Corticospinal excitability is enhanced after visuomotor adaptation and depends on learning rather than performance or error.

Hamid F. Bagce, Soha Saleh, Sergei V. Adamovich, John W. Krakauer, Eugene Tunik

1Department of Rehabilitation and Movement Science, School of Health Related Professions and
2Graduate School of Biomedical Sciences, University of Medicine and Dentistry of New Jersey,
3Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, New Jersey,
4Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland

Corticospinal excitability changes following gain adaptation

Eugene Tunik, Department of Rehabilitation and Movement Science, UMDNJ School of Health Related Professions, 65 Bergen St. 7th Floor, Newark, NJ 07107 (e-mail: tunikeu@umdnj.edu).

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Abstract

We used adaptation to high and low gains in a virtual reality set-up of the hand to test competing hypotheses about the excitability changes that accompany motor learning. Excitability was assayed through changes in amplitude of motor evoked potentials (MEPs) in relevant hand muscles elicited with single pulse transcranial magnetic stimulation (TMS). One hypothesis is that MEPs will either increase or decrease, directly reflecting the effect of low or high gain on motor output. The alternative hypothesis is that MEP changes are not sign-dependent but rather serve as a marker of visuomotor learning, independent of performance or visual-to-motor mismatch (i.e. error). Subjects were required to make flexion movements of a virtual forefinger to visual targets. A gain of 1 meant that the excursions of their real finger and virtual finger matched. A gain of 0.25 (“low-gain”) indicated a 75% reduction in visual versus real finger displacement, a gain of 1.75 (“high-gain”) the opposite. MEP increases (>40%) were noted in the tonically activated task-relevant agonist muscle for both high- and low-gain perturbations after adaptation reached asymptote with kinematics matched to veridical levels. Conversely, only small changes in excitability occurred in a control task of pseudorandom gains that required adjustments to large errors but in which learning could not accumulate. We conclude that changes in corticospinal excitability are related to learning rather than performance or error.

Keywords

sensorimotor; primary motor cortex; transcranial magnetic stimulation; motor evoked potential; virtual reality
Introduction

The performance versus learning distinction is a particularly vexing issue when studying changes in the brain after training in healthy subjects or in patients (Krakauer 2007). One approach to assess the state of the motor system is to measure corticospinal excitability with transcranial magnetic stimulation (TMS)-induced motor evoked potentials (MEPs) (Pascual-Leone et al. 1994; Pascual-Leone et al. 1995; Muellbacher et al. 2001). MEPs serve as both an assay for internal changes in inhibitory/excitatory balance that cannot be accessed directly and as a measure of the integrity of a motor output pathway. For example, a lower conduction time (Vang et al. 1999), a higher baseline MEP amplitude (Rapisarda et al. 1996) and a greater change in MEP amplitude (Koski et al. 2004; Swayne et al. 2008) after stroke are thought to be beneficial to voluntary action, i.e., performance. Alternatively, increases in MEP have been associated with motor learning (Jensen et al. 2005; Koeneke et al. 2006; Gallasch et al. 2009; Cirillo et al. 2011) rather than changes in motor output although in these studies the learning-related changes in performance were not controlled for. One study, however, demonstrated that as subjects learned to increase peak pinch force to maintain a target EMG profile, motor cortex excitability scaled up in a linear fashion (Muellbacher et al. 2001). On follow-up measurements, MEP amplitude returned to baseline levels but subjects retained the learned peak force profile, which suggested that the increased MEP was not necessary to maintain performance but instead was associated with learning. Alternative explanations for the excitability changes in this study are possible however. One is that the transient increase in MEP amplitude was a result of the ramping up of force (i.e. motor output) from low to high (versus high to low) during adaptation, rather than learning per se. Another potential explanation is that the increased excitability was the result of error: once the mismatch between produced and optimal force was zero, excitability normalized.
Thus whether excitability changes are truly learning-related rather than related either to the
direction of the change in the magnitude of motor output or to errors remains unresolved.

Here we sought to directly test whether learning affects corticospinal excitability, independent of
performance or error, by studying gain adaptation of finger movements in a novel virtual reality
(VR) environment. Gain adaptation is ideally suited to test the competing hypotheses that
changes in M1 excitability relate to changes in performance or error, versus that they relate to
learning. In the low-gain condition (gain=0.25), a 75% reduction in visual versus real finger
displacement occurs requiring an increase in finger excursion, whereas the high-gain condition
(gain=1.75) results in the opposite. It has been shown that some motor cortical cells linearly
scale firing rate with speed and distance (Paninski et al. 2004). As a gain change requires either a
higher or a lower peak velocity and displacement for a given visual excursion compared to
baseline, then M1 excitability changes might reflect changes in firing rate. That there is a
relationship between excitability and firing rate has been known from classic animal studies
investigating the effect of anodal DC-current on motor cortex (Creutzfeldt et al. 1962; Bindman
et al. 1964; Purpura and McMurtry 1965).

In this study, we measured MEPs with the assumption that any modulation of corticospinal
excitability following adaptation represents an excitability change at the level of primary motor
cortex, as recent animal studies suggest that the locus of learning-related synaptogenesis and
reorganization is in M1 and not at spinal-level synapses (Remple et al. 2001; Kleim et al. 2002;
Adkins et al. 2006). If excitability changes parallel the performance change required by the
visuomotor gain relationship then excitability should increase for the low-gain condition and
decrease for the high-gain condition. If, however, excitability is a marker for motor learning then excitability increases should be seen for both gain conditions.

