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Taccola, G, Ichiyama, RM orcid.org/0000-0002-7640-8032, Edgerton, VR et al. (1 more author) (2022) Stochastic spinal neuromodulation tunes the intrinsic logic of spinal neural networks. Experimental Neurology, 355. 114138. ISSN 0014-4886

https://doi.org/10.1016/j.expneurol.2022.114138

Stochastic spinal neuromodulation tunes the intrinsic logic of spinal neural networks

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Highlights

- Spinal sensorimotor networks are stochastically modulated, particularly by highly varying proprioceptive input.
- The continuous arrival of multiple sensory modalities provides highly redundant neural networks that, at the systems level, are continuously in highly dynamic states.
- An epidural interface can be used as an effective site to trigger spinally evoked potentials to probe activity levels of multiple motor pools simultaneously via muscle specific EMG.
- Sub-motor threshold spinal neuromodulation amplifies and enables a wide range of voluntarily triggered motor activities after spinal cord injury.
- Neuromodulation can acutely and selectively elevate the excitability of different neuronal networks to facilitate use-dependent mechanisms
- Use-dependent mechanisms can then be engaged to transform the reorganization of spinal-supraspinal networks to higher functional states.

Abstract

The present review focuses on the physiological states of spinal networks, which are stochastically modulated by continuously changing ensembles of proprioceptive and supraspinal input resulting in highly redundant neural networks. Spinal epidural interfaces provide a platform for probing spinal network dynamics and connectivity among multiple motor pool-specific spinal networks post-injury under *in vivo experimental conditions*. Continuous epidural low-frequency pulses at low intensity can evoke motor responses of stochastically changing amplitudes and with an oscillatory pattern of modulation. The physiological significance of this oscillatory pattern, intrinsic to "resting" spinal networks and observed in both uninjured and injured locomotor circuits, is unclear. This neural variability among spinal networks appears to be a fundamental mechanism of the network's design and not a "noise" interfering with movement control. Data to date also suggest that the greater the level of stimulation above motor threshold, the greater the loss of modulation over the motor output that is physiologically provided by interneuronal networks, which integrate naturally occurring proprioceptive and cutaneous input generated during movement. Sub-motor threshold spinal electrical stimulation experiments demonstrate a range of functional improvements of multiple physiological systems when used in concert with sensorimotor training after spinal cord injury. Although our understanding of the systemic, cellular and molecular modulatory mechanisms that trigger these activity-dependent adaptive processes remain incomplete, some basic physiological principles have evolved, at least at the systemic and neural network levels and to some degree at the cellular level.

Keywords: Spinal cord; motor control; electrical stimulation; spinal cord injury; electromyograms; motor reflex.

Abbreviations: CDP, Cord Dorsum Potential; CST, Corticospinal Tract; CV, Coefficient of Variation; eEmc, electrical Enabling motor control; ECG, Electrocardiography; EMG, Electromyography; ERs, early responses; Quip, quipazine; L, Lumbar; l, left; LRs, late

responses; MRs, middle responses; r, right; Sol, Soleus; Strych, strychnine; TA, Tibialis Anterior; Th, Thoracic.

1. Introduction

The most basic, overarching physiological principles of movement control must take into account that 1) the resultant of all modes of sensory input to all motor pools defines the constantly changing physiological state of the nervous system and 2) given these physiological states, the net resultant of the input contributes to defining the pattern of net excitation of each motor pool and how the motor pools are coordinated. To generate the vast array of potential movements an extensive transformation of sensory input to motor neurons must occur in real time largely "automatically". Further, for this real time to occur automatically as effectively and smoothly as it normally does, the neural networks must function largely in a feedforward manner.

This review is focused on probabilistic logic of the control of movement at the spinal level in the uninjured as well as in the injured spinal cord and highlights how the physiological states of neural networks can be optimally neuromodulated using highly varying, but with relatively low levels of spinal neuromodulation. We recognize that the organismic effects of spinal stimulation techniques are mediated at levels of functional reorganization ranging from systems to synapses. Here, we will emphasize data demonstrating extensive integration of multiple spinal and supraspinal neural networks functioning across multiple levels of physiological systems.

A significant degree of variability in the outcome of every motor task reflects the complexity and the number of variables that affect the outcome. The anatomical and cellular similarities of spinal cord stimulation with peripheral nerve stimulation alone are consistent with the intrinsic variability of motor output in general, as was so succinctly demonstrated by Bernshteĭn in 1967. It was suggested that afferent stimulation elicits fluctuating motor outputs, resulting from multi-level modulatory mechanisms derived from a diffuse spinal interneuronal system (Gossard et al., 1994) as well as descending influences from supra-spinal centers (Moreno-Lopez et al., 2021).

The goal of this review is to characterize the complexity of the design of spinal networks that executes the final stages of postural and locomotor control. The enormity of this

function is illustrated by the fact that the spinal cord receives all proprioceptive signals from our lower body continuously from the earliest prenatal stages to death. In addition, it must be able to interpret continuous ensembles of supraspinal input consisting of other sensory modalities in order to successfully perform all necessary tasks for survival. Some of this sensory input must be stored in some form of memory that can be accessed as needed, while weighing the importance of this sensory information automatically. This automaticity assumes a capability to anticipate oncoming needs, commonly referred to as feedforward processing. Another design feature of control of movement relates to multiple sources and strategies of control, with their relative importance dependent on the task at hand. A design feature, not commonly recognized, is the rather substantial redundancy of the neural networks that can perform essentially the same motor task. Given these design features we have presented experimental strategies that yield new insights as to how we can use newly developed technologies to effectively interface with unique physiological properties of spinal networks and how they function synergistically with supraspinal input. These concepts largely focus on systems level physiological processes that become evident largely only under *in vivo* conditions. Finally, we briefly propose how these concepts in concert with use-dependent mechanisms can be used to develop more effective recovery strategies following paralysis.

2. Assessment of unique dynamics of spinal networks under neural modulation during stepping

Given the variability that clearly is an intrinsic characteristic of sensorimotor networks, it is inevitable that the predicted output generated from a given motor pool at any given spinal response is highly probabilistic. The magnitude of this probabilistic phenomenon is a fundamental feature of the motor pool's output, which varies from millisecond to millisecond within a single burst of EMG activity which is tightly linked to the proprioceptive ensembles at every phase of the motor task. In neurologically intact, awake rats at rest, sub-threshold pulses directly applied to the spinal cord through chronically implanted epidural micro-scaled electrode arrays elicit motor responses that are highly modulated in amplitude (Taccola et al., 2021). A similar variability in the amplitudes and timing of

spinally evoked potentials on consecutive step cycles is shown in an adult rat with a complete spinal transection (Fig.1).

During rhythmic activities such as stepping, the formation of EMG bursts is also directly linked to the intensity of spinal stimulation delivered. Three components of spinally evoked responses with different latencies occur during an EMG burst: early (ER; latency 1-4 ms), middle (MR; latency 5-10 ms), and late responses (LRs; latency > 11 ms). ERs reflect the direct supra-threshold input to motoneurons, while MRs correspond primarily to monosynaptic reflexes and LRs to polysynaptic interneuronal spinal networks (Gerasimenko et al., 2006; Lavrov et al., 2006). Specifically, in the soleus (Sol) and tibialis anterior (TA) muscles, the genesis of a burst involved the modulation of both MR`s and LR's, but the modulation of the LRs was phase-dependent only in the Sol. In contrast, EMG activity in both the flexor and extensor muscles showed only MR during the interburst interval. The modulation of different spinally evoked potentials based on the latencies and muscle groups during stepping are highly phase dependent and are considered to reflect the dynamic and functional physiological state of spinal networks that are unique under *in vivo* conditions.

During tonic neuromodulation of the lumbosacral spinal segments, the amplitude and timing of evoked potentials also changed as a function of the level of weight bearing, speed of stepping and pharmacological activation. The latencies and amplitudes of the evoked potentials to epidural stimulation were modulated in a phase-dependent manner during bipedal stepping on a treadmill. These patterns were modulated to a large extent based on the presence or absence of an EMG burst (see light and dark gray shaded areas in Fig. 1A). Averages of all responses during (intraburst) and between (interburst) EMG bursts of the TA and Sol are shown in Fig. 1B. The amplitudes of the responses are 5- to 10-fold higher during than between bursts. In addition, the number of LRs is greater during compared to between bursts. The spinally evoked responses are further modulated as a function of whether they are induced in the early vs. late phase of the flexor (TA) or extensor (Sol) EMG (see orange and green in Fig. 1C), demonstrating the continuing, but predictable changes of the excitability of locomotor networks (Gerasimenko et al., 2006).

Varying the speed of stepping largely affects the EMG burst duration of ankle extensors but not flexors in intact (Roy et al. 1991) and spinal (Courtine et al. 2009) rats. We examined the behavior of motor-evoked potentials in flexor and extensor muscles during stepping at different treadmill speeds. Plotting the evoked responses to consecutive 25-ms epochs between stimuli for a single step cycle at different speeds demonstrates different patterns of changes in the amplitudes and durations for the LRs compared with the MR (Fig. 1D). Increased treadmill speed resulted in a decrease in the number of epochs during the stance phase but no change in the number of epochs during the swing phase. The amplitude of the MR in the TA increased with speed of stepping, whereas the LRs were small and occurred randomly during the swing phase at all speeds tested. In the Sol, a prominent MR was present and occurred only during the stance phase. The largest MR amplitudes generally occurred at the beginning of stance and progressively decreased throughout the remainder of the stance phase. The LRs in the Sol also were present only during the stance phase. At the initiation of stance, LRs were sustained throughout each 25-ms epoch but with succeeding epochs the initiation of the LRs had a progressively longer delay following each stimulus. The amplitudes of the individual LR did not seem to vary consistently across the speeds tested. Another expected feature of the LRs in the Sol was the inverse relationship between the speed of stepping and the number of 25-ms epochs in which LRs occurred, given that the stance phase shortens as speed of locomotion increases.

