Multisystem Neurorehabilitation in Rodents with Spinal Cord Injury

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Abstract

A number of neurorehabilitative strategies have demonstrated efficacy in enhancing the recovery of sensorimotor function after a spinal cord injury (SCI). Combinations of task-specific motor training, epidural electrical stimulation of the spinal cord, and pharmacological interventions such as the administration of serotonergic agonists have resulted in remarkable improvements of locomotor and/or postural functions in rats with a complete SCI. Similar results are emerging in human patients with severe spinal cord damage. Synergistic amelioration of the loss of sensorimotor function through combinatorial approaches, i.e., the use of two or more interventions simultaneously, indicates that individual interventions can have both specific and complementary influences. For example, electrical stimulation applied at distinct rostrocaudal locations or agonists to specific receptor subtypes administered systemically tune unique aspects of locomotor movements. When administered simultaneously, the effects of these interventions can combine synergistically and result in significantly greater improvements in locomotor performance than either intervention alone. In addition, the use of robotic assistance during motor training, in particular in an "assist-as-needed" mode that allows a normal amount of variability in performing the task as opposed to a repetitive rigid training mode, can strongly enhance the effect of locomotor rehabilitation. We suggest that all

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of these interventions are enabling factors. They enable spinal neural circuitries to interpret task-specific sensory input and use this information in a feedforward manner to produce appropriate motor responses. Continued advancement in the development and refinement of such neurorehabilitative interventions will ensure progress towards improving the quality of life of individuals with a SCI or other severe sensorimotor dysfunctions.

Keywords
Spinal cord injury • Epidural electrical stimulation (EES) • Monoamine administration • Robotic training • Rehabilitation

1.1 Introduction

Severe spinal cord injury (SCI) significantly impacts the ability of affected individuals to generate functional standing and walking movements. A century of research on the organization of the neural processes that control movements in mammals, however, has demonstrated that the basic neuronal circuitries sufficient to generate efficient stepping patterns and independent standing are embedded within the lumbosacral segments of the spinal cord [1–3], i.e., caudal to the level of most human SCI. Indeed, current views on motor control suggest that the descending systems provide excitatory and modulatory drives to spinal circuits, but the operations underlying the elaboration of motor patterns for walking and standing are essentially achieved by the neuronal networks in the spinal cord. Therefore, the question becomes: how can we transform nonfunctional spinal motor circuitries into highly functional and adaptive networks after a severe SCI to enable motor control during neurorehabilitation and thus restore functional capacities in paralyzed subjects?

In this chapter, we briefly summarize the basic historical concepts underlying the control of locomotion and the plasticity of spinal neuronal networks with neurorehabilitation. We then show how this fundamental knowledge can be exploited to design enabling multisystem interventions after a severe SCI, i.e., combinations of electrical and pharmacological stimulation paradigms, robotic devices, and sensory-based motor training that are capable of restoring motor control abilities after the loss of descending input (Fig. 1.1). We describe recent experiments in animal models of SCI that demonstrate the impressive capacity of this multisystem approach to improve motor functions after the complete interruption of supraspinal information. Next, we describe current efforts for the development of technologies to optimize this approach. Finally, we discuss the potential of this technologically intensive but physiology-based neurorehabilitation approach to crystallize into fully operative neuroprosthetic systems and robotically assisted training procedures capable of restoring useful functional capacities in humans with severe spinal cord damage.

1.2 Experimental Concepts Underlying Activity-Dependent Plasticity After a SCI

At the beginning of the past century, Philippson [2] and Sherrington [1] reported unexpected observations that revolutionized our conception of the neural control of movements. They showed that after a complete transection of the thoracic spinal cord in cats and dogs, the hindlimbs could still exhibit a range of motor patterns in response to changing sensory inputs. These observations led Sherrington to conceive the production of locomotor movements as “a train of motor acts resulting from a train of successive external situations.” [1] Sherrington aimed to emphasize the crucial importance of afferent information in

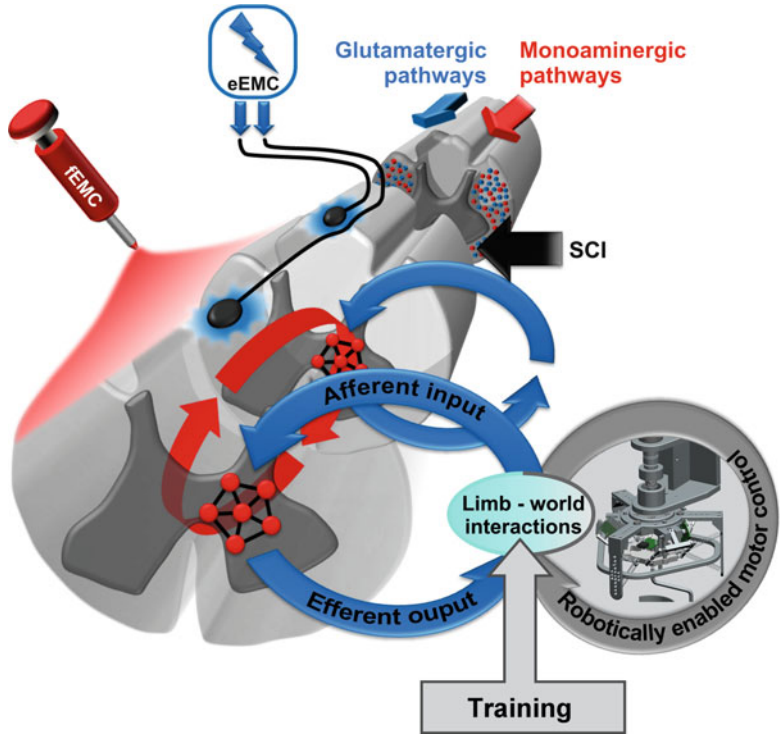

Fig. 1.1 Multisystem neurorehabilitation to restore motor functions after a severe SCI. Schematic drawings of locomotor circuits are shown after a SCI at the thoracic level that interrupts both glutamatergic (*blue*) and monoaminergic (*red*) descending pathways originating from various brainstem areas. The combination of monoamine receptor agonists and epidural electrical stimulation at the L2 and S1 levels can tune the physiological state of the spinal circuits to a level sufficient for motor control to occur. Therefore, these interventions are termed pharmacologically (*fEMC)* and electrically (*eEMC*) enabled motor control. The generation of efficient locomotor movements under their combined influences, termed e*fEMC*, results from the ability of spinal circuitries to ensure a continuous match between afferent input and efferent output defining optimal motor states. To ensure appropriate interactions between the locomotor system and the external world during training, robotic interfaces can be interposed to provide robotically enabled motor control conditions. Such robotic systems can assist limb movements for propulsion as well as trunk motion for balance. Finally, these various *motor control-enabling systems* can be used in combination to facilitate neurorehabilitation

allowing, selecting, and controlling spinal motor outputs after the loss of supraspinal influences (see discussions in [4]). How can this conceptual view be exploited to improve functional capacities after a SCI?

