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Report

Motor Recovery after Spinal Cord Injury Enhanced by Strengthening Corticospinal Synaptic Transmission

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Summary

The corticospinal tract is an important target for motor recovery after spinal cord injury (SCI) in animals and humans [1–5]. Voluntary motor output depends on the efficacy of synapses between corticospinal axons and spinal motoneurons, which can be modulated by the precise timing of neuronal spikes [6–8]. Using noninvasive techniques, we developed tailored protocols for precise timing of the arrival of descending and peripheral volleys at corticospinal-motoneuronal synapses of an intrinsic finger muscle in humans with chronic incomplete SCI. We found that arrival of presynaptic volleys prior to motoneuron discharge enhanced corticospinal transmission and hand voluntary motor output. The reverse order of volley arrival and sham stimulation did not affect or decreased voluntary motor output and electrophysiological outcomes. These findings are the first demonstration that spike timing-dependent plasticity of residual corticospinal-motoneuronal synapses provides a mechanism to improve motor function after SCI. Modulation of residual corticospinal-motoneuronal synapses may present a novel therapeutic target for enhancing voluntary motor output in motor disorders affecting the corticospinal tract.

Results

Deficits in motor function are one of the most devastating and to date incurable problems after spinal cord injury (SCI). Voluntary motor function is largely controlled by the corticospinal tract, which is a major descending motor pathway in mammals [9]. A role of the corticospinal tract in functional recovery after SCI has been proposed for animals and humans [1–5]. However, interventions that successfully engage the corticospinal tract in motor function recovery after an injury to the spinal cord remain sparse. Corticospinal transmission largely depends on the strength of synaptic connections between corticospinal drive and spinal motoneurons. Long-lasting potentiation of synaptic strength can be induced by precisely timing the arrival of presynaptic action potentials prior to postsynaptic depolarizing action potentials (a process known as spike timing-dependent plasticity (STDP) [6, 7]), which Taylor and Martin [8] showed to enhance voluntary motor output when targeting the spinal cord in intact humans. The corticospinal tract is a likely candidate for inducing synaptic plasticity, considering its remarkable pattern of connections at the spinal cord level after SCI [2, 10]. Thus, we hypothesized that arrival of corticospinal volleys prior to motoneuron discharge at residual corticospinal-motoneuronal synapses will enhance voluntary motor output in humans with chronic incomplete SCI.

To test our hypothesis, we developed tailored noninvasive brain and peripheral nerve stimulation protocols using onset latencies of electromyographic (EMG) responses to stimulation at different levels of the corticospinal pathway in 19 participants with cervical SCI (Table S1 available online) and 14 age-matched healthy controls. Corticospinal neurons were activated at the cortical level via transcranial magnetic stimulation (TMS) delivered over the hand representation of the motor cortex. Spinal motoneurons were activated antidromically by peripheral nerve stimulation (PNS) delivered to the ulnar nerve at the level of the wrist.

Paired-Pulse Stimulation Protocols

We tested the less affected side in individuals with SCI as determined by the level of force exerted during maximal voluntary contraction (MVC) by the index finger and right dominant side in healthy controls. The interstimulus interval (ISI) at which descending volleys elicited by TMS and antidromic volleys elicited by PNS would arrive at corticospinal-motoneuronal synapses of the first dorsal interosseous (FDI) muscle was estimated in both groups of subjects (Table S2 and Figure S1). A significantly longer conduction time from motor cortex to synapse ($p = 0.001$) and from ulnar nerve at the wrist to synapse ($p < 0.001$) was found in participants with SCI compared to healthy controls. Considering these differences, in one protocol, the ISI between paired pulses allowed descending volleys to arrive at the presynaptic terminal of corticospinal neurons 1–2 ms before antidromic volleys in motoneurons reached the dendrites (protocol referred as to STDP, SCI = 1.5 ± 0.6 ms, healthy controls = 1.5 ± 0.3 ms; $p = 0.68$; Figure 1A). In a second reversed protocol, the ISI allowed antidromic volleys to reach motoneuron dendrites 5 ms before the descending volleys reached the presynaptic terminal (protocol referred to as control, SCI = 5.0 ± 0.3 ms, healthy controls = 5.3 ± 0.4 ms, $p = 0.14$; Figure 1B). We applied 100 pairs of TMS and PNS pulses at 0.1 Hz. The amplitude of the F wave was larger during the control compared to the STDP protocol in both groups of subjects [$F_{(1,21)} = 22.2$, $p < 0.001$; Figures 1E and 1F] suggesting that spinal motoneurons were differentially driven by our interventions.