Based on these findings, LRs probably reflect the dynamics of activation intrinsic to spinal networks involved in motor programs, such as coordination of precise movements during stepping. Based on the data shown in Fig 1 it seems likely that the dynamics of these spinal networks play a role in defining the probability of the activation patterns of motor pools that are generated at different phases of a step cycle.

During the swing phase, these networks can plan the position of the foot for the next stance phase based on afferent information received during the previous step and/or from the contralateral hindlimb of an uninjured cat (McVea and Pearson, 2006). During the beginning of the stance phase, when the foot touches a surface, it seems likely that the afferent information processed in the spinal cord helps in maintaining balance, posture, coordination of different hindlimb muscles, and the ability to make appropriate

adjustments. These “planning” events during stepping have been demonstrated in a decerebrated and spinalized cat (Musienko et al. 2012) and in a chronic spinal cat when performing a step after being tripped during the preceding swing phase (Zhong et al. 2012). We speculate that during this adaptive state, the neural networks can modulate the amplitude and duration of MRs and LRs to accommodate and generate the contralateral limb kinematics in a manner commensurate with the previous proprioceptive ensembles.

Relative to spinal neuromodulation alone, multiple pharmacological interventions have been studied to excite the spinal neural networks. Two families of drugs that have been studied extensively in rodents after severe spinal injuries includes serotonergic agonist quipazine and glycinergic antagonist strychnine. These pharmacological interventions either independently or in combination have resulted in functional improvements and can be synergistic with spinal electrical neuromodulation. Quipazine increased the MR and LRs in the TA to a greater extent than that observed with strychnine. The largest qualitative difference in the Sol between quipazine and strychnine was the greater prominence of the MR relative to the LR with quipazine as observed via the frequency domain analysis. In addition, the MR and LRs occurred over a more prolonged period during quipazine compared with strychnine, resulting in a significantly longer stance phase with quipazine. Based on these one might predict that the combined effects of these two drugs could be complementary or even synergistic given that their mechanisms of neuromodulation of the locomotor networks have fundamentally different characteristics (Gad et al., 2015). Essentially, we propose that these pharmacological interventions neuromodulate the physiological state of the spinal networks resulting in the ensemble of sensory information being translated to a different kinetics and kinematics of stepping consistent with the new modulated state. The modified kinetics and kinematics generated in the subsequent step will then generate a different proprioceptive ensemble and thus a different pattern of activation of motor pools appropriate for the next step cycle. In essence, the combination of the immediately changed physiological state of the spinal networks and the consequential different sensory ensembles will be translated in real time into the next unique, but predictable, “footprint” among the relevant motor pools. This constantly changing physiological state and the predictable motor response can be

attributable to the “awareness” of the chain of networks recognizing the previous state and the high probability of the next appropriate motor event, i.e., which motor pools will be activated. This chain of events is routine for CPG networks (Edgerton,1976).

These observations are consistent with the notion that the sensory-to-motor translation to an outcome is a probabilistic, not a deterministic phenomenon. A classic example of this phenomenon is the experiment with the blacksmith, performed by Bernshteĭn (1967) demonstrating the accuracy of the skilled worker with the hammer consistently hitting his target although this was accomplished with a seemingly challenging number of degrees of freedom generated by the constantly changing joint torques being controlled within and among consecutive movements (English, 1979; Latash and Zatsiorsky, 2016). Another strategy to test the probabilistic hypothesis was used by Pham and collaborators (2020) by having the same mouse running on a treadmill for 30 min on two occasions, but 1-2 weeks apart. Lumbosacral neurons throughout the gray matter were labeled with c-fos (a marker of neuronal activity) after each period of stepping at a speed of 20 cm/s, during which each performance consisted of taking approximately 7000 steps. The key observation was that only 20% of the activated neurons were double labeled.

We suggest that the sources of variability and probabilistic strategy reflects a consistently observed feature of sensory-motor networks that have evolved phylogenetically, ontogenetically and epigenetically.

This interpretation is strongly supported by numerous observations describing a highly probabilistic process that occurs in the translation of sensory- to- motor outputs across multiple species and at multiple sites in the spinal cord as well as the brain (Ivanenko et al., 2013; Bizzi et al., 2000; Cai et al., 2006a, b; Ziegler et al., 2010). The importance of these observations leading to concepts such as “primitives” or “motor modules” is that this design feature of neural networks simplifies the ample variability in actionable options, although inherent in this design feature is some loss in the precision in performing a task. This basic physiological design, however, provides a neural network mechanism that seems to be highly use-dependent, reducing the variability that can yield a high predictability of successful execution of the planned task by both spinal and/or supraspinal networks.

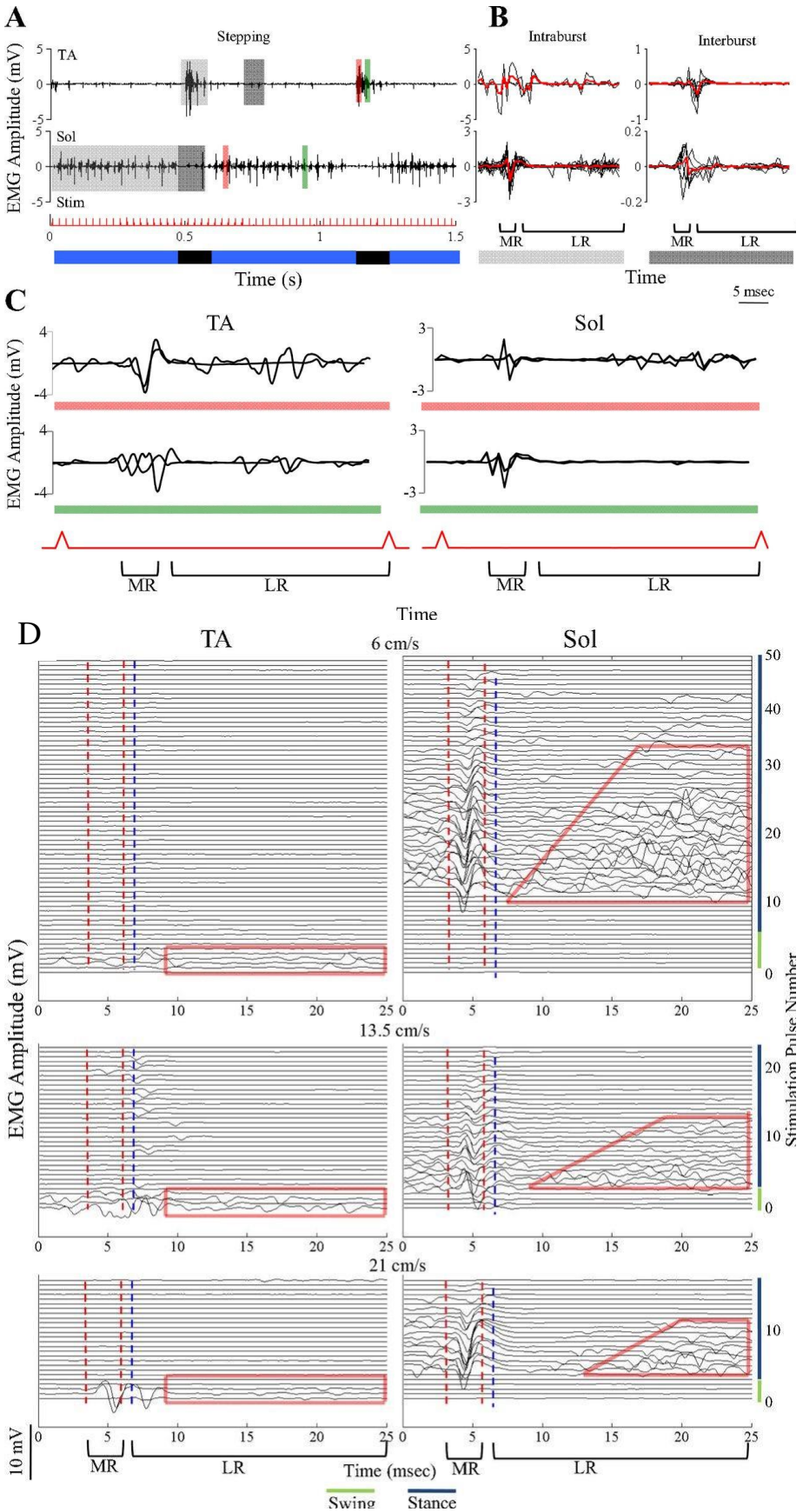

A
Stepping
TA
Sol
Stim
EMG Amplitude (mV)
Time (s)
B
Intraburst
Interburst
MR
LR
Time
5 msec
C
TA
Sol
EMG Amplitude (mV)
MR
LR
Time
D
TA
Sol
6 cm/s
13.5 cm/s
21 cm/s
EMG Amplitude (mV)
Stimulation Pulse Number
10 mV
MR
LR
Time (msec)
Swing
Stance

Figure 1: Modulation of spinally evoked potentials during stepping. A) TA and Sol EMG during stance (blue) and swing (black) phases of stepping on a treadmill at 13.5 cm/s with partial weight bearing under the influence of epidural stimulation (40 Hz between L2 and S1). Light gray highlight, intraburst interval; dark gray highlight, interburst interval; red and green highlights, early and late phases of the EMG burst, respectively; Stim, eEmc pulse. B) the MR and LRs for all motor-evoked potentials during the intraburst interval (left plots for the areas highlighted in light gray in A) and during the interburst interval (right plots for the areas highlighted in dark gray in A) are shown as black traces, and the red bold line shows the average of all potentials. C) zoomed-in view of the early (top traces) and late (bottom traces) phases of the TA and Sol EMG bursts highlighted in A. Note the presence of both an MR and LRs during both phases of the EMG burst in the TA and for the early phase in the Sol but only an MR for the late phase in the Sol. eEmc evoked potentials during stepping at different treadmill speeds. D) the effect of treadmill speed on the modulation of the evoked potentials generated for each stimulation pulse in the TA and Sol muscles for a single step cycle. Modified from Gad et al., 2013.

