Dose-response curve of associative plasticity in human motor cortex and interactions with motor practice

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Submitted 22 October 2012; accepted in final form 19 October 2013

Dose-response curve of associative plasticity in human motor cortex and interactions with motor practice. J Neurophysiol 111: 594–601. 2014. First published November 6, 2013; doi:10.1152/jn.00920.2012.—Associative plasticity is hypothesized to be an important neurophysiological correlate of memory formation and learning with potentials for applications in neurorehabilitation and for the development of new electrophysiological measures to study disorders of cortical plasticity. We hypothesized that the magnitude of the paired associative stimulation (PAS)-induced long-term potentiation (LTP)-like effect depends on the number of pairs in the PAS protocol. We also hypothesized that homeostatic interaction of PAS with subsequent motor learning is related to the magnitude of the PAS-induced LTP-like effect. We studied 10 healthy subjects. In experiment 1a, subjects received 90 (PAS90), 180 (PAS180), or 270 (PAS270) pairs of stimuli, followed by a dynamic motor practice (DMP) 1 h after the end of the PAS protocols. In experiment 1b, the DMP preceded the PAS protocol. In experiment 2, the time course of PAS270 was studied. We found that PAS270 resulted in greater increase in motor evoked potential (MEP) amplitude compared with protocols with fewer pairs of stimuli. Moreover, the interaction between PAS protocols with motor learning differed depending on the number of stimulus pairs used to induce PAS. While DMP alone increased MEP amplitudes, DMP during the LTP-like effects induced by PAS270 led to a long-term depression (LTD)-like effect (homeostatic interaction). This homeostatic interaction did not occur after PAS90 and PAS180. In conclusion, we found a dose-dependent effect of the number of stimulus pairs used in the PAS protocol on cortical plasticity. Homeostatic interaction between PAS and DMP was observed only after PAS270.

Hebbian plasticity; motor cortex; motor learning; paired associative stimulation; transcranial magnetic stimulation

LONG-TERM POTENTIATION (LTP) likely plays an important role in motor learning. One method of studying plasticity in human motor cortex is paired associative stimulation (PAS). This method pairs transcranial magnetic stimulation (TMS) and peripheral nerve stimulation to alter cortical excitability. The PAS protocol has been shown to result in both LTP- and long-term depression (LTD)-like plasticity depending on the time interval between TMS and peripheral nerve stimulation (Stefan et al. 2000). To explain the relationship between pre- and postsynaptic activity and strength of synaptic transmission, Hebb (1949) and later Bienenstock, Cooper, and Munro (BCM) showed in their models that the efficacy of a given synapse depends not only on instantaneous pre- and postsynaptic activities but also on a slowly varying time-averaged value of the postsynaptic activity (Bienenstock et al. 1982). One approach to test this model is to pair activation of pre- and postsynaptic neurons at different intervals and record synaptic activity after a certain number of stimuli (Bortolotto et al. 2011). PAS can be used to test rules governing cortical plasticity in humans. It is important to note that the BCM model suggested a nonlinear (sigmoidal) relationship between pre- and poststimulation time intervals but made no assumptions regarding the relationship between the number of stimulus pairs and plasticity. The role of the number of stimulation pairs is assessed in the present study (Rubin 2001; Song et al. 2000; van Rossum et al. 2000).

Abraham and Bear (1996) introduced the concept of “metaplasticity,” which refers to a higher-order form of synaptic plasticity in which pre synaptic activity leads to a persistent change in the direction or magnitude of subsequent activity-dependent plasticity, without affecting the actual synaptic efficacy. Metaplasticity serves as a homeostatic factor because it ensures that plasticity is kept within a working range and away from saturation. Although the BCM model of synaptic plasticity explains the synaptic behavior of neurons in response to their activities, the rules governing these metaplastic interactions have not been clearly defined. In the present study, we investigated the effects of three different PAS interventions with different numbers of stimulation pairs and their interactions with subsequent motor learning.

We hypothesized that PAS effect is dose dependent. We also examined the metaplastic interactions between PAS effects and motor learning. We tested the hypothesis that homeostatic response occurs after a certain threshold of PAS-induced LTP has been exceeded.

MATERIALS AND METHODS

Subjects

Ten healthy subjects (6 men and 4 women) with no history of neurological or psychiatric disorders and with normal neurological examination were recruited. The majority of the participants were right-handed (8 right-handed, 2 left-handed), with a mean age of 39.6 ± 14 yr (range 20–58 yr). The University Health Network Research Ethics Board approved the study, and all subjects provided written informed consent.