In the early 1980s, Edgerton and Rossignol reasoned that if sensory input can access and control spinal circuits deprived of brain input, the repetitive exposure to organized patterns of sensory input with training might promote beneficial functional changes in the activated neuronal networks. Their work clearly demonstrated the potential utility of intense daily exercise on a treadmill for improving the stepping capacities of adult cats with a complete spinal cord transection at the thoracic level. They further reported that after several months of daily step training, the spinal cats regained an impressive ability to produce full weight-bearing locomotion for extended periods of time [5, 6]. Fueled by these findings, Edgerton and his team evaluated the potential of rehabilitative training and weight-bearing afferent input to improve function after a SCI by evaluating the ability of spinal cats to develop the capacity to stand [7]. They discovered a surprising property of spinal circuitries: cats that had been trained intensely to stand, developed the remarkable ability to support their entire body weight for up to 1 h, but they stepped very poorly on the treadmill, i.e., the spinal cord learned the sensorimotor task

that was specifically practiced and trained [8, 9]. These results led to the concept of spinal learning via activity-dependent plasticity: as repetitive activation of a synapse can change its properties within a timeframe that ranges from milliseconds to months [10], the repetitive and simultaneous activation of certain sensory and motor pathways with task-specific training can select and reinforce those circuits and connections in a way that significantly improves their ability to perform the practiced movement successfully [11, 12]. This Hebbian-type plasticity at a systems level predicts that the outcome of a neurorehabilitative program will strongly depend upon the type and quality of the motor function that is trained. Moreover, this concept emphasizes the crucial importance of concurrent sensory information in shaping the functional remodeling of spinal circuitries with training.

Following these observations, there has been substantial success in translating activity-based rehabilitation therapies from cats to humans with a partial SCI [13, 14]. Improvements of ambulatory function in response to locomotor training in patients with an incomplete SCI have been reported in several studies from different laboratories [15–18]. A clinical trial demonstrated that with weight-bearing training, 92% of subjects with an incomplete SCI (ASIA C or D) regained the ability to walk at a functional speed within 3 months [19]. In contrast, in individuals with a severe SCI classified as ASIA A, B, and most Cs with low lower limb motor scores [20], locomotor training has not resulted in successful overground walking, even with the aid of any walking device. Why does locomotor training fail to significantly ameliorate motor functions in severely affected individuals?

The answer may be deceptively simple: robust neural activity needs to be present for activity-dependent plasticity to occur, i.e., some critical level of excitability must be present within the locomotor networks to respond to proprioceptive input. In contrast to individuals with an incomplete SCI who progressively regain basic walking capacities after recovering from the initial spinal shock, patients with a severe SCI exhibit limited or no residual function to be trained [18], and locomotor rehabilitation thus fails to promote useful plasticity in the sensorimotor pathways [21]. Therefore, given the assumption that the locomotor networks remain functional in the lumbosacral spinal cord after these severe injuries, the next logical step was to develop interventions to gain access to the dormant spinal locomotor circuitries after a SCI, with the aim of enabling motor control during rehabilitation to mediate use-dependent plasticity in the trained neuronal networks.

1.3 Motor Control–Enabling Systems After a SCI

A severe lesion of the spinal cord significantly compromises the degree of sustainable excitability in the lumbosacral circuitries. Thus, the inability to produce standing and stepping patterns after a severe SCI is not due only to the interruption of the descending motor commands, but also, and above all, to the markedly depressed state of the spinal neuronal networks [21]. Consequently, in the past decade, much effort has been focused on developing paradigms to tune the physiological state of the spinal circuits to a level sufficient for stepping and standing to occur. Various strategies including electrical stimulation of the muscles [22, 23] or dorsal roots [24], epidural [25–27] or intraspinal [22, 24] electrical spinal cord stimulation, administration of a variety of pharmacological agents [28–32], and smart robotic systems [33, 34] have shown the capacity to facilitate standing and/or stepping after a severe SCI. Since these interventions are not used to induce but rather to allow the production of movements, we term these paradigms *motor control–enabling systems* (Fig. 1.1).

1.3.1 Electrically Enabled Motor Control (*eEMC*)

Weight-bearing locomotion and standing have been induced in complete spinal mammals by electrical stimulation, using both penetrating electrodes inserted into the spinal cord tissue and electrodes placed on the surface of the dura. Using penetrating microelectrodes, Shik and colleagues [35] originally observed that stimulation of the dorsolateral funiculi at the cervical and thoracic spinal cord levels initiates stepping in decerebrate cats via activation of intraspinal

fibers. More recently, the Mushahwar, Prochazka, and Rossignol research teams have developed systems of intraspinal stimulating microelectrodes for rats and cats whereby a set of penetrating electrodes is inserted in the ventral horn to facilitate the activity of the neuronal networks that control stepping [36–38]. Using a less invasive technique, Garcia-Rill and colleagues [39] reported that epidural electrical stimulation of both the cervical and lumbar enlargements with plate electrodes induces locomotion in decerebrate cats. Since then, tonic *eEMC* applied over the dorsal surface of virtually any lumbar or sacral segment [40] has shown the ability to facilitate stepping on a treadmill as well as standing in rats [29, 41], rabbits [42], cats [42], and humans with a severe SCI [43, 44].

While intraspinal microstimulation offers the advantage of closer juxtaposition of the electrode to motoneurons and interneurons in the intermediate and ventral laminae, the insertion of multiple penetrating electrodes into the spinal cord is a complex procedure [22] that can inflict significant tissue damage [45]. Their placement may be difficult to maintain in ambulatory individuals, particularly for very long periods. In addition, recent evidence suggests that many of the beneficial effects of intraspinal microstimulation may rely on the same mechanisms as epidural electrical stimulation (EES) [46]. While the direct stimulation of muscles using computer-controlled patterns of activation has had some success in the recovery of hand control [47], acceptability by individuals with a SCI has not been high. One limitation is the absence of feedback mechanisms for maintaining adaptive control. We therefore focus this section on the principles of and mechanisms through which EES enables motor control after a SCI while retaining some adaptive features.

The mechanisms underlying the facilitation of motor activities with *eEMC* are not yet fully understood [48]. Electrophysiological recordings [49] and computer simulations [46, 50] suggest that EES can directly engage spinal circuits primarily by recruiting posterior root fibers at their entry into the spinal cord, as well as along the longitudinal portions of the fiber trajectories. When the stimulation is used to actually induce evoked potentials during quiet standing, 3–4 well-defined motor responses in lower limb muscles can be classified based on their respective latencies and threshold (Fig. 1.2). The early response (ER), which only

