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Acute neuromodulation restores spinally-induced motor responses after severe spinal cord injury

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30 **Abstract**

31

32 Epidural electrical spinal stimulation can facilitate recovery of volitional motor control in
33 individuals that have been completely paralyzed for more than a year. We recently
34 reported a novel neuromodulation method named Dynamic Stimulation (DS), which short-
35 lastingly increased spinal excitability and generated a robust modulation of locomotor
36 networks in fully-anesthetized intact adult rats. In the present study, we applied repetitive
37 DS patterns to four lumbosacral segments acutely after a contusive injury at lumbar level.
38 Repetitive DS delivery restored the spinally-evoked motor EMG responses that were
39 previously suppressed by a calibrated spinal cord contusion. Sham experiments without
40 DS delivery did not allow any spontaneous recovery. Thus, DS uniquely provides the
41 potential for a greater long-term functional recovery after paralysis.

42

43 *Keywords:* neuromodulation, asynchronous noisy stimulation, spinal contusion, multi-
44 electrode array, epidural interface, spinal reflexes.

45

46 **Introduction**

47

48 A spinal cord injury significantly reduces the resting level of activity in caudal spinal neural
49 networks (Frigon and Rossignol, 2008) and may reduce or even suppress evoked
50 potentials that are spinally-induced from lesioned motor networks (Courtine et al., 2009).
51 Likewise, excitability of networks caudal to the lesion are also largely altered, even in
52 segments not visually affected by the initial trauma (Taccola et al., 2010).

53 However, their baseline excitability can be modified by neuromodulation via tonic
54 electrical epidural or transcutaneous spinal cord stimulation and/or pharmacological
55 activation. These protocols can modulate the excitability farther or closer to the motor
56 threshold needed to generate action potentials within and among sensory-motor and
57 autonomic networks in response to other sources of stimulation (Gerasimenko et al.,
58 2015). Thus, tonic neuromodulation of spinal networks changes their physiological state,
59 augmenting the probability to exceed the threshold of excitation. Changes in the basal
60 excitability of spinal networks explain how cutaneous and proprioceptive input, as well as

61 input from descending motor pathways, allow to recover supra spinal-spinal connectivity
62 after severe paralysis (Gad et al., 2013). These critical amounts of sensory excitation
63 and/or supraspinal input, added to the elevation of baseline excitability levels, are the two
64 main conditions to reach motor threshold and therefore generate movement (Taccola et
65 al., 2018). Indeed, spinal cord networks have been converted from a non-responsive state
66 to one that can generate sufficient depolarizing currents to induce action potentials among
67 interneurons projecting to motoneurons of multiple motor pools.

68 To elicit locomotor-like patterns from spinal networks, a unique stimulating paradigm
69 characterized by a noisy waveform was developed *in vitro* to optimally recruit neonatal
70 spinal neuronal networks (Taccola, 2011; Dose et al., 2016). This stochastic pattern of
71 modulation was then delivered dynamically to distinct sites of the spinal cord of fully
72 anesthetized adult *in vivo* rats (Taccola et al., 2020). This method was named Dynamic
73 Stimulation (DS), as opposed to the more static profile of trains of stereotyped pulses. DS
74 generated patterns of muscle bursting followed by short-lasting rhythmic discharges
75 (Taccola et al., 2020). Moreover, DS augmented distinct components of the EMG
76 responses elicited by segmental epidural weak pulses, during and after the end of DS
77 protocol delivery. Repetitive delivery of DS (rDS) further increased the amplitude of
78 spinally-induced EMG responses (Taccola et al., 2020).

79 However, along with reduced background activity in spinal networks, we have
80 demonstrated reduced amplitudes in spinal evoked motor responses within one week
81 after a severe SCI (Lavrov et al., 2008). Further, the response intensities and latencies
82 vary based on site of stimulation and duration of the injury (Gad et al., 2013). In addition,
83 the time course of the reemergence of spinally-induced responses were similar to the
84 recovery of stepping after a severe SCI, indicating that evoked responses from hindlimb
85 muscles can represent a potential biomarker of the functional recovery after SCI (Gad et
86 al., 2015). However, the mechanism linking the modulation of background activity in the
87 spinal networks with the modulation of motor-evoked responses still remains poorly
88 understood.

