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Spike-Timing-Dependent Plasticity in Primate Corticospinal Connections Induced during Free Behavior

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SUMMARY

Motor learning and functional recovery from brain damage involve changes in the strength of synaptic connections between neurons. Relevant in vivo evidence on the underlying cellular mechanisms remains limited and indirect. We found that the strength of neural connections between motor cortex and spinal cord in monkeys can be modified with an autonomous recurrent neural interface that delivers electrical stimuli in the spinal cord triggered by action potentials of corticospinal cells during free behavior. The activity-dependent stimulation modified the strength of the terminal connections of single corticomotoneuronal cells, consistent with a bidirectional spike-timing-dependent plasticity rule previously derived from in vitro experiments. For some cells, the changes lasted for days after the end of conditioning, but most effects eventually reverted to preconditioning levels. These results provide direct evidence of corticospinal synaptic plasticity in vivo at the level of single neurons induced by normal firing patterns during free behavior.

INTRODUCTION

Synaptic plasticity is a key mechanism mediating reorganization of neural circuits during learning and recovery from injury (Cramer et al., 2011; Kleim, 2012; Wolpaw and Tennissen, 2001). In vitro studies have shown that the synaptic connection between two neurons can be strengthened or weakened when the presynaptic neuron fires before or after the postsynaptic neuron, as described by spike-timing-dependent plasticity (STDP) rules (Bi and Poo, 2001; Caporale and Dan, 2008; Markram et al., 2011). Most of these studies have involved slice preparations of cerebral or hippocampal cortex and elicited STDP using controlled stimulation patterns that differ from those typically occurring during normal behavior. Such studies have revealed that STDP rules for different synapses can differ with regard to polarity of the synaptic changes and width of the effective windows (Caporale and Dan, 2008). Moreover, the STDP rule for a given synapse can be significantly altered by the presence and concentration of neuromodulators (Salgado et al., 2012; Seol et al., 2007). STDP is typically documented with repetitive pairing of pre- and postsynaptic spikes at regular intervals, but when tested with more complex spike patterns, STDP is a nonlinear and variable function of the pattern of pre- and postsynaptic activity (Froemke et al., 2010). In vitro experiments have confirmed that the conditioned effects can last for up to several hours in the absence of additional activity. These observations raise several questions addressed in this study: can plasticity of primate corticospinal cell terminals be induced during free behavior by using the cell's normal firing patterns to trigger stimulation of target motoneurons? What are the STDP rules pertaining to monosynaptic and disynaptic connections of primate corticomotoneuronal cells? How long do the conditioned effects last after the end of conditioning and the resumption of normal neuronal activity?

These questions were addressed by establishing an artificial connection between cortical neurons and spinal cord with a recurrent brain-computer interface. Experiments using a similar paradigm to deliver spike-triggered stimulation within motor cortex produced synaptic plasticity for spike-stimulus delays about less than 50 ms (Jackson et al., 2006a; Rebesco et al., 2010). In these studies, the conditioning effects were documented indirectly, through changed outputs evoked by intracortical microstimulation (Jackson et al., 2006a) or through inferred functional interactions between the recorded populations (Rebesco et al., 2010). Since stimuli could only be delivered after the triggering spikes, only the facilitation phase of the STDP function was explored. Here we report direct evidence of changes in the synaptic strength of single corticospinal cells in monkeys induced by spike-triggered spinal simulation through an autonomous recurrent neural interface operating during free behavior. The finite conduction time from cortex to spinal cord allowed stimuli to be delivered before the arrival of the corticospinal impulses, which produced decreases in synaptic strength, consistent with a bidirectional STDP rule.

RESULTS

Corticomotoneuronal (CM) cells, which connect cerebral cortex to spinal motoneurons, are an important subset of