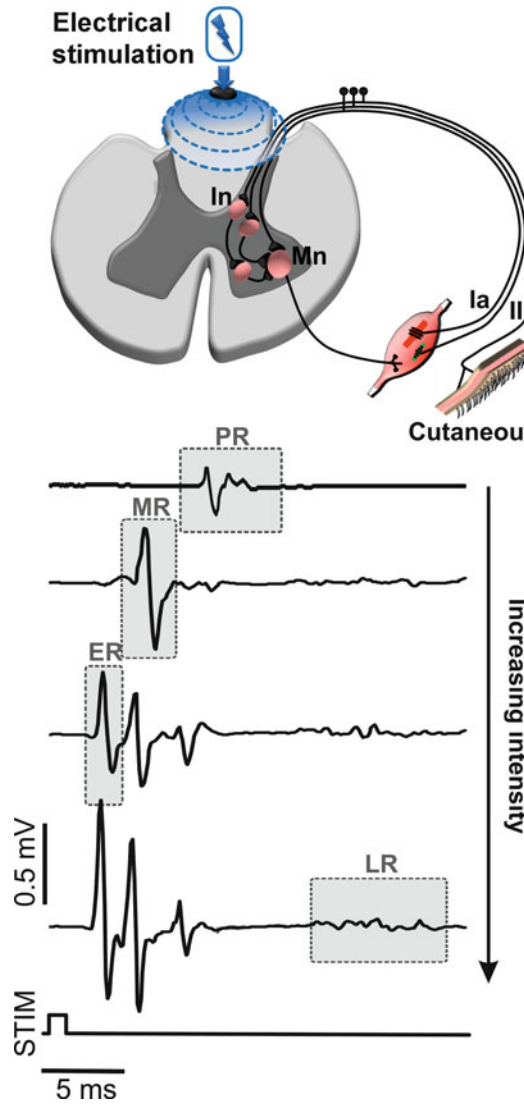

Fig. 1.2 EES elicits distinct motor responses through the recruitment of specific pathways. Schematic illustration of the afferent systems putatively recruited when delivering single-pulse EES over spinal segment S1. When applied over the dorsal aspect of the spinal cord, the electrical stimulus typically elicits three or four responses in all hindlimb muscles. The responses are termed early response (*ER*), middle response (*MR*), polysynaptic response (*PR*), and late response (*LR*) based on their respective latencies and thresholds (see text for details). *In* interneuron, *Mn* motoneuron

appears at higher intensities when stimulating the more caudal segments, results from the direct stimulation of motoneurons and/or motor nerves. The middle (MR) response is essentially mediated by the monosynaptic connections between Ia fibers and motoneurons, i.e., a response equivalent to the H-reflex [49, 51] (Fig. 1.2). The neural elements associated with the polysynaptic response (PR) and long latency response (LR) remain undetermined but are likely to rely on multiple afferent systems. Based on the electrophysiological signature of these responses, we argue that the PR relies in part on the disynaptic and/or oligosynaptic connections between group II fibers and motoneurons [25, 49] (Fig. 1.2). We also surmise that EES recruits large-diameter cutaneous afferent fibers that contact multisensorial interneurons (Fig. 1.2), facilitate transmission in group Ib and II pathways [52], and can elicit coordinated bilateral motor responses in flexor and extensor muscles [53]. Cutaneous sensory systems may contribute to both PR and LR responses. It is worth noting, however, that this intuitive explanation is not clearly applicable to the "enabling" mode of stimulation whereby modest stimulation levels induce little or no measurable evoked potentials. At this intensity, the stimulation instead modifies the physiological state of the locomotor circuitry via the activation of proprioceptive input associated with standing and stepping [54].

How do electrically induced motor responses translate into functional patterns of electromyographic (EMG) activity during stepping and standing? When a spinal rat is positioned bipedally on a stationary treadmill belt, continuous EES applied at the sacral level (S1) induces tonic levels of EMG activity in extensor muscles, which enables the maintenance of a continuous standing posture (Fig. 1.3) [28]. A close inspection of muscle EMG traces reveals that the sustained EMG activity in extensors is composed of a succession of motor responses that are closely linked to the electrical stimulation (Fig. 1.3). When treadmill belt motion is initiated, all hindlimb joints undergo changes toward extension (limb moving backward), creating dynamic proprioceptive input that immediately transforms the motor patterns from a tonic to a rhythmic state (Fig. 1.3). Under such locomotor states, we found that EMG bursts are essentially built from a sequence of MR responses in extensor muscles and MR and PR responses in flexor muscles (Fig. 1.3) [25]. Both responses are markedly modulated in amplitude throughout the gait cycle according to the phase of the movement [25, 49, 51] (Fig. 1.3). This phase-dependent modulation of electrically evoked motor responses in flexor and extensor muscles creates rhythmic and alternating bursts of EMG activity sufficient to sustain continuous hindlimb locomotion on a treadmill [25]. MR and PR motor components show similar behaviors when eliciting step-like patterns with epidural stimulation in the paralyzed legs of human subjects [43]. Together, these data indicate that central mechanisms dynamically update the level of excitability in motor pools and strictly tune the gain in afferent pathways based on the current sensory and motor states of the locomotor apparatus [55]. Although experimental evidence is still incomplete, *eEMC* seems to play a crucial role in augmenting the excitability of the spinal circuitries that underlie and control postural and locomotor tasks.

Analysis of EMG activity during standing and stepping showed that EES engages motor pools through the recruitment of afferent pathways, which follow a strict muscle-specific architecture along the rostrocaudal extent of the spinal cord [56], consequently, it is plausible to determine whether *eEMC* delivered at specific locations elicits distinct patterns of motor responses in lower limb muscles. To address this issue, we applied *eEMC* over lumbar (L2) versus sacral (S1) segments during both standing and stepping in spinal rats [28]. Consistent with the rostrocaudal anatomical gradient of flexor and extensor motor pools, we observed a facilitation of flexion with lumbar EES, whereas stimulation applied at the sacral level primarily facilitated extension, both during standing (Fig. 1.4a) and stepping (Fig. 1.4b). Moreover, the combination of two [28], and even more efficiently three [41], sites of *eEMC* promoted