3. Continuous subthreshold spinal stimulation enables functional recovery

Continuous epidural electric stimulation at subthreshold intensity (20% below motor threshold) greatly increases spontaneous motor activity of paralyzed rats (Gad et al., 2013a). When protracted for long sessions (six hours), continuous sub-threshold epidural electric stimulation not only significantly increased flexor and extensor hindlimb EMG activity, but also enabled more frequent spontaneous stepping movements and forelimb movements during occasional rearing (Gad et al., 2013a). These findings demonstrate that sub-motor threshold stimulation modulates the neural networks into a greater state of readiness. In turn, patterns of spontaneous cutaneous and proprioceptive input, triggered by the dragging movement of the hindquarters occurring when the rat is successfully, intentionally, moving the upper limbs. This dragging of the hindquarters increases the level of excitation above the motor threshold of motor neurons that control the lower limbs (Taccola et al., 2018).

In this way the ensemble of cutaneous-proprioceptive inputs at multiple levels along the spinal cord form the source for primary control of movement. As a result, the residual spinal circuitry caudal to a spinal cord lesion has the potential to be activated, even when critically dysfunctional after prolonged disuse and severe loss of supraspinal input.

One interpretation of the human clinically motor complete lesion is that they are often not anatomically complete, with some residual, but nonfunctional networks remaining anatomically connected caudal to lesion but their level of functional connectivity is

insufficient to generate enough excitation to perceive any sensation or generate any motor response. One of the neuromodulation mechanisms to regain functional connectivity across the lesion is to elevate the spinal networks' basal level of excitability to a "state" closer to the motor threshold. This can be accomplished by applying a sub-threshold electric current that moves the excitability close to, but not beyond the motor threshold. When sufficient current is delivered to the networks that have some ascending or descending residual connection, the amount of motor activity strongly increases and enables more robust weight-bearing activities of voluntary stepping and standing (Harkema et al., 2011, Angeli et al., 2014; Gerasimenko et al., 2015; Grahn et al., 2017; Angeli et al., 2018, Sayenko et al., 2018, Gill et al., 2018, Wagner et al., 2018; Gad et al., 2019; Kandhari et al., 2022).

Given that some level of voluntary movement of the lower limbs has been recovered in a relatively high percentage of individuals paralyzed in the lower body, some controversy has emerged as to whether this newly acquired movement is voluntarily or "reflexly" controlled. A voluntary component has been demonstrated clearly in most of these individuals. For example, they can generate force levels that induce a movement as instructed and can even modulate the level of force, which was tested by varying amplitude and timing of an auditory stimulus (Angeli et al., 2014). As discussed above, however, attributing these changes solely to voluntary control in unlikely given that there are multiple sources of control in generating such improvement. It seems highly likely that there is some contribution of multiple sensory inputs directly or indirectly, in virtually all movements in the uninjured as well as in the injured individual. The question is how many of these sources, and to what degree have been lost and which ones can be recovered. Undoubtedly a significant source of control can be derived from proprioception, emphasizing, however, that there are multiple mechanisms and sources of control to generate a wide variety of movements. Based on these concepts, the feasibility of human subjects with functionally complete motor paralysis to regain voluntarily initiated and sustained independent, full weight-bearing standing and stepping using epidural or transcutaneous stimulation combined with activity-dependent mechanisms has repeatedly been demonstrated in recent years (Angeli et al., 2014; Grahn et al., 2017; Gill et al., 2018; Angeli et al., 2018, Gad et al. 2019, Kandhari et al., 2022).

We have defined this method of spinal stimulation as electrical Enabling motor control (eEmc), as opposed to directly inducing a specific stereotyped movement for each set of stimulation parameters as occurs at increasingly higher amplitudes of currents, changing sites of stimulation, etc. The greater the level of stimulation above motor threshold, the greater the loss of potential control that is normally derived from the spinal interneurons that translates the proprioceptive and cutaneous input in patterns that generate stepping. With eEmc, combined with practice, i.e. when combined with proprioceptive and cutaneous input from lower limbs during load bearing, subjects learned to voluntarily perform bilaterally standing and stepping movements. These results provided some of the first evidence that in individuals diagnosed with complete paralysis for more than a year, eEmc can enable neuronal circuits, by exploiting proprioceptive and cutaneous information, as well as newly acquired input from descending motor signals (presumably residual, but previously incompetent without stimulation).

This potential to functionally re-connect the cord caudal to a lesion with supraspinal pathways is consistent with cadaveric studies of 564 SCI individuals, demonstrating that some white matter was still preserved through the lesion in subjects considered to have a clinically motor-complete lesion (Kakulas, 1999). Although these anatomical data are in line with the functional responses observed with eEmc, the mechanism of eEmc seems more likely linked, at least in part, to guiding a spinal-supraspinal re-connectivity and organization of spinal and propriospinal networks. So far, the potential of spinal neuromodulation has been exploited by eEmc via epidural and transcutaneous stimulation. Of further interest, is the magnitude of the effects on other organ systems that regained function in response to both spinal stimulation procedures.

The significance and relevance of these concepts in regaining locomotor function after severe spinal injuries might be expected given the progressive, conservative and similar evolutionary adaptations across many species in controlling movements. After observing the initial improvements in the postural and locomotor functions in paralyzed human subjects using spinal stimulation techniques combined with use-dependent interventions, there has been a rapid increase in awareness of complementary neuromodulatory principles in controlling locomotor function that have not been widely recognized. The net

effect of a wide range of sensory-motor functions linked to multiple physiological systems is that the neural control of movement has evolved to function largely automatically.

A review of these observations go well beyond what can be addressed in the present manuscript. But a list of those significantly improved functions from either spinal epidural or transcutaneous neuromodulation are: upper limb and hand function (Gad et al., 2018), trunk stability (Rath et al., 2018), independent standing (Angeli et al., 2014, Rejc et al., 2017, Grahn et al., 2017; Gill et al., 2018, Sayenko et al., 2018), breathing and coughing (Gad et al., 2020), bladder, bowel (Gad et al., 2018; Kreydin et al., 2022) and sexual function (Harkema et al., 2011), prevention of hypotonic responses and normalized blood pressure (Phillips et al., 2018). In addition, there are recent examples of similar restorative procedures that have been successful in improving function in individuals with Parkinson's (Samotus et al., 2020), cerebral palsy (Solopova et al., 2017, Gad et al., 2021), stroke and multiple sclerosis (Kreydin et al., 2020).

4. Variability of motor output in an anesthetized rat in response to a constant-stimulus intensity delivered via a spinal epidural electrode array.

The inter-subject variability in motor output in response to spinal stimulation have been extensively described, (Murg et al., 2000; Gerasimenko et al., 2006; Lavrov et al., 2006). The variability of motor output to spinal stimulation at very low intensities has not been explored as much as high-intensity pulses, although it has been clearly shown to enable relatively effective modulation of the physiological states of spinal locomotor networks after transection (Gossard et al., 1994, Gad et al., 2013a).

In fully anesthetized intact rats, weak pulses locally applied to the spinal cord evoked small, apparently randomly-modulated EMG responses (Taccola et al., 2020a). Variations of motor output in response to constant, low-frequency epidural stimulation was not due to temporary adjustments in the position of epidural electrodes over the spinal cord. Indeed, respiratory and cardiac pulsations could cause small electrode shifts that alter the efficacy of stimulation. However, amplitude of Sol and TA EMGs was not significantly different from when an ECG spike occurred close to the stimulus artifact (unpublished observations), demonstrating that mechanical artifacts secondary to

respiratory and cardiac cycles do not play a significant role in the variation seen in motor responses.

In the sample experiment reported in Fig. 2 A, the intensity of stimulation was the lowest to induce appreciable deflections in the baseline from right TA and Sol, with the appearance of MRs. Hereafter, we define as “sub motor-threshold stimulation” an electrical stimulation delivered at intensities around the motor threshold value, namely the lowest amplitude able to elicit at least one electrophysiological motor response during its repetitive supply. Note that, at this sub motor-threshold intensity, no visible muscle twitches were observed, which would usually occur at about 100 µA in this particular type of experiment.