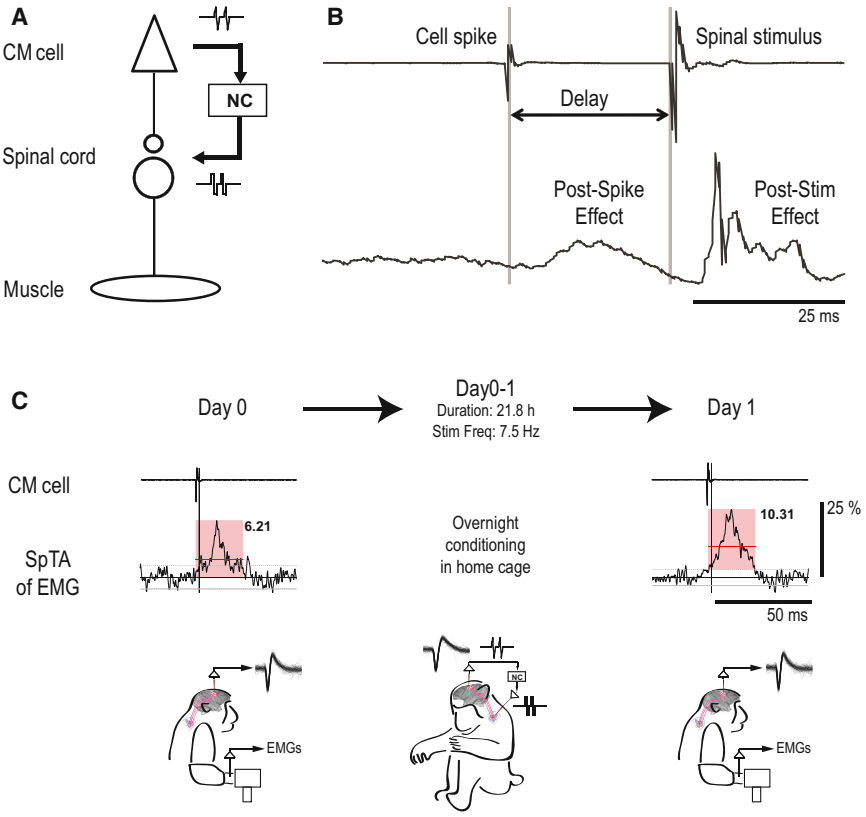

Figure 1. Experimental Design and Protocol

(A) Schematic showing action potentials of CM cell triggering intraspinal stimuli via neurochip (NC).

(B) Cortical recording (top) and SpTA of EMG (bottom) for CM spikes followed after delay of 25 ms by spinal stimulus. SpTA shows postspike facilitation and poststimulus response in same target muscle.

(C) SpTAs of EMG acquired before (day 0) and after (day 1) a 22 hr period of conditioning, showing analysis interval (pink square), baseline ± 2 SD of SpTA (horizontal gray lines), and MPI above baseline of feature (horizontal red lines and black numbers). Conditioning increased MPI by 66% ($p = 0.0003$). Vertical bar calibrates 25% of baseline. Drawings represent monkey performing task on days 0 and 1 and behaving freely during 21.8 hr of conditioning, with mean spike frequency of 7.5 Hz.

primate motor cortical neurons, playing a crucial role in fine control of digits (Porter and Lemon, 1993). Their output effects on target motoneurons can be documented in spike-triggered averages (SpTAs) of rectified electromyographic (EMG) activity and their response properties documented during performance of motor tasks (Fetz and Cheney, 1980; Jackson et al., 2003; Schieber and Rivlis, 2005). We have recorded the activity of the same CM cells over successive days of free behavior, using an implanted electrode array (Jackson and Fetz, 2007) positioned to record CM cells located along the bank of the precentral gyrus (Rathelot and Strick, 2006; Smith and Fetz, 2009). The spinal cord was stimulated with a second set of microwires implanted at fixed depths in the cervical enlargement (Mushahwar and Horch, 1998). An artificial corticospinal connection between a CM cell and its target spinal site was produced with an autonomous head-fixed neural interface that operated continuously during free behavior in the monkeys' home cage (Jackson et al., 2006b, 2007). The so-called "Neurochip" was programmed to detect action potentials of a single CM cell and deliver an intraspinal stimulus after a fixed delay. We chose a spinal site where trains of stimuli evoked responses in one or more of the CM cell's target muscles (Figures 1A and 1B), thus confirming the activation of the cell's postsynaptic motoneurons. During conditioning the spike-triggered spinal stimuli were above threshold for activating motoneurons but too weak to disrupt normal movements or to activate the recorded CM cell, either antidromically or orthodromically (Figure 1B).

The synaptic efficacy of the CM cells' corticospinal terminals was assessed by the magnitude of spike-related effects (SpREs) in SpTAs, compiled while the monkey performed a standard isometric wrist target-tracking task. The magnitudes of the SpREs can be a function of behavior and the amount of EMG activity (Bennett and Lemon, 1994; Davidson et al., 2007), so we compiled the SpTAs using spikes occurring during comparable levels of EMG activity. As discussed in the Supplemental Information available online, under these conditions the main determinant of the changes in magnitude of PSpE is the strength of the mediating postsynaptic potentials. Excitatory SpREs consist predominantly of post-spike facilitation (PSpF), mediated by sufficiently strong synaptic connections of the triggering CM cell to spinal motoneurons and interneurons and beginning at an appropriate latency after the spike (Figures 1B and 2C) (Fetz and Cheney, 1980; Schieber and Rivlis, 2005). Some SpREs can also include an earlier component due to synchrony with other CM cells, as discussed below. The magnitude of the SpRE was quantified by the mean percent increase (MPI) of the feature above baseline (Fetz and Cheney, 1980) and was used to document the change in efficacy of corticospinal terminals after 3.5– 62 hr of conditioning with spike-triggered stimulation (Figure 1C). Postspike suppression effects, as seen in ECR of Figure 2A, are probably mediated by a disynaptic link via inhibitory interneurons, and their MPI is a negative number.