Stimulation

Four Magstim 200 stimuli (Magstim, Whitland, UK), connected to a 4-in-1 combining box to deliver pulses with different

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intensities, and a figure-of-eight-shaped coil (outside diameter of each loop was 9.5 cm) were used to apply TMS to the left motor cortex (M1). The trigger pulses for TMS were delivered from a Micro1401 interface (Cambridge Electronics Design) controlled by Signal Software (version 3.07). The coil was held with the handle pointing laterally at a 45° angle to the sagittal plane at the optimal scalp site to evoke a motor evoked potential (MEP) in the relaxed abductor pollicis brevis (APB) muscle of the right hand. The motor hot spot was then marked and was used for the rest of the session. MEP was recorded through surface bipolar Ag-AgCl electrodes from the right APB at rest. EMG was continuously monitored with visual and auditory feedback to ensure complete muscle relaxation. The EMG signals were amplified 1,000 times, filtered (2 Hz–2 KHz), and digitized through a CED 1401 interface.

**Paired Associative Stimulation**

PAS involves a series of paired peripheral and cortical stimuli. Electrical stimuli were delivered to the median nerve of the right wrist at an intensity sufficient to produce a small motor response in the APB muscle. The median nerve was stimulated with a constant-current stimulator (Digitimer DSTA, Digitimer, Welwyn Garden City, UK) through bipolar electrodes at the wrist with a 200-μs square wave pulse. The cathode was positioned proximally. The median nerve stimulus was followed by suprathreshold TMS to the left M1 adjusted to produce 1-mV peak-to-peak MEP amplitude in the APB muscle. The interval between median nerve stimulation and TMS was set to 25 ms to induce facilitatory PAS. We used three different durations of PAS with 90, 180, and 270 pairs of stimuli delivered at 0.25 Hz. These interventions are termed PAS90, PAS180, and PAS270.

**Dynamic Motor Practice**

The subjects performed brisk isometric abductions with their right thumb to a target force window. The subjects’ hands except the thumb were fixed with the palm down and the forearm resting on a table. The thumb abduction force was recorded by a high-accuracy force transducer (model LCCA-25, Omega, Stamford, CT). The tactile “Go” signal was generated by an electrical stimulation of the median nerve through bipolar electrodes at the wrist at 200% of perceptual threshold through bipolar electrodes brevis (APB) muscle of the right hand. The motor hot spot was then marked and was used for the rest of the session. MEP was recorded through surface bipolar Ag-AgCl electrodes from the right APB at rest. EMG was continuously monitored with visual and auditory feedback to ensure complete muscle relaxation. The EMG signals were amplified 1,000 times, filtered (2 Hz–2 KHz), and digitized through a CED 1401 interface.

**Experiments**

The four sessions of experiments 1a (3 sessions) and 1b (1 session) occurred at least 1 wk apart and in random order. In all experiments, MEP changes were captured with both input-output (I/O) curve and single-intensity TMS. I/O curve in APB muscle was measured at four time points: before (Tpre), at 15 and 30 min after the first (priming) intervention (T15 and T30), and at 15 min after the second intervention (T’15) (Fig. 1). The I/O curves were obtained from four intensities at 100%, 110%, 120%, and 130% of baseline resting motor threshold (RMT) with 10 TMS pulses for each intensity. RMT was defined as the minimum stimulator output that evoked MEPs of >50 μV in at least 5 of 10 trials with the APB muscle completely relaxed. Single-intensity TMS at 130% of RMT was used to capture changes immediately after the priming intervention (T0) and immediately after the second intervention (T’0). Twenty TMS pulses were used to produce the average MEP for the single-intensity measure, and the results were compared to 10 TMS pulses at 130% of RMT at baseline. Intensities for both I/O curve and single-intensity TMS were adjusted to RMT at baseline and remained constant for the rest of the experiment.

**Experiment 1. PAS and motor learning interactions.** EXPERIMENT 1A. EFFECT OF NUMBER OF PAS PAIRS ON LTP-LIKE PLASTICITY AND INTERACTIONS WITH MOTOR LEARNING. The experimental design is outlined in Fig. 1. Subjects received PAS90, PAS180, or PAS270, each followed by DMP at 1 h after the end of the PAS, or experiment 1b (DMP followed by PAS90) in four separate visits at least 1 wk apart in random order.