Effects of Paired-Pulse Stimulation Protocols on Electrophysiological Recordings

Changes in transmission in the corticospinal pathway were assessed by examination of the size of motor evoked potentials (MEPs) elicited in the resting FDI muscle by TMS and transcranial electrical stimulation (TES) before and after each protocol. Participants with SCI and healthy controls showed an increase in the size of MEPs in the FDI muscle elicited by TMS [$F_{(4,88)} = 12.1$, $p < 0.001$] and TES [$F_{(4,44)} = 4.2$, $p = 0.006$; Figure 2 and Table S3] after the STDP, but not the control protocol. MEP size returned back to baseline between 50 and 120 min (mean = 81.7 ± 31.2 min, n = 7) after stimulation. Similarly, when MEPs were elicited by stimulation of the cervicomedullary junction, the size of the responses was increased after the STDP protocol in both groups of subjects [$F_{(4,24)} = 3.0$,

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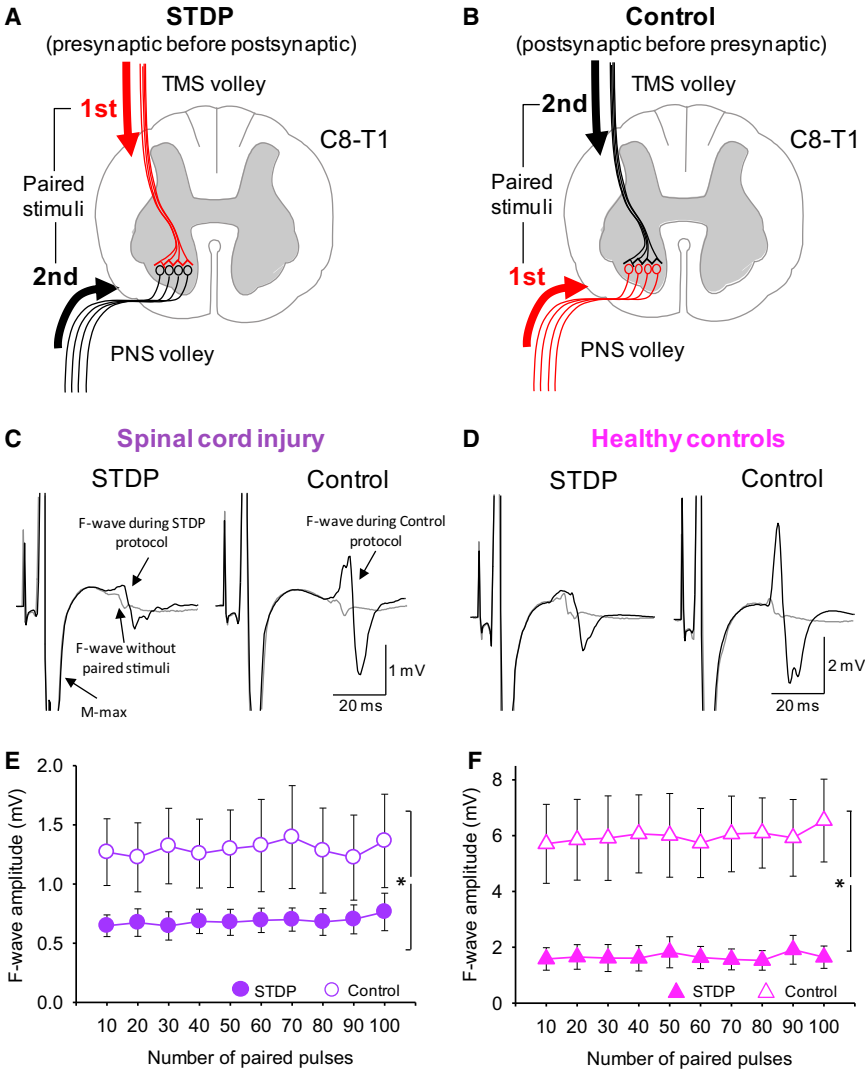