Strengthening of Corticospinal Connections through Spike-Triggered Stimulation

Figure 2A illustrates the output effect in SpTAs of three muscles from a representative CM cell that had an excitatory effect on two flexor muscles and an inhibitory linkage to an extensor muscle. The magnitude and shape of SpREs from this CM cell were stable for 2 days prior to conditioning (cf. day −1 and day 0). After the first 22 hr of spike-triggered stimulation, there was

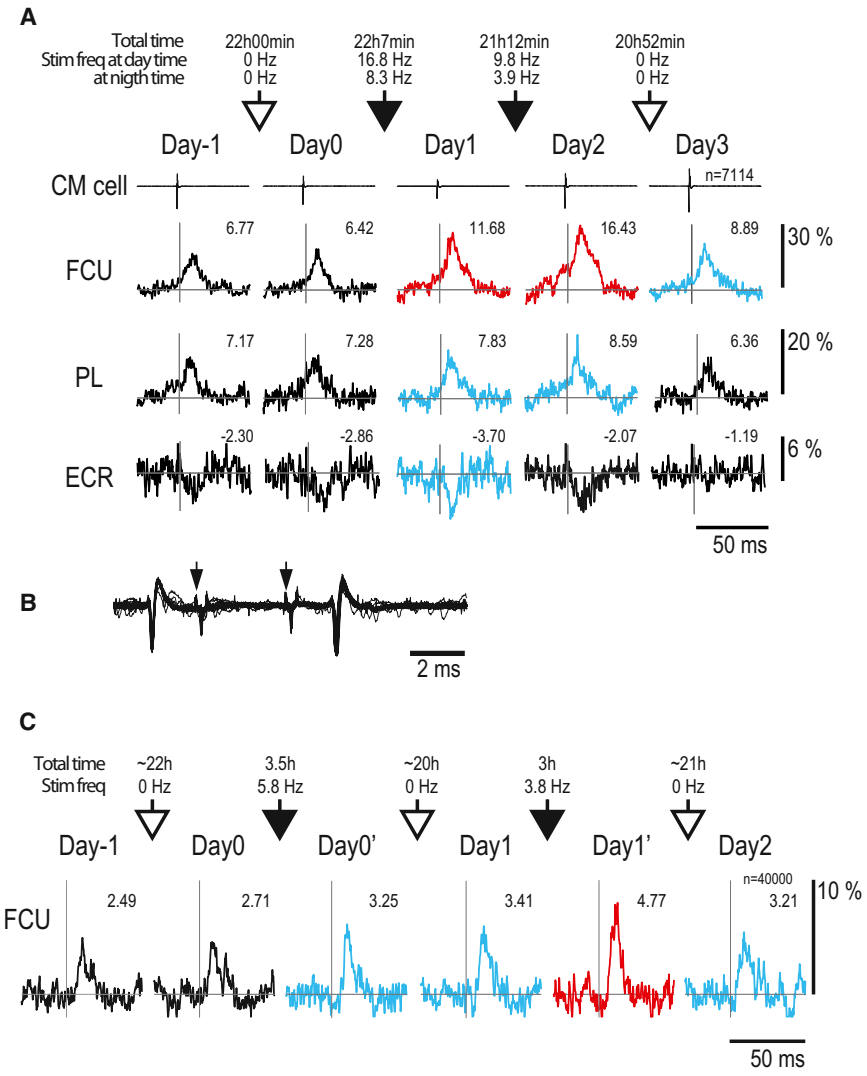

Figure 2. Strengthening of Corticospinal Connections

(A) SpTAs show PSpF in two flexor muscles and PSpS in an extensor. The PSpFs are superimposed on facilitatory synchrony effects in both flexor muscles. Numbers give MPI of the SpREs; vertical line corresponds to trigger event. First two columns show repeatability of SpREs for 2 successive days without intervening conditioning (day −1 and day 0). Open arrows designate periods without conditioning. Solid black arrows designate conditioning periods (50 μA stimuli and 12 ms delay); duration and average stimulus frequencies are given above arrows. Colors of SpTAs for subsequent days indicate significance of changes relative to day 0: red, $p < 0.01$; blue, $p < 0.05$; and black, no significant difference. Scales at right calibrate percentage of baseline. Numbers give MPI. FCU, flexor carpi ulnaris; PL, palmaris longus; ECR, extensor carpi radialis; n, number of triggering spikes for all SpTAs.

(B) Collision test for CM cell in (A). Spontaneous spikes in cell triggered two spinal stimuli (arrows) with intensity sufficient to trigger antidromic spikes (seen after second stimulus); antidromic response for first stimulus is absent due to collision. The current intensity (360 μA) was higher than that used during conditioning.

(C) PSpEs for another CM cell, showing pure PSpF without synchrony. Conditioning times are 3.5 hr on day 0 and 3 hr on day 1.

a statistically significant increase in all the SpREs, both facilitation and suppression (cf. day 1 versus day 0). The facilitation increased further after a second day of conditioning (day 2). The day after the end of conditioning (day 3), the SpREs in one muscle (FCU) remained above preconditioning levels.

The SpREs of CM cells could include "synchrony effects," whose onsets were too early relative to the triggering spike to be mediated by the triggering cell, as seen in FCU and PL muscles of Figure 2A. These early effects are mediated by other CM cells that are synchronized with the trigger cell. Cross-correlograms of synchronized CM cells typically show central peaks straddling the reference cell (Jackson et al., 2003; Smith and Fetz, 2009; Figure S1B), so the average time of the synchronized spikes would be the same as those of the recorded CM cell. Thus, the conditioning paradigm would also strengthen terminals of the synchronized cells and lead to increases in the synchrony effects (see Supplemental Material for further discussion). Additional examples of increases in both PSpF and synchrony effects are shown in Figure S2.