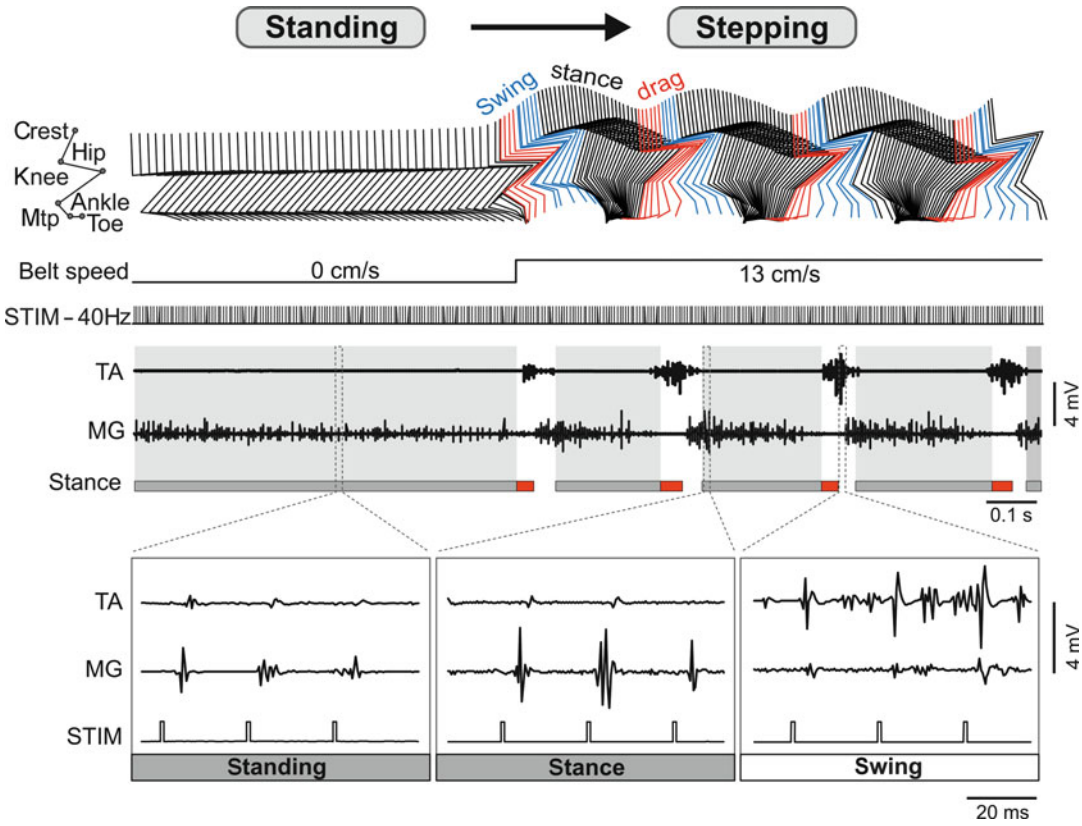

Fig. 1.3 Modulation of spinal circuits with EES during stepping in spinal rats. Hindlimb kinematics and EMG activity from tibialis anterior (*TA*) and medial gastrocnemius (*MG*) muscles are shown for a spinal rat receiving continuous (40 Hz) EES at the sacral (S1) level. During the represented sequence, the treadmill belt abruptly switches from static (no motion) to a dynamic (13 cm/s) condition. The lower insets display the responses occurring during the highlighted region of the EMG recordings. During standing, the sustained EMG activity in extensor muscles (*left inset*) is composed of a succession of MR responses that are locked to the stimulation. The emergence of the dynamic state (belt motion) induces the immediate modulation of motor evoked responses whereby the MR in the MG is facilitated during stance (*middle inset*) and inhibited during swing (*right inset*), whereas the MR and LR are suppressed in flexor muscles during stance, but substantially facilitated during swing

clear synergistic facilitation of stepping which was evident in the increased consistency of hindlimb kinematics and enhanced weight-bearing capacities.

Under normal conditions, glutamatergic reticulospinal neurons provide the tonic excitatory drive to engage spinal locomotor networks [57]. Here, we summarize results from various studies that collectively demonstrate the powerful ability of basic spinal cord electrical stimulation to replace the descending source of tonic excitation to enable standing and stepping in paralyzed subjects with a severe SCI. We therefore term this intervention *electrically enabled motor control* or *eEMC* (Fig. 1.1). In the complete absence of monoaminergic input, however, *eEMC* alone fails to promote substantial levels of weight bearing with plantar placement of the feet on the treadmill belt [28]. Similarly, descending glutamatergic input alone fails to elicit long-lasting step-like patterns in mice without the presence of monoamines [57]. We show in the next section that to attain robust stepping capacities after a severe SCI, *eEMC* needs to be combined with pharmacological agents that replace the lost modulatory monoaminergic input.

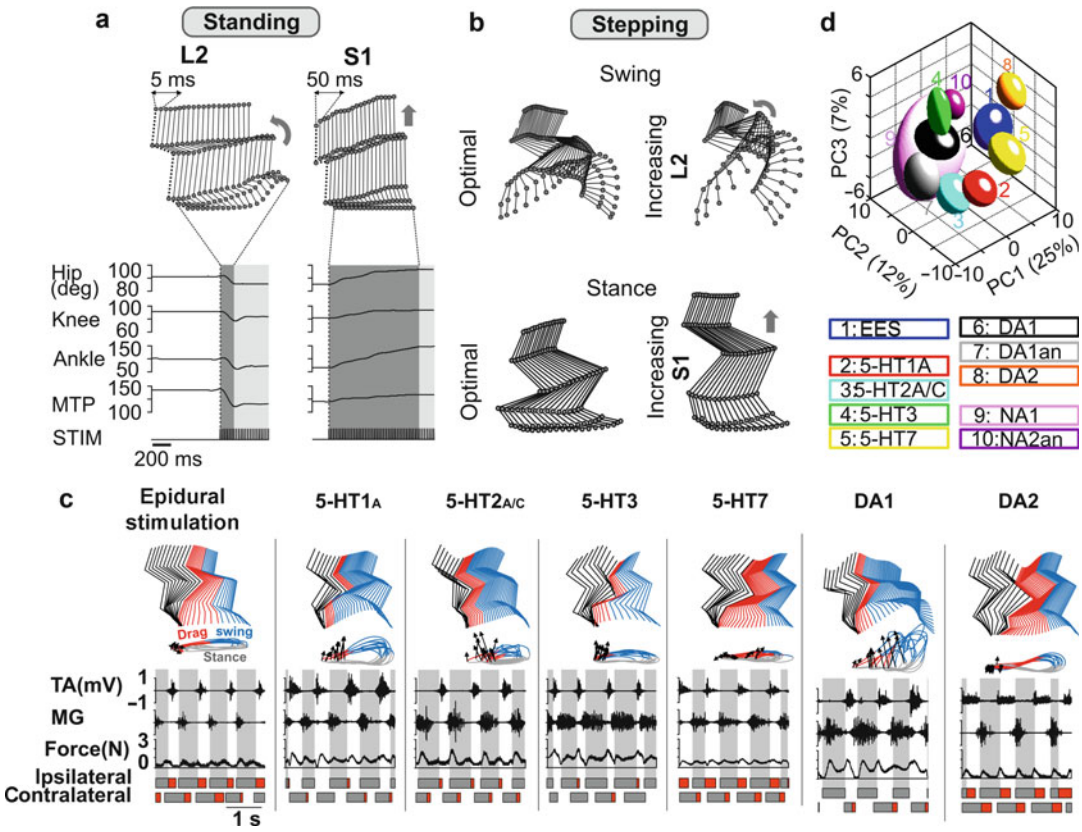

Fig. 1.4 Specific modulation of hindlimb movements mediated by EES and monoaminergic agonists during standing and stepping. (**a**) Stick diagram decomposition of hindlimb movements and associated time course of changes in hindlimb joint angles (increase toward extension) when delivering EES at L2 (*left*) or S1 (*right*) during standing. (**b**) Effects of increasing stimulation intensity at L2 during swing (*top*) and at S1 during stance (*bottom*) on hindlimb stepping movements enabled by dual-site EES and serotonin agonists. (**c**) Representative features of locomotion recorded in spinal rats under EES at L2 + S1 and agonists to various monoaminergic receptors (*indicated above*). A representative stick diagram decomposition of hindlimb motion during swing is shown for each condition with the successive color-coded trajectories of limb endpoint. Vectors represent the direction and intensity of the limb endpoint velocity at swing onset. A sequence of raw EMG activity from TA and MG muscles is displayed at the bottom. Gray and red bars indicate the duration of stance and drag phases, respectively. (**d**) Three-dimensional statistical representation of locomotor patterns based on principal component analysis applied on a large number of gait parameters ($n = 135$). Gaits associated with a given monoaminergic receptor clustered in a distinct location, revealing that each receptor promoted unique stepping patterns [61]

1.3.2 Pharmacologically Enabled Motor Control (*fEMC*)

Spontaneous locomotor activity is associated with a substantial release of monoamines within most laminae of the lumbosacral segments [58]. These monoaminergic inputs are not restricted to the classical, hardwired synaptic communication but primarily operate perisynaptically through three-dimensional chemical diffusion, i.e., volume transmission [59]. Monoaminergic neurotransmitters easily escape the synaptic cleft, enter the extracellular space, and reach extrasynaptic G-protein–coupled receptors located on the surface membrane of neighboring cells. This signaling transduction pathway profoundly alters cell properties over timescales that span from minutes to hours [59]. Volume transmission communication suggests that pharmacological agents mimicking the action of monoamines

could act in concert with EES to orchestrate the functional tuning of spinal circuitries in SCI subjects [60].