Figure 1. Paired-Pulse Stimulation Protocols

(A) Illustration of the spike time-dependent plasticity (STDP) protocol. Here, corticospinal neurons were activated at a cortical level by using transcranial magnetic stimulation (TMS volley) delivered over the hand representation of the motor cortex and spinal motoneurons were activated antidromically by peripheral nerve stimulation (PNS volley) delivered to the ulnar nerve at the wrist. The interstimulus interval between paired pulses was designed to allow descending volleys, elicited by TMS, to arrive at the presynaptic terminal of corticospinal neurons (1st, red arrow) 1–2 ms before antidromic PNS volleys in the motoneurons reached the dendrites (2nd, black arrow).

(B) Illustration of the control protocol. Here, antidromic PNS volleys were timed to reach motoneuron dendrites (1st, red arrow) 5 ms before the TMS volleys reached the presynaptic terminal (2nd, black arrow). In both protocols, 100 pairs of TMS and PNS pulses were applied at 0.1 Hz for ~17 min.

(C and D) Electromyographic recordings from the first dorsal interosseous (FDI) muscle showing a representative average of the maximal motor response (M-max) and a subsequent F wave during each paired-pulse stimulation protocol (black traces) and during isolated PNS without paired-pulse stimulation (gray traces) in a participant with SCI and in a healthy control.

(E and F) The graphs show the group data in SCI participants (n = 18) and in healthy controls (n = 10). The abscissa shows the number of paired pulses measured applied during each protocol (a total of 100 paired pulses). At each point, the average of ten F waves is shown. The ordinate shows the size of the F wave in millivolts. The F wave amplitude was significantly larger during the control (open purple circles, SCI; open pink triangles, healthy controls) compared to the STDP (closed purple circles, SCI; closed pink triangles, healthy controls) protocol at all points in both groups of subjects as indicated by the asterisk. Note the difference in scale in traces and graphs. Error bars indicate the SE. *p < 0.05.

p = 0.03]. Overall, the increases in the size of MEPs after the STDP protocol were present in 89% of SCI participants and in 90% of healthy controls. In an additional experiment, we found that when antidromic action potentials elicited by PNS arrived at corticospinal-motoneuronal synapses 15 ms before TMS-induced presynaptic potentials, the size of MEPs elicited by TMS [$F_{(4,28)}$ = 9.09, p < 0.001] and by stimulation of the cervicomedullary junction [$F_{(4,16)}$ = 5.3, p = 0.04] was decreased in both groups of subjects (Figure S2). Changes in motoneuronal excitability could also contribute to the changes observed in MEP size. We found that STDP and control protocols had no effects on F wave amplitude [$F_{(4,60)}$ = 0.2, p = 0.92] or persistence [$F_{(4,60)}$ = 0.4, p = 0.83; Figure S3 and Table S3].

Effects of Paired-Pulse Stimulation Protocols on Voluntary Motor Output

We examined whether changes in corticospinal transmission elicited by the STDP protocol affected voluntary motor output in the hand that received the stimulation. After the STDP, but not the control protocol, the magnitude of force exerted by the index finger [$F_{(4,72)}$ = 6.1, p < 0.001; Figures 3A, 3C, and 3D] and mean rectified EMG activity in the FDI muscle [$F_{(4,72)}$ = 6.6, p < 0.001; Figure 3B, 3E, and 3F] was increased in both groups. The increments in force and EMG activity (combined) were present in 80% of SCI participants and 85% of healthy controls. These changes were still present at 85.0 ± 7.1 min after stimulation. A positive correlation was found between changes in mean rectified EMG and MEP size after the STDP (SCI: r = 0.78, p < 0.0001 and healthy controls: r = 0.47, p = 0.01; Figures S4A and S4B) but not the control protocol. Changes in mean force and MEP size positively correlated after the STDP, but not the control protocol, in healthy controls (r = 0.57, p = 0.01, p < 0.001; Figure S3D) but not SCI individuals (r = 0.12, p = 0.50; Figure S4C).

Manual Dexterity Improved after the STDP Protocol in Humans with SCI

Figure 4A shows pictures of the tasks completed during the nine-hole peg test (9HPT) used to examine manual dexterity only in individuals with SCI. The time to complete the 9HPT decreased after the STDP but not the control protocol [$F_{(4,28)}$ = 3.9, p = 0.01; Figure 4B]. The improvements in the 9HPT were present in 87% of the participants with SCI.