**Experiment 1B. EFFECTS OF MOTOR LEARNING ON PAS90.** The design was similar to that of experiment 1a except that we changed the order of DMP and PAS. Subjects underwent DMP first, followed by PAS90 1 h later. This study was designed to assess the effect of motor learning on MEP and to evaluate the effect of motor learning on PAS. T0 and T’0 are the immediate measurements after DMP and after PAS. I/O curve was measured at four time points: before DMP (Tpre), 15 and 30 min after DMP (T15 and T30), and 15 min after PAS90 (T’15) (Fig. 1). The four sessions of experiments 1a (3 sessions) and 1b (1 session) occurred at least 1 wk apart and in random order.

**Experiment 2. Time course of PAS.** The effect of PAS270 without DMP was assessed in six subjects, who also participated in experiment 1. After PAS270, the subjects were followed for 75 min and MEP and I/O curve were measured at the same time points as in

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Fig. 1. General outline of studies. Sessions in experiments 1a (3 sessions) and 1b (1 session) were conducted in random order. Experiment 2 was performed in a separate session from that of experiment 1. RMT, resting motor threshold; I/O, input-output; MEP, motor evoked potential; PAS, paired associative stimulation.
experiment 1a (Fig. 1). Experiment 2 was separated from experiment 1 by at least 1 wk.

Statistical Analysis

Repeated-measures analysis of variance (RM ANOVA) was used to compare means in a general linear model using SPSS 19.0. Sphericity of variance was assumed in RM ANOVA when Mauchly’s test of sphericity was not significant. If sphericity was violated, Greenhouse and Geisser correction was applied (Greenhouse and Geisser 1959). In all statistical analyses of cortical excitability the ratio of MEP amplitude to baseline (Tpre) was calculated. The use of the ratio to baseline values reduces the effects of baseline MEP differences by normalizing the values across subjects. However, it is important to note that in analysis of I/O curves the ratios show changes from baseline and they do not represent MEP amplitudes. Therefore, I/O curve slopes were analyzed further by calculating the slope of each curve with a linear regression model and line fitting for intensities at each time point. The calculated slopes were normalized to baseline time point and were used in RM ANOVA to detect changes in I/O curve slope by the factors “Time” (3 levels: Tpre–T30) and “PAS duration” (3 levels: PAS90, PAS180, and PAS270). Post hoc analyses of significant findings were performed with paired t-test and corrected for multiple comparisons with Bonferroni correction.

The factor “Time” represents the time point at which measurements were made. For the intensity of 130%RMT, two extra time points (T0, T’0) were measured immediately after the first or second intervention (Fig. 1). The intensity of 130%RMT for the remaining time points was measured in combination with other intensities in I/O curves. In experiment 1a, “PAS duration” (3 PAS interventions with 90, 180, and 270 paired stimuli), “Time,” and “Intensity” were used as within-subjects factors. Post hoc analyses of significant findings were performed with paired t-test and corrected for multiple comparisons with Bonferroni correction. To investigate the interaction between “PAS duration” and DMP on MEP amplitudes induced at the intensity of 130%RMT, a separate RM ANOVA with factors “Time” and “PAS duration” was performed. The intensity of 130%RMT was used because the extra time points show the time course with greater detail. For experiments 1b and 2, two separate RM ANOVAs, one for the I/O curves with factors “Time” and “Intensity” and one for intensity of 130%RMT with factor “Time,” were used. Student’s t-test was used for post hoc comparison of significant effects. To assess the contribution of background muscle activity to changes in MEP amplitudes, root mean square (RMS) amplitude EMGs for 40 ms just before TMS were calculated. RM ANOVA was used to assess the changes in background EMG activity over time and in different studies.

To investigate whether PAS durations (PAS90, PAS180, PAS270) and the level of LTP-like plasticity after PAS can predict the degree of homeostatic interaction, we used multivariable linear stepwise regression analysis with factors “PAS duration” (3 levels) and “Maximum MEP amplitude” (maximum value of the average of 10 stimuli for each intensity between T0 and T30) and “Maximum MEP amplitude ratio” [maximum value of the average of 10 stimuli for each intensity between T0 and T30 as a ratio to baseline values (Tpre)] and “homeostatic response” as dependent variable. “Homeostatic response” was calculated by subtracting the average of MEP amplitude ratios to baseline (Tpre) after the second interventions (T’15) from average of MEP amplitude ratios of I/O curves of T15 and T30 (Fig. 1). When this value is positive it means the direction is in favor of homeostatic changes, and larger positive values mean larger homeostatic interaction. On the other hand, negative values suggest nonhomeostatic interaction.