We directly tested this hypothesis in adult rats with a complete SCI [28]. We selected agonists to 5-HT_{1A} and 5-HT_7 (8-OHDPAT) and 5-$HT_{2A/C}$ (quipazine) receptors since these pharmacological agents have previously shown the ability to facilitate locomotion in rodents with a SCI [29, 30]. In the subacute phase after the injury, the functional state of the spinal circuitries is markedly depressed. Accordingly, neither electrical stimulation nor serotonin agonists could induce functional states that would enable stepping movements on the treadmill at 1 week post-injury. In striking contrast, the combination of dual-site EES and serotonin agonists promoted coordinated locomotion with plantar placement and substantial levels of weight bearing on the treadmill. Detailed statistical analyses revealed that each pharmacological or electrical intervention modulates distinct aspects of the locomotor movements, suggesting a fine-tuning of selective functional circuits (Fig. 1.4d). For example, 5-$HT_{2A/C}$ receptors primarily facilitated extension and weight-bearing capacities, whereas 5-HT_{1A} and 5-HT_7 receptors facilitated rhythmic components and promoted stepping patterns biased towards flexion (Fig. 1.4c). The functional specificities of electrical and pharmacological stimulations, in turn, provided the means for the exquisite synergy between the two paradigms, such that only a combination of serotonin agonists and EES was able to engage spinal locomotor networks as early as 1 week after a complete SCI. We recently investigated whether this receptor-specific functional tuning of gait could apply to a broader range of monoaminergic receptors. Using advanced neurobiomechanical analyses (Fig. 1.4c), we demonstrated the intriguing ability of serotonergic, dopaminergic, and noradrenergic receptor subtypes to modulate stepping behavior in qualitatively unique ways in adult spinal rats [61]. Thus, stimulation of spinal monoaminergic receptors pharmacologically and recruitment of spinal circuits electrically can modulate recognizable qualitative features of locomotion independently as well as collectively in rats deprived of any supraspinal influences. Since the beneficial influences of *fEMC* and *eEMC* do not simply sum algebraically but actually enable novel and specific motor control states, we term this synergistic combination *efEMC* for *electropharmacologically enabled motor control* (Fig. 1.1).

1.3.3 Robotically Enabled Motor Control (*rEMC*)

There are various lessons to be learned on the advantages of developing the engineering aspects of robotic technologies in coordination with input from neurophysiologists and rehabilitative specialists [62]. One example of this multidisciplinary perspective is the importance of the type of control that is designed to operate a robot when attempting to assist in the recovery of stepping after a SCI [11, 12, 33, 34, 54, 63]. More specifically, we first observed that adult mice with a complete midthoracic SCI could learn to step more successfully when there was no continuous and rigid control of the movements of the limbs by the robotic arms, i.e., the mice were allowed to step independently at intervals throughout a given robotically controlled training session [34]. Subsequently, a similar experiment was performed with spinal mice in which the control of the robotic arm was programmed to "assist-as-needed." The robotic arm would move the limb within a preselected window size to accommodate the variation that is intrinsic to every movement of the gait cycle [33]. Those mice that were trained with the robotic arms controlled in an "assist-as-needed" mode learned to step better than those trained with rigid control of the trajectory of the legs during stepping. Further investigation identified the probable reason for this improved stepping with the "assist-as-needed" control algorithms [64]. Detailed analysis of the EMG patterns revealed that the rigid control scheme intermittently interrupts the alternate recruitment of flexor and extensor muscles; the neural control system operates in a continuous correction mode. In contrast, by enabling variability in the limb trajectory, the "assist-as-needed" control mode does not constrain the timing of the movement, thereby allowing the appropriate recruitment of flexor muscles during swing and extensor

muscles during stance, as required to produce a coordinated stepping pattern [64].

Collectively, these data emphasize the importance of designing smart robotic interfaces to enable the spinal locomotor system to generate appropriate stepping movements as opposed to building robots that move the limb along fixed trajectories. Consequently, we term this concept *robotically enabled motor control* or *rEMC* (Fig. 1.1). There is growing evidence that *rEMC* not only applies to limb movements but also to the trunk–limb system for the control of balance and weight bearing [65].

1.3.4 Sensory-Enabled Motor Control (*sEMC*)

Under normal conditions, the descending systems control the general features of locomotor movements, i.e., gait initiation, speed of progression, and direction of walking. A key issue for the design of clinically relevant neurorehabilitation procedures is the identification of an alternative source of adaptive control for stepping when these pathways are interrupted by a SCI. As summarized in the first section of this chapter, Sherrington originally introduced the idea that sensory ensembles dictate the properties of spinal locomotion *in vivo* [1]. This viewpoint, historically reduced to the "chain of reflex" hypothesis, predicts that the succession of external situations detected by afferent systems allows, determines, and actually controls the characteristics of centrally generated motor outputs. Currently, sensory input is instead regarded as part of reflex subsystems that modulate, but are under the control of, central pattern generator (CPG) networks [53, 66]. Here, we provide a few examples that illustrate the ability of multisensory information to control spinal motor outputs with an astonishing degree of precision, a capacity that can be exploited to produce flexible and adaptive patterns of locomotion after a SCI.

In the absence of treadmill motion, but under weight-bearing conditions, electrical and pharmacological stimulations allow spinal rats to maintain a tonic posture behaviorally visible as standing (Fig. 1.5a). When the treadmill belt motion is initiated, however, the spinal circuits detect the emergence of dynamic conditions and immediately transform the motor patterns from a tonic to a rhythmic state [28]. Likewise, spinal locomotor systems can accommodate limb kinematics and EMG patterns to changing treadmill belt speeds within a single step, even at running velocities (Fig. 1.5a). Strikingly, while spinal rats are running on the treadmill, the sudden stop of the belt abruptly terminates flexor bursting and results in sustained tonic activity of extensor muscles [28]. Spinal sensorimotor systems are thus capable of recognizing a deviation from expected task-specific patterns of proprioceptive input within milliseconds, hence allowing the immediate switch from a running to a standing state without any supraspinal influence. Similar modulation of locomotor patterns can be found in decerebrate and spinal cats [42] as well as humans with a severe SCI during manually assisted stepping on a treadmill [67, 68]. Along the same line, spinal rats show the remarkable ability to adjust limb movements to a sudden change in the direction of the treadmill belt from forward to backward, or to a progressive rotation of the body in a sideward direction (Fig. 1.5b). In both situations, spinal circuitries respond to changing external conditions with a complete reorganization of hindlimb kinematics and muscle activity patterns to produce continuous locomotion in virtually any direction in space [28].

During the execution of these various motor tasks, we found that there was often a continuous match between the spatiotemporal patterns of sensory inputs (external situations) and the characteristics of the motor outputs [28] (Fig. 1.5b). The precision and versatility of these complex tuning patterns cannot be explained by any of the spinal reflex responses that have been described to date. Together, these data suggest that the ensemble of afferent systems sensitive to load, direction, and velocity collectively contribute to elaborate a detailed representation of the locomotor state that allows for the continuous selection of the combination of motor circuits appropriate to perform the current task successfully. These observations imply that after the loss of brain input, sensory information is instructive in a functional, primarily feedforward manner [12].