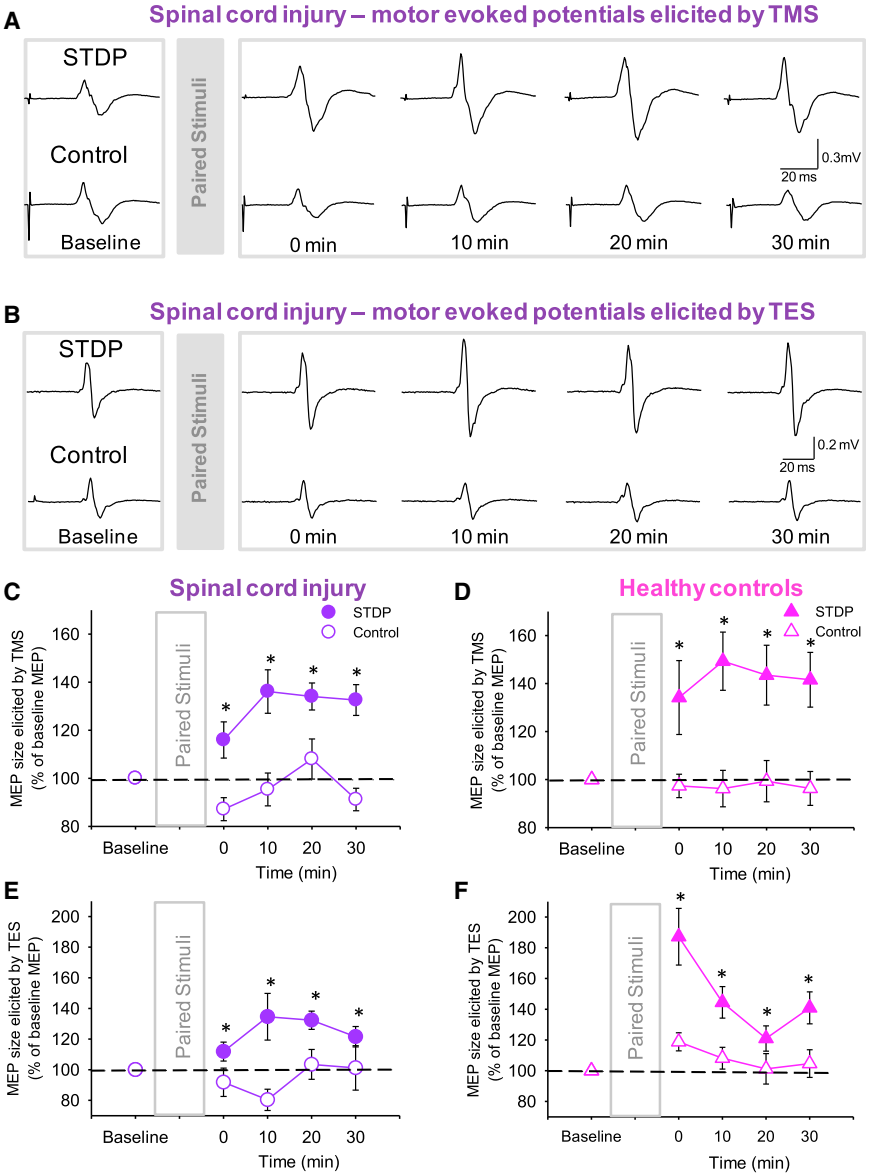

Figure 2. Motor Evoked Potentials

Transmission in the corticospinal pathway was assessed by examination of the size of MEPs elicited in the resting FDI muscle by TMS and transcranial electrical stimulation (TES) before (Baseline) and after (0, 10, 20, and 30 min) each paired-pulse stimulation protocol. Raw traces from a representative participant with SCI shows an average of 30 MEPs elicited by TMS (A) and 10 to 20 MEPs elicited by TES (B). The gray bar represents the pair stimulation (paired-pulse stimuli; 100 paired pulses at 0.1 Hz for ~17 min). Note that the size of MEPs evoked by TMS and TES was increased at all times after the STDP (upper traces) but not after the control (lower traces) protocol. Graphs show group data. The abscissa shows the time of measurements, and the ordinate shows the peak-to-peak amplitude of the MEPs elicited by TMS and TES in the FDI muscle as a percentage of the baseline MEP in participants with SCI (C and E; closed purple circles, STDP; open purple circles, control; n = 18) and in healthy controls (D and F; closed pink triangles, STDP; open pink triangles, control; n = 10). Note the increase in the size of FDI MEP elicited by TMS and TES at all times in both groups of subjects. Also note that we did not observe a significant difference between the effects reported at time 0 and later time points in (C–F). Error bars indicate the SE. *$p < 0.05$.