89 The objective of this study is to determine the efficacy of dynamic noisy patterns in
90 restoring motor control after a calibrated spinal cord injury.

91

92 Experiments were performed on 11 adult female Sprague Dawley rats (250–300 g body
93 weight). All procedures have been approved by the Animal Research Committee at UCLA
94 and are in accordance with the guidelines provided by the National Institutes of Health
95 (NIH) Guide for the Care and Use of Laboratory Animals and with the European Union
96 directive for animal experimentation (2010/63/EU).

97 Firstly, animals were sedated with isoflurane gas at a constant flow of 1.5%-2.5%,
98 followed by urethane (1.2 mg/Kg, *i.p.*).

99 Subsequently, recording wire electrodes (AS 632, Cooner Wire Co, Chatsworth, CA,
100 USA) for intramuscular electromyography (EMG) were implanted bilaterally in the tibialis
101 anterior (TA) and soleus (Sol) muscles. EMG signals were band-pass filtered (gain 1000,
102 range 10 Hz to 5 KHz and notched at 60 Hz), amplified (A-M Systems Model 1700
103 differential AC amplifier, A-M Systems, Sequim, WA, USA), and finally digitalized at 10
104 kHz (Digidata® 1440, Molecular Devices, LLC, CA, USA).

105 Delivery of signals was performed using a high-density platinum based multi-electrode
106 array, structured in three longitudinal columns and six horizontal rows of paired electrodes
107 (Chang et al., 2014; Taccola et al., 2020). Array implantation in the epidural dorsal space
108 was performed after a T12 to L2 laminectomy, to dorsally expose the spinal cord.

109 To determine threshold intensity for each preparation, a train of 40 rectangular pulses at
110 0.3 Hz was adopted. Five sweeps were delivered for each stimulation amplitude, moving
111 up by 100 μ A increments, ranging from 100 to 800 μ A. Threshold was defined as the
112 minimum intensity for eliciting a detectable EMG response from any muscle. As recently
113 reported (Taccola et al., 2020), DS consists of an EMG segment (29.5 s long) collected
114 from the Sol muscle of a neurologically-intact adult rat during stepping. The trace, once
115 acquired in AC mode (gain 1000, filter range 10 Hz to 5 KHz notched at 60 Hz) through
116 an A-M Systems Model 1700 differential AC amplifier (A-M Systems, Sequim, WA, USA),
117 was digitalized at 10 kHz (Digidata® 1440, Molecular Devices, LLC, CA, USA) and then
118 reduced off-line at a sampling rate of 2000 Hz, using Clampfit® 10.3 software (Molecular
119 Devices, LLC, CA, USA). Afterwards, the original EMG segment was duplicated, applying
120 a staggered onset of 0.5 s and then exported (as an ASCII text file) to a programmable
121 stimulator (STG 4002®; Multi Channel Systems, Reutlingen, Germany) to be applied to
122 different electrode combinations within the array. The protocol was delivered to the two

123 lateral columns of electrodes in the array with opposite rostro-caudal cathode/anode
124 polarity.

125

126 Spinal cord functionality was tested under urethane by applying trains of electrical pulses
127 (test pulses) (0.1 ms duration, 0.3 Hz frequency). Pulse amplitude was increased after 5
128 sweeps in the range of 100 – 800 μ A in order to define threshold intensity and trace a
129 recruitment curve after injury. Severe spinal cord injuries abolishing spinally induced
130 motor responses, were performed using a calibrated customized device, composed of a
131 steel rod of 33.0 g weight dropping on the exposed cord from 5 cm of height. The end of
132 the rod is a cylindrical protrusion of 1 mm radius to directly impact on the dorsal spinal
133 midline at L4/L5. The impounder was left on the original injury site for 10 seconds before
134 being carefully raised from the cord surface. During the impact, the trunk was stabilized
135 by supporting the animal's belly with a rod, 2 cm high, under the chest. After 40-90 min
136 from lesion, spinal cord functionality was tested under urethane by applying trains of
137 electrical pulses (test pulses) (0.1 ms duration, 0.3 Hz frequency). Pulse amplitude was
138 increased after 5 sweeps in the range of 100 – 800 μ A in order to define threshold intensity
139 and trace a recruitment curve after injury. The entire protocol for assessing spinal cord
140 functionality spanned 40 min and was replicated twice before DS delivery. Repetitive DS
141 consisted in the delivery of eight consecutive DS patterns of 30 s with 1 min intervals for
142 a total duration of 11 min.