Materials and Methods

Subjects

Nine subjects participated in Exp. 1 (5M, mean age ± 1 SD, 25.9 ± 4.6 years old), nine in Exp. 2 (5M, 29.2 ± 7.0 yo), six in Exp. 3 (4M, 32.0 ± 6.9 yo), and fourteen in Exp. 4 (9M, 26.8 ± 6.2 yo). All subjects were right-handed (Oldfield 1971), free of neurological or orthopedic conditions that could interfere with the experiment, were safe to receive TMS (Keel et al. 2000), and provided written and verbal institutionally-informed consent. All protocols were approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey.

Setup

Subjects were seated with forearms (semi-pronated) and hands hidden from direct line-of-sight under a widescreen monitor oriented horizontally (Fig. 1B). Real-time visual feedback of hand motion was conveyed on the monitor as motion of VR-rendered hand models (Virtools software, Dassault Systems) actuated by kinematic data streaming from data gloves (5DT-16MRI) worn by subjects. The display was angled and magnified such that VR hand size, position, and orientation matched a 1st person perspective vantage.

Task
Upon a visual cue, subjects flexed the right index finger metacarpophalangeal (MCP) joint to a virtual target represented on the screen as an arrow (i.e. subjects were asked to completely cover the virtual arrow with the virtual finger), and then returned to a fully extended position. Subjects were asked to complete the task as fast as possible, but without sacrificing accuracy or precision. Each trial lasted 3.5 seconds (inter-trial rest interval: 2.5 seconds). Depending on the experiment (see below), the motion of the VR hand was scaled in real-time relative to the kinematic data streaming from the glove. One of three scaling factors were applied to the VR hand motion: 1.00 (G1.00, veridical), in which virtual hand motion corresponded to actual motion, 0.25 (G0.25), in which virtual hand motion was 25% of actual motion, and 1.75 (G1.75), in which virtual hand motion was 175% of actual motion (Fig. 1A). Thus, a 45° actual movement would produce a virtual movement of 11.25° (G0.25), 45° (G1.00), or 78.75° (G1.75).

**Experiment 1**: This experiment tested for changes in corticospinal excitability following a block of adaptation to low-gain visual feedback. Subjects performed the task in three blocks. Feedback was G1.00 in Block 1 and Block 3, and G0.25 in Block 2 (Fig. 1C, top). Each block consisted of 42 movements performed toward three physical angles (45°/65°/85°, 14 trials per angle). Three targets were used to keep subjects engaged in the task. To ensure that any change in excitability could be attributed to a learned remapping and not just changes in movement amplitude, movements were kinematically-clamped, which is to say that subjects’ physical target angles were 45°, 65°, and 85° at the end of adaptation. This was achieved through systematic manipulation of the perceived angular position of the target in virtual space: it was either kept the same (G1.00 blocks: 45°/65°/85°) or adjusted to be of smaller visual excursion (G0.25 block: 11.25°/16.25°/21.25°). As detailed below, MEPs were measured after each movement block.
Experiment 2: This experiment tested for changes in corticospinal excitability following a block of adaptation to high-gain visual feedback. The protocol was similar to Experiment 1, except here movement amplitude in all three blocks was clamped at 20°/30°/40°, with visual excursion reaching 20°/30°/40° in G1.00 and 35°/52.5°/70° in G1.75. The nature of the high-gain visual feedback was the reason why different movement amplitudes were used in Exp. 2. For example, implementing an 85° movement amplitude in Exp. 2 would have required an unnatural 148.75° virtual joint angle.

Experiment 3: This was a control experiment to address the potential confound that changes in MEPs might relate to progressive increases in peak velocity (i.e. motor output) associated with gain adaptation rather than learning per se. Subjects performed movements with veridical feedback in three separate blocks. During Blocks 1 and 3 (B1G1.00, B3G1.00), subjects made 42 movements to one of three pseudorandomly presented target angles (35°/45°/55°). During Block 2 (B2G1.00), on the other hand, the target angle was increased from 10° to 55°, simulating a “ramping-up” of motor output throughout the block. To prevent subjects from anticipating subsequent targets during Block 2, angles of five-degree increments were pseudorandomly presented in a progressively increasing fashion throughout Block 2 (i.e. 10°…20°…15°…30°…). The last ten trials during Block 2 were identical to Blocks 1 and 3, ensuring that subjects reached similar asymptotic levels of motor output.

Experiment 4: The block experiments described above were designed to test the hypothesis that corticospinal excitability changes are the result of accumulated learning rather than motor output.
A counter-hypothesis would be that these changes arise because of perceived error and their associated corrections. This control experiment tested excitability during trial-by-trial error correction in the absence of a constant systematic perturbation that would allow accumulation of learning. Subjects flexed the MCP joint over a single block (66 total trials). In pseudo-randomly interleaved trials, feedback was either G1.00, G0.25, or G1.75 (22 trials/condition). In this experiment, an explicit visual target was NOT presented to the subjects at the beginning of each trial, as was the case in Experiments 1, 2 and 3. Rather, subjects were instructed to produce movements to a kinesthetically-defined 45° target angle. To aid subjects in identifying the end of movement, the virtual finger turned red when subjects achieved the 45° angle. Preplanned movements to a proprioceptive 45° target angle minimized between-condition differences in kinematic traces during the early phase of the trial, while still allowing subjects enough time to fully perceive the altered visuomotor feedback. As the subjects initiated movement, however, they were expected to adjust kinematics on-line in response to the altered visuomotor feedback. Compare Exp. 4 to Exp. 1-3, in which explicit visual feedback of the target was provided at the very beginning of the trial, allowing subjects in Exp. 1-3 to preplan movements to various visual target angles.