To confirm that the terminals of the triggering CM cell alone could be strengthened, we also measured the effects of conditioning on a pure serial PSpF, shown in Figure 2C. In this case, the PSpF was remeasured after a relatively brief conditioning period of 3.5 hr, which proved sufficient to enhance the PSpF (cf. day 0′ with day 0). Moreover, in this case, the increase in PSpF lasted over 24 hr after conditioning (cf. day 1 with day 0). These changes were confirmed a second time on the subsequent days after further conditioning (day 1 and day 2).

We investigated the effects of this conditioning paradigm in 19 different sessions, using 15 different pairs of CM cells and spinal sites in two monkeys. Conditioning produced significant changes in the 16 sessions that had appropriate spike-stimulus delays (for details of all sessions, see Table S1). The amplitudes of spike-related facilitation and suppression were both found to increase after overnight conditioning with spike-stimulus delays between 12 and 25 ms. The increased output effects were sustained through multiple successive days of conditioning (Figures 2A and S2). Figure 3 plots the sizes of the SpREs before and after conditioning in all the sessions, obtained with spike-stimulus delays between 0 and 500 ms. For delays between 12 and 25 ms, the points are distributed above the unity line, indicating increases in the SpRE for both facilitation (Figure 3Aa) and suppression (Figure 3Ab). These changes tended to be

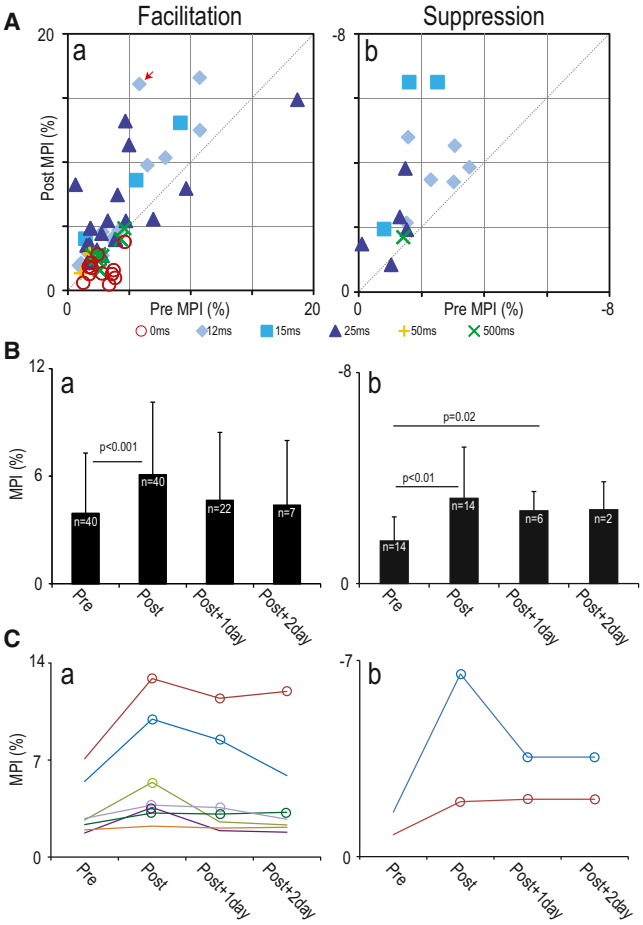

Figure 3. Summary of Conditioning Effect on Output

(A) MPI of spike-related effects in SpTAs compiled before and after conditioning (pre-MPI and post-MPI). Facilitation is shown in (a); suppression is shown in (b). Data points are color coded for different spike-stimulus delays. Data points above the dashed line indicate SpREs that increased after conditioning. The red arrow identifies session in Figure 2A. For sessions involving multiple days of conditioning, the postconditioning values were obtained after the last conditioning period.

(B) Average MPI on pre- and postconditioning day, and 1 and 2 days after the end of conditioning, for facilitation (a) and suppression (b). Error bars show SD. Significance of comparisons is given above horizontal bars.

(C) Individual MPI values in successive days for cell-muscle pairs that were documented for 2 postconditioning days, for facilitation (a) and suppression (b). Open circles for subsequent days indicate significant changes ($p < 0.05$) relative to day 0.

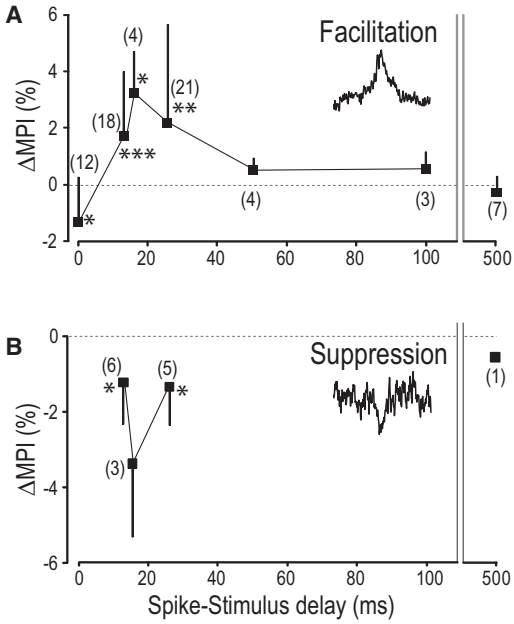

Figure 4. Changes in Spike-Related Effects as Function of Delay between Spikes and Stimuli

The graphs plot average differences in MPI (ΔMPI) for pre- and postcondition days (including the unchanged cases). Numbers of separate observations are shown in parentheses. Error bars show SD. Asterisks denote values significantly different from 0 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

(A) Spike-related facilitation.