143

144 Ninety minutes after the lesion, spinally-induced responses in TA and Sol muscles were
145 suppressed (Fig. 1 A). Single pulses delivered at maximal intensity (800 μ A) to the
146 segment just below the injury site elicited no responses (Fig. 1 A₁). About three hours
147 after injury, the rDS protocol was applied at the intensity of 600 μ A (Fig. 1 B) followed by
148 a long resting phase. Fifty minutes after the end of the protocol (Fig. 1 C), the same test
149 pulses, delivered with the cathode on the right side, produced a consistent response from
150 the muscles of the left leg, without any output from the right side (Fig. 1 C₁). Conversely,
151 by inverting the cathode/anode polarity of the test stimuli (Fig. 1 D, cathode on the left
152 side), TA and Sol on the right leg showed large muscle contractions without any
153 responses from the left leg (Fig. 1 D₁). Similar observations were made in four animals,

154 when rDS was applied 190 ± 17 min after the impact. Likewise, spinally-evoked responses
155 that were not present before rDS, reappeared when tested 228 ± 18 min after the impact.
156 Restored responses elicited by 750 ± 100 μ A, showed a mean amplitude of 0.23 ± 0.28
157 mV for Sol and 0.13 ± 0.15 mV for TA and a time to peak of 5.4 ± 1.0 ms for Sol and 5.6
158 ± 1.3 ms for TA (n=4). Responses evoked in intact cords with the same strengths of
159 stimulation and electrode location showed amplitude and time to peak values similar to
160 restored responses recorded from injured animals after rDS (for intact cords, amplitude =
161 0.32 ± 0.54 mV for Sol and 0.09 ± 0.08 mV for TA; time to peak = 6.6 ± 2.3 ms for Sol
162 and 5.8 ± 1.9 ms for TA; n=3).

163 Further assessments were made to assure that recovery of spinally-induced responses
164 was enabled by rDS and not by a spontaneous recovery over longer resting periods.
165 Therefore, four sham experiments were performed, to replicate the same experimental
166 procedures without any delivery of DS (Fig. 1 E-G). In a sample experiment, the lack of
167 EMG responses from the injured cord segment was confirmed by continuous testing for
168 up to 250 min after injury (Fig. 1 E₁, G₁, H₁). Moreover, suppression of spinally-induced
169 responses extended also to more rostral and caudal segments, eventually demonstrating
170 a worsening of the functional deficit within the first few hours after initial compression
171 (data not shown).

172
173 In the present study, we exploited a recently designed protocol of multisite stimulation
174 with noisy patterns, named Dynamic Stimulation, and its delivery through an epidural
175 interface consisting in a multi-electrode array. Recently, we proved that these two
176 resources modulate locomotor networks and facilitate the motor output induced by
177 subthreshold cortical input. Here, in fully anesthetized animals, we demonstrated that the
178 rDS paradigm of stimulation was linked to patterns leading to a greater recovery of motor
179 output after a severe spinal cord injury.

180 Unlike many studies involving neurorehabilitation, our strategy did not target the lumbar
181 central pattern generator for locomotion (Kiehn and Butt 2003), but was centered at the
182 site of lesion to promote reconnection along adjacent segments. Another original point of
183 this research was that the continuous electrostimulation of the lesioned cord was
184 performed in an acute setting (in the first three hours after injury). This finding suggests

185 the possibility to employ novel dynamic stimulation paradigms, epidurally and/or
186 transcutaneously, to the lesioned spinal cord as a first surgical intervention to limit the
187 loss of functions following a spinal cord injury.

