained orthostatic challenges in rat and NHP models, as well as in humans[136,137]. Large clinical trials are underway to turn this strategy into a treatment for people who suffer from severe orthostatic hypotension.

Perspectives on neuromodulation therapies

Neuromodulation of anatomically intact circuits in the brain, brainstem and spinal cord have shown realistic promise to improve neurological functions after SCI. However, current approaches exclusively based on electricity are poorly specific. Even when the biophysical and/or functional properties of neural tissues steer electrical currents toward specific neuronal subpopulations, the resolution of these physiological filters remains limited regarding the highly specialized flow of communication that circulates in the intact nervous system. Identification of the neuronal subpopulations that are engaged and remodeled in response to electrical stimulation might open avenues for more targeted interventions that may be combined with pharmacological agents. However, neuromodulation therapies mediate improvements that remain incomplete, and thus not fully satisfying. Consequently, the reconstitution of circuits with biological repair strategies remains essential to maximize recovery.

Biological strategies to reconstitute circuits

The reconstitution of circuits following spinal cord damage comes in several interpretations, each targeting specific mechanisms and comprising unique challenges (Box 2). The more accomplishable interpretation consists of densifying the projectome of neurons that retain axons below the injury. When the SCI is complete, however, reconstitution of circuits implies the regeneration of severed axons through hostile tissues to repair damaged circuits or form new relay circuits that bypass the lesion and restore communication across the injury. Alternatively, these hostile tissues can be repopulated with grafts that reconstitute functional spinal cord environments. Below, we summarize the biological strategies that target each of these interpretations,

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BOX 2

Specific challenges in each compartment of the injured region

Damage to the spinal cord is typically inflicted by mechanical insults such as laceration or contusion. This primary injury tears axons apart and causes severe bleeding, which triggers a secondary injury response involving a cascade of cellular and molecular events such as immune cell infiltration, cell death, demyelination and scar formation. These secondary events lead to the formation of three cellularly distinct compartments, starting from a core fibrotic scar or cavity that is surrounded by an astrocyte border, and spared but reactive and reorganizing neural tissue[109].

Fibrotic scar (core)

Severe inflammation provokes the formation of the fibrotic scar, which is composed of fibroblasts, pericytes, endothelial and inflammatory cells. While the origin of the cell types responsible for fibrotic scarring has been intensely debated[138–140], we know that the origin is neither neuronal nor glial; therefore, the fibrotic component of the scar must be distinguished from the glial scar. Because the fibrotic scar has been ascribed an inhibitory role, many interventions sought to ablate the cellular constituents of the fibrotic scar with the goal of decreasing the extracellular matrix[110,141]. Partial ablations have been reported to result in decreased extracellular matrix deposition and improved behavioral outcomes, while complete ablation of the same cells resulted in larger tissue damage and worsened behavioral outcomes[141]. In striking contrast, recent studies revealed that the fibrotic scar can act as a permissive scaffold that supports stimulated axons to regrow through this scar[115,121]. Differences between these studies are likely to result from the specific types of extracellular matrix that are upregulated and deposited by each intervention. Deconstructing the biology of fibrotic scarring will provide actionable pathways to manipulate the extracellular matrix in order to foster the repair of damaged circuits.

Astrocyte border (surrounding the core)

Fibrotic scars are surrounded by a thin and densely packed border of astrocytes, termed the astrocyte border, which is regulated through signal transducer and activator of transcription 3 (STAT3)[142] and leucine zipper-bearing kinase (LZK) signaling[143]. Astrocyte borders are almost exclusively composed of newly proliferated and reactive astroglia[144,145]. This border was long regarded as a physical and chemical barrier to axon growth. However, the supposedly negative properties of border-forming astrocytes have now been dismantled. We know that they are not the principal cause of regenerative failure[121], but instead restrict the spread of inflammation into viable neural tissue[121,144,146]. Moreover, they are not the primary producers of purportedly inhibitory chondroitin sulfate proteoglycans (CSPGs)[121], but can instead support the growth of stimulated axons[115,121]. These observations imply that border-forming astrocytes support neural repair and that hindering astrocyte border formation is likely to be detrimental to recovery.

Spared but reactive neural tissue (neighboring the core)

Adjacent to the astrocyte borders lies spared but reactive neural tissue, which contains hypertrophic reactive astroglia combined with neurons forming circuits and undergoing synaptic turnover.

with a focus on the reconstitution of circuits after SCI to adhere to the theme of this review (Fig. 4).