Time courses of MR amplitude for 700 consecutive sweeps (total duration = 35 min) were plotted from TA (Fig. 2 C) and Sol (Fig. 2 D). All responses were characterized by a large variability in amplitude, as indicated by the high CVs (MR TA = 0.22; MR Sol = 0.21). As a reference, variability of responses evoked at supramaximal strengths has been reported significantly lower than in the presence of sub motor-threshold pulses (Taccola et al., 2020a). Nevertheless, peak variability was not affected by epidural pulses happening either close to or far (± 20 ms) from the occurrence of a heartbeat (Fig. 2 C, D). Furthermore, this variability did not reflect any patterned modulation, common among extensor and flexor muscles, as confirmed by the coefficient of correlation among paired peaks of MR responses expressed from TA and Sol (-0.07; Fig. 2 E). Moreover, comparison of the cumulative peak amplitude of consecutive TA and Sol responses (Fig. 2 F) indicates that the neuronal spinal networks projecting to these two motor pools reflect increasingly larger motor units in the TA motor pool compared to the Sol motor pool, for increasing strengths of stimulation (Henneman et al., 1965).

Furthermore, Taccola and Sayenko showed that the continuous epidural stimulation of intact spinal cords of adult rats fully anesthetized with ketamine, elicits EMG responses characterized by an intrinsically random amplitude, but which also follow a spontaneous oscillatory rhythm of modulation with a lower frequency than the one supplied by the stimulating pattern. Pharmacological interventions aimed at blocking inhibitory adenosinergic receptors not only increase the peak amplitude of single motor evoked potentials, but also magnify the level of response variability, moving the rhythmic pattern

418 of amplitude modulation toward higher frequencies. They concluded that this endogenous
419 pattern of modulation might represent both an intrinsic and rhythmic tone able to set the
420 subthreshold excitability of propriospinal circuits, as well as a potential pharmacological
421 target for facilitating network functionality (Taccola et al., 2020c).
422 Reduced preparations of isolated spinal cords from neonatal rats showed that, in a more
423 controlled experimental environment, continuous dorsal stimulation evoked responses
424 from motoneuronal pools that were highly variable in amplitude (Lev-Tov and Pinco, 1992;
425 Pinco and Lev-Tov, 1993; Taccola et al., 2012; Dose et al., 2014). This concept is
426 replicated in the time course illustrated in Fig. 2 H, showing motor responses that are
427 intrinsically modulated in amplitude (CV = 0.15) during 900 stereotypical electrical pulses
428 continuously supplied at threshold intensity to the homologous dorsal root.
429 These differences become even more informative when this experiment is performed in
430 the awake rat (see Fig. 2, 3) and even in spinally injured individuals when stepping and
431 receiving epidural stimulation (Huang et al., 2006).

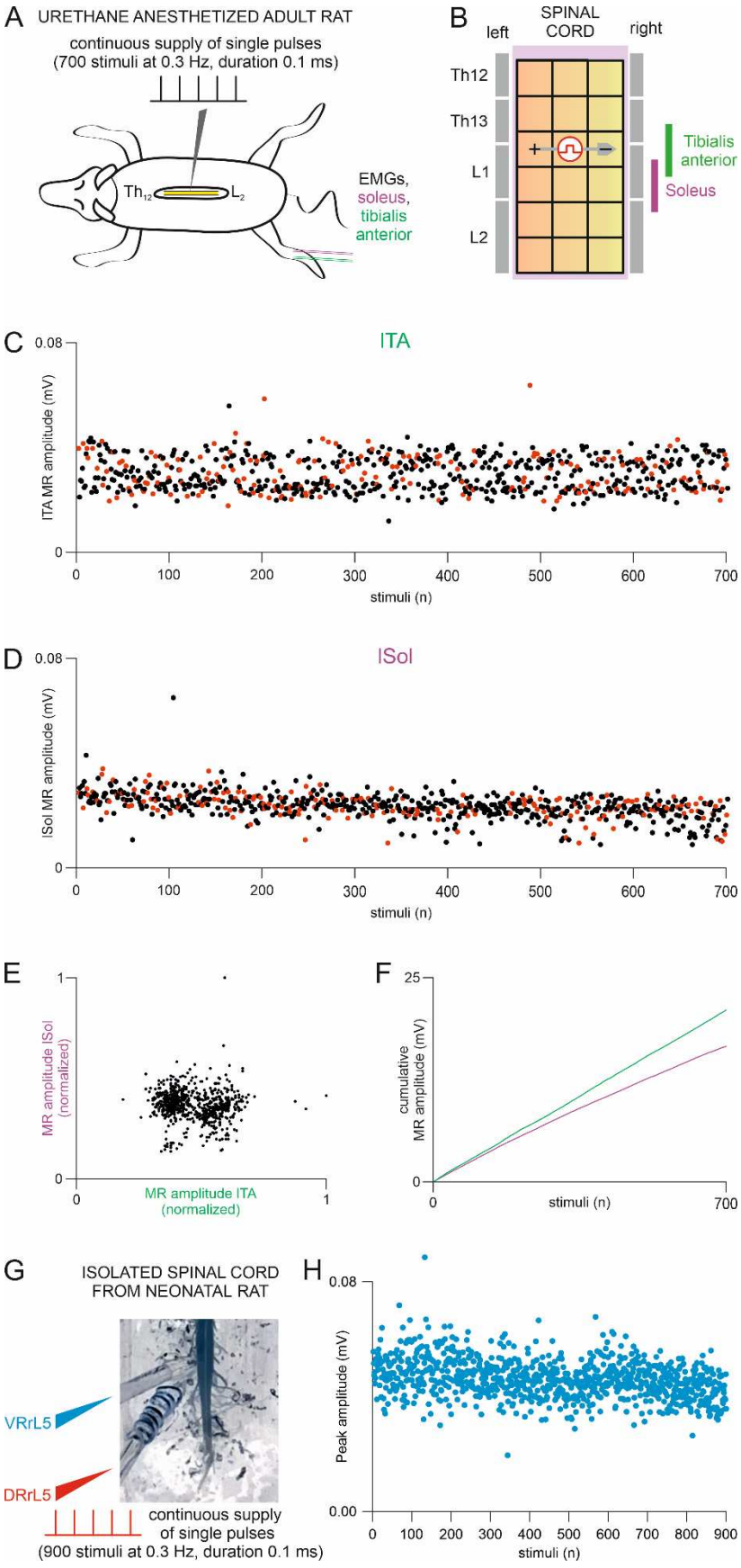

A
URETHANE ANESTHETIZED ADULT RAT
continuous supply of single pulses
(700 stimuli at 0.3 Hz, duration 0.1 ms)
Th12
L2
EMGs,
soleus,
tibialis
anterior
B
SPINAL
CORD
left
right
Th12
Th13
L1
L2
Tibialis
anterior
Soleus
C
ITA
ITA MR amplitude (mV)
0.08
0
0
100
200
300
400
500
600
700
stimuli (n)
D
ISol
ISol MR amplitude (mV)
0.08
0
0
100
200
300
400
500
600
700
stimuli (n)
E
MR amplitude ISol
(normalized)
1
0
0
1
MR amplitude ITA
(normalized)
F
cumulative
MR amplitude (mV)
25
0
0
stimuli (n)
700
G
ISOLATED SPINAL CORD
FROM NEONATAL RAT
VRrL5
DRrL5
continuous supply
of single pulses
(900 stimuli at 0.3 Hz, duration 0.1 ms)
H
Peak amplitude (mV)
0.08
0.00
0
100
200
300
400
500
600
700
800
900
stimuli (n)

Figure 2. Continuous supply of weak single pulses delivered through the epidural electrode array generate small and variable motor output. The acute recording set-up from fully anesthetized rats is schematized in A. Epidural stimulation was provided by continuously delivering single pulses to the cord through a pair of independent electrodes in the planar and flexible stimulating array (each rectangular electrode = 500 x 200 µm; Gad et al., 2013b; Chang et al., 2014), while EMG responses were continuously collected from TA and Sol. In B, the cartoon indicates the continuous supply of square monophasic weak impulses (0.3 Hz, 300 µA, single pulse duration 0.1ms) to the central sites of the array (Th13/L1 vertebral level, spinal level = L5, cathode on the left). In C and D, every dot in the figure corresponds to the peak of motor (EMG) signals that were recorded from the left TA and Sol muscles, respectively, in response to 700 consecutive suprathreshold stimuli (300 µA) applied to the dorsum of the spinal cord. Red dots in C and D correspond to the 190 EMG responses generated by epidural pulses occurring in coincidence with spontaneous ECG events (cardiac rate = 7.5 Hz). Amplitude of peaks elicited by epidural pulses close to a heartbeat was not significantly different from the one occurring with stimulation during a pause between two cardiac events, for both TA ($p = 0.850$) and Sol ($p = 0.546$). In E, the plot indicates a poor correlation between the amplitude of each stimulus evoked from TA and Sol. In F, the cumulative amplitude of evoked MR responses from lTA (green) and lSol (purple) indicates a near-linear correlation with increasing number of pulses (modified from Taccola et al., 2020a). In an isolated spinal cord from a neonatal rat (G, picture from a 3-day old animal), 900 serial single pulses (0.3 Hz, single pulse duration 0.1 ms) applied at threshold intensity (12 µA) to a dorsal root (DR) elicit motor responses from a ventral root (VR) that are highly variable in peak amplitude (H; modified from Taccola et al., 2012; Dose et al., 2014).