(B) Spike-related suppression. For comparison, plots that include only cases in which the PSpEs changed significantly are shown in Figure S3.

sustained 24 hr after the termination of conditioning (Figure 3B), despite the resumption of normal activity. The SpREs remained larger than their original amplitudes 24 hr after the end of conditioning in 71.4% of the cases tested (facilitation: 16 of 22; suppression: 4 of 6) (Figures 2A and 3B). The SpREs for those cells tested after a second postconditioning day are plotted individually in Figure 3C. The SpREs remained larger than their original amplitudes 48 hr after the end of conditioning in 44.4% of the cases tested (facilitation: 2 of 7; suppression: 2 of 2).

Dependence of Conditioning Effects on Delay between Spikes and Stimuli

To document the dependence of the conditioning effects on the time between the CM spikes and the spinal stimuli, we performed the conditioning with different spike-stimulus delays. The overall results, plotted in Figure 4, indicate that significant increases in amplitudes of SpREs could be produced only with delays of 12–25 ms between the action potentials and stimuli, consistent with the effective window of the standard STDP rule (Bi and Poo, 2001; Caporale and Dan, 2008; Markram et al., 2011). Delays of 50 ms and longer were ineffective in producing significant changes. Note that these latter cases involved essentially the same amount and patterns of spinal stimulation as with effective delays, so the absence of synaptic changes serves as a control for nonspecific effects of stimulation.

A particularly significant observation was the reduction of SpREs with zero delays between the spikes and stimuli. This average reduction in MPI was significant at the $p < 0.05$ level. Two examples, obtained in separate monkeys, are illustrated in Figure 5. In these cases the conduction time along the corticospinal pathway caused the CM action potentials to arrive at the stimulation site after the intraspinal stimuli had activated the cells' target motoneurons. Interestingly, two cases show a clear synchrony component prior to conditioning (ED45 in Figure 5A and PL in Figure 5B) that was also reduced. In these cases, the earliest synchrony spikes could arrive at the spinal cord before

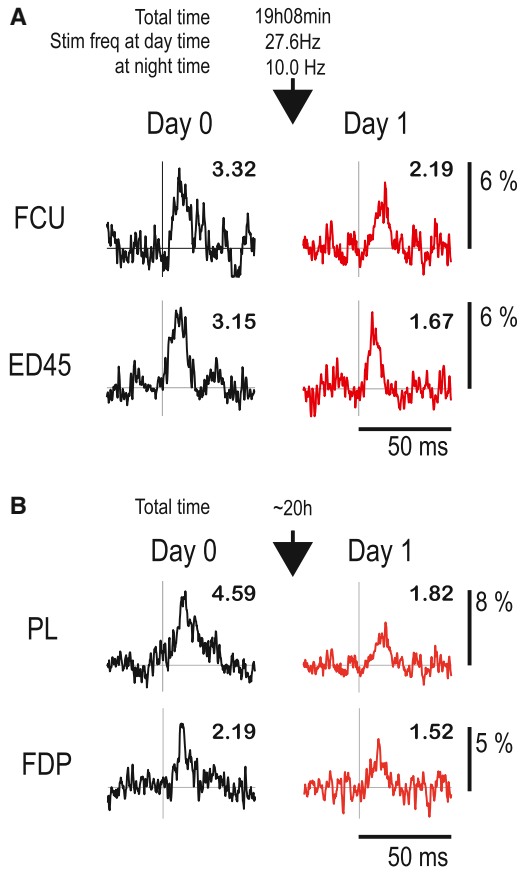

Figure 5. Decreases in SpRE Produced by Intraspinal Stimuli with Zero Delay after Spike

The two cells were recorded in different monkeys. Data were obtained in Sessions 3 (A) and 4 (B) in Table S1. Note the decrease in MPI values after conditioning. ED45, extensor digitorum 4 and 5; FDP, flexor digitorum profundus.

the stimulus, but most spikes of the synchronized cells would not, because they are distributed around the trigger spikes, as shown by central peaks in cross-correlograms of CM cell pairs (Jackson et al., 2003; Smith and Fetz, 2009) (Figure S1B). Therefore, on average, the synchronized spikes would have the same net firing times relative to the spinal stimuli as the trigger spikes and most would arrive after the stimulus. The decrease in the synchrony component therefore confirms the consequent reduction of synaptic efficacy of the synchronized cell(s). The decrease in SpREs when the postsynaptic cells are activated before arrival of the presynaptic input is consistent with a bidirectional STDP rule for excitatory synapses (Caporale and Dan, 2008).