**Neuronavigated Transcranial Magnetic Stimulation (TMS)**

Single-pulse TMS (Magstim Rapid2, 70mm double AFC coil) was applied at 110% resting motor threshold (minimum intensity required to elicit MEPs >50μV in the right first dorsal interosseous (FDI) muscle on 5/10 consecutive trials). Each subject’s high-resolution anatomical MRI scan (3T Siemens Allegra) was used to render a 3-dimensional cortical surface. Fiducial locations on the MRI were co-registered with the subject’s head to allow frameless
neuronavigation (Visor, Advanced Neuro Technology). The stimulated ‘hotspot’ of motor cortex was marked on the MRI scan. The coil was held tangentially with the handle facing posteriorly 45° off the sagittal plane, and was tracked on-line to be over the hotspot.

Experiments 1, 2, and 3: Twenty MEPs were recorded immediately after each block over a period of two minutes. To maintain consistent EMG activity across trials and conditions, subjects were asked to lightly squeeze a force transducer (FlexiForce, Tekscan Inc.). Force feedback was displayed on the monitor as a rectangular horizontal bar. TMS was triggered by a TTL signal when a target force of 2.5±0.25 N was maintained over a period of 100ms. This particular target force was used because the EMG activity required to attain this force level matched the EMG activity produced in Exp. 4, allowing us to draw direct comparisons between the results of these experiments and Exp. 4.

Experiment 4: MEPs were recorded during each movement. TMS was triggered by a TTL when the MCP angle reached 40° (i.e. immediately prior to reaching the 45° target angle). Keeping joint angle constant at the time of MEP measurement assured that between-condition MEP differences would not be confounded by discrepancies in muscle length-dependent stretch reflexes (Raptis et al. 2010).

Analysis

Kinematics: The MCP joint angle data was filtered (2nd order Butterworth: 10 Hz low-pass) and analyzed offline with custom-written MATLAB software (The Mathworks, Inc.). For each trial, movement onset and offset were defined as the time at which the angular velocity exceeded and
fell below 5% of peak angular velocity for >60 ms. In Experiments 1, 2 and 3, peak angular velocity served as a marker of performance because it reliably represented subjects’ adaptation to the visuomotor discordance. In Experiment 4, instantaneous angular velocity (at the time of TMS stimulation) and the peak initial angular acceleration within 100ms of movement onset were analyzed as performance parameters. Angular acceleration was analyzed on trials following each condition (‘n+1’ trials) to determine whether on-line adaptation had affected forward planning on subsequent trials.

Electrophysiology: Electromyographic (EMG) activity of the FDI and abductor digiti minimi (ADM) muscles was acquired with a 4-Channel Bagnoli EMG System (Delsys, Inc.). Raw analog EMG signal was amplified (x10), streamed to a data acquisition card (NI USB-6221, National Instruments Corp., 2 kHz sampling frequency), and analyzed offline with custom-written MATLAB software. We computed: (1) motor evoked potential (MEP), the peak-to-peak amplitude of the EMG signal 20-50 ms after the TMS pulse (Fig. 1D); and (2) background EMG, calculated after filtering (2nd order Butterworth: 5-250 Hz band-pass, 55-65 Hz notch), full-wave rectifying, and enveloping (20 Hz low-pass) the EMG signal. From this, the average EMG signal 50ms preceding TMS stimulation was extracted for analysis. Because empirical data suggest that background EMG covaries with MEP amplitude for dynamic, low-force contractions (Aranyi et al. 1998; Kasai and Yahagi 1999; Ni et al. 2006), such as those used in our protocol, we compared background EMG levels across conditions (Datta et al. 1989; Flament et al. 1993) to assure that between-condition differences in MEP amplitude were due to learning and not confounded by motor output.
Coil Position: To ensure that coil position did not differ among conditions, a 3-dimensional coil position error was calculated as the distance between TMS coil position at the time of stimulation and the ‘hotspot’.

Statistics

Electrophysiological variables (MEP, EMG) were averaged across trials for each condition and subject. Means were submitted to a repeated measures analysis of variance (rmANOVA). For kinematic analysis of Experiments 1 and 2 a three-way rmANOVA with factors [levels]: time [early,late], target [angle1,angle2,angle3], and condition [B1Veridical, B2Gain, B3Veridical] was calculated. The first and last trials for each angle of each condition were used for the ‘early’ and ‘late’ data, respectively. For kinematic analysis of Experiment 3, a one-way rmANOVA with levels [B1Veridical, B2Veridical, B3Veridical] was calculated for both early- and late-stage adaptation, in which the average of the first and last three trials was used for the ‘early’ and ‘late’ data respectively. Finally, a mixed linear regression model was also performed for Experiment 4 to characterize the degree to which each variable contributed to the MEP effect. For this, MEP was defined as the dependent variable and coil displacement error, background EMG, angular velocity, and gain feedback (G0.25, G1.75) as independent variables. Data was analyzed with PASW Statistics 18 (SPSS Inc.). Repeated measures analysis of variance was used to test for main effects and interactions. Statistically significant interaction effects were tested post-hoc using Tukey’s Honestly Significant Difference (HSD) Test. Significance threshold was set at p < 0.05.
Results