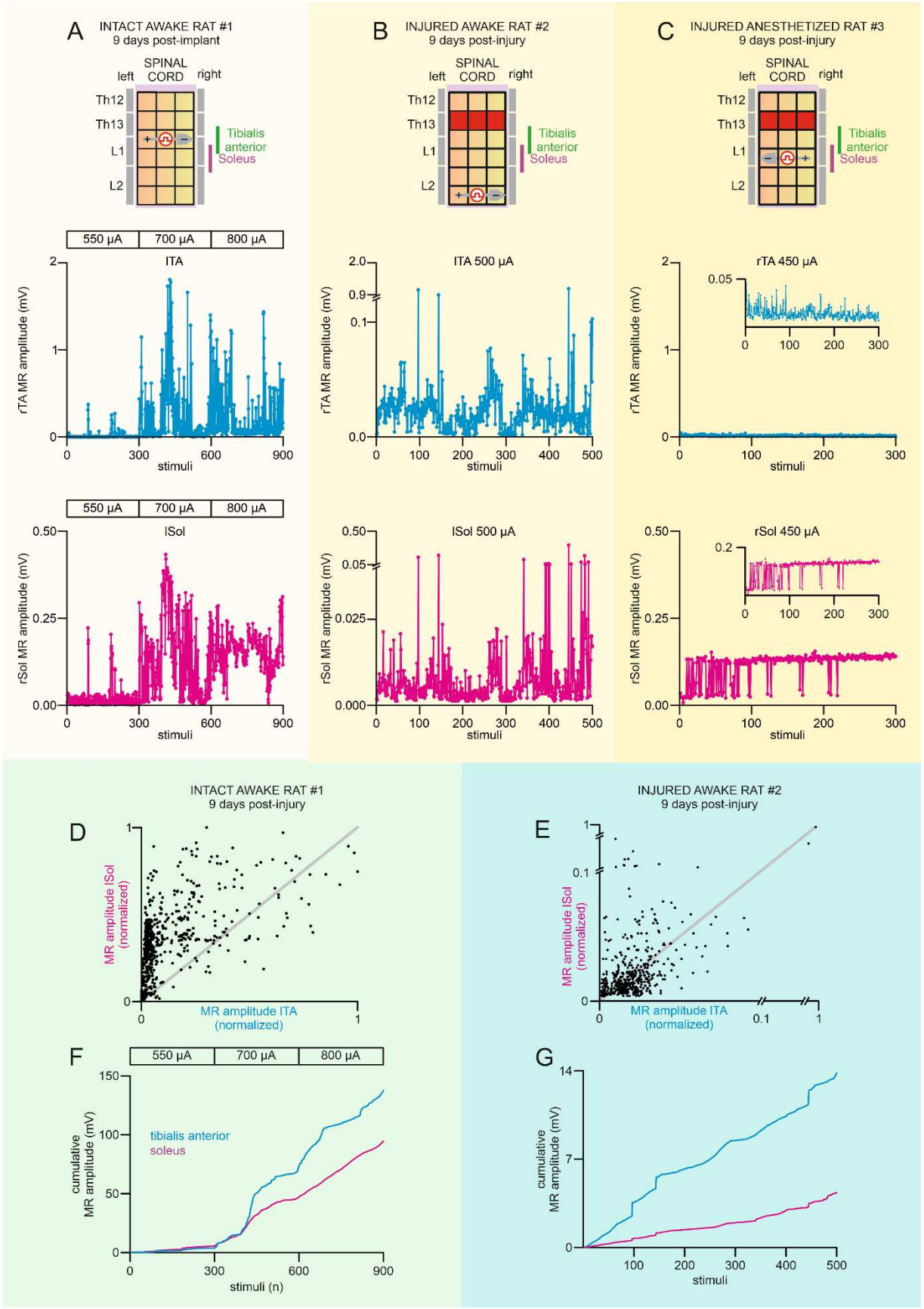
5. Variability in excitability of spinal networks that occurs in an awake rat

To clarify whether the intrinsic variability of the motor output observed in acute recordings from fully anesthetized rats impacts on the motor behavior elicited by epidural stimulation, we recently applied a continuous protocol of sub motor-threshold stimulation to awake adult rats (Taccola et al., 2021). In intact rats at rest, in response to single sub-motor threshold pulses continuously supplied to the dorsal cord at low-frequency, the motor output from flexor and extensor hindlimb muscles showed a marked amplitude variation (Fig. 3 A). At lower stimulation intensities, small responses seldom appeared, while a slight increase in strength reduced the variability of responses (Fig. 3 A).

Nonetheless, amplitude responses in TA and Sol were poorly correlated, as reported by the scattering plot in Fig. 3 D. This phenomenon is also represented in Fig. 3 F by the distinct point of diversion of the cumulative amplitudes of the two muscles when stimulation intensities were increased to 700 and then to 800 microamperes. These latter findings, collected in adult awake animals at rest, suggest the existence of a continuously

changing pattern of modulation that includes an inherent variability in the motor responses affecting the yield of epidural stimulation, especially at low intensities.

After SCI, evoked responses from flexor and extensor muscles of awake rats at rest showed a large variability and a mainly synchronous modulation, with similar peaks occurring in response to the same stimuli in both Sol and TA (Fig 3 B). Indeed, notwithstanding the compromised descending drive due to injury, a modulatory system, mainly composed of afferent inflow, was still able to induce changes in peak responses, with a non-linear profile of cumulative peak amplitudes (Fig. 3 E, G). This effect was largely reduced under full anesthesia (Fig. C). However, albeit about 10 times lower than in the awake state, patterned baseline changes were still present, unveiling an intrinsic stochastic tone modulating the amplitude of the motor output.


A
INTACT AWAKE RAT #1
9 days post-implant
SPINAL CORD
left
right
Th12
Th13
L1
L2
Tibialis anterior
Soleus
550 µA
700 µA
800 µA
ITA
rTA MR amplitude (mV)
stimuli
lSol
rSol MR amplitude (mV)
B
INJURED AWAKE RAT #2
9 days post-injury
ITA 500 µA
lSol 500 µA
C
INJURED ANESTHETIZED RAT #3
9 days post-injury
rTA 450 µA
rSol 450 µA
D
INTACT AWAKE RAT #1
9 days post-injury
MR amplitude lSol (normalized)
MR amplitude ITA (normalized)
E
INJURED AWAKE RAT #2
9 days post-injury
F
cumulative MR amplitude (mV)
tibialis anterior
soleus
stimuli (n)
G
stimuli

483

Figure 3. Variability of spinal reflexes elicited by weak stimulation at rest in both intact and paralyzed rats and under full anesthesia.

In A, an uninjured (RAT #1) rat is implanted with an electrode array for epidural stimulation 9 days earlier. In response to continuous epidural electrical stimulation with 900 square impulses (duration = 0.1 ms, frequency = 0.3 Hz), EMG output is recorded from left TA and Sol. Stimulation is delivered as indicated in the cartoon (Th13/L1 vertebral level, L5 spinal level, cathode on the left) at growing intensities (left to right). Amplitude of spinally-induced EMG responses with an intermediate latency (5 to 10 ms; middle response, MR) has been calculated for each single event to describe the time-course lasting 45 consecutive minutes. Starting from the lowest intensities of stimulation (first 300 pulses, 550 µA), motor responses sporadically occur on both muscles at the same time. By increasing stimulation (around motor threshold, from the 301st pulse, 700/800 µA), EMG responses seldom appear and display a highly variable amplitude, although with a similar trend among the two muscles. In D, the plot indicates an inverse correlation between the amplitudes of each stimulus evoked from TA and Sol (stimulus strengths = 700/800 µA), with higher Sol peaks mainly occurring for lower TA responses. In F, cumulative amplitude of evoked MR responses from lTA (sky blue) and lSol (magenta) traces a step-like correlation for the increasing number of pulses, indicating a non-linear processing of subsequent pulses.

In B, in a second rat (RAT #2) the spinal cord was severely injured at vertebral level Th 13 (spinal level L3/L4, cathode on the left) just before implanting the epidural array. Consistently with a moderate injury, the locomotor behavior is largely impaired when tested on a treadmill (belt speed = 13.5 cm/s) one week after lesion. Nine days later, single weak pulses are continuously delivered (duration = 0.1 ms, frequency = 0.3 Hz; S1) and the amplitude of single EMG responses (time to peak = 5 to 10 ms; middle response, MR) is calculated for 500 consecutive pulses to define a time course for TA (sky blue dots) and lSol (magenta dots) when the rat was quietly resting. In E, the amplitude of spinally-induced reflex responses is subject to a modulatory pattern occurring synchronous between Sol and TA as illustrated by the plot arrangement around the idealized diagonal line in F. In G, cumulative amplitude curves for MRs from lTA (sky blue) and lSol (magenta) reveal a non-linear profile, highlighting the patterned modulation of MRs evoked by consecutive pulses.

In C, in a fully anesthetized animal (RAT #3), nine days later after injury (L3/L4 spinal level), single pulses are continuously delivered at the L5. The amplitudes of spinal reflexes from right TA (sky blue dots) and Sol muscles (magenta dots) are illustrated in the time courses for all successive pulses (300). In the insert, a magnification of the time course of rTA indicates the clear stochastic fluctuations of baseline responses under anesthesia (modified from Taccola et al., 2021).