RM ANOVA with factors “Intervention” (PAS90, PAS180, and PAS 270 conditioning and no PAS conditioning) and “Trial block” (blocks of motor practice) as within-subject variables was used to investigate the performance in DMP with a preceding PAS paradigm (experiment 1a) or without a conditioning paradigm (experiment 1b).

RESULTS

RMT at baseline was 51.12 ± 1.5% (mean ± SE) of stimulator output. RM ANOVA showed no significant difference in RMT in different study sessions. The mean MEP amplitudes for the baseline (Tpre) I/O curve were 0.1, 0.33, 0.75, and 1.19 mV for 100%, 110%, 120%, and 130% of RMT, respectively. Mean stimulus intensity to generate 1-mV amplitude was 70.2 ± 17.6% of stimulator output. RM ANOVA showed no significant difference of intensity among study sessions.

Background Muscle Activities

The RM ANOVA on the prestimulus EMG RMS amplitudes showed no modulation of background APB muscle activity over time \(F(3,0.35), P = 0.741\). The grand average of background RMS EMG was 7 μV. Therefore, the changes in MEP amplitudes cannot be explained by changes in background muscle activities.

Experiment 1a. Effects of Three Different PAS Durations on Corticospinal Excitability

RM ANOVA for the factors “PAS duration” (3 levels: PAS90, PAS180, PAS270), “Time” (3 levels: Tpre, T15, T30), and “Intensity” (4 levels: 100–130%RMT) as within-subject factors for ratio of MEP to baseline measured in I/O curves of APB muscle after PAS showed a significant effect of “PAS duration” \(F(2,4.292), P = 0.032\). “Time” \(F(2,5.932), P = 0.012\), and “PAS duration” × “Time” interaction \(F(4,1.631), P = 0.016\) and no significant effect of “Intensity.” It should be noted that since the MEP amplitudes were normalized to baseline for each intensity in the I/O curve, an effect of stimulus intensity within each “Time” point is not expected. The fact that we did not detect a significant “Intensity” × “Time” interaction suggests that the shape of the I/O curve was stable over time (Fig. 2).

Post hoc paired t-test of I/O curves showed significantly higher APB MEP amplitude ratio to baseline for all time points and intensities after PAS270 compared with PAS90 (\(P = 0.022\)) and a trend for higher MEP amplitude ratio compared with PAS180 (\(P = 0.073\)). No significant difference between PAS90 and PAS180 was detected. This finding supports our primary hypothesis that increasing the number of PAS repetition pairs increases the LTP-like effects produced by PAS. Post hoc analysis for the factor “Time” showed that significant increase in MEP amplitude was observed for T15 (\(P = 0.05\)) and T30 (\(P = 0.021\)) compared with Tpre. No significant differences were detected between T15 and T30. Figure 2 shows that the significant “PAS duration” × “Time” interaction is due to increased effect of PAS270 from T15 to T30, whereas the effect of PAS90 decreased from T15 to T30. This finding indicates that the duration of LTP-like effect is also influenced by PAS duration.

Analysis of I/O Curve Slope

I/O curve slopes at baseline for PAS90, PAS180, and PAS270 protocols were 1.197 ± 0.45, 1.22 ± 0.49 and 1.23 ± 0.48, respectively. No significant difference was found between I/O curve slopes at baseline. The slope of each I/O curve normalized to baseline was used in RM ANOVA with factors “Time” (3 levels: Tpre, T15, T30) and “PAS duration” (3

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levels: PAS90, PAS180, PAS370). This analysis showed a significant effect of “Time” \(F(2,3.5), P = 0.047\), a trend for “PAS duration” \(F(2,3.21), P = 0.063\), and no “PAS duration” \(\times\) “Time” interaction. Post hoc analysis for “Time” showed a significant increase in I/O curve slope ratios from baseline \((P = 0.049)\) and a significant change increase in I/O curve slope in PAS270 compared with PAS90 \((P = 0.045)\) (Fig. 2D).