Discussion

Our results demonstrate for the first time spike timing-dependent plasticity of residual corticospinal-motoneuronal synapses in humans with chronic incomplete SCI and their functional consequences. We found that when TMS-induced presynaptic volleys arrived 1–2 ms before antidromic volleys, induced by PNS, at corticospinal-motoneuronal synapses of an intrinsic finger muscle, corticospinal transmission, index finger force, and EMG increased for up to 80 min in participants with SCI and in healthy controls. Importantly, our tailored protocol resulted in improvements in manual dexterity in SCI participants. The changes in corticospinal transmission were positively correlated with enhancements in voluntary motor ouput in both injured and healthy persons, suggesting an association between motor output and strength in the induced plasticity. MEPs evoked by TES and cervicomedullary stimulation increased after the STDP protocol, suggesting that our effects are less likely to be related to changes in corticocortical synapses. We argue that residual corticospinal-motoneuronal synapses present a novel therapeutic target for enhancing voluntary motor function after SCI.

Changes in Corticospinal-Motoneuronal Synapses in Humans with SCI

Three lines of evidence support our argument that the most likely mechanism contributing to our results are changes at corticospinal-motoneuronal synapses. First, we found that the size of MEPs in the FDI muscle elicited by TMS and TES increased after the STDP protocol. At the stimulus intensities used during MEP testing, TMS probably activated corticospinal axons transynaptically, while TES activated the axons of pyramidal tract cells in the subcortical white matter [11, 12]. Furthermore, MEPs evoked by stimulation of the corticospinal tract at the cervicomedullary junction were also increased after the STDP protocol; these MEPs are not influenced by the classical presynaptic inhibiton [13] and are likely to be altered by changes occurring at the corticomotoneuronal synapse [14, 15]. Second, we found that the amplitude and persistence of F waves tested in the FDI muscle remained unchanged after the STDP protocol, suggesting that the increase in MEP size was not related to increases in the excitability of spinal motoneurons. Although some limitations have been described in the extent to which F wave measurements can assess motoneuron excitability [16, 17], motor units of all sizes seem capable of contributing to F wave activity [18–20], and this measure can detect changes in motoneuronal excitability in healthy controls and after SCI [18–22]. Third,