Densification of projections from spared circuits

Natural recovery from SCI largely correlates with the relative number of spared neuronal pathways[106]. This trivial etiology makes it abundantly clear that augmenting recovery after SCI will require the densification of projections from spared circuits. Neutralizing inhibitory molecules or targeting intrinsic neuronal growth competence promotes this remodeling (Box 3).

When axons are severed within the adult CNS, they fail to regenerate. Extrinsic inhibitors found in degenerating myelin and astrocyte borders were once thought to be responsible for this failure[6]. This understanding launched a race to identify inhibitory molecules and develop strategies to neutralize them. Initial experiments suggested that these therapies act by promoting the regeneration of severed axons across the site of injury[107]. However, modern methods revealed that these interventions do not promote the regeneration of corticospinal tract axons through scars, but rather mediate short-distance growth of axon collaterals over short distances in healthy tissue[6,108]. Early efforts were conducted with the hope that a singular solution would be sufficient to neutralize inhibition and thus overcome regenerative failure. These hopes have declined with the realization that reducing inhibition does not promote sufficient regrowth of severed axons[6,108].

In parallel, others have focused on identifying regeneration-associated genes to reactivate intrinsic growth programs of neurons[109] and stabilize growth cones[110] (Box 3). Regrowth of severed axons from corticospinal, retinal and propriospinal neurons has been achieved with the manipulation of the phosphatase and tensin homolog (*PTEN*)[111], suppressor of cytokine signaling 3 (*SOCS3*)[112], *STAT3* (ref. [112]), *SOX11* (ref. [113]), osteopontin (OPN)[114,115], insulin growth factor 1 (IGF-1)[114,115], ciliary neurotrophic factor (CNTF)[115] and Krüppel-like family of transcription factors (KLFs)[116] and neuronal RHOA[117], among others[118]. Transcription and growth factors can be manipulated concurrently to augment this regrowth. Activation of *mMTOR* and *STAT3* pathways with co-deletion of *PTEN* and *SOCS3*, or overexpression of the growth factors IGF-1 and OPN, induced synergistic regrowth of axons across astrocyte bridges and into topologically relevant targets below the injury, which was sufficient to restore some function[114]. Transient exposure to reduced oxygen levels, called intermittent hypoxia, is an alternative strategy to modulate raphe serotonergic neurons, trigger brain-derived neurotrophic factor synthesis, and promote the growth of serotonergic projections below the injury[119]. Likewise, neuronal activity promotes the release of various neurotrophic factors that augment the growth of spared neuronal projections. Neurorehabilitation leverages this mechanism to improve functional recovery[42].

While impressive, these circuit reconstitutions present limitations. First, the regrowth of severed axons was obtained only for axons originating from specific populations of neurons. The remarkable diversity of projection neurons in the brain, brainstem and spinal cord suggests that unique combinations of transcription and growth factors may be necessary to induce axon regrowth from each subpopulation[115,120]. Second, the accessibility of active gene regulatory regions may limit the efficacy of transcription factor manipulation. Understanding spatial and temporal changes in transcriptional and chromatin environments in neuronal subpopulations could lead to the development of targeted, temporally controlled strategies to improve chromatin accessibility and transcriptional initiation in recovery-organizing neurons. Third, increasing the intrinsic growth competence of neurons promotes axon repair through healthy spinal cord tissue, but stimulated axons abruptly stop when encountered with fibrotic scar tissue[115,121]. Therefore, the reconstitution of damaged circuits or formation of new circuits that reestablish communication across complete SCI remains a formidable challenge.