Interactions Between PAS and DMP

We used MEP amplitudes generated by 130%RMT to investigate PAS-DMP interactions (Fig. 3A). RM ANOVA for MEP ratio to baseline with factors “PAS duration” (3 levels: PAS90, PAS180, PAS270) and “Time” (5 levels: T0, T15, T30, T0, T15) showed a significant “PAS duration” \(\times\) “Time” interaction \(F(8,5.34), P < 0.001\). No significant effects for factors “Time” and “PAS duration” were detected. The significant interaction was because of the reduction of MEP amplitude after DMP in the PAS270 group, whereas MEP amplitude increased after DMP in the PAS90 and PAS180 groups. These findings indicate homeostatic interaction between PAS270 and DMP, and no such interaction was found in PAS90 and PAS180 groups (Fig. 3A).

Peak MEP Amplitude and Type of Priming Intervention Predict Magnitude of Homeostatic Interaction

When the variables “PAS duration,” “Maximum MEP amplitude,” and “MEP amplitude ratio” were entered into a multiple linear regression model, 57% of the variation of “Homeostatic response” was explained. The correlation with “MEP amplitude ratio” \((P = 0.34)\) was not significant, whereas the correlations with “Maximum MEP amplitude” \((P = 0.01)\) and “PAS duration” \((P < 0.001)\) were significant. When “MEP amplitude ratio” was removed from the model, the remaining two variables explained 56% \((P < 0.001)\) of the variation of “Homeostatic response.” “PAS duration” alone explained 39% and “Maximum MEP amplitude” accounted for 17% of this variation. Because there was a significant effect of “PAS duration,” each duration was examined separately. The Pearson correlation coefficients were significant only for PAS270 \((R^2 = 0.59, P = 0.006;\) Fig. 4).

Experiment 1b. Effect of DMP on MEP and PAS90

RM ANOVA with “Time” (4 levels: Tpre–T15) and “Intensity” (4 levels: 100–130%RMT) as within-subject factors for MEP amplitude ratios in I/O curve of APB showed a significant effect of “Time” \(F(3,4.227), P = 0.016\) and no significant effect for “Intensity” and “Intensity” \(\times\) “Time.” Similarly, RM ANOVA for intensity of 130% RMT with “Time” (6 levels: Tpre–T15) showed a significant effect of “Time” \(F(5,4.5), P = 0.001\), and pairwise comparison of the MEP amplitude at different time points showed a nonsignificant MEP increase at T15 \((P = 0.178)\) compared with Tpre and

Fig. 2. Changes in MEP amplitude ratios to baseline (Tpre) over time in experiment 1a. Each line represents 1 intensity in the I/O curves. Each PAS protocol is shown in a separate graph: PAS90 (A), PAS180 (B), and PAS270 (C). Values above 1 indicate an increase in MEP amplitude ratio [long-term potentiation (LTP)-like effect], and values below 1 indicate reduction in MEP amplitude ratio [long-term depression (LTD)-like effect] compared with baseline. Dr: changes in I/O curve slope. For each time point, slope of the I/O curve was calculated by linear regression, was normalized to the baseline slope, and is plotted against time. Error bars represent SE.

Fig. 3. Changes in MEP amplitude ratio to baseline (Tpre) over time in experiment 1b. Each line represents 1 intensity in the I/O curves. Each line represents 1 PAS session in experiment 1a (A) or experiment 1b (B), with the dynamic motor practice (DMP) followed by PAS90 just before T0. Error bars represent SE.
a significant decline in MEP amplitudes from T15 to T'15 (P = 0.02) and T'30 (P = 0.006) (Fig. 3B).

Experiment 2. Time Course of PAS270 Effects

The data are shown in Fig. 5. RM ANOVA for MEP amplitude ratio to baseline (Tpre) of I/O curves showed a trend for factor “Time” (4 levels: Tpre, T15, T30 and T'15) $[F(3,2.8), P = 0.07]$ and no significant effect of “Intensity” (4 levels: 100–130%) but significant “Time” × “Intensity” interaction $[F(9,3.2), P = 0.004]$. The significant interaction was due to different modulation of MEP amplitude ratios measured by different intensities. Intensities of 100, 110, and 130%RMT showed reduction while the intensity of 120%RMT showed increase after T15. Moreover, the intensity of 130%RMT showed larger increase in MEP amplitude ratios compared with intensities of 100–120%RMT after 1 h (T'0–T'15) (Fig. 5B). RM ANOVA of MEP amplitude ratios to baseline (Tpre) for 130%RMT with factor “Time” (6 levels: Tpre–T'15) confirmed the significant effect of “Time” $[F(5,7.89), P = 0.033]$. Post hoc t-tests showed significant increase at T15 ($P = 0.04$) and T'15 ($P = 0.002$) compared with baseline and a significant increase from T30 and T'0 to T'15 ($P = 0.007$), indicating an early and a later phase of increase in MEP amplitude ratio after PAS270.