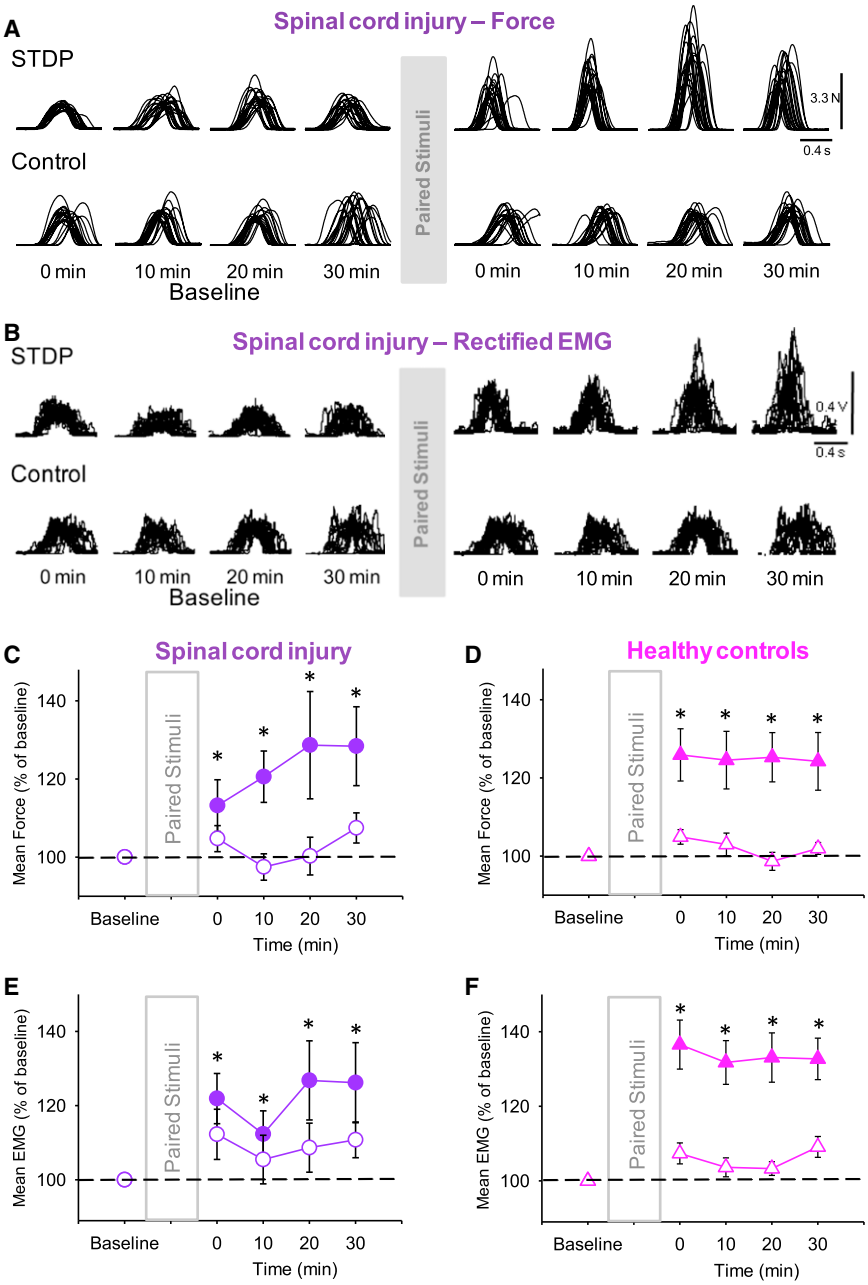

Figure 3. Voluntary Motor Output

Voluntary motor output was assessed by examination of changes in mean force and mean rectified EMG during brief, fast, index finger voluntary contractions in the abduction direction before (Baseline) and after (0, 10, 20, and 30 min) the paired-pulse stimulation protocols. Raw force (A) and EMG (B) traces from a representative participant with SCI. At each time point, 20 raw traces are overimposed. The gray bar represents the paired-pulse stimulation (paired-pulse stimuli; 100 paired pulses at 0.1 Hz for ~17 min). Graphs show group data. The abscissa shows the time of measurements, and the ordinate shows the mean force measured during index finger abduction and mean rectified EMG activity in the FDI muscle as a percentage of the baseline in participants with SCI (C and E; closed purple circles, STDP; open purple circles, control; n = 10) and in healthy controls (D and F; closed pink triangles, STDP; open pink triangles, control; n = 10). Note the parallel increase in mean force and EMG activity after the STDP, but not the control, protocol in both groups of subjects. There were no significant differences between the effects reported at time 0 and later time points in (C–F). Error bars indicate the SE. *p < 0.05.

some features of the changes observed in our study are consistent with spike timing-dependent changes at synapses described in animal models. We found that MEPs were facilitated when presynaptic volleys arrived before motoneuronal discharge. It is known that presynaptic activity preceding postsynaptic firing or depolarization induces long-term synaptic potentiation [6, 7]. After SCI, axonal loss and demyelination [23] may affect the temporal dispersion of descending volleys to recruit spinal motoneurons [24]. Then, it is possible that the onset of postsynaptic excitation of motoneurons may build up more slowly after SCI compared to healthy controls, and in this case postsynaptic events might be preceding presynaptic inputs. However, when volleys reached the spinal motoneurons before the presynaptic terminal, we observed no changes in MEP size at a short interval or inhibition at a longer interval, which is in agreement with previous results obtained in humans [8]. These results suggest that the inhibitory effects of STDP protocols might have a specific window for temporal plasticity at different synapses [25]. For example, in animal studies, a narrow transition zone at a short interval of around 5 ms has been reported between potentiation and depression [6]. The effects of our STDP protocol on physiological and behavioral outcomes occurred after 100 pairs of stimulus at 0.1 Hz and lasted for up to 80 min, which is also consistent with timing-dependent changes reported in animal models [6].