To summarize the data shown in figures 2 and 3, the outputs of motor pools are defined by: 1. the physiological states (net state of excitability) of the neural networks that project to a given combination of motor pools prior to the signal that triggers the execution of a motor task, i.e., feedforward control; 2. these physiological states can be modulated continuously by changes in the level and sources of inhibitory or excitatory input to the

motor pools which can be varied via electrical neuromodulation of the spinal cord and pharmacologically as occurs during anesthesia. Finally, regardless of these many input-output variables the predominant phenotype of the motor units within a motor pool also play a role in their responsiveness. More specifically, comparisons of Fig. 3 D versus E and F versus G reflects the responses of an uninjured rat compared to an injured rat, and the rat is awake during the recording for both experiments. The most dramatic difference is that the amplitude of the responses is about tenfold greater in the uninjured rat when compared at a stimulation intensity of 550 versus 500 µA. A unique and distinctive feature of the distribution of the relative responses of the two muscles demonstrate that at the lower intensities of stimulation the highest amplitudes are highly biased toward the Sol in the uninjured state, whereas after the injury, the larger responses occurred predominantly in the TA. The marked inflections in the TA of Fig. 3 F and G seem to reflect only one or a few responses that could be attributable to the fact that the rats are awake, although resting. There are many published examples of the output of motor pools when performing a task in one physiological state can be the direct opposite when the physiological state has been converted to one which “anticipates” a more appropriate outcome (Hultborn, 2001). It is of high importance to recognize the fact that the spinal circuitry is so markedly suppressed in the anesthetized state that it seems highly likely that the classical assessments of evoked potentials under those conditions falls far short of the true potential of these networks in awake, *in vivo* conditions.

The spectral analysis for all time courses reported in Fig. 2 and 3 help to clarify how an intrinsic stochastic tone and a rhythmic pattern of changing amplitudes coexist in the modulation of the motor output. In the power spectra illustrated in Fig. 4, the intrinsic stochastic background of amplitude modulation of motor responses is reported as a relatively flat portion of the frequency domain in the isolated spinal cord (Fig. 4A), as well as in acute recordings from a fully anesthetized rat (Fig. 4 B). Interestingly, in awake intact animals at rest, intrinsic random modulation is superimposed onto sharp peaks of the spectrum, corresponding to the main frequency of oscillatory patterns of modulation (Fig. 4 C, note the different scale on the y axis). After spinal injury these oscillatory patterns of modulation are largely reduced in the presence of anesthesia (Fig. 4 D, E). Oscillatory

patterns of modulation are more evident in the awake state of intact animals, as opposed to after SCI where they are largely depressed. We speculate that oscillatory patterns of activity, likely triggered by descending input from suprapinal structures to rhythmogenic spinal circuits, depend on the distinct physiological (and pathological) states experienced by the spinal cord (Cuellar et al., 2009). Furthermore, the intrinsic tone of modulation that appears to be stochastic, mostly arises from background fluctuations in the excitability of propriospinal networks subjected to a continuously varying synaptic input and afferent inflow (Radosevic et al., 2019). Both contributions are, however, affected by the presence of anesthesia.

Accordingly, Burke and collaborators concluded that, in cats, variability of lumbar evoked potentials follows a predictable rhythmic pattern that becomes generally stochastic after spinal transection (Chang et al., 1994).

Moreover, from an integrative point of view, these intrinsic patterned and stochastic mechanisms of modulation potentially reverberate from a multi-level interaction among the entire central nervous system and the organism, including circulating and hormonal factors (Ono et al., 1990), circadian variations (Vakhrameeva and Finkel, 1977; Wolpaw and Seegal, 1982) and other vital functions, such as ongoing respiration (Kitahata et al., 1969).

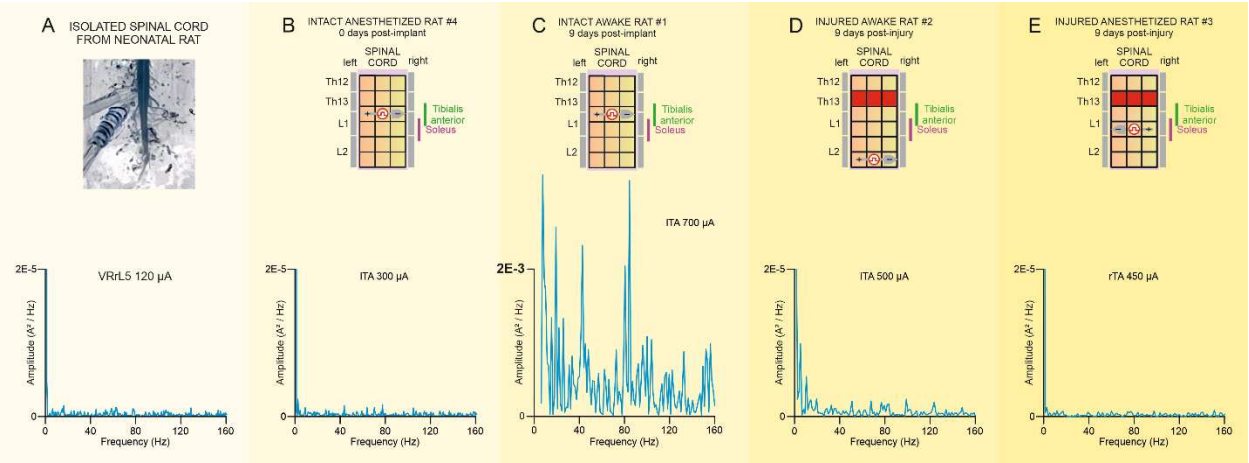


Figure 4. Spectral analysis of the time course of spinal reflex variability reveals the coexistence of an intrinsic stochastic tone merged to rhythmic patterns of changing amplitudes.

Power spectra are traced for the time courses reported in Fig. 2 and 3. In A, the flat portion of the frequency domain from the isolated spinal cord reveals the stochastic nature of

modulation of motor reflexes elicited by dorsal root pulses. In B, a similar domain is reported for a fully anesthetized intact rat. In C, in an awake intact animal, strong frequency components emerge from a more random background (note the y-axis scale is 100 times higher than other spectra). In D, a spinal cord injury largely suppressed oscillatory patterns in the spectra, albeit still maintaining few rhythmic components at low frequencies of the domain. In E, when motor responses are induced from a spinalized animal under anesthesia, the flat spectrum only describes the intrinsic stochastic background of stimulation.

6. Variability is always present in the control of stepping

Even after extensive practice there will be some variability in the neural mechanisms in generating any movement. While there have been numerous studies demonstrating this phenomenon, the apparent necessity of this as a fundamental and essential feature of motor control is poorly understood. Many experiments have demonstrated that there are numerous combinations of networks that can generate essentially, the same motor task, regardless of the level of practice and training. This outcome is not unexpected given the large number of variables of different components of the sensorimotor system, ranging from the physiological states of individual synapses within and among the multiple neurons in the spinal cord and brain that are involved in a given movement. There is a significant level of variability from millisecond to millisecond in the excitability of a highly selective neuronal network that projects to a single combination of motor pools in a live animal under *in vivo* conditions, when stepping at a relatively constant speed on a treadmill (Pham et al. 2020).

Many controlling events occur within the nervous system between the initiation of the intent and the actual completion of a movement, which render it a highly probabilistic phenomenon. As noted earlier, Bernshteĭn (1967) reasoned that the challenge of achieving a desirable level of predictability requires a reduction in the degrees of freedom being involved in a given movement and characterized this phenomenon as one of redundancy that the nervous system has to resolve.

The concepts presented here, only represent a small part of the components that make up the design of the spinal cord that makes it possible to “accurately” perform movements, even for a task as fundamental as stepping. Given the probabilistic phenomena at multiple levels along the motor axis, it has been suggested that there are “families of solutions that are able to solve the problem.” (Latash and Zatsiorsky, 2016). This feature has led

to the principle of “motor equivalence” in that this variability provides access to multiple options to perform the same task (Lashley, 1930; Hebb, 1949). Thus, whether the high variability is perceived as a problem of either redundancy or abundance raises the fundamental biological question of what are the architectural design features that enable these solutions to emerge so instantaneously and automatically in our daily movements. These two qualities, instantaneous and automatic, are incompletely understood in the control of movement. Further, an obvious question is whether a design feature that embraces such enormous degrees of variability would be useful when precision is required in so many movements.

Based on the data discussed above and related data on the issue of variability and the concept of redundancy of neural networks, there is no controversy regarding the fact that it is a fundamental design feature of sensory-motor systems, phylogenetically, ontogenetically and epigenetically. Some of the consequential questions raised by this conclusion are: 1) How extensive is the variation in movements when performing the same task? 2) To what extent is the variation a function attributable to neural, biomechanical, etc. features? 3) What are the advantages and disadvantages of a control system with such “randomness”?