Dynamic Motor Practice

RM ANOVA with “Intervention” (4 levels: sessions 1–3 of experiment 1a and experiment 1b) and “Trial block” (6 levels) as within-subject factors showed a significant effect for “Trial block” $[F(5,7.89), P = 0.007]$ on the percentage of correct responses. No significant effect was observed for different “Interventions” or “Trial block” × “Intervention” interaction. Pairwise comparisons for “Trial blocks” showed a significant increase in the percentages of correct responses between first and second blocks ($P = 0.033$), third and fourth blocks ($P = 0.037$), and fourth and fifth blocks ($P = 0.035$). This comparison indicates similar increase in the percentages of correct responses over the course of the DMP in all “Interventions” (Fig. 6).

DISCUSSION

PAS

Associative plasticity is thought to be an important neurophysiological correlate of memory formation and learning (Classen et al. 2004). Neuroplasticity encompasses both synaptic plasticity and nonsynaptic plasticity and refers to changes in neural pathways and synapses (Pascual-Leone et al. 2011). In this study we defined plasticity by changes in cortical
excitability that were measured by recording changes of I/O curve slopes or in MEP amplitude ratios at particular stimulation intensities. We showed that the duration of PAS induction, defined as the number of paired stimuli, influences the plasticity outcome. We also found that the interaction of PAS intervention with DMP depends on the state of cortical excitability before the motor practice paradigm and PAS duration. The LTP-like effect observed after PAS270 was reversed by DMP, which produced a LTD-like effect after PAS270 (homeostatic interaction). This type of interaction was not seen after PAS90 and PAS180. Since the DMP by itself increases MEP amplitudes, the LTD-like effect that we observed is related to the priming effect of PAS on DMP. We speculate that homeostatic interaction occurs in the setting of increased excitability. PAS270, as we hypothesized, induced a longer-lasting effect that resulted in homeostatic response by DMP. The time course of PAS270 was also studied with more time points than previous studies for 75 min after PAS, and we found that a LTD-like effect did not occur. In contrast, we found a late phase of increase in cortical excitability after PAS270. This finding is in line with previous studies that showed gradual increase in MEP after PAS for duration of >1 h (Elahi et al. 2012; Morgante et al. 2006; Stefan et al. 2000). To our knowledge, the role of different levels of LTP-like plasticity in homeostatic and nonhomeostatic responses has not been studied in human motor cortex. We showed graded response after PAS and its interactions with DMP.

It has been postulated that the PAS LTP-like effect is due to spike timing-dependent plasticity (STDP) (Stefan et al. 2002). STDP works by correlating pre- and postsynaptic neuronal spikes and can result in strengthening or weakening of synapses, depending on the temporal order of spiking. Most experimental studies of STDP have focused on the timing of spike pairs (Markram et al. 1997). Although the BCM model (Bienenstock et al. 1982) explains the main aspects of the synaptic plasticity at the microscopic level, certain limitations exist for this model at the networks level. Application of STDP to a larger system-level scale requires certain considerations. Reorganization of the network, the nature of the collective responses measured through the network output, and propagation of the effect to other parts of the network are important issues that are not included in the BCM model of STDP. This includes the effect of the number of stimulus pairs and its relation to plasticity induction. In the present study, we found increasing MEP amplitude in response to longer duration of PAS induction. However, the synaptic or nonsynaptic mechanisms of these changes need to be elucidated.

MEP amplitude is an established marker for the degree of cortical and pyramidal tract activation (Abbruzzese and Trompetto 2002). There are at least two possible explanations for training-induced increases in MEP amplitudes. MEP amplitude increase could reflect 1) improved connectivity of interneurons to pyramidal tract neurons in the motor cortex, 2) an increase in the number of cortical neurons of similar excitability activated by TMS (excitability threshold), or a combination of both. In a previous study (Elahi et al. 2012), we showed no significant changes in response to PAS in resting and active motor thresholds.