The recovery of hindlimb locomotion in animals with a SCI is usually attributed to the

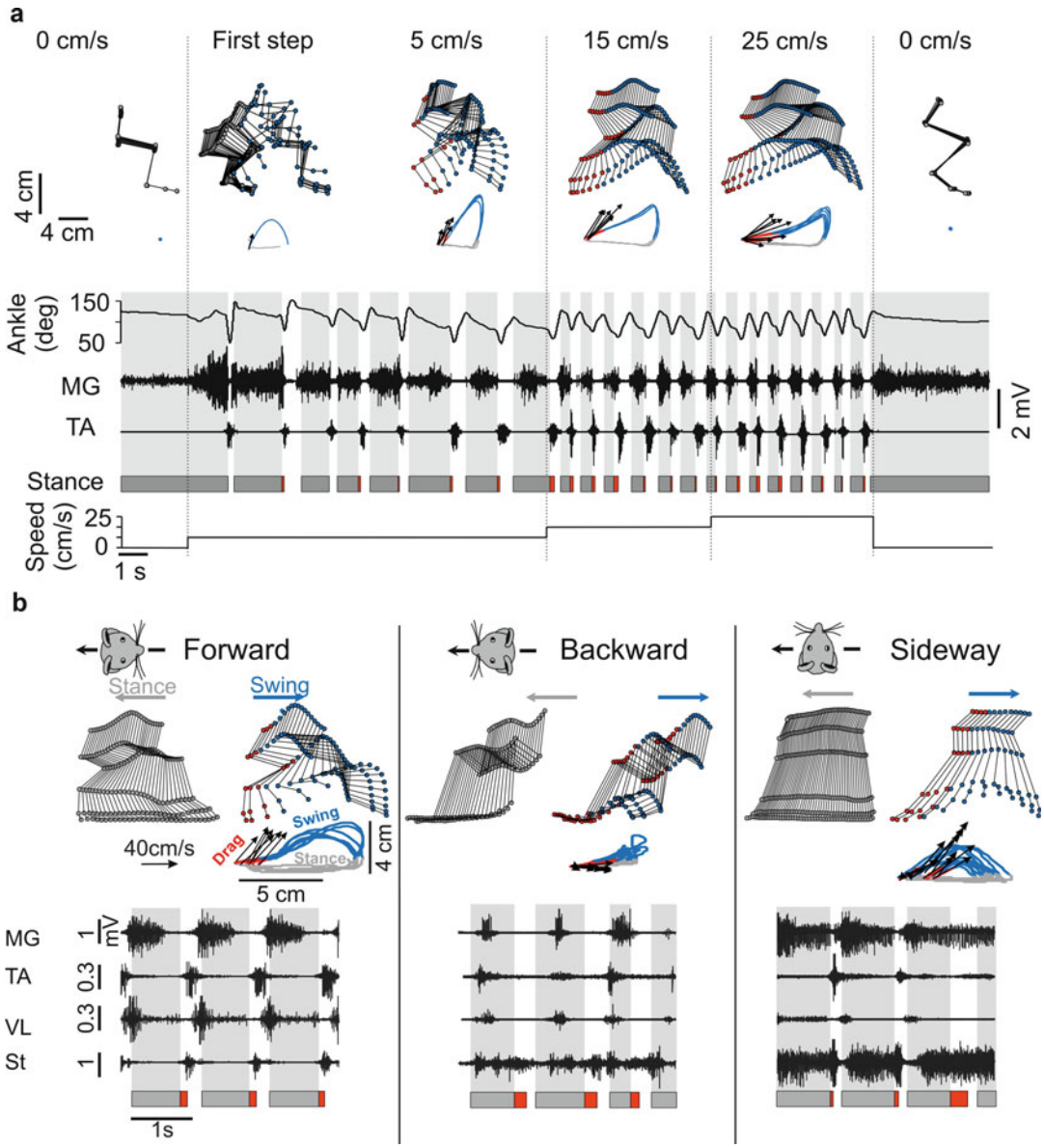

Fig. 1.5 Effects of velocity- and direction-sensitive afferent input on the characteristics of hindlimb movements in spinal rats. (**a**) Representative example of hindlimb kinematics and EMG activity recorded from a continuous sequence of steps during a gradual increase of treadmill belt speed including running velocities. Stick diagram decomposition of the first step shows the smooth transition from standing to stepping. Conventions are the same as in Fig. 1.3. (**b**) Representative example of hindlimb kinematics and EMG activity recorded during continuous locomotion in the forward (*left*), backward (*middle*) and sideway (*right*) direction. The same limb from the same rat corresponding to the leading (*front*) limb during sideway stepping is shown for the three conditions. Data are represented as in Fig. 1.3, except that stick diagrams are shown in three dimensions, with the main plane oriented with the direction of treadmill belt motion. *VL* vastus lateralis, *St* semitendinosus muscles [28]

recovery of neuronal networks responsible for central pattern generation, i.e., CPG networks [69, 70]. Even in humans, the recovery of locomotor function after a severe SCI is still thought to heavily rely on CPGs present in the human spinal cord [71]. We instead argue that the recovery of impressive locomotor capacities with step training (see Sect. 1.5) under the presence of

electrical and/or pharmacological stimulation relies on the ability of the spinal circuitries to utilize sensory ensembles as a continuing source of motor control and as a substrate for learning [12, 72]. Indeed, the data presented in this review show that the spinal cord acts as a smart processing interface that continuously integrates multisensory input to control its motor output, both acutely and chronically. Thus, beyond representing an automated machinery that produces stereotyped reflexes and CPG-like activity, we argue that evolutionary pressures engineered the spinal brain to process complex patterns of afferent input and utilize this information to make decisions about how to maintain successful locomotion. Moreover, repetitive exposure to specific sensory patterns with practice allows for the significant optimization of these sensori-motor processes whereby spinal circuitries can learn to produce optimal motor states in the total absence of brain input.

Here, the concept of optimal motor states is not restricted to stereotyped stepping patterns with alternation between extensor and flexor muscles, but instead it encapsulates the rich repertoire of motor behaviors underlying activities of daily living. In fact, even when deprived of any supraspinal influence, spinal circuitries can recognize task-specific sensory input and instantly modulate or transform the patterns of muscle activity to execute a variety of motor tasks ranging from standing, walking, running, stepping backward, or even stepping in a sideward direction [28]. Currently, the power of *sEMC* for the production and training of motor functions after SCI is not well recognized or exploited to the level of its potential [44] (Fig. 1.1).

1.4 Impact of Chronic SCI on the Function of Spinal Circuitries

What is the impact of the chronic absence of weight-bearing and normal activation patterns on the functional capacities of spinal locomotor systems? In general, it is thought that severe spinal cord damage induces a short period of complete paralysis, which is followed by a slow and incomplete recovery of function that eventually reaches a plateau in the chronic state of the injury. Overwhelming evidence against this oversimplified view, however, has accumulated in recent years. A large number of detrimental changes in cell properties and circuit connectivity have been described in the chronic state of SCI. For example, Vinay and his coworkers [73] found that a complete SCI leads to a downregulation of the potassium-chloride co-transporter-2 (KCC2) in motoneuron membranes, which, in turn, results in a substantial positive shift in the membrane equilibrium potential of chloride. This shift has a dramatic impact on neuronal function by changing the effect of inhibitory input into excitatory input, which could contribute to the development of spasticity [74].

