To investigate whether visuomotor integration processes induce long-term potentiation (LTP) and depression (LTD)-like plasticity in the primary motor cortex (M1), we designed a new paired associative stimulation (PAS) protocol coupling left primary visual area (V1) activation achieved by hemifield visual evoked potentials (VEPs) and transcranial magnetic stimulation (TMS) over the left M1, at specific interstimulus intervals (ISIs), delivered at 1 Hz (V-PAS). Before and after V-PAS, we measured motor evoked potentials (MEPs). To clarify the mechanisms underlying V-PAS, we tested the effect of 1-Hz repetitive TMS (rTMS). 0.25-Hz V-PAS and rTMS, and a shorter 0.25-Hz V-PAS protocol. To examine V-PAS with contralateral V1 activation, we delivered V-PAS activating the right V1. To clarify whether V-PAS increases V1 activity or parieto- and premotor-to-M1 connectivity, before and after V-PAS, we examined VEPs and MEPs evoked by paired-pulse techniques. V-PAS increased, decreased, or left MEPs unchanged according to the ISI used. After 1-Hz rTMS MEPs decreased. Although 0.25-Hz rTMS elicited no aftereffect, 0.25-Hz V-PAS modulated MEPs according to the ISI used. The short 0.25-Hz V-PAS protocol left MEPs unchanged. Contralateral V1 inhibited MEPs. After V-PAS, VEPs remained unchanged and the premotor-to-M1 inhibitory connections decreased. V-PAS induces M1 LTP/LTD-like plasticity by activating premotor-to-motor connections.

Keywords: paired associative stimulation, primary motor cortex, rTMS, visual evoked potentials, visuomotor integration

Introduction

Experimental studies in humans and nonhuman primates have demonstrated early cortical motor area activation, time-locked with direct or indirect primary visual area (V1) activation, reflecting early visuomotor integration processes (Schroeder et al. 1998; Lamme and Roelfsema 2000; Saron et al. 2001; Ledberg et al. 2007; Gaillard et al. 2009; Casali et al. 2010). Early visuomotor integration is important in several motor control processes including alerting functions, motor attention, and motor preparation (Foxe and Simpson 2002; Michel et al. 2004; Pruszynski et al. 2008; Kirchner et al. 2009; Makin et al. 2009, 2012; Katsuki and Constantinidis 2012).

Increasing evidence suggests that motor control depends on long-term potentiation (LTP)- or depression (LTD)-like plasticity in the primary motor cortex (M1) (Rioult-Pedotti et al., 2000; Sanes and Donoghue 2000). Several researchers have investigated LTP/LTD-like mechanisms with repetitive transcranial magnetic stimulation (rTMS) techniques by measuring rTMS-induced long-term changes in motor evoked potential (MEP) amplitudes (aftereffects) (Ziemann et al. 2008). Among the rTMS techniques, paired associative stimulation (PAS) entails M1-rTMS coupled with electric stimulation applied to a contralateral peripheral nerve, at specific interstimulus intervals (ISIs). PAS aftereffects depend on the specific ISIs used; MEP sizes increase after 25 ms ISI but decrease after 10 ms ISI suggesting PAS-induced aftereffects are due to associative plasticity in M1 that would normally be driven by sensorimotor integration processes (Stefan et al. 2000; Wolters et al. 2003). Research over recent years has developed new PAS protocols by coupling rTMS with stimulation that directly or indirectly activates remote cortical areas including the contralateral M1 (Koga-nemaru et al. 2009; Rizzo et al. 2009), ipsilateral nonprimary motor areas (Araki et al. 2011; Buch et al. 2011) and even nonmotor cortical areas such as those responsible for pain perception (Suppa et al. 2012). Overall these studies support the hypothesis that associative plasticity operates in M1 regardless of the specific PAS protocol used, and underline the importance of sensorimotor integration processes in motor control. Precisely whether and through which physiological mechanisms early visuomotor integration processes contribute to motor control remains unclear.