Excitability increased following gain adaptation

Subjects showed complete adaptation to visuomotor gain when visual motion of the VR finger was scaled to either 25% (Exp. 1, G0.25 condition) or 175% (Exp. 2, G1.75 condition) of actual finger motion. Figures 2A (Exp. 1) and 3A (Exp. 2) show a representative subject’s mean joint angle (top) and angular velocity (middle) trace for early and late adaptation stages. While early adaptation was characterized by initial hypometria (Exp. 1) or hypermetria (Exp. 2) with subsequent on-line adjustments, finger position traces during late adaptation were comparable to those in the veridical block. For kinematic analysis of Experiments 1 and 2 a three-way rmANOVA with factors [levels]: time [early,late], target [angle1,angle2,angle3], and condition [B1Veridical, B2Gain, B3Veridical] was calculated. The first and last trials for each angle of each condition were used for the ‘early’ and ‘late’ data, respectively. For kinematic analysis of Experiment 3, a one-way rmANOVA with levels [B1Veridical, B2Veridical, B3Veridical] was calculated for both early- and late-stage adaptation, in which the average of the first and last three trials was used for the ‘early’ and ‘late’ data respectively. It should be noted that in Experiment 2, there was a significant drop in peak velocity below baseline at around trial number eight (see third time bin in Figure 3B), likely due to subjects’ tendency in early trials to overshoot the visual target but in later trials to adjust on-line kinematics by moving at a slower speed. We quantified the degree of adaptation by calculating the peak velocity in each trial. Figures 2B and 3B show similar behavior at the group level, in which subjects progressively adapted their peak velocity to match that of the veridical blocks. As expected rmANOVA revealed a significant main effect of target angle on peak velocity for both the low- and high-gain learning paradigms (Exp. 1: $F_{2,16}=122.41, p<0.001$; Exp. 2: $F_{2,16}=40.73, p<0.001$). Notably, a
significant main effect of visual feedback condition on peak velocity was observed for both the low- and high-gain learning paradigms (Exp. 1: F_{2,16}=11.94, p=0.002; Exp. 2: F_{2,16}=4.36, p=0.038), and as displayed in Figures 2B and 3B, the TIME*CONDITION interaction effect was also significant (Exp. 1: F_{2,16}=5.552, p=0.016; Exp. 2: F_{2,16}=5.29, p=0.023). To confirm that the observed effects were a result of learning, we averaged raw velocities across all three angles and performed two separate one-way rmANOVAs for both the early and late interactions. Sub-analysis revealed a significant main effect of visual feedback condition on peak velocity during the early stage of adaptation (Exp. 1: F_{2,16}=20.208, p<0.001; Exp. 2: F_{2,16}=6.438, p=0.011), however, similar effects were not seen during the latter phase of each block (Exp. 1: F_{2,16}=1.073, p=0.363; Exp. 2: F_{2,16}=1.171, p=0.334), suggesting that subjects fully adapted to the visuomotor gain. Post-hoc pairwise comparison confirmed that velocity effects were driven by significant differences between the early gain block and first veridical block (Tukey’s corrected; Exp. 1: t_8=-4.038, p=0.001; Exp. 2: t_8=3.4685, p=0.004), but not between the two veridical blocks (Tukey’s corrected; Exp. 1: t_8=-2.277, p=0.10; Exp. 2: t_8=-0.294, p=0.221). Overall, these findings demonstrate that kinematic performance in finger space post-adaptation was similar to that seen pre-adaptation.

MEPs were measured in the FDI muscle for a period of two minutes following each training block. A representative subject (Fig. 2A and 3A) and group data (Fig. 2B and 3B) demonstrate that corticospinal excitability was increased for a period of two minutes following adaptation to both low- and high-gain feedback (Mean Percent Change SE; Exp. 1: 53.3% 16.2; Exp. 2:...
40.9% 17.1), relative to the veridical. rmANOVA confirmed a significant MEP effect for both experiments (Exp. 1: $F_{2,16}=4.618$, $p=0.026$; Exp. 2: $F_{2,16}=4.901$, $p=0.042$). Post-hoc pairwise comparisons revealed that the effects were driven by significant differences between MEPs following the adaptation block and MEPs following the first veridical block (Tukey’s corrected; Exp. 1: $t_8=2.694$, $p=0.044$; Exp. 2: $t_8=2.511$, $p=0.038$), but not when comparing MEPs following the first and second veridical blocks (Tukey’s corrected; Exp. 1: $t_8=-0.035$, $p=0.999$; Exp. 2: $t_8=-0.021$, $p=1.000$). No significant between-condition differences were noted in background EMG activity (Exp. 1: $F_{2,16}=2.321$, $p=0.147$; Exp. 2: $F_{2,16}=0.647$, $p=0.519$), suggesting that any difference in motor output could not account for the MEP effects.

We performed a post-hoc sub-analysis of MEPs collected after the low-gain (Exp. 1) and high-gain (Exp. 2) blocks to discern whether post-adaptation increases in excitability were relatively stable over the entire two minutes of MEP data collection. Pairwise t-tests revealed non-significant differences between the first and last ten MEPs following both the low-gain block (MEP SE; Early: 2.27 0.48 mV; Late: 2.26 0.47mV, $t_8=0.070$, $p=0.946$) and the high-gain block (MEP SE; Early: 2.63 0.44 mV; Late: 2.30 0.37mV, $t_8=1.286$, $p=0.234$), suggesting that only a slight drop in excitability occurred during the second half of MEP data collection.

We performed a second post-hoc analysis on MEP and EMG variance to ensure that the above effects were not driven by outlier trials. For this, the variance of these two outcome measures (MEP and EMG) was calculated for each experiment (Exp 1 and Exp 2) for each subject (S1...
through S9) for each condition (Veridical-Gain-Veridical). Variances were submitted to a repeated measures ANOVA, which confirmed no significant main effect of condition on MEP variance (Exp. 1: $F_{2,16}=0.16$, $p=0.850$; Exp. 2: $F_{2,16}=1.50$, $p=0.253$) or EMG variance (Exp. 1: $F_{2,16}=1.49$, $p=0.255$; Exp. 2: $F_{2,16}=0.88$, $p=0.432$).