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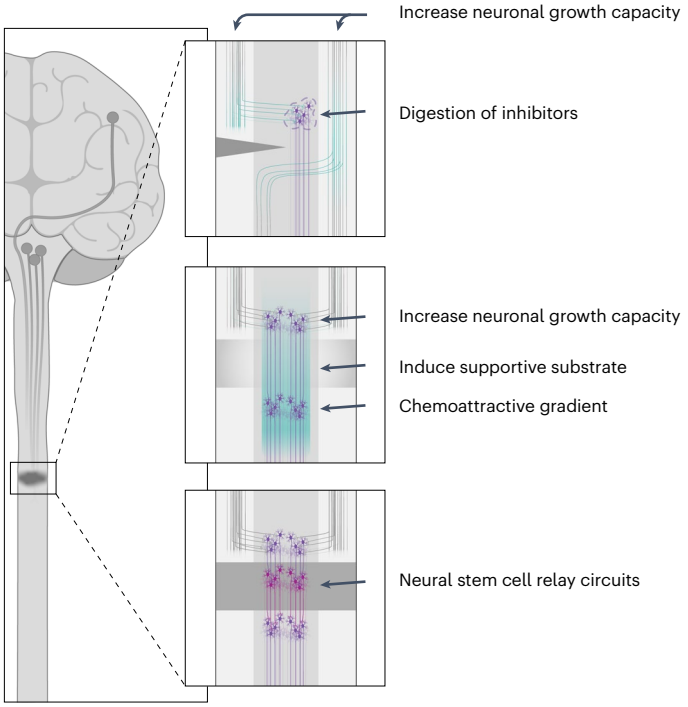

Fig. 4 | Biological strategies to reconstitute damaged circuits. Schematic summarizing the primary biological strategies to reconstitute circuits after SCI. The reconstitution of circuits following spinal cord damage depends on the severity of injury. For incomplete injuries, regeneration of severed axons is not required, as a sufficient number of axon projections remain intact. Densifying the projectome of neurons that retain axons below the injury can be achieved by either activating intrinsic neuronal growth competence or digesting inhibitors associated with degenerating myelin or chondroitin sulfate proteoglycans (top). When the SCI is complete, reconstitution of circuits necessitates regrowing severed axons across injury sites to restore connectivity. This regrowth can be achieved for propriospinal neurons by deploying a multipronged, developmentally inspired repair strategy that sequentially upregulates intrinsic neuronal growth competence, induces supportive substrates and provides chemoattraction (middle). Alternatively, these hostile tissues can be repopulated with the engraftment of supportive neural stem cells that receive input from key supraspinal centers and relay information across injury sites to restore connectivity (bottom).

BOX 3

Overcoming inhibition versus reactivation of growth programs

Neutralizing inhibitory molecules and targeting intrinsic neuronal growth competence have been the main strategies to promote the anatomical remodeling of residual neuronal projections after SCI.

Neutralizing inhibitory molecules

The most established strategy involves the delivery of the enzyme chondroitinase (ChABC), which digests the glycosaminoglycan side chain of CSPGs. CSPGs are the key constituents of perineuronal nets that regulate the opening and closing of the neurogenesis critical period. Devoid of perineuronal nets, neurons become available to receive synapses from newly formed axons of residual projections. Multiple independent laboratories have demonstrated that ChABC mediates functional improvements in both rodent and NHP models[20,147]. Activity-dependent neurorehabilitation further enhances this recovery[41]. Equally popular are strategies targeting the nogo receptor reticulon 4 (RTN4), which neutralizes myelin-associated inhibition[107]. Positive outcomes in rodent and NHP models have led to a multinational clinical trial that is evaluating the efficacy of antibodies against this receptor to augment manual dexterity after incomplete SCI (NCT03935321).

Targeting intrinsic neuronal growth competence

One of the transformative moments for neural repair came from systematic screenings in optic nerve injury models. It was found that the genetic deletion of *PTEN* induces unprecedented regrowth of injured axons from retinal ganglion cells[111]. Suppressing this transcription factor in cortical neurons induced long-distance regrowth of corticospinal tract axons through spared astrocyte bridges following SCI[148]. These findings revealed that reactivating intrinsic neuronal growth mechanisms enables true regeneration of severed axons that become insensitive to extrinsic inhibitors. Many discoveries followed, as summarized in the main text.

Reconstitution of damaged circuits

A complete SCI not only interrupts communication across the injury, but also provokes the formation of fibrotic scar tissues with different lesion compartments that each present challenges to reconstitute spinal circuits (Box 2). Cajal posited that regrowing axons through such hostile tissues would require recapitulation of the conditions that stimulate and guide the growth of axons during development[122]. Certain components of this hypothesis were documented[123–125], culminating in a developmentally inspired, multipronged strategy that reactivated the intrinsic growth capacity of neurons, induced the formation of substrates to support axon growth within the core lesion compartment, and guided axons downstream using spatially and temporally controlled release of growth factors[115]. Stimulated, supported and chemoattracted axons regrew through astrocyte borders, across the fibrotic scar, and into healthy tissues where they formed contacts with neurons below the SCI (Fig. 4). This strategy targeted propriospinal neurons with the assumption that these cells would become relay circuits capable of mediating recovery. Despite the pronounced regrowth of electrophysiologically conductive axons, no recovery of function was observed[115]. Various mechanisms may explain this lack of recovery, including the absence of activity to shape these new circuits, innervation of improper targets, poor remyelination and even axon retraction. Equally mysterious is whether other neuronal subpopulations located in the brain and brainstem could be similarly guided through complete SCI and to relevant targets below the injury.