We designed this study to investigate whether early visuomotor integration contributes to motor control by eliciting LTP/LTD-like plasticity in the human M1. For this purpose, we designed and tested in healthy subjects a modified PAS protocol that couples left M1-rTMS with ipsilateral V1 activation (V-PAS), elicited by hemifield pattern-reversal visual evoked potentials (VEPs). We first tested postintervention changes in MEP amplitudes after delivering V-PAS at specific ISIs. To clarify MEP amplitude changes after M1-rTMS given alone, we delivered rTMS at 1 Hz and with the same pulse number and intensity used for V-PAS. To exclude possible confounding due to stimulation frequency, we compared the effect of V-PAS and rTMS given alone at a frequency lower than 1 Hz (0.25 Hz) that is known to elicit no aftereffects on MEP amplitudes per se (Fitzgerald et al. 2006). To clarify a possible 0.25-Hz V-PAS-induced “dose effect” (Kang et al. 2011), we compared 0.25-Hz V-PAS with a shorter 0.25-Hz V-PAS protocol delivering fewer stimuli. To verify whether V-PAS elicited MEP changes also when we activated the contralateral V1, we delivered a modified V-PAS protocol designed to activate the right V1. Finally, to clarify whether V-PAS elicits increased activity in V1 or in functional connections between parietal or premotor areas with M1, we examined VEPs and MEPs elicited by standardized paired-pulse protocols designed to test functional connectivity between posterior parietal cortex (PPc), dorsal premotor (PMd), and ventral prefrontal cortex (PMv) and ipsilateral M1 (Civardi et al. 2001; Koch et al. 2007a,b; Davare et al. 2008; Batümner et al. 2009), before and after V-PAS.
Materials and Methods

Subjects
Fourteen healthy right-handed volunteers (6 men and 8 women, mean age: 27 ± 2.9 years) were enrolled. None of the subjects had a history of neurological disorder or used drugs active on the central nervous system and all of them had normal or corrected to normal visual acuity. All subjects gave informed consent and all experimental protocols conformed with the Declaration of Helsinki and were approved by the institutional review board.

Visual Evoked Potentials: Stimulation and Recordings
Subjects were comfortably seated in an armchair, in a dimly lit room, with their right eye covered by an eyepatch. They were instructed to fully relax and look at a fixation point (small red cross) at the exact center of a computer screen (15°, maximum luminance 370 cd/m²; distance from subject’s eyes: 70 cm). The pattern-reversal visual stimuli consisted of black-and-white checks (individual check visual angle 30°; field size 24°; luminance contrast 80%) that changed phase at one reversal per second (Geesela and Brigell 1999; American Clinical Neurophysiology Society 2006) using Stim2 software (Compumedics USA, Ltd.). Half the visual field was stimulated by dividing the screen vertically into a static left half that produced a constant checkerboard image, and a dynamic right half that changed phase, thus activating the left temporal hemiretina.

Bipolar electroencephalographic (EEG) activity was recorded from 5 scalp sites using silver plated Ag/AgCl electrodes positioned over the midoccipital (MO, 5 cm above nasion), left lateral occipital (LLO, 5 cm lateral to MO), right lateral occipital (RLO, 5 cm lateral to MO), left temporal (LT, 5 cm lateral to LLO), and right temporal areas (RT, 5 cm lateral to RLO); all the electrodes were referenced to a common reference electrode placed at midfrontal position (MF, 12 cm above nasion). One additional electrode, positioned over the right distal ulna, was used for grounding.

The impedence between the electrodes was kept below 5 kΩ. A notch filter (50 Hz) and a bandpass filter (0.5–1 kHz) were implemented with a Digitimer D360 (Digitimer Ltd, Welwyn Garden City, UK). The signal was amplified, acquired at 5 kHz through a 1401 plus AD laboratory interface (Cambridge Electronic Design, Cambridge, UK) and stored on a personal computer for off-line analysis (Signal software). Effective hemiretina stimulation was confirmed by specific response components to hemifield stimulation (N75, P100, N145; at MO and LLO ipsilateral to the half-field stimulated; P75, N105, and P135 at LLO and temporal electrode contralateral to the half-field stimulated). The reversal rate and the high luminance contrast elicited a reproducible P100 component. The peak P100 latency was calculated from MO for each subject by averaging at least 100 traces (Table 1).