Finally, to ensure an appropriate sampling size for the remaining experiments (Exp. 3 and 4), we performed a sample-size calculation for the two primary outcome variables (MEPs and angular velocity) of Exp. 1 and 2 using an ANOVA with a significance threshold set to 0.05 and a power of 0.8. To be more conservative, we used data from the high-gain adaptation experiment (Exp. 2), which had the smaller effects in both variables. For an effect size of 2.61 [MEPs: $(2.38-1.78)/0.23]$ and 4.15 [Velocity: $(2.32-1.78)/0.13$], the sample-size calculation indicated a minimum of 5 and 3 subjects, respectively, to yield significant effects.

Control experiment: “Ramping-up” motor output did not augment excitability

We performed a control experiment (Experiment 3) to rule out the possibility that the post-adaptation M1 excitability increases observed in Experiments 1 and 2, which were in the same direction (increases) were the result of the similar “ramping-up” of peak velocity (i.e. motor output) to the final asymptotic level. Fig. 4 shows that peak velocity in B2G1.00 pseudorandomly increased to the asymptotic level of B1G1.00 and B3G1.00 (Velocity Early: $F_{2,10}=30.632$, $p<0.001$; Velocity Late: $F_{2,10}=1.300$, $p=0.309$). The inset of Fig. 4 shows that when M1 excitability was probed at the end of each block, we observed no between-block MEP effects ($F_{2,10}=0.605$, $p=0.565$), suggesting that increases in M1 excitability observed in Exp. 1 and 2
were a result of visuomotor learning rather than associated changes in motor performance (see Table 1).

*Control experiment: Observed errors and their associated corrections did not augment excitability*

We observed increases in corticospinal excitability following the low- and high-gain conditions independent of performance. The possibility remains that these changes in excitability resulted from a period of observing and correcting errors in extent rather than learning the gains themselves. Experiment 4 was designed to address this possibility. Subjects were exposed to a block of trials in which they had to make finger flexion movements to a 45° physical angle. Three different visual gains were pseudo-randomly interleaved across trials: veridical (G1.00), low-gain (G0.25) or high-gain G1.75). Figure 5A shows a typical subject’s mean joint angle (top) and angular velocity (middle) trace for each condition. Instantaneous angular velocity immediately prior to the TMS pulse (i.e., at 40° flexion) was calculated to confirm that subjects showed kinematic evidence of a differential response to the gain changes. At the group level (see Figure 5B, left plot), angular velocity significantly differed between conditions (F2,26=18.843, p<0.001), with *post-hoc* pairwise comparisons revealing that subjects significantly sped up in the G0.25 condition (group mean=1.996 rad/s, t13=3.187, p=0.007) and slowed down in the G1.75 condition (1.531 rad/s, t13=4.270, p=0.001), relative to the veridical condition (G1.00: 1.822 rad/s). Thus subjects were seeing large errors in every trial and attempted to correct them. We confirmed this further by analyzing peak angular acceleration in the first 100ms of the
subsequent (‘n+1’) trial (i.e., before visual feedback contaminated the feed-forward plan developed in the ‘n’ trial). Significant differences were noted for the ‘n+1’ trials (mean±SE for G1.00, G0.25, and G1.75: 12.6±1.1 rad/s², 13.3±1.1 rad/s², 11.5±0.9 rad/s²; F²,26=4.418, p=0.023), with post-hoc comparisons demonstrating a slight, although non-significant, increase in acceleration for post-G0.25 trials (t₁₃=1.25, p=0.232) and slight decrease for post-G1.75 trials (t₁₃=1.58, p=0.139), relative to post-G1.00, but a significant difference between post-G0.25 and post-G1.75 trials (t₁₃=3.14, p=0.008). This suggests that subjects partially preplanned movements based on the feedback they received in the previous trial. A control analysis of the same kinematic variable for trials preceding the G1.00, G0.25, and G1.75 (‘n-1’) trials expectedly did not show this effect (12.0±0.9, 12.6±1.1, 12.8±1.1 rad/s²; F²,26=1.897, p=0.174). These kinematic analyses revealed that subjects were overtly responding on-line to the observed visual errors and partially adapting to each perturbation, evidenced by the ‘n+1’ analysis, though they never reached full adaptation due to the pseudo-random ordering of the trials.

MEPs were evoked on-line as the subjects’ actual MCP angle reached 40°. Figure 5A (bottom plot, typical subject) and Figure 5B (right plot, group mean) show that MEP amplitude was affected by visual feedback (main effect: F²,26=5.934, p=0.008). Post-hoc pairwise comparisons confirmed that the 12.1% (+/-2.9) MEP increase was significant in the G0.25 over the G1.00 condition (t₁₃=2.965 p=0.011), but not between the G1.75 and G1.00 condition (p=0.993). Thus only small MEP effects were seen in the trial-by-trial experiment when compared to the blocked paradigm despite larger perceived errors and continuous corrections in velocity. The effect seen in the low-gain condition is likely a small learning effect because we still found a small MEP
effect after binning velocity across conditions. To do this, G0.25 trials with velocities exceeding 1SD and G1.75 trials with velocities below 1SD of each individual subject’s global mean velocity were excluded from analysis (rmANOVA for angular velocity following trial exclusion: F2,26=1.080, p=0.348). Despite equalized velocity across all three visual feedback conditions, MEP amplitude remained 12.2% higher in the G0.25 condition (rmANOVA for MEP following trial exclusion: F2,26=3.931, p=0.038).