188 The manner in which acute multiple depolarizations, as the ones induced by rDS,
189 counteract early functional impairments after SCI has not been explored so far and,
190 likewise, the mechanisms of such recovery are far from being elucidated.

191 On the contrary, though, a spreading depolarization along the cord has been reported so
192 far to contribute to secondary damage after an impact injury (Gorji et al., 2004), since it
193 releases additional glutamate that reaches a toxic level for cells and leads to functional
194 deficits (Hinzman et al., 2015). In the present study, the continuous delivery of DS, acutely
195 applied across the lesion site, generated additional depolarizations that, paradoxically,
196 not only did it not worsen the functional deficit, but in fact consistently facilitated the
197 recovery of motor output.

198 This effect was robust, also, in spite of the possible spreading depolarization induced by
199 damage had already concluded when we delivered rDS (3 hours after lesion).
200 Alternatively, rDS might have confined the spreading depolarization triggered by the
201 trauma, by generating multiple after-hyperpolarizations of cell membranes in response to
202 the insurgence of action potentials evoked by rDS in the network neurons. Indeed, these
203 asynchronous and diffused hyperpolarizing events throughout the network could act as
204 unexcitable nodes along the path of spreading depression, limiting the massive
205 propagation.

206 Moreover, the acute delivery of rDS might regress acute phenomena of network
207 dysfunction (Taccola et al., 2010) by promoting activity-based plastic events (Ganguly
208 and Poo, 2013). Indeed, DS provides a pattern of phasic stimulation derived from
209 sampling traces from hind limb muscles during real locomotion. This pattern of input
210 varies in amplitude and frequency and is comparable to that of afferent feedback during
211 gait (Prochazka et al., 1976). According to this view, application of rDS a few hours after
212 the trauma might promote activity-like signals mimicking a locomotor training session long
213 before the subject is stabilized and prepared for neurorehabilitation protocols aimed at
214 facilitating recovery of locomotion.

215 Extensive amounts of data are accumulating which points to activity-dependent
216 mechanisms in play ranging from RNA expression and synaptic proteins to system-level
217 learning phenomena within and among spinal networks (Kobayakawa et al., 2019) as well
218 as the transformation of dormant to competent spinal connectivity in response to epidural
219 and transcutaneous stimulation when combined with sensory-motor training (de Leon et
220 al., 1998). Perhaps these phenomena can become even more robust in a time frame
221 closer to the moment of injury.

222 Moreover, motor output potentiation winds up when DS delivery is repeated at short-time
223 intervals (Taccola et al., 2020), as performed in this study. This event is likely due to the
224 accumulation of a molecular factor released by rDS. For example, BDNF is upregulated
225 following standard epidural electrical stimulation (Baba et al., 2009) and, in turn, spinal
226 BDNF modulates the motor output (Côté et al., 2011). Interestingly, BDNF is involved in
227 promoting recovery after lesion (Kim et al., 1996; Jakeman et al., 1998; Boyce et al.,
228 2007). It is not known, however, whether the restorative effect of rDS can be replicated in
229 the absence of BDNF.

230 The recovery of functions observed after an acute application of rDS is robust and adds
231 a new element to the advantages of noisy stimulation (Taccola et al., 2020). Indeed,
232 variable stimulation can work both toward increasing connectivity among spinal neurons
233 spared by a lesion and toward rescuing them from the spinal shock.

234 These promising data, collected from terminal recordings in fully anesthetized animals,
235 suggest the need for further studies to translate this neuromodulating strategy both in the
236 acute stage of a spinal injury and in chronic scenarios in absence of anesthesia, to confirm
237 the ability of rDS to restore functions. Collectively, these data provide compelling reasons
238 why DS should be explored as a possible critical component in the acute phase of
239 treatment that might limit the severity that emerges after a spinal cord injury.