At the network level, a series of anatomical and neurophysiological observations in animals [75–77] and humans [78, 79] suggest that after a severe SCI the spinal circuitries responsible for the control of stepping and standing undergo a major remodeling, a process that continues to evolve for years after the SCI [80]. After the interruption of descending pathways, the severed axonal fibers degenerate, creating vacant synaptic territories that become partially reoccupied by sprouting intraspinal fibers [75, 77]. These new synaptic connections likely lead to the formation of aberrant circuits that may misdirect neural information towards inappropriate motor networks during movement [54, 81]. Indeed, we observed that rats with a complete SCI show a significant deterioration of stepping capacities in the chronic state of the injury [28]. Whereas the combination of electrical and pharmacological stimulations enabled coordinated locomotion with plantar placements at 1 week after the injury, the same rats exhibited poorly coordinated stepping patterns with large variability when tested at 9 weeks post-lesion (Fig. 1.6a, b). Compared to noninjured rats, these animals displayed a large increase in the expression pattern of the activity-dependent neuronal marker c-fos in all lumbar and sacral segments (Fig. 1.6b, d) [28]. This marked increase in the number of cells contributing to stepping in chronic spinal animals suggests that new nonfunctional circuits progressively form after a severe SCI, and that these abnormal connections engage inappropriate circuits to produce locomotor

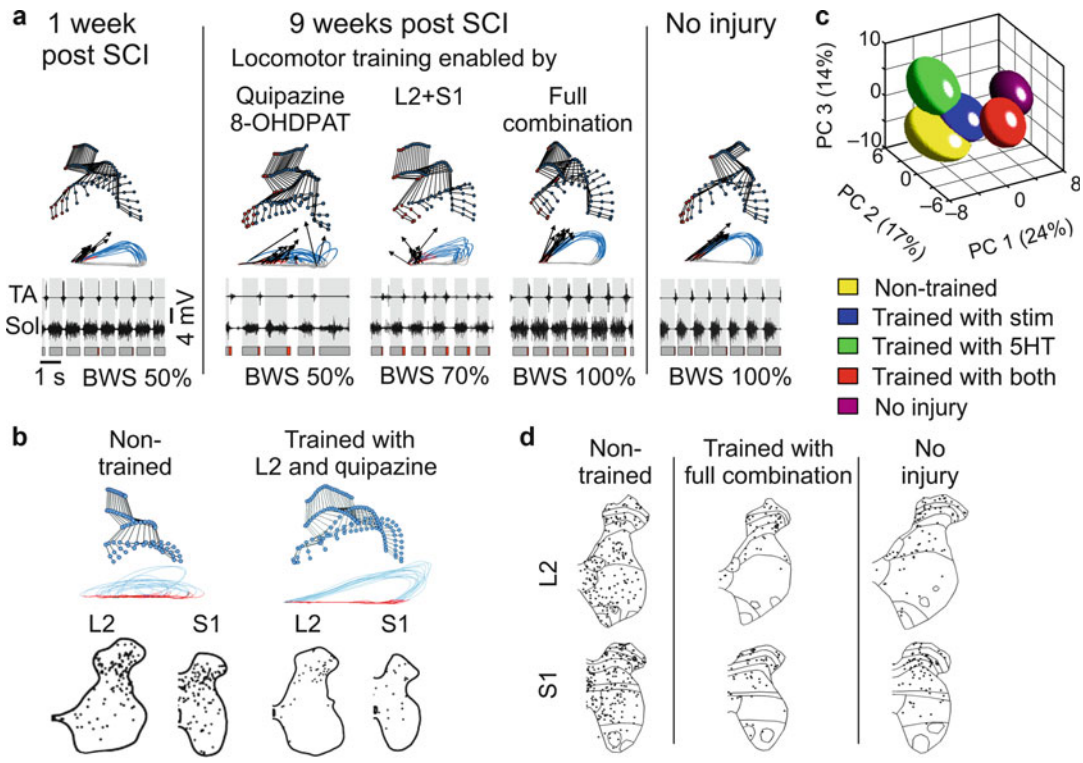

Fig. 1.6 Locomotor training enabled by selective pharmacological and/or electrical stimulation paradigms promotes the recovery of intervention-specific gait patterns in rats deprived of supraspinal input. (**a**) Representative illustrations of EMG and kinematic features during stepping under the full combination (stimulation at S1 plus L2 and quipazine plus 8-OHDPAT) 1 week post-injury (before training; *left*) and after 8 weeks of training enabled by pharmacological and/or electrical stimulation (*middle*). A similar representation is shown for a noninjured rat (*right*). Conventions are the same as in Fig. 1.3. (**b**) Representative illustrations of kinematic features during stepping in nontrained rats and rats trained with EES at L2 and quipazine administration. Below representative camera lucida drawings of FOS-positive neurons in spinal segments L2 and S1 of a nontrained SCI rat and a SCI rat trained with stimulation at L2 and quipazine administration. (**c**) Three-dimensional statistical representation of locomotor patterns based on principal component analysis applied on a large number of gait parameters ($n=135$). Each group ($n=5$–7 rats) clustered in distinct locations, revealing that each locomotor training paradigm promoted the recovery of unique stepping patterns. (**d**) Representative camera lucida drawings of FOS-positive neurons in spinal segments L2 and S1 of a nontrained SCI rat (*left*), a SCI rat trained with the full combination (*middle*) and a noninjured rat (*right*) [28]

patterns when pharmacological and/or electrical interventions are administered. These results are compatible with the emergence of abnormal reflexes [78, 79], unintended movements [81] and spasticity [82] in the chronic state of the injury in humans. Together, these results show that spared neuronal circuitries below a complete SCI do not remain unchanged. Instead, major plastic changes progressively take place post-lesion, which lead to a deterioration of the neuronal function in the chronic state of the injury.

In light of these changes, can step training enabled by locomotor permissive interventions direct the chaos of plasticity that spontaneously occurs after a SCI and can this use-dependent plasticity lead to useful changes associated with improved functional capacities?

1.5 Neurorehabilitation with Motor Control–Enabling Systems

Intensive rehabilitative training has shown the capacity to prevent deterioration of function and improve stepping and standing capacities in cats

with a complete SCI [83]. Similar activity-based approaches alone, however, failed to promote similar improvements in rats [84] and humans [21] with a severe SCI. As mentioned in the first section of this chapter, we surmised that the absence of robust activity during locomotor training is largely responsible for the poor effects of rehabilitation. We directly tested this hypothesis by training spinal rats on a treadmill under the presence of *efEMC* interventions, which encourage coordinated patterns of locomotion in the paralyzed hindlimbs.

In our first attempts, we only used a combination of lumbar (L2) EES and 5-$HT_{2A/C}$ agonist (quipazine) administration to facilitate locomotion during the training of spinal animals [85]. As mentioned above, each locomotor permissive system modulates distinct features of stepping behaviors. Accordingly, this specific combination promotes unique patterns of locomotion including enhanced extension components, in particular, in the distal extremities [29]. After 2 months of training, the rats displayed improved locomotor movements characterized by a low variability in kinematics features and the capacity to step for an extended period of time on the treadmill under the presence of pharmacological and electrical interventions. The rats, however, developed exaggerated stance phases with marked extension of the foot and toes during swing (Fig. 1.6b). The chronic repetition of a certain type of movement thus reinforced and indeed amplified the specifically trained stepping behavior. More recently, we tested the therapeutic potential of locomotor training enabled by lumbar (L2) plus sacral (S1) EES and agonists to 5-HT_{1A}, 5-$HT_{2A/C}$, and 5-HT_7 receptors (quipazine and 8-OHDPAT) [28]. Compared to lumbar stimulation and quipazine alone [85] (Fig. 1.6b), this combination enabled more normal stepping patterns and effectively promoted locomotion as early as 1 week post-injury (Fig. 1.6a). In contrast, the combination of lumbar stimulation and quipazine was not effective in encouraging locomotion until 2–3 weeks post-SCI [86]. After 9 weeks of neurorehabilitation, the spinal rats recovered the impressive capacity to perform full weight-bearing locomotion with features that were nearly indistinguishable from those underlying walking patterns of the same rats recorded before the injury (Fig. 1.6b, c). Rats trained with electrical stimulation alone or serotonin agonists alone developed specific patterns of locomotion, but these interventions failed to prevent the deterioration of functional capacities at the chronic state of the injury (Fig. 1.6a–c). Collectively, these results suggest that the repetitive activation of unique combinations of sensorimotor circuits under the influence of distinct electrical and pharmacological stimulations and through task-specific sensory patterns lead to the selection and reinforcement of those neuronal networks in an activity-dependent manner [12]. As exemplified in cats [7–9, 87], the rodent spinal motor circuitries deprived of any supraspinal influences can learn the task that is trained and practiced.