To characterize the relative contribution of each independent variable on MEP amplitude, we performed a mixed linear regression analysis. MEPs were significantly correlated with expected variables such as coil displacement error (β=-0.194, p<0.001), background EMG (β=0.212, p=0.001), and the G0.25 condition (β=0.120, p=0.003), but not with angular velocity (β=0.041, p=0.262) or the G1.75 condition (β=-0.057, p=0.171). No significant between-condition differences were noted in background EMG activity (p=0.406), suggesting that MEP effects were not confounded by differences in motor output.

Accuracy of TMS stimulation
The 3-dimensional error between the coil’s position at the time of stimulation and the M1 hotspot was not significantly different between conditions (Exp. 1: p=0.286; Exp. 2: p=0.155), suggesting that MEP measurements were elicited from a consistent region of M1.
Discussion

Here we tested whether changes in corticospinal excitability are related to learning, to changes in performance (motor output) or motor error. We found increases in excitability for both low and high gains, rather than an increase and a decrease respectively, and no increase in excitability in a performance-matched control experiment in which subjects were required to progressively ramp up motor output, suggesting that excitability changes do not directly relate to changes in performance. In addition, control experiments showed that the increases in excitability could not be attributed to correcting visual errors or changes in kinematics or EMG. Overall, the results are consistent with the idea that changes in corticospinal excitability are the result of learning rather than performance changes or errors.

Many studies have measured MEP changes after various forms of intervention with respect to motor output, including motor learning. The critical question examined here is what do changes in excitability signify? Do changes in MEP amplitude relate to behavioral changes, to learning, or are they merely epiphenomenal? The experimental appeal of MEPs is they may be measured non-invasively and thus can be used in human studies. The pervasive assumption appears to be that an increase in MEP is behaviorally relevant because it should make it easier to then volitionally recruit motor neurons for execution. Interestingly, however, this assumption has been surprisingly difficult to prove. In several studies, repetitive TMS (rTMS) has been used to alter cortical excitability with inconsequential effects on motor performance (Muellbacher et al. 2000). For example, in one study subjects made repeated fast index finger abductions after 5hz rTMS over contralateral M1. The rTMS did not enhance motor performance on any kinematic variable compared to a sham group despite significant differences in MEPs for the two groups.
(Agostino et al. 2007). In another study, low frequency rTMS over M1 reduced MEP amplitude in the first dorsal interosseous muscle but had no effect on maximal finger tapping speed, on performance on a grooved pegboard test, on an object grip and lift task, or on visuomotor tracking (Todd et al. 2009). Another study failed to find a relationship between increments in voluntary muscle contraction and changes in cortical excitability (Gelli et al. 2007). Conversely, however, in a study that modulated excitability using practice and ischemic nerve block, motor output was enhanced (Ziemann et al. 2001). Those few studies that arguably have shown a disruptive effect of rTMS over M1 on motor performance have related to higher-order processes rather than execution itself. For example, rTMS over M1 led to impaired grip-force scaling – subjects appeared to have disruption of their memory of the previously lifted object weight (Nowak et al. 2005). Notably, subjects generated forces larger than was required and the effects were bilateral, which suggests that input onto motor neurons may not have been the relevant factor. M1 excitability was not assessed in this study and the authors conjectured that rTMS was disrupting sensory inputs into M1 rather than its output. This grip-force scaling effect is consistent with a more recent study that showed larger MEPs when an object that was lifted had been preceded by a heavy rather than a light object; this difference had no apparent effect on ability to scale grip-force in the subsequent lift (Loh et al. 2010). Thus review of the literature presents conflicting evidence with regard to any causal relationship between changes in corticospinal excitability and changes in motor output or performance.

Studying gain adaptation offered the ideal opportunity to dissociate performance- versus learning-related changes in excitability because low and high gains require opposite changes in motor output. For a given target in extrinsic space, after adaptation to a low gain, a larger
movement with a higher peak velocity is required compared to baseline, and vice-versa for a high gain. If MEP amplitude reflects the magnitude of output from M1 then we should have seen opposite changes in excitability in the relevant agonist muscle at low and high gains. Instead we saw large excitability increases for both low- and high-gain adaptation and no excitability changes in a performance-matched training protocol; a result consistent with the studies cited above that showed no clear relationship between excitability and motor output (Muellbacher et al. 2000; Agostino et al. 2007; Gelli et al. 2007; Todd et al. 2009). We conclude that excitability increases following adaptation reflect something other than altered motor output. In order to attribute these changes to accumulated learning, it was first necessary to control for the possibility that these changes resulted from observation of large sensory prediction errors. We found that in a pseudo-random gain condition, in which subjects had to make continuous on-line corrections in the setting of large errors, there was only minimal change in excitability. It therefore appears that changes in corticospinal excitability reflect accumulated learning-based modifications in a controller. Interestingly, our analysis of the pseudo-random control task revealed that the modest increases in MEPs in the low-gain condition were likely attributed to the partial adaptation that was evident in the n+1 trial. Overall, these data are consistent with findings in single unit recording experiments in primates that have shown that motor cortical map expansion does not occur when movements are just repeated; skill learning is also required (Plautz et al. 2000).