Although we did not record directly at the synapse, using electrophysiological measurements by stimulating different levels of the corticospinal pathway in individual subjects, we could generate accurate estimates of the time of arrival of action potentials to the muscle; indeed latencies of EMG responses are dependent on the generation of action potentials in motoneurons. Importantly, these measurements have been shown to be sensitive to detect changes in clinical diagnostic procedures [26]. The consistency between electrophysiological measurements across sessions, the use of a figure-of-eight coil in a posteromedial orientation to reliably elicit D waves (direct waves) [27, 28], and the specificity of our results support the view that human noninvasive electrophysiology can be successfully used to guide interventions after SCI.

Neuronal Mechanisms

STDP is thought to depend on NMDA receptor activation and the timing of action potential back propagation through the dendrites of the postsynaptic neuron [29]. In our study,

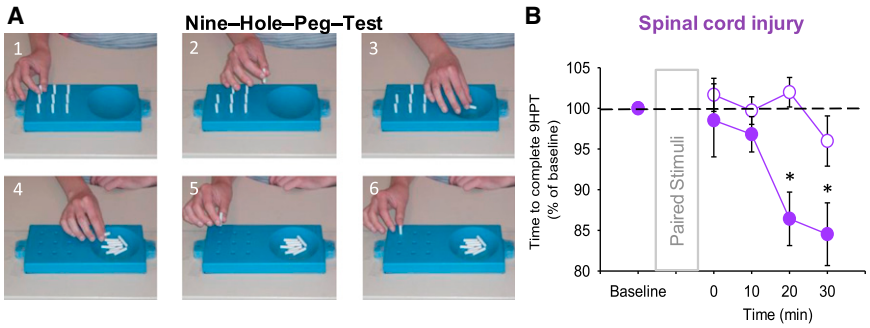

Figure 4. Manual Dexterity

Manual dexterity was assessed by examination of changes in the speed to complete the nine-hole peg test (9HPT) before (Baseline) and after (0, 10, 20, and 30 min) the paired-pulse stimulation protocols in participants with SCI.

(A) Individual pictures showing the steps to complete the 9HPT. Note that pictures 1–3 show the part of the test were each pin is lifted by a precision grip between the index and thumb and deposited into the reservoir located on the side, while pictures 4–6 show that each pin is pick up and repositioned back into each hole by a precision grip between the index and thumb.

(B) Graph shows group data in participant with SCI (n = 8). The abscissa shows the time of measurements, and the ordinate shows the time to complete the 9HPT as a percentage of the baseline after the STDP (closed purple circles) and control (open purple circles) protocols. Note the improvements to complete the 9HPT after the STDP but not the control protocol. Error bars indicate the SE. *p < 0.05.

during paired-pulse stimulation, the size of the F wave was increased compared to rest in both protocols. This is in agreement with the results by Nielsen and collaborators [30], who showed in an upper-limb muscle that motoneuronal excitability increased if paired volleys elicited by PNS and TMS arrived at the spinal cord, at similar intervals used in our study. The distinct pattern of increased activation of spinal motoneurons during the control compared to the STDP protocol indicates that motoneurons were differentially driven by the stimulation. One possibility is that activation of spinal motoneurons first, in the control protocol, resulted in increased sensitivity to excitatory inputs. In agreement, previous results showed that a decrease of the threshold of motoneurons results in larger activation by the descending drive in healthy controls [28], and to a lesser extent after SCI [31]. Physiological and behavioral changes were absent after the control protocol, although during paired-pulse stimulation the size of F wave responses were larger than in the STDP protocol. The mechanism contributing to this effect is unclear. Though, the pronounced increase in F waves size during the control protocol, reaching values of up to several millivolts, might have limited their responsiveness to plasticity.