This concept of Hebbian plasticity among spinal sensorimotor pathways is consistent with the changes in c-fos expression patterns underlying continuous locomotion of trained rats. Regardless of the intervention used to facilitate stepping, we found that rats exposed to locomotor rehabilitation exhibited a substantial decrease in the number of c-fos positive neurons compared to nontrained animals [28, 85] (Fig. 1.6b–d). However, the detailed features of c-fos expression patterns in the lumbar and sacral segments depended significantly on the selective intervention provided during training, i.e., each neurorehabilitation procedure promoted specific gait patterns that were presumably produced by unique combinations of neuronal networks (Fig. 1.6c). These results demonstrate that the recovery of stepping ability after a complete SCI does not result from the activation of an ontogenetically defined hardwired circuitry that persists and recovers post-injury. Instead, specific combinations of locomotor training, pharmacological, and electrical stimulation interventions induce novel activity-dependent anatomical states that reflect the ability of spinal circuits to learn and that can promote high levels of functional recovery without any supraspinal input in adult rats.

1.6 Development of Operative Neuroprosthetic Systems

As described above, different stimulation parameters and sites of EES can modulate specific aspects of the spinal locomotor output. In addition, with varying levels of activation of specific pharmacological receptors, *fEMC* strategies can be used to selectively activate different combinations of locomotor circuits within the lumbosacral spinal cord. For an individual to take full advantage of this modularity, however, semiautomated control systems including feedback loops will be necessary [88]. The flexible manipulation of *eEMC* and *fEMC* to modulate movements will further require the development of a device that can receive mechanical and/or biological signals that, in turn, can modulate an output of chronically implanted epidural electrode arrays capable of achieving the desired movement. There are multiple solutions with varying degrees of complexity and sophistication that can be utilized to achieve this goal. As a starting point, we have developed an on–off system that can detect the intent of a rat with a complete thoracic spinal cord transection to step based on EMG signals from the forelimbs [89]. Once the criterion EMG pattern from multiple forelimb muscles is recognized, an output signal is sent to a stimulator that activates the lumbosacral spinal cord epidurally with a preselected frequency and voltage level. This approach needs further development so that different combinations of electrodes from the chronically implanted epidural electrode array can be activated at a selective stimulation intensity and frequency to achieve the most effective standing or stepping in a subject at any given time during the recovery from injury. In humans, the neural interface must be able to accommodate differing levels and types of dysfunction within and across subjects. Thus, this interface must have different degrees of automaticity in the interpretation of feedback signals. For human subjects, a hand-controlled "joystick" could be designed so that the user could manually control the stimulation parameters (with predefined limits for safety) when the person intends to stand, walk, or perform other sensorimotor tasks.

A more advanced but complex and invasive approach could capitalize on established concepts from brain–machine interface systems. Neural states can be readily extracted from the modulation of cortical ensembles to detect the intent to perform a range of tasks [90–92]. In turn, these neural states can be readily exploited to modulate the patterns of stimulation in a neuroprosthetic epidural electrode array to stand, walk, or adjust locomotor movements to the requirements of the external world, e.g., cross an obstacle or, climb stairs.

In the technical development of interventions to facilitate motor recovery after a SCI and many other degenerative neuromotor disorders, there will inevitably be the need for a paradigm shift in the ability to monitor and quantify a wide range of motor tasks, including postural control, locomotion, and fine motor skills. Although the technical capability and expertise to accomplish such assessments is well established in basic research laboratories, realization of these technical capabilities in clinical rehabilitation settings is minimal. This limitation, in itself, has minimized advances that could be made from a research, and also a patient's, perspective. For example, it is clear that the technical capabilities exist to quantify all of these types of movements and to provide immediate feedback to the patient that can serve as a major motivational stimulus and also immediate knowledge of whether a certain intervention has any impact on the ability to perform a given motor task. This type of information is equally available to the researcher, clinician, and patient. A key to capitalize on this type of technology will involve the design of smart robotic interfaces to enable the performance of movements in severely affected individuals (see Sect. 1.3.3).

1.7 Perspectives for Viable Clinical Applications

We are approaching a new and exciting era for the capability to recover significant levels of motor control after a severe SCI and the onset of a variety of degenerative motor diseases. This optimism is based on years of progression of the

evolution of new perspectives and concepts related to how the nervous system controls movement. These new fundamental concepts provide the basis for developing new strategies that combine biological and technical breakthroughs. For example, we know that very complicated and detailed motor tasks can be performed with little or no supraspinal control due to the fact that most of the neurophysiological details are embedded and accomplished within the circuits of the spinal cord [93]. Furthermore, we now understand that these neural circuits remain functional after most spinal cord injuries and that they can be revived with appropriate activity-dependent interventions [28, 83]. In this chapter, we have documented various observations supporting these positions, and we have demonstrated that access to this surviving circuitry can be gained by electrically stimulating the lumbosacral spinal cord epidurally and by facilitating the spinal circuitry pharmacologically. Most importantly, however, a central component in realizing improved motor control using these *motor control–enabling strategies* is the potent activation of the circuitries underlying the motor task that is being relearned. Specifically, the strategies will have minimal or no positive effect in relearning a motor skill if the circuitry that generates that motor skill is not recruited in the presence of EES and/or pharmacological facilitation. Our challenge in the near future is to develop procedures that will improve the efficacy of these interventions by understanding in more detail the basic biology of these enabling techniques. Which circuits within the spinal cord are being activated to perform a given task and what neurotransmitter systems are critical for these circuits to successfully generate the desired movement with the patient having the control and confidence necessary to execute the strategies in day-to-day activities? The application of these strategies with further developments in robotics will have to occur to fully realize the impending, remarkable potential that remains after even some of the most severe injuries to the neuromotor system.

Conclusions

Spinal cord damage severely impacts sensorimotor function and thus the quality of life of affected individuals. After a SCI, improvement in sensorimotor functions can be achieved via a number of activity-dependent rehabilitative strategies, e.g., task-specific sensorimotor training, robotic interface systems, pharmacological facilitation of the spinal neural circuitries, and spinal cord epidural stimulation. Although each of these interventions can have a positive impact on the recovery process after a SCI, the efficacy of these interventions can increase tremendously when they are administered in combination. Consequently, future efforts should consider a multidimensional approach in developing and refining neurorehabilitative approaches for individuals with severe sensorimotor dysfunctions after a SCI or other debilitating conditions.

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