DISCUSSION

Our results provide a direct demonstration that the synaptic strength of single primate CM cells can be systematically changed during unrestrained behavior. We used a recurrent brain-computer interface to trigger intraspinal stimuli from action potentials of single CM cells and activate the cells' target motoneurons at fixed delays. This conditioning paradigm produced changes that correspond with a bidirectional STDP rule previously documented for excitatory synapses from in vitro experiments.

Neural Mechanisms of Conditioning Effect on Corticospinal Connections

The synaptic efficacy of the CM cells' corticospinal terminals was documented by the magnitude of SpREs seen in SpTAs of rectified muscle activity. The SpRE often include two distinguishable components that are superimposed (e.g., Figures 1C, 2A, and S2) and are produced by different mechanisms. A postspike facilitation (PSpF) with an onset time consistent with serial conduction times from cortex to muscles (i.e., greater than 5 ms) is likely to be mediated by monosynaptic or disynaptic linkages from the trigger cell to motoneurons of the target muscle (Fetz and Cheney, 1980; Porter and Lemon, 1993). In addition, many SpREs show a "synchrony facilitation" evident in an increase above baseline that begins earlier and that would be produced by other CM cells that fire synchronously and have some spikes that occur prior to the trigger cell spikes (Jackson et al., 2003; Smith and Fetz, 2009; Davidson et al., 2007). Many of the SpREs show evidence of a clear PSpF superimposed on a broad synchrony peak (Figures 2A, 5B, S1C, and S2). Sometimes these two features look distinct enough to invite separation, but reliable quantification of the separate components is problematic for several reasons. The separation would require an accurate estimate of the time course of the synchrony component beneath the PSpF, which is ultimately unknown. Even if it could be reliably estimated, subtraction of the synchrony component would assume linear summation, which is not the case for averages of rectified EMG (Baker and Lemon, 1995). Moreover, in many cases, these two components are hard to distinguish because the rise of the serial PSpF merges with the synchrony peak, as in Figure S2. For these reasons, we have not attempted to separate and extract quantitative measures of these superimposed components.

We found that the conditioning paradigm changed the size of the synchrony component similarly to the changes in the pure PSpF. This would be predicted from the fact that spikes of synchronized cells are distributed around the trigger cell and therefore have the same average timing relative to the spinal stimuli. Thus, spike-triggered stimulation would affect the spinal terminals of other corticospinal neurons firing synchronously with the trigger cell in the same way as terminals of the trigger cell. This was seen with the increase in the size of the early synchrony component, similar to the increase in the serial PSpF (e.g., Figure 2A, FCU, day 2, and Figure S2; see discussion in Supplemental Materials), as well as the decrease in the synchrony component for zero delays (Figures 5A and 5B).

Figure 6 illustrates the likely mechanism producing conditioned increases in the strength of corticospinal synapses and in the resultant postspike effects for positive spike-stimulus delays. When the spinal neurons were activated 12–25 ms after arrival of the descending action potential, both excitatory and inhibitory SpREs were enhanced. Increases in the excitatory SpREs probably involve the strengthening of corticomotoneuronal terminals of the CM cells on target motoneurons, as

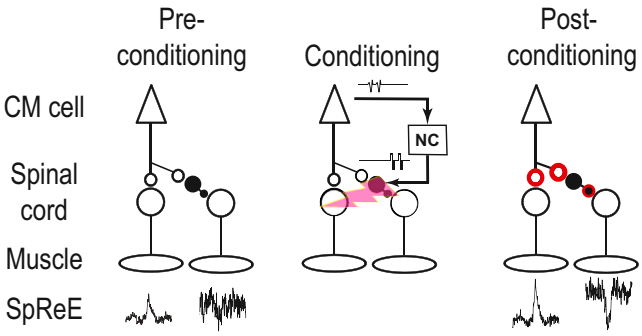

Figure 6. Proposed Mechanisms for Conditioning Effect
Spike-triggered stimulation with appropriate positive delay produces a strengthening of CM cell terminals, increasing direct excitatory postspike effects. Changes in inhibitory effects could involve terminals of CM cell and/or inhibitory interneuron. The strengthened terminals are illustrated in red.

illustrated. The mechanisms for enhancing the disynaptically mediated postspike suppression could involve changes at two sites: in the terminals of the CM cell or terminals of the mediating inhibitory interneurons (IINs) or both. Previous studies found that the polarity of the bidirectional STDP rule is reversed for some synapses from excitatory to inhibitory neurons (Bell et al., 1997; Tzounopoulos et al., 2004). This would produce decreases in suppression, counter to our observation, but the rule for descending excitatory connections to spinal IINs remains to be determined. Given that postspike suppression appeared in antagonists of the facilitated muscles, the underlying pathway probably involved Ia inhibitory interneurons (Jankowska et al., 1976), which are glycinergic (Fyffe, 1991). Other spinal IINs are GABAergic. In any case, assuming that stimuli activated the IINs, our evidence suggests the standard STDP rule for positive delays would apply to the corticospinal input to spinal IINs. Consistent with this, Iriki et al. found that tetanic stimulation of the pyramidal tract produced increases in corticospinal postsynaptic potentials in all of their unidentified spinal interneurons (Iriki et al., 1990). Our conditioning protocol could also strengthen the synaptic connection of the IINs to motoneurons, consistent with the STDP rule for GABAergic synapses (Haas et al., 2006; Woodin et al., 2003). Future experiments are needed to resolve the plasticity rules at these two synapses. Whatever the outcome, our results show that the net effect mediated via the disynaptic inhibitory linkage is enhanced by appropriate spike-triggered stimulation. Simultaneously strengthening both excitatory outputs to agonist muscles and inhibitory linkages to antagonists makes functional sense in maintaining the balance of effects that CM cells have on their reciprocal targets.