Engraftment of new circuits

A cure for SCI will require reconstituting the natural topology of the CNS. However, demonstrating that intraspinal neurons may relay sufficient information downstream to produce behaviors led to a more optimistic definition of the requirements for meaningful recovery after SCI.

Efforts to repopulate the hostile lesion environment with relay circuits followed. Various types of cells, including neural progenitor cells (NPCs), have been grafted into the injured spinal cord with widely differing success[125] (Fig. 4). Only when NPCs were harvested from homologous spinal cord tissue, supported with growth factors and embedded in stabilizing matrices or scaffolds, did they survive the engraftment. NPCs matured into glial cells and neurons that filled the fibrotic tissue lesion and propelled thousands of axons along the entire rostrocaudal extent of the rodent and primate spinal cords[126,127]. Differentiated neurons attracted projections from various host neurons located in the brain and brainstem, thus establishing relay circuits

that could restore electrophysiological connectivity across complete SCI[126]. However, these new circuits mediated limited recovery[126–128]. Various mechanisms may be invoked to account for this discrepancy, including the importance of activity-dependent factors, guidance of axons to relevant targets, and even the necessity to differentiate NPCs into specific neuronal subpopulations. For example, transplantation of NPCs enriched with V2a interneurons into the injury site improves recovery of diaphragm activity[129].

Perspectives on biological strategies to reconstitute circuits

All of these interpretations on the requirements for reconstituting circuits after SCI led to considerable advances[42]. We now understand how to densify the projections from spared neurons, regrow severed axons over long distances, and repopulate the injured spinal cord with viable circuits. However, the resulting recovery has so far been insufficient to translate into impactful clinical applications. We surmise that directing interventions to specific neuronal subpopulations[118] and guiding their regrowing axons to relevant targets may be necessary to achieve recovery. Single-cell methods are opening a path to support this shift of resolution. Even following pronounced spinal circuit repair, however, complementary interventions must counteract the maladaptive changes that take place in circuits located below a severe SCI, as observed following multiple sclerosis[130].

Molecular choreography of recovery from SCI

The cure for SCI is unforeseeable, but we can already forecast that current strategies will not complete this long-haul quest. Throughout this review, we have argued that we must operate a radical transition in the resolution of our CNS interrogation. Instead of focusing on vaguely defined circuits and pathways, we must establish multi-omic cartographies at single-cell resolution over the entire CNS to map the landscape of neurons that can orchestrate the natural recovery of function after SCI—thus capturing the changing properties of recovery-organizing neuronal subpopulations. These large-scale, multi-omic cartographies will also identify whether and how other cell types and epigenetic mechanisms contribute to improving or impairing recovery.

Imaging whole nervous systems[131], single-cell technologies[132] and cell-specific interrogations[133] have established the analytical tools to catalog the molecular choreography of recovery from spinal cord damage with exquisite detail. We now possess technologies to dissect the molecular signatures and growth requirements one neuron at a time, across all the neurons of the CNS and over timescales ranging from minutes to months. The resulting space–time catalogs of recovery from SCI are poised to uncover the transcriptional programs that will support the regrowth of any neuronal subpopulation from the brain, brainstem and spinal cord. We must then harness this knowledge to inform these evidence-based repair programs that engage and reconstitute the projections from each recovery-organizing neuronal subpopulation to their respective targets.

This new era will unlock the blueprints to reconstitute the natural topology of all the relevant recovery-organizing neuronal populations, with the hope that the development of a cure for SCI will then follow.

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Competing interests

G.C., J.B. and J.W.S. hold various patents in relation with some of the present work. G.C. and J.B. are consultants of ONWARD medical. G.C. and J.B. are minority shareholders of ONWARD, a company with partial relationships with some of the presented work. The remaining authors declare no competing interests.

Additional information

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