Discussion of these questions, even very superficially, is not possible in this review. We will, however, briefly review some of the questions regarding the range of possible sources that are essentially neural, with a focus on, to what extent spinal networks can contribute to this variation in how we perform the same movement task (Feeney et al., 2018; Hamilton et al., 2019). The variability that is intrinsic to motor control could be an advantage in providing 1) adaptive mechanism to perturbations that require differing kinetics and kinematics for the same task, 2) a mechanism to avoid fatigue and 3) a physiological and anatomical potential for reorganization of networks after a neuromuscular injury. But perhaps, more importantly, this variability may be an essential feature of the architectural design in order for it to perform almost an infinite number of movements for a huge number of environmental challenges (Cai et al. 2006; Ziegler et al. 2010; Christou, 2011; Howard et al., 2020).

7. Sources of modulation of motor responses in the presence of constant stimulation parameters

Data reviewed above demonstrate the intrinsic variability of the spinally evoked motor output. Variability of the motor output at rest has already been extensively reported from reflex responses induced by electrical stimulation of peripheral nerves (Chen et al., 2001). Thus, deciphering the origin of the variability of the motor output elicited by direct spinal stimulation might benefit from the plethora of evidence collected about the neurophysiological mechanisms involved in the variability of H-reflex responses. Indeed, the continuous application of single stereotyped pulses to a peripheral nerve generates intrinsically-variable motor responses in amplitudes and latencies and occasional failures (Lloyd and McIntyre, 1955; Rall and Hunt, 1956; Rudomin and Dutton, 1967; Gossard et al., 1994). In this section, we explore the origin of the variability of the motor output elicited by serial stereotyped pulses by analyzing its neuronal sources. As peripheral and spinal stimulations share a common neuronal infrastructure (Brooks and Eccles, 1947; Eccles et al., 1954), we review herein the sources of variability of H-reflexes, as a prototypical model that describes how numerous and simultaneous contributions shape the network output in a probabilistic manner.

7.1 Variability of the motor output derived from the recruitment of afferent terminals

Changes in the amplitude of motor reflexes are correlated to fluctuations in the membrane potential of afferent terminals. Indeed, an ensemble of dorsal interneurons produces spontaneous rhythmic field potentials (cord dorsum potentials, CDPs; Cuellar et al., 2009) that are recorded as low frequency waveforms from the dorsal surface of the cord. These oscillations provide a pre-synaptic inhibition of concurring input from afferent stimulation (Rudomin and Dutton, 1967; Rudomin and Dutton, 1969a, b; Rudomin and Madrid 1972, Contreras-Hernández et al., 2015). Generally, the peak of each spontaneous CDP corresponds to the maximal modulation of motor reflex amplitude (Manjarrez et al., 2000). Along with the pre-synaptic inhibition from afferent terminals, other potential pre-synaptic sources of amplitude variability refer to the type of fibers recruited by afferent pulses. Indeed, epidural stimulation activates not only large cutaneous afferent Aβ-fibers, but also large, myelinated proprioceptive fibers (Capogrosso et al., 2013; Formento et al., 2018).

In addition, these fibers have multiple post-synaptic targets, which thus increase the variability of the motor output, although the convergence from distinct types of afferents onto common interneurons has also been reported (Jankowska et al., 1981).

Nonetheless, motor output variability might also depend on the random recruitment of different number and type of afferents. In turn, spontaneous changes in the activation threshold of afferents can be affected by a stochastic variation in the properties of Na^+ channels in nodes of Ranvier of single axons (Hales et al., 2004), particularly after stimulation. According to this hypothesis, random changes in the motor output might additionally derive from the impossibility to temporally summate specific afferent input travelling through fibers with different conduction velocities and from multiple sources. However, the consistency of afferent pre-synaptic input elicited by stereotyped electrical stimulation has been observed in experiments where the *"stability of the motoneuron's surface membrane"* was increased using high concentrations of anesthetics (Eccles, 1946). In this case, electrical stimulation of afferents evoked excitatory post-synaptic potentials on motoneurons, which were stereotyped in terms of latency and amplitude (Eccles, 1946), demonstrating that, in a finely controlled and therefore highly reduced preparation, a minimal afferent stimulation can induce a more consistent synaptic input to motor neurons.

Another hypothesis considers the variability of the motor output as caused at pre-synaptic level by probabilistic changes in neurotransmitter release from individual afferent synapses (Ribrault et al., 2011). However, in anesthetized animals, only little variation of the motor output has been ascribed to an individual event of transmitter release (Harrison et al., 1989). It has been suggested, however, that the number of synapses recruited by weak electrical pulses is likely to be sufficient (Prodanov and Feirabend, 2007) to minimize any contribution of random variations in the release from single synapses.

7.2 Variability of the motor output derived from post-synaptic afferent targets

A main contribution to the stochastic variability of the motor output generated by weak electrical stimulation of afferents has a post-synaptic origin. Indeed, weak afferent stimulation determines a sub-maximal neurotransmitter release from a wide array of afferents with differing responsiveness to mechanical events even at low intensities.

Thus, the probability to reach the threshold for generating an action potential in post-synaptic targets mostly relies on the probability to receive and integrate, with an optimal timing, additional converging synaptic input. Furthermore, due to input being sub-threshold, the motor output is influenced more by spontaneous variations in the membrane potentials of each element of the circuit, including motoneurons (Berg, 2017; Radosevic et al., 2019). Spontaneous fluctuations in the baseline of motoneuronal membrane potentials affect the threshold for generating an action potential (Harrison et al., 1989; Gossard et al., 1994; Manuel et al., 2009). Intrinsic changes in resting potentials also derive from: the random gating of voltage-gated ion channels (channel noise); the synaptic noise that collectively defines the stochastic nature of quantal release; the probabilistic nature of diffusion; the probabilistic nature of chemical reactions within the synaptic cleft; the unpredictable responses of ligand-gated ion channels (White et al., 2000). However, given their large number and small magnitude, these cellular events are uncorrelated among neuronal networks and would not significantly affect the overall variability of motor output (Chang et al., 1994).

In addition, different populations of interneurons are randomly activated at each consecutive pulse, each one with its own intrinsic fluctuations of background activity, in turn evoking input of variable strength directed to motoneurons (Parker, 2015). Additional variability in motor neuronal output arises from the non-linear summation of synaptic currents (Cushing et al., 2005; Berg, 2017; Radosevic et al., 2019) and from physiological fluctuations in biophase composition and electrolytic concentrations of the extracellular milieu (Ventriglia and Di Maio, 2002; Venton et al., 2003; Kuwabara et al., 2007). Interestingly, the number and identity of recruited motoneurons within the same motor pool differ from pulse to pulse (Rall and Hunt, 1956; Gossard et al., 1994). It is noteworthy how a sub-group of spinal motoneurons shows two stable membrane potentials, which can be alternatively selected using brief afferent synaptic input (Hounsgaard et al., 1988; Lee and Heckman, 1998). This 'bistable' behaviour of distinct motoneurons provides an additional non-linear contribution to changes in cell excitability.

7.3 Descending and proprioceptive sources of modulation of motoneurons

741 Motoneuronal output is also regulated by descending serotoninergic (Fung and Barnes,
742 1989) and noradrenergic neurons originating in supraspinal structures (Fung et al., 1994).
743 For instance, electrical stimulation in the brainstem of the Raphe nucleus (Kaneko et al.,
744 1984) and the locus coeruleus (Strahlendorf et al., 1980; Chan et al., 1986; Fung et al.,
745 1991) modulates spinal reflexes by putatively exploiting tonically active descending
746 pathways (Engberg et al., 1968; Jones and Gebhart, 1987). Indeed, supraspinal centers
747 provide a tonic release of neurotransmitters that impacts on spinal reflex amplitude, which
748 reacts to random fluctuations that increase output variability. Indeed, studies in reduced
749 *in vitro* preparations have clearly shown that intact descending projections from the brain
750 increase the variability of electrically-induced motor responses (Mullins and Friesen,
751 2012*).* At the same time, also volitional descending commands from supraspinal sources
752 contribute to modulate the motor output (Erbel et al., 1970), by selecting one among a
753 broad range of physiological states. For instance, the ability to voluntarily condition the H-
754 reflex following spinal transection of the corticospinal tract (CST) in animals is lost (Chen
755 and Wolpaw, 1997; Guzmán-López et al., 2015). Moreover, the execution of complex
756 motor tasks, ranging from posture (Cecen et al., 2018) to running (Ferris et al., 2001;
757 Courtine et al., 2007; Lavrov et al., 2015, Shah et al., 2012), contributes to modulating
758 the amplitude of evoked responses. In line with the task-dependency of the motor output,
759 in our experiments we accounted for an additional source of variability in response to
760 protracted continuous stimulation. During our awake recordings, rats, even if constrained
761 in a small cage, showed slight changes in the position of limbs over the entire period of
762 observation potentially affecting the amplitude of spinal reflexes. Indeed, in response to
763 changes in position, the proprioceptive input detecting the position of hindlimbs provides
764 a strong mechanism of modulation, as confirmed by the amplitude changes of responses
765 in correspondence to the many different limb positions occurring during repetitive
766 stimulation. Data from Bizzi and colleagues have shown the interdependence between
767 resting position of the limb and the movement vectors of the limb (d'Avella et al., 2003).
768 Recently, it has been demonstrated that pathways descending from the cortex have a
769 specific role in modulating proprioceptive input through a class of lumbar interneurons
770 located in laminae IV–VI, thus suggesting a crucial role for cortico-spinal tracts in
771 modulating sensory input from limbs (Jankowska, 2021; Moreno-Lopez et al., 2021).