In vitro studies have elucidated the properties of the bidirectional STDP rules in a variety of supraspinal synapses (Bi and Poo, 2001; Caporale and Dan, 2008; Markram et al., 2011). The long corticospinal conduction time allowed demonstration that stimulation of postsynaptic cells prior to the arrival of the presynaptic impulses produced decreases in synaptic efficacy, consistent with bidirectional STDP documented in in vitro experiments. Evidence for such decreases in synaptic efficacy when postsynaptic responses precede presynaptic input has been found in sensory systems of other in vivo preparations (Jacob et al., 2007; Meliza and Dan, 2006; Mu and Poo, 2006). In most previous studies, the conditioning protocol employed paired electrical stimulation. In contrast, we triggered stimuli from action potentials generated during normal behavior, which may produce more effective conditioning (Jackson et al., 2006a; Rebesco et al., 2010), perhaps due to the enhanced efficacy of natural activity patterns, known to affect plasticity (Caporale and Dan, 2008; Sjöström et al., 2001) and possible activity-dependent delivery of facilitating neuromodulators.

Duration of Conditioned Effects

In most in vitro experiments the duration of so-called "long-term" potentiation and depression has typically been tested for up to several hours and found to last that long in the absence of neural activity. In most in vivo studies, the conditioned effects decay over periods of hours after the end of conditioning (Lucas and Fetz, 2013; Rebesco et al., 2010; Wolters et al., 2003). An exceptionally long-lasting effect of many days was seen with spike-triggered stimulation of nearby motor cortical sites (Jackson et al., 2006a). We found that conditioned changes in the strength of corticospinal connections lasted for variable times, in some cases up to at least 2 days postconditioning. The conditioned changes of connections to different motoneuron pools from the same CM cell could survive for different durations (e.g., Figure 2A). Thus, after the end of spike-triggered stimulation, the natural activity patterns of the pre- and postsynaptic neurons tend to eventually drive synaptic strengths back to preconditioning levels. The synaptic strengths can be influenced by two opposing factors. Under purely random pre- and postsynaptic timings, the strength of synapses would tend to decrease because the two components of the bidirectional STDP function are usually asymmetric, with greater net suppression. However, this effect is countered when presynaptic inputs tend to occur more often before the postsynaptic activity. Thus, under normal behavioral conditions, the balance of these and possibly other factors would determine the strength of the "steady-state" synaptic connections. Differences in the decay rate of the conditioned changes would reflect differences in the relative efficacy of these factors.

Clinical Relevance

Toward promoting neural plasticity in the motor system, electrical stimulation has been delivered in cerebral cortex (Brown et al., 2003) and spinal cord (Courtine et al., 2009; van den Brand et al., 2012) during movement. Such externally programmed stimulation has shown promise for facilitating recovery from the deficits of stroke (Brown et al., 2003) and spinal cord injury (Edgerton et al., 2008). The underlying mechanisms are likely to involve Hebbian strengthening of the neural connections that are activated during movements. Delivery of repetitive stimulation has typically been preprogrammed. Recent evidence suggests that a more effective strategy to promote long-lasting plasticity may be prolonged activity-dependent stimulation (Jackson et al., 2006a; Edwardson et al., 2013).

The success of our protocol suggests that neurorehabilitative treatment for patients with damaged corticospinal pathways could exploit similar paradigms to strengthen spared

corticospinal connections (Jackson and Zimmermann, 2012). Previous studies have documented spinal cord conditioning produced by pairing peripheral nerve stimulation with cortical stimulation or with voluntary activation in able-bodied subjects (Khaslavskaia and Sinkjaer, 2005; Stefan et al., 2000). Pairing transcranial magnetic stimulation with antidromic activation of motoneurons at appropriate times enhanced the motor potentials evoked by stimulating corticospinal axons and improved motor performance in normal subjects (Taylor and Martin, 2009) and in patients with spinal cord injury (Bunday and Perez, 2012). A similar mechanism appears to play a role in the lasting therapeutic effects of functional electrical stimulation applied to peripheral nerves to overcome foot drop (Everaert et al., 2010).

The translation of our activity-dependent stimulation paradigm to practical clinical applications would be promoted with less invasive procedures. Instead of recording neural activity with intracortical electrodes, it is possible to record activity-dependent cortical potentials less invasively through surface electrodes (Miller et al., 2012; Nishimura et al., 2013) or to use muscle activity as a surrogate of cortical activity (Lucas and Fetz, 2013; Nishimura et al., 2013). The intraspinal stimulation that we used is known to activate many afferent fibers of passage (Gaunt et al., 2006), suggesting that less invasive spinal surface stimulation could also be effective in cortically triggered conditioning.