240

241

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248

249 *Author Disclosure Statement*

250 VRE, researcher on the study team hold shareholder interest in NeuroRecovery
251 Technologies and hold certain inventorship rights on intellectual property licensed by The
252 Regents of the University of California to NeuroRecovery Technologies and its
253 subsidiaries. VRE, and PG, researchers on the study team hold shareholder interest in
254 Spinex. Wentai Liu, researcher on the study team holds shareholder interest in Niche
255 Biomedical Inc.

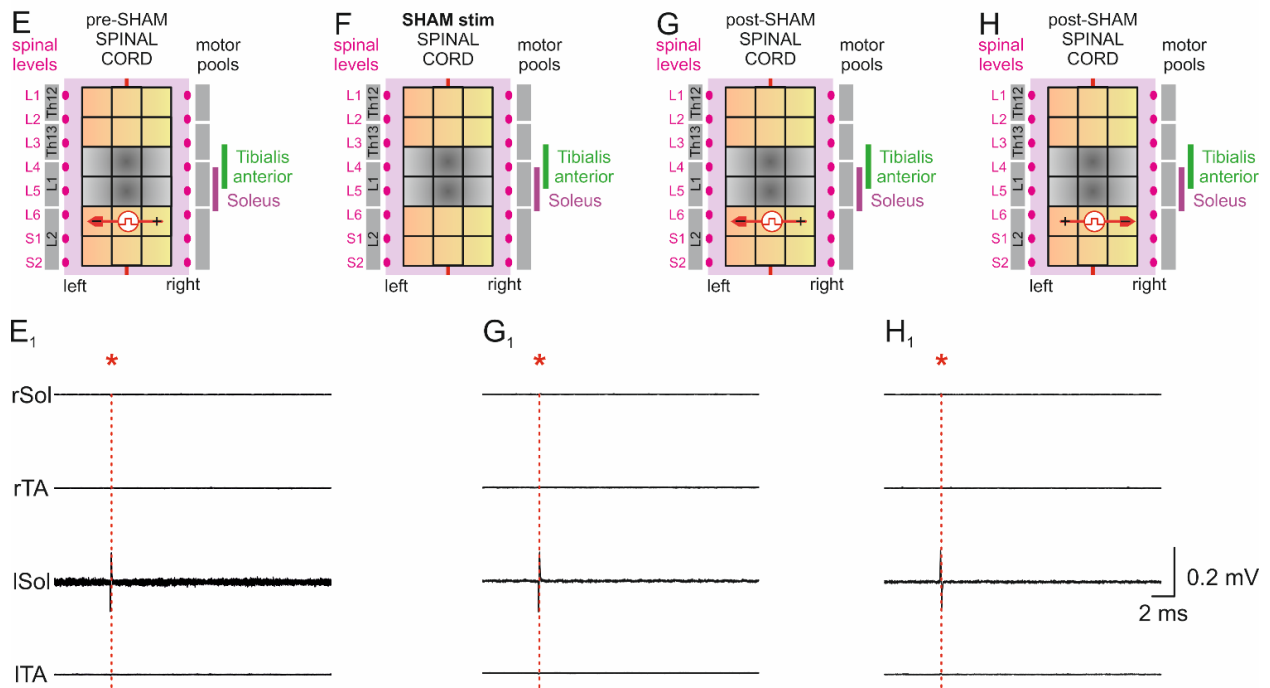
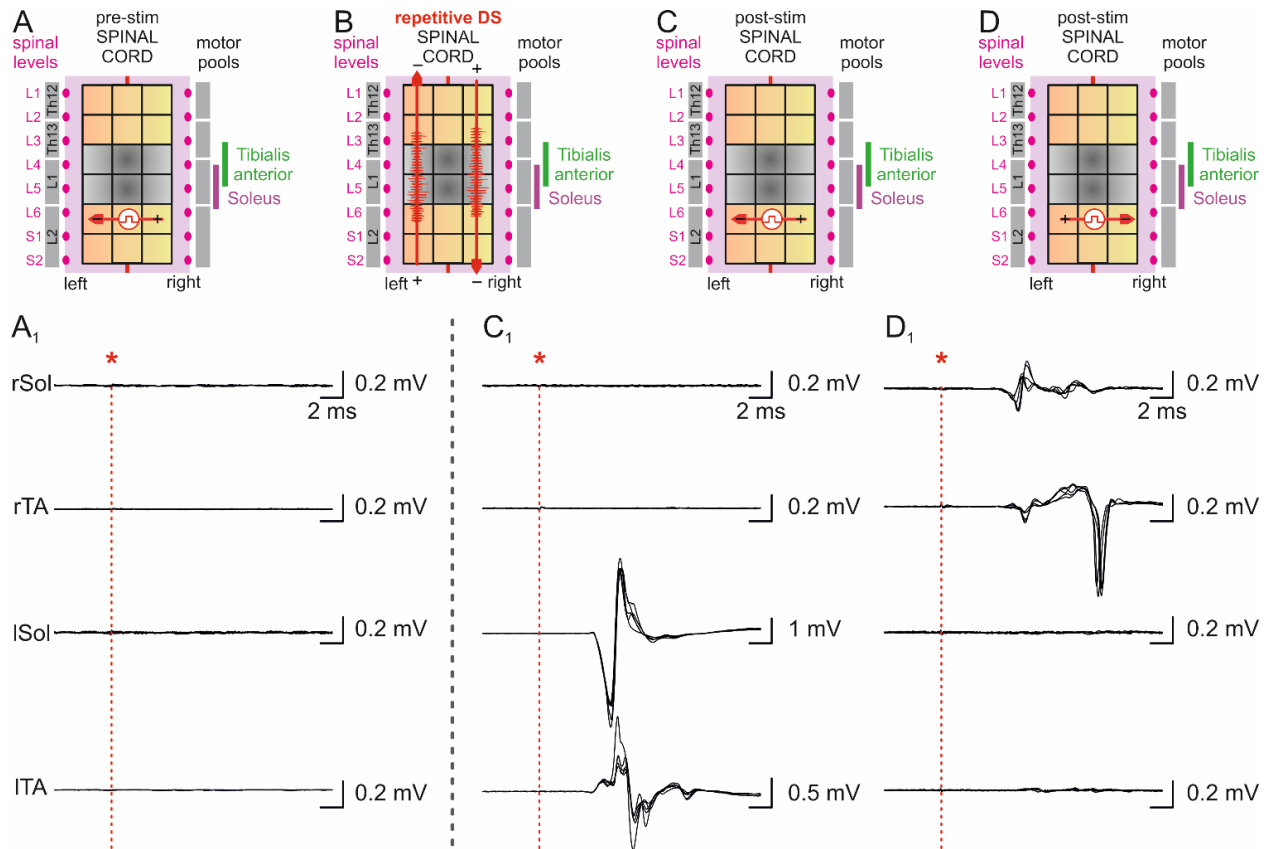


Figure 1. rDS restores spinally-induced responses after acute spinal contusion.

In A, the cartoon summarizes the stimulation setting, with a calibrated representation of the array width and the distance between homosegmental dorsal roots. 90 mins after a calibrated compression to the spinal cord at L4/L5, single pulses (red star and dotted line; intensity = 800 μ A; duration = 0.1 ms) applied to L6 (cathode on the right) are unable to elicit any bilateral EMG responses from Sol and TA muscles (A₁). After additional 90 mins, rDS was supplied through the lesioned spinal cord (B) and 50 mins later (C, D), the same stimulation delivered in A now evokes spinally-induced responses from both the left leg (C₁, cathode on the right) and from the right one, as well, by swapping the position of the poles (D₁, cathode on the left). In E-H the same protocol in A-D was followed in an animal that did not receive any rDS. In the latter case, no spontaneous recovery was reported for either configuration of stimulation (G₁, H₁). In each panel A₁, C₁, D₁, E₁, G₁, H₁, five consecutive traces are superimposed.

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