It has been shown that there is trial-to-trial motor adaptation under conditions of random perturbation and that such learning is captured by state-space models just as well as learning of a constant perturbation (Donchin et al. 2003; Diedrichsen et al. 2005). Here we found that a
pseudo-random gain did not lead to appreciable changes in corticospinal excitability despite
evidence for within-trial updating of motor commands. The lack of an effect of visual-to-motor
errors on excitability is perhaps unsurprising given the lack of direct visual inputs to M1
(Felleman et al. 1997; Lewis and Van Essen 2000). But this finding is also consistent with a
variety of studies in human and model systems that report an absence of learning effects in M1 if
they are tested before adaptation reaches a steady state; a point when the learned movement is
repeated with low variability. Force-field adaptation is only disrupted by TMS over M1 when
adaptation had reached asymptote (Orban de Xivry et al. 2011). Similarly, transient disruption of
M1 using single-pulse TMS, time-locked to the perturbation of a graspable object, did not impair
the on-line reach-to-grasp correction (Tunik et al. 2005). Anodal transcranial stimulation (tDCS)
over contralateral M1 had an effect on retention of the asymptotic level reached after adaptation
to a visuomotor rotation but not on the rate of acquisition (Galea et al. 2011). In a functional
MRI study, a learning effect was not detected in M1 for adaptation to either random rotations or
force fields; the authors concluded that changes in M1 may only occur when adaptation is
allowed to accumulate (Diedrichsen et al. 2005). Similarly, in a single-unit recording study in
monkeys, delay-period activity (Paz and Vaadia 2004) and reduced variability in firing rate
(Mandelblat-Cerf et al. 2009) in M1 cells was only seen after adaptation to rotation had reached
steady-state. We have recently proposed that these results can be explained by positing that a
second form of repetition-associated reinforcement learning in M1 occurs in adaptation
paradigms and that it may occur in M1 (Huang et al. 2011). In animal models, multiple plastic
changes in M1 have been described for skill learning tasks (Li et al. 2001; Kleim et al. 2004;
Molina-Luna et al. 2009).
All these results in human and non-human animals can be unified by positing that the late reinforcement process in adaptation paradigms and skill learning are mechanistically similar, namely that they require synaptic changes (in M1 or elsewhere) and that such changes require short- and long-term LTP-like processes apparent as increases in cortical excitability (Castro-Alamancos and Connors 1997; Ziemann et al. 2004). Thus the increases in corticospinal excitability that we found here for accumulated gain adaptation are consistent with the idea that the changes in motor output required to maintain stable performance may be associated with plastic changes in the motor neuraxis, regardless of the sign of the gain change. Because excitability was assayed through changes in MEPs, it is possible that the effects observed in the current experiment represent changes either at the level of M1 cell body synapses, along the length of upper motor neuron (UMN) axons, or at the level of the UMN–αMN (alpha motor neuron) synapse in the spinal cord. Indeed, there is persistent debate with regard to which level of the neuraxis is the locus for such learning-dependent changes. It is known from recent magnetic resonance spectroscopy work that focal reductions in GABA inhibition occur in sensorimotor cortex during motor learning (Floyer-Lea et al. 2006) and stimulation studies have shown that the degree of learning in M1 is correlated with the ability to modulate GABA inhibition (Stagg et al. 2011), which may be artificially inhibited using stimulation techniques such as anodal tDCS (Stagg et al. 2009). Likewise, some have shown that learning has little effect on corticofugal axonal excitability (Classen et al. 1998) or spinal excitability (Remple et al. 2001; Kleim et al. 2002). Learning-related changes have been detected, however, at spinal levels too, characterized by modulated changes in the H-reflex (Meunier et al. 2007; Wolpaw 2010; Winkler et al. 2012). So far, the specific involvement of cortical versus spinal levels in motor learning remains unknown. The evidence clearly supports the notion that spinal level
reflex circuitry can be conditioned in relatively simple tasks (Thompson et al. 2009) or even by stimulation of the motor cortex (Wang et al. 2012). Empirical data suggest that voluntary acquisition of complex visuomotor skills relies on the direct involvement of motor cortex (Pruszynski et al. 2011; Pruszynski and Scott 2012). Our study was limited by the absence of spinal-level excitability measurements, limiting our ability to isolate the precise location in the neuraxis at which our corticospinal excitability changes occurred. Regardless of the location of excitability change (M1 vs. UMN axon vs. UMN–αMN synapse) or the underlying neuronal mechanism (increased excitation versus decreased inhibition), the current study demonstrates that visuomotor adaptation induces short-term sign-independent, facilitation of the corticospinal system. An interesting follow-up study would be to use the same gain adaptation paradigm (along with spinal-level assays of excitability) to test for longer-lasting effects across separate days, at varying stimulation intensities (i.e. 115% vs. 120% RMT), and background EMG levels (i.e. force output at 0N [rest], 1N, 3N, etc.).

Our finding of increases in corticospinal excitability for both increases and decreases in gain is consistent with those previous studies that have shown no behavioral consequence of changes in MEP magnitude (Agostino et al. 2007; Gelli et al. 2007; Todd et al. 2009). We conclude that caution is required when reporting changes in MEP magnitude as having behavioral significance, especially in the context of neurorehabilitation. Had adaptation to the two oppositely signed gains shown a differential effect on excitability then perhaps more of a case could have been made for using manipulations of visual feedback to enhance cortical excitability and perhaps aid voluntary recruitment. Given our results, however, we conclude that changes in corticospinal excitability are a marker for learning-related processes and should not in-and-of-themselves be
considered relevant to motor performance. That said, it is possible that if excitability is
abnormally depressed, like after stroke, then methods to enhance excitability might improve
2009). This consideration leads to the interesting possibility that VR adaptation paradigms might
indirectly benefit patients after stroke by increasing excitability, which may then enhance
performance in unrelated motor tasks.

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Disclosures
No conflicts of interest, financial or otherwise, are declared by the authors.

Author Contributions
and E.T. approved final version.