A second issue for clinical application is the duration of effects. In our experiments, the synaptic changes could be rapidly induced, and many eventually reverted in the days after the end of conditioning. The changes could be maintained by continuing the conditioning protocol for several days. It is possible that more prolonged conditioning could lead to more permanent changes. In future studies, longer-lasting changes might also be induced by combining this paradigm with additional methods to strengthen the effects and make them more permanent (Jefferson et al., 2011; van den Brand et al., 2012). These include simultaneous DC polarization (Delgado-Lezama et al., 1999) or pharmacological intervention (Alilain et al., 2011; van den Brand et al., 2012). Another key factor in obtaining long-term effects is prolonged conditioning during volitional movements (Everaert et al., 2010), which could lead to structural changes and can be implemented with the sort of portable recurrent neural interface described here.

EXPERIMENTAL PROCEDURES

Subjects

Experiments were performed with two male *Macaca nemestrina* monkeys (4–5 years old, weight 4.0 and 5.5 kg). All procedures were approved by the University of Washington Institutional Animal Care and Use Committee.

Recording

Activity of single CM cells in the precentral gyrus was recorded over successive days using chronically implanted, hand-moveable tungsten microwire electrodes (Jackson and Fetz, 2007) (diameter 50 μm; impedance 0.5 MΩ; interelectrode spacing 500 μm). The electrodes could be positioned along the bank of the precentral gyrus, where many CM cells reside (Rathelot and Strick, 2006; Smith and Fetz, 2009). Each session began by recording the cell's responses during an isometric, 2D eight-target wrist torque tracking task. Action potentials of cells were initially identified by a spike-sorting device based on a template-matching algorithm (MSD, Alpha Omega Engineering). The output effects of CM cells on forearm muscles were identified by perispike features in SpTAs of rectified EMG. Between these sessions of task performance, lasting about 3–5 hr, the monkey was returned to the home cage, where movements were unrestricted.

Stimulation

Spinal stimuli were delivered through polyurethane-coated platinum iridium wire electrodes (diameter 30 μm; impedance 200–600 kΩ) implanted chronically in the cervical spinal cord at the levels of C6-T1 (Mushahwar and Horch, 1998). Sites from which trains of stimuli evoked responses in one or more target muscles of the CM cell were chosen for conditioning. During conditioning, stimulation intensities were subthreshold for generating antidromic or orthodromic responses in the recorded cells but activated the cells' target motoneurons.

Conditioning

The Neurochip was programmed to generate acceptance pulses when the waveform of the CM cell exceeded a threshold above the noise level and passed through two time-amplitude windows (Jackson et al., 2006b; Mavoori et al., 2005). After a specified delay after the action potential, the Neurochip delivered a biphasic, constant-current stimulus pulse (0.2 ms/phase) to a wire electrode in the cervical spinal cord. To confirm unit isolation and reliable triggering during free behavior, we stored short sections of raw recording (sampled at 11.7 kHz) to on-board memory, interspersed with stimulation rate in 1 s bins during the conditioning period (Jackson et al., 2006b; Mavoori et al., 2005).

Analysis

We compiled the SpTA when the cell and its target muscles were coactivated during performance of a center-out eight-target acquisition task. To facilitate comparison of SpTAs from successive days, we accepted trigger spikes for SpTAs only when they occurred within the same range of EMG activity. The numbers of triggers were equalized to the minimum number "n" obtained for any of the SpTAs by accepting only the first n triggers for all SpTAs. To detrend the baseline from raw SpTA, we fitted the baseline trends by a polynomial of appropriate order (1 to 4) and subtracted this from the original SpTA trace. The features in the SpTAs were identified by consistent changes in values above or below 2 SD of baseline for facilitation and suppression, respectively. Baseline was defined as the interval from 30 to 10 ms preceding the trigger spike. Onset and offset of the facilitation (or suppression) were defined as the first and last value above (or below) 2 SD of baseline. The mean percent increase (MPI) measured the average values between onset and offset of the feature minus baseline, divided by baseline (Fetz and Cheney, 1980). The MPI had negative values for suppression effects. The significance of the MPI features was determined by means of a t test.

Significant differences in MPI produced by conditioning were determined by comparing MPI on postconditioning days with MPI on day 0 (Figures 1C, 2, 3C, and 5), using one-way ANOVA with repeated measures. Post hoc multiple comparisons were conducted using the Bonferroni test (Figure 3B). To document the significance of the changes in MPI (ΔMPI, Figures 4 and S3), we confirmed for each spike-stimulus delay whether the mean of the distribution was significantly different from zero as follows. We first tested whether the sample sets for each delay were more consistent with a normal distribution or an exponential distribution using the Lilliefors test ($p > 0.05$). For the normally distributed values, the single-tailed Student's t test was used to determine whether the mean of the distribution was significantly different from zero. For the exponentially distributed values, the Wilcoxen signed-rank test was used to determine whether the distribution median was significantly different from zero. The p values for differences from zero are designated by asterisks. For points designated by "(1)," the number of samples was too small to provide meaningful statistics.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Material, three figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2013.08.028.

AUTHOR CONTRIBUTIONS

Y.N. and R.W.E. conducted the experiments and analyzed the data. E.E.F., Y.N., and S.I.P. conceived the study and wrote the manuscript.

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