PAS in this study induced weaker responses than in some previous studies (Classen et al. 2004; Ziemann et al. 2004). An inverse correlation between PAS response and RMT, age, or minimum stimulus intensity to elicit MEPs of 1 mV has been reported in a previous study (Muller-Dahlhaus et al. 2008). The weaker responses we observed for PAS90 and PAS180 may be due to the relatively high RMT and stimulus intensity required to generate 1 mV or the older age of our study population. The faster rate of the PAS used in this study compared with some previous studies may be another factor that contributed to the efficacy of the PAS protocols inducing the LTP-like effect. This issue needs further evaluation.

**Dynamic Motor Practice**

The behavioral paradigm used in the present study was not simply motor practice. Subjects were provided with both the knowledge of their performance by observing their force graphs and feedback on the success and failure of each trial. Furthermore, fixation of the hand in our measurement device and the use of extensors to produce accurate responses were, for the majority of subjects, an unnatural task. Thumb extension was selected for this study because it was an unfamiliar task for most subjects to produce accurate force responses. This paradigm, at least to some extent, required skill acquisition. Learning may occur during and after motor practice sessions or during the process of adaptation. Adaptation or the process of trial-to-trial error reduction is relevant to learning in general (Hallett and Grafman 1997). The assumption that adaptation, unlike acquiring a new motor skill, does not require the learning of new pattern of muscle activation but rather a new mapping between well-learned movements and spatial goals (Hallett and Grafman 1997) is relevant here and is another possible underlying mechanism of performance changes in DMP used in this study. In a recent study (Cantareno et al. 2013), similar to our findings, LTP-like effect was blocked by a motor learning task but not after simple motor practice (without learning); this observation supports our assumption that learning must have played a role in the DMP task used in the present study.
In experiments 1a and 1b, each subject had four sessions of DMP. Although we cannot completely exclude a small carryover effect from one session to the next affecting our results, we tried to minimize this effect. 1) We separated each session by at least 1 wk (in most cases it was longer). 2) We chose a short duration of DMP. There is no evidence that the carryover effect of short DMP lasted more than a few days. 3) The sessions were tested in random order, and therefore the carryover effect cannot explain the differences that we observed within experiments 1a and 1b.

The fact that we found no changes in accuracy of the performed task but others have found modulation of acceleration of movement after PAS can be partly explained by Fitts’ law indicating a trade-off between motor speed and accuracy (Fitts 1954). It is possible that we missed capturing small improvements in motor accuracy as a result of modulation of movement acceleration after stimulation as shown in other studies. Furthermore, changes in the accuracy of movement measured in DMP may occur in a much longer timescale or after multiple sessions. Motor skill learning can be divided into a fast (early) stage, in which significant improvements can be seen within a single training session, and a later, slow stage, in which further gains can be achieved across multiple practice sessions or between training sessions (off-line learning). Other studies also support different time courses for PAS-induced changes in cortical excitability and motor performance improvements. For example, in one study, motor performance improvement continued while PAS-induced plasticity was blocked (Stefan et al. 2006). In another study (Rajji et al. 2011), improvement in motor accuracy in a rotary pursuit task was observed 1 wk after the facilitatory PAS. In the present study we examined the early stage of motor practice effects, and therefore it is possible that late changes in movement accuracy were undetected.

Interactions Between PAS and DMP

PAS has been shown to both improve and worsen performance in various motor tasks. Jung and Ziemann (2009) observed that PAS-induced LTP-like effect on motor practice could in part be nonhomeostatic because motor practice performance could be enhanced after PAS LTP. In the same experiment they showed that a delay between PAS and motor practice session can result in worsening of performance. This effect could be due to nonsaturated priming LTP and delayed initiation of homeostatic mechanisms. Studies in mouse and rat hippocampus showed that saturated LTP occludes (Madronal et al. 2007) while nonsaturated LTP facilitates subsequent learning (Berger 1984). However, we did not observe modulation of the DMP performance by prior PAS, as all experimental groups showed similar improvement in DMP. Our results also show that PAS duration and peak MEP amplitude after PAS are predictors of homeostatic response. Although it is not known why the peak MEP amplitudes predict the homeostatic response better than the average MEP amplitude, we speculate that the peak MEP amplitude at any time point (T15 or T30 in the present study) plays a more important role in the homeostatic process than the overall increase in cortical excitability.