7.4 Contribution of spinal neuronal networks to the variability of the motor output

An additional important source of modulation of the motor output comes from the spontaneous rhythmic activity of spinal networks, which coordinate the activity of multiple motoneuronal pools along the lumbosacral cord (Edgerton et al., 1976). This is consistent with our experiments, in which a synchronous timing of modulation was observed in both extensors and flexors motoneuronal pools located in different segments (Fig. 3 B, D). Likewise, Cuellar and colleagues (2009) recorded from the dorsum of the cord spontaneous tonic discharges at rest that became sinusoidally-like modulated during the activation of spinal central pattern generators for rhythmic movements (Cuellar et al., 2009). The spontaneous pattern could originate from the intrinsic rhythmic activity of a propriospinal network impinging onto motoneuronal pools (Jankowska et al., 1974; Yakovenko et al., 2007) and thus affecting the electrically-induced motor output, as well. Indeed, in our recordings, a rhythmic amplitude modulation seems to have a slow periodicity, which could not be clearly identified even using our long-term recordings (over 30 min), likely highlighting the need for even longer periods of observation.

In summary, the variability of motor reflexes induced by electric stimulation of peripheral nerves is a complex phenomenon that relies on multiple targets of modulation occurring both at pre- and post-synaptic levels and involving descending, afferent and propriospinal input converging onto spinal motoneurons. In line with peripherally-evoked motor responses, our recent data displayed a rhythmic pattern of modulation following epidurally-delivered weak pulses. This is not surprising, considering that direct stimulation of the dorsal spinal cord shares common features with stimulation of peripheral nerves, as they both inevitably recruit dorsal root afferents with a lower activation threshold (Struijk et al., 1993; Rattay et al., 2000). However, as opposed to peripheral stimulation, direct stimulation of the spinal cord allows the current flow to orthogonally spread across the cord from the epidural electrode, eventually affecting the entire spinal circuitry (Swiontek et al., 1976). This might account for the involvement of a propriospinal network-mediated contribution to variability. We have, however, observed a highly predictable amplification in modulation of motor output among primary muscles involved in

802 locomotion with a more dynamic modulation, derived from EMG activity as compared to
803 tonic pulses, of spinal networks in an awake uninjured adult rat (Taccola et al., 2020a).
804
805 **8. Challenging factors in shaping the physiological states of spinal networks**
806 **to optimize outcomes**
807 Can the sources that contribute to the electrically-evoked motor potentials variability in *in*
808 *vivo* conditions, be determined predominantly by the size of axons of the dorsal root as
809 modeled routinely? The widespread opinion based largely on modeling experiments is
810 that the electrophysiological responses to spinal epidural stimulation is basically attributed
811 to the relative size of dorsal root fiber diameters that contribute to determining the
812 threshold of responses. Additionally, dorsal root fibers of different diameter are assumed
813 to correspond to distinct spinal pathways, each projecting to a specific type of interneuron.
814 While there is little doubt that the modeling is theoretically sound, the common
815 interpretation in using this model to understand the mechanisms of spinal epidural and/or
816 the non-invasive transcutaneous neuromodulation, however, is critically limited,
817 compared to the large number of variables that contribute to the input/output ratio in *in*
818 *vivo* behaving conditions (Moore et al., 2017; Greiner et al., 2021). Furthermore, the
819 assumption that peripheral input selectively reaches distinct spinal interneurons is
820 challenged by evidence showing numerous types of afferents that converge onto the
821 same spinal interneurons (Selzer and Spencer, 1969; Jankowska et al., 1981; Honda,
822 1985; Pinto et al., 2008; Jankowska, 2021).
823 After a spinal cord injury, the activity of spinal circuits controlling stepping and standing
824 can be strengthened by performing and practicing distinct functional tasks, i.e., increasing
825 the activation of task-specific circuits (Edgerton et al., 2008; 2018). A key question
826 remains, however, regarding how specific should the networks that are involved in the
827 practice and training be relative to those that are critical in the primary motor skill of
828 interest (de Leon et al., 1998, Shah et al., 2013, Rejc et al., 2017).
829 The lumbosacral spinal circuitry can be optimally activated, also when there are
830 proximally located injuries, using short-termed and prolonged electrical stimulation of the
831 spinal cord with varying and highly interactive combinations of stimulation parameters
832 (“noisy” and “dynamic” stimulation; Taccola, 2011; Taccola et al., 2020a, b; Howard et al.,

2020). However, based on the variability of the motor output induced by spinal stimulation sub-motor threshold intensities, there are many questions remaining, including the challenge of optimizing stimulation parameters to reach and sustain the optimal outcome, particularly in realizing that the optimal is a moving target as there can be a continuous reorganization of key neuronal networks that are linked directly and/or indirectly to activity dependent-mechanisms.

The physiological states of spinal networks continuously change according to its milieu as reflected in the membrane potentials of selected areas of dendrites compared to the soma of the same neuron in mice performing a specific motor task. Mehta and colleagues (Moore et al., 2017), for example, showed in behaving mice that the dendrites of cortical pyramidal neurons play a dominating role in the generation of somatically derived action potentials. These subthreshold fluctuations emphasized the potential for the dendrites, which receive the dominating source of input, and act as an analog code in addition to the binary information derived from an action potential. Observations such as these demonstrate the need for considerable caution in attributing the size of axons within dorsal roots, based on simulation model as a dominant factor among the multiple mechanisms that define the responsiveness of a motor pool or of interneurons projecting directly or indirectly to motor neurons under *in vivo* conditions. It seems unlikely that the outputs of motor neurons as reflected in EMG signals as demonstrated in multiple experiments shown in Figs. 1-3 could have been generated only by, or at least predominantly by, the action potentials projecting directly to motor neurons via monosynaptic inputs from primary afferents located in the dorsal roots. In addition, it seems remote that continuous modulations of physiological states are based only or predominantly on a binary function (“all or none”) for generating axonal action potentials in Ia fibers evoked from electrodes placed near a specific dorsal root.

We suggest that this concept is severely limited and is due to a comprehensive and fresh analyses with respect to the physiological mechanisms that are modulated with different technical strategies, such as epidural or transcutaneous stimulation under *in vivo* conditions. The seemingly widespread opinion that the neural mechanisms embedded in the nervous system that defines spinal motor responses *in vivo* can be attributed to axonal size within dorsal roots seems inconsistent with the multiple mechanisms known to be

present that are modulated in *in vivo* conditions (Taccola et al., 2018). Thus, we suggest that attributing the size of the axon as a dominating factor in determining how electrical neuromodulation shapes the electrophysiological dynamics of spinal networks under *in vivo* conditions is an oversimplification.

9. Conclusions

In the development of spinal neuromodulatory techniques designed to recover function following paralysis, the primary focus in most cases has been to optimize the quantity and quality, and spatial distribution of the current applied to spinal networks. There is a growing recognition, however, that optimizing the physiological states of the neural networks that drives the intended (planned) motor output is an important source of control in defining any movement. In general, when applying currents at levels exceeding the motor threshold, the dominant control strategy is derived from input parameters which are sufficient to induce specific movements. The mechanism of control of a movement when applying currents less than the motor threshold provides a strategy for the control mechanisms that are intrinsic to the spinal neuronal networks in translating proprioception to generate the “intended” behavior. Thus, the outcome of using high levels of current emphasizes the ability to induce a movement, while using low levels of current changes the responsiveness of neural networks and has an enabling effect in recovering a wide range of planned, intended movements that can be initiated predominantly under voluntary control, given the level of automaticity that is built into those networks. The present review focuses on the dynamics of the physiological states of spinal networks which is being continuously modulated by a continuously changing ensemble of proprioceptive input as well as supraspinal drives. Using a spinal epidural interface to probe the dynamics of multiple motor pool-specific spinal networks *in vivo,* we examined the sources of input that drive the dynamics of spinal networks into physiological states ranging from a relatively quiet anesthetized state compared to a highly dynamic state that is observed in an unanesthetized resting awake rat before and after a complete mid-thoracic spinal cord transection. The dynamics of spinal motor pool connectomes pre- and post-injury, and with and without anesthesia reflects a continuously changing stochastic modulation, with an oscillatory pattern of amplitudes of evoked potentials.

Given the extent of the known dynamics and variance of the physiological states of spinal networks and the numerous sources of cellular and synaptic variability among these networks, the challenge is to define the mechanisms through which an effective level of accuracy is achieved. A better understanding of this highly probabilistic design that is an intrinsic feature of the neural control of movement could expand the possible strategies for achieving higher levels of functional recovery after a wide range of neurological dysfunctions.

Declaration of interest: VRE, researcher on the study team hold shareholder interest in Onward and hold certain inventorship rights on intellectual property licensed by The Regents of the University of California to Onward. VRE, and PG, researchers on the study team hold shareholder interest in SpineX.

Acknowledgments: GT is also grateful to Dr. Elisa Ius for her excellent assistance in preparing the manuscript.

Funding: GT is supported by funding from the European Union's Horizon 2020 Research and Innovation Program under the Marie Sklodowska-Curie (grant agreement No. 661452). This research was also funded in part by NIH/NIBIB U01EB007615, NIH/NINDS 1U01NS113871-01, International Spinal Research Trust (STR104), Walkabout Foundation, Dana & Albert R. Broccoli Charitable Foundation and Nanette and Burt Forester, including matching by PwC LLP and Roberta Wilson.

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