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Table 1

Summary of the group mean (±1SEM) kinematic and electrophysiological data. MEP, motor evoked potential (mV) after (Exp. 1, 2, 3) or during (Exp. 4) the motor task; EMG, background electromyographic activity (mV) immediately before the MEP; VEL, peak (Exp 1, 2, 3) or instantaneous (Exp 4) angular velocity (rad/s). Peak velocity for Exp 1 and 2 are averaged across all three target angles.

**Experiment 1 (Low-Gain Adaptation)**

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<th></th>
<th>B₁G₁₀₀</th>
<th>B₂G₀₂₅</th>
<th>B₃G₁₀₀</th>
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<tr>
<td>MEP</td>
<td>1.615 ±0.382</td>
<td>2.262 ±0.473</td>
<td>1.625 ±0.394</td>
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<tr>
<td>EMG</td>
<td>0.031 ±0.005</td>
<td>0.037 ±0.007</td>
<td>0.030 ±0.004</td>
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<tr>
<td>VEL Early</td>
<td>2.189 ±0.134</td>
<td>1.614 ±0.106</td>
<td>2.464 ±0.096</td>
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<tr>
<td>VEL Late</td>
<td>2.692 ±0.220</td>
<td>2.418 ±0.222</td>
<td>2.719 ±0.268</td>
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**Experiment 2 (High-Gain Adaptation)**

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<td>MEP</td>
<td>1.778 ±0.229</td>
<td>2.380 ±0.327</td>
<td>1.781 ±0.261</td>
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<td>EMG</td>
<td>0.054 ±0.012</td>
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<td>0.051 ±0.009</td>
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<tr>
<td>VEL Early</td>
<td>1.780 ±0.130</td>
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<td>VEL Late</td>
<td>1.971 ±0.173</td>
<td>1.857 ±0.192</td>
<td>2.031 ±0.145</td>
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**Experiment 3 (Asymptote Control)**

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<td>MEP</td>
<td>1.894 ±0.484</td>
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<td>VEL Early</td>
<td>2.139 ±0.222</td>
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<tr>
<td>VEL Late</td>
<td>3.131 ±0.434</td>
<td>3.362 ±0.500</td>
<td>3.725 ±0.479</td>
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**Experiment 4 (Trial-By-Trial Control)**

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<th>G₁₇₅</th>
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<tbody>
<tr>
<td>MEP</td>
<td>2.175 ±0.538</td>
<td>2.392 ±0.556</td>
<td>2.182 ±0.534</td>
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<td>EMG</td>
<td>0.039 ±0.005</td>
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<td>0.040 ±0.006</td>
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<td>VEL</td>
<td>1.822 ±0.165</td>
<td>1.996 ±0.159</td>
<td>1.531 ±0.181</td>
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Figure Captions

Figure 1.
A: Actual finger position (left) and virtual finger position (right) for a single 45° physical angle. Physical angle (performance) was identical and virtual angle (feedback) was augmented (in real-time) for each condition within each experiment. Virtual target arrows (for Experiments 1, 2, and 3) and virtual finger color change (Experiment 4) are shown. B: Virtual reality setup. C: Condition and trial structure for each experiment. MEP acquisition is indicated by the arrows either after a block of movements (Experiment 1, 2, 3) or during a given trial (Experiment 4). D: Raw and filtered/rectified (thin line) EMG signal acquired from a typical subject in Exp. 4.

Figure 2.
Kinematic and electrophysiologic data in Experiment 1. A (subject): Blue line and shaded region represents the mean (±1SD) MCP flexion angle (top) and peak angular velocity (middle) for a typical subject, averaged across B1G1.00 and B3G1.00. Mean traces of the first three (solid red) and last three (dashed red) trials of B2G0.25 are superimposed. Mean (±1SD) MEP bar plot (bottom) for this subject demonstrates increased M1 facilitation immediately following B2G0.25 (red) relative to B1G1.00 (dark blue) and B3G1.00 (light blue). B (group): Group mean peak velocity (±1SEM), as a percent change relative to the average veridical trials for each angle for each block. Trials for all three target angles are binned together, thus the 42 total trials are represented by 14 bins on the x-axis. Subjects adapted to B2G0.25 by normalizing movement
velocity to the level observed in the veridical blocks. Also shown (inset) is group mean (±1SEM) MEP, as a percent change relative to veridical.

Figure 3.
Kinematic and electrophysiologic data in Experiment 2. (See Figure 2 Caption).

Figure 4.
Kinematic and electrophysiological data in control Experiment 3 (see Methods). Labels are as in Figure 2B. The gold color shows the incrementally increasing angular velocity over the course of the block, without any requirement for visuomotor adaptation (as was necessary in Experiments 1 and 2). Note the absence of any modulation of M1 excitability (inset).

Figure 5.
Kinematic and electrophysiologic data in Experiment 4. A (subject): Mean MCP flexion angle (top plot) and angular velocity (middle plot) for G1.00 (thin line), G0.25 (thick line), and G1.75 (dashed line) trials. The bottom plot shows the same subject’s mean MEP traces, which have been re-aligned in time according to when TMS was triggered. Dotted line marks the time at which the subject attained a 40° flexion angle, resulting in a trigger to signal the TMS pulse. B (group): Group mean (±1SEM) instantaneous angular velocity (left plot) and MEP (right plot). Asterisk denotes significant effects in a one-sample paired t-test. To rule out velocity-based confounds on MEPs, the data was reanalyzed by excluding G0.25 trials in which angular velocity exceeded 1SD of the global mean velocity, and similarly, excluding G1.75 trials in which
velocity was below 1SD of the global mean. Blinded re-analysis of MEP data after velocity equalization revealed that MEPs were unaffected.