Modulation of PAS by motor practice has also been demonstrated. Several studies showed that prior motor learning produced a homeostatic effect on the subsequent associative plasticity in healthy subjects. Motor practice decreases subsequent PAS-induced LTP plasticity but increases LTD plasticity of the trained M1 (Stefan et al. 2006; Ziemann et al. 2004). This is consistent with our findings. Facilitatory PAS resulted in a paradoxical LTD in previously trained M1. It is interesting to note that even the shortest PAS duration used in this study (PAS90) resulted in paradoxical LTD-like plasticity in a previously trained subject. However, we did not investigate longer PAS durations after DMP to determine whether longer PAS durations can also produce similar interactions. It should be noted that the mechanisms underlying PAS-induced plasticity are not necessarily the same as those for motor training. When inhibitory PAS10 was applied immediately after motor training, the efficacy of PAS25 to enhance cortical excitability was maintained (Stefan et al. 2006). This finding suggests that the mechanisms supporting PAS-induced plasticity remained intact after motor training. Similarly, our data show that although cortical excitability measured through MEP was returned to baseline after DMP, the homeostatic interaction with PAS remained. These findings indicate that homeostatic responses and changes in MEPs are different processes.

The delay between priming stimulation (PAS) and motor practice seems to play an important role in induction of the homeostatic responses (Jung and Ziemann 2009). One study showed that homeostatic and nonhomeostatic interaction between PAS and subsequent learning of rapid thumb flexion movements depended on the time delay between priming stimulation (PAS) and motor practice (Jung and Ziemann 2009). Facilitatory PAS improved motor acceleration immediately after the LTP induction (nonhomeostatic) and reduced the motor acceleration with a 90-min delay (homeostatic). Therefore, we delayed the second intervention by 1 h to allow sufficient time for the metaplastic mechanism to occur.

The longer PAS duration (PAS270) in this experiment showed an early and a late phase of increased cortical excitability (Fig. 5B). This late increase in excitability after PAS had also been observed in previous studies (Morgante et al. 2006; Stefan et al. 2000). In contrast to PAS270 alone (Fig. 5B), the PAS270-DMP combination (Fig. 3A) resulted in significant reduction of MEP amplitude. This interaction cannot be explained by the time course of PAS270 alone or the effect of DMP alone. The mechanism of reversal of synaptic potentiation could be explained by depotentiation or synaptic metaplasticity. The role of depotentiation is not well known. It has been suggested that both depotentiation and synaptic metaplasticity prevent unnecessary information from being stored by keeping neuronal firing rate within a physiological range (Abraham and Bear 1996; Zhou and Poo 2004). A few studies have shown deficits of depotentiation processes in a mouse model of schizophrenia (Shamir et al. 2012) and homeostatic responses in diseases such as dystonia (Quartarone et al. 2005). Understanding homeostatic metaplasticity in both healthy subjects and disorders of cortical plasticity is important because it may lead to development of new electrophysiological biomarkers or diagnostic tools for these conditions.

Conclusions

We found graded LTP-like response to PAS duration and showed that homeostatic interaction between PAS and subse-
quent motor practice is related to the maximum amount of MEP amplitude achieved before the motor training and the duration of PAS intervention. Understanding the rules of synaptic plasticity at the systems level will ultimately help us to develop efficient protocols to modulate the motor cortex or new biomarkers to capture abnormalities of cortical plasticity in patients with neurological and psychiatric disorders.

GRANTS

The study was supported by the Canadian Institutes of Health Research (CIHR, Grant MOP 62917). R. Chen was supported by a CIHR Industry Partnered Investigator Award and the Catherine Manson Chair in Movement Disorders. E. Elahi was supported by a CIHR-Dystonia Medical Research Foundation of Canada fellowship in the field of dystonia.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: B.E., W.D.H., Z.J.D., and R.C. conception and design of research; B.E. and C.G. performed experiments; B.E., C.G., and R.C. analyzed data; B.E., W.D.H., Z.J.D., and R.C. interpreted results of experiments; B.E. and R.C. prepared figures; B.E., Z.J.D., and R.C. drafted manuscript; B.E., Z.J.D., and R.C. edited and revised manuscript; B.E., W.D.H., Z.J.D., C.G., and R.C. approved final version of manuscript.

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