Did we target corticospinal neurons with direct or indirect inputs to motoneurons? Corticospinal neurons that innervate hand muscles make monosynaptic connections with spinal motoneurons and their activity is highly modulated during independent finger movements [9, 32]. Since SCI participants were able to elicit finger movements, most likely, we targeted corticospinal neurons with direct inputs to motoneurons. However, MEP sizes were increased after the STDP protocol; these responses probably involve an early component by direct activation of the motoneurons by corticospinal neurons and later components due to indirect activation through excitatory inputs from the brainstem and/or spinal cord [14]. Damage or reorganization in corticospinal and propriospinal neurons [5] could affect the final outcome. The PNS used in our study would also activate sensory fibers including group Ia afferent inputs onto motoneurons. Orthodromic inputs from these afferents or interneurons, including changes in presynaptic inhibition [33], might contribute to the increases in MEP size by adding inputs to the corticospinal pathway. Regardless of the type of corticospinal cells mediating our results, or the possible additional contribution of other descending or sensory pathways, our findings clearly demonstrate functionally relevant plasticity at the spinal cord level.

STDP Enhanced Voluntary Motor Output and Manual Dexterity after SCI

Our results agree with previous evidence indicating a central role of the spinal cord in restoring useful function after SCI [11, 18, 34, 35] and add the novel finding that synaptic plasticity between residual corticospinal projections and spinal motoneurons is an important target to maximize motor recovery after SCI.

To date, multiple noninvasive approaches have been used to alter corticospinal transmission after SCI, including repeated electrical stimulation of a peripheral nerve [36], repetitive TMS of the motor cortex [37], and paired associative stimulation targeting the motor cortex [38]. Others have proposed that repeated noninvasive activation of more direct and indirect corticospinal volleys to spinal motoneurons might influence motor outcomes after SCI by favoring spinal plasticity [39, 40]. However, since transmission of these different corticospinal volleys to spinal motoneurons is altered after SCI [41, 42], their use might be problematic in patients. The effects of the STDP protocol lasted for 80 min after stimulation. A more prolonged use of this technique and/or their combination with other strategies might increase their therapeutic efficacy and may present a mechanism to enhance voluntary motor output in humans with SCI and other motor disorders affecting the corticospinal tract.

Experimental Procedures

The study was performed in accordance with the Declaration of Helsinki. All subjects gave their informed consent to the experimental procedures, which was approved by the local ethics committee at the University of Pittsburgh. Participants with SCI were recruited from the Department of Physical Medicine and Rehabilitation research registry at the University of Pittsburgh. Nineteen participants with SCI (mean age = 47.8 ± 12.5 years, two female, Table S1) and 14 right-handed age-matched healthy controls (mean age = 39.4 ± 17.8 years, eight female; p = 0.12) participated in the study. Participants with SCI had a chronic (≥1 year), cervical injury (C4–C8), and residual sensory and motor hand and arm motor function.

Recordings

EMG was recorded by surface electrodes secured to the skin over the muscle belly (Ag–AgCl, 10 mm diameter). The signals were amplified, filtered (20–1,000 Hz), and sampled at 2 kHz for off-line analysis (CED 1401 with Signal software, Cambridge Electronic Design, Cambridge, UK). Forces exerted at the proximal interphalangeal joint of the index finger were measured by load cells (Honeywell, range ± 498.1 N, voltage ± 5 V, high-sensitivity transducer 0.045 V/N). Force was sampled at 200 Hz and stored on a computer for off-line analysis.

Procedures

During testing, subjects were seated in an armchair with both arms relaxed and flexed at the elbow by 90° with the forearm pronated and the wrist and forearm restrained by straps. Subjects participated in two paired-pulse stimulation protocols (i.e., STDP and control). First, subjects were randomly tested on different days for the effects of each paired-pulse protocol on electrophysiological measurements, including MEPs elicited by TMS, TES, cervicomedullary junction stimulation, and F waves. Second, we examined whether changes in corticospinal transmission observed after the STDP protocol influenced voluntary motor output. Subjects were randomly tested on different days for the effects of each paired-pulse protocol on voluntary motor output (i.e., EMG and force) and manual dexterity (i.e., 9HPT). Sessions were separated by at least 2 days. Subjects were unaware of which stimulation protocol was used at each session. All measurements were tested before (baseline), immediately after (Time 0), and 10, 20, and 30 min after the STDP and control protocols. In a subset of subjects, the effects on electrophysiological outcomes were followed for up until measurements returned to baseline and the interval between TMS and PNS was changed (15 ms; see the Supplemental Experimental Procedures).

Data Analysis

See the Supplemental Experimental Procedures.

Supplemental Information

Supplemental Information includes Supplemental Results, Supplemental Experimental Procedures, four figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.10.046.

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