<table>
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<th>Subjects</th>
<th>N75 lat. (ms)</th>
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Note that each value refers to the first VEP recording obtained in each subject. lat., latency; amp., amplitude; AV, average; SD, standard deviation.

Motor Evoked Potentials: Stimulation and Recordings
TMS was delivered through a bihaptic magnetic stimulator (Magstim SuperRapid, The Magstim Company Ltd, Whitland, South West Wales, UK) connected to a figure-of-eight coil with a mean loop diameter of 9 cm. The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane. The optimal position (hot spot) of the coil for eliciting a twitch in the resting right dorsal interosseus muscle (FDI) was marked directly on the scalp with a soft-tip pen. Resting motor threshold (RMT) was calculated as the lowest stimulus intensity able to evoke MEPs of at least 50 µV in 5 of 10 consecutive trials. Active motor threshold (AMT) was calculated during a 20–50% maximum voluntary target muscle contraction as the lowest intensity able to evoke an MEP of at least 200 µV in 5 of 10 consecutive trials. RMT and AMT were calculated with step width of 1% maximum stimulator output.

MEPs were recorded from the FDI muscle, using a belly-tendon montage, with 2 Ag/AgCl electrodes connected to the Digitimer D360 with the notch filter on (50 Hz) and the bandpass filter implemented at the 20-1 kHz band. The EMG signal was amplified, digitalized, and stored on a laboratory computer for off-line analysis (Signal software).

Experimental Design
The study included 6 experiments and at least 7 days elapsed between 2 consecutive sessions (Fig. 1).

Experiment 1: Effect of V-PAS at Different ISIs on MEP Amplitudes
The experimental design consisted of a conditioning-test stimulation protocol. In Experiment 1, baseline measures (T0) included 100 VEP recordings to calculate VEP P100 peak latency for each subject. We also recorded 20 test MEPs at T0 by applying single TMS pulses able to evoke MEPs of 1 mV peak-to-peak in amplitude. Conditioning V-PAS consisted of 600 MEPs elicited at 1 Hz frequency (10 min intervention), and each VEP preceded a single TMS pulse given over the left M1 at ISIs ranging from VEP P100 peak latency + 40, 60, 80, 100, 120, and 140 ms (V-PAS). ISIs were selected according to several previous animal and human studies demonstrating frontal area activation as early as 150 ms after visual stimulation (Schroeder et al. 1998; Lamme and Roelfsema 2000; Sarson et al. 2001; Ledberg et al. 2007; Gaillard et al. 2009; Casali et al. 2010). During V-PAS, we delivered the same TMS intensity used for testing MEP size at T0 and used a single ISI throughout conditioning. As a measure of LTP/LTD-like plasticity, we recorded MEPs elicited by single pulse TMS at 5 (T1), 15 (T2), 25 (T3), 35 (T4), 45 (T5), and 55 min (T6) after conditioning. The TMS intensity was similar to that used at T0. During MEP recordings, subjects looked at the fixation point at the center of a static checkerboard image on both sides of the display. To examine possible changes in MEPs during V-PAS, we also recorded the 600 MEPs elicited during V-PAS and averaged them in 30 bins of 20 MEPs each. Fourteen subjects were randomized and counterbalanced to participate in the 6 experimental sessions, and at least 1 week elapsed between sessions. In a subgroup of 5 subjects, we collected MEPs also at 65 (T7), 75 (T8), 85 (T9), and 95 min (T10) after V-PAS.

Experiment 2: Effect of 1-Hz rTMS on MEP Amplitudes
During Experiment 2, subjects looked at the fixation point at the center of the static checkerboard image on both sides of the display. Conditioning rTMS consisted of 600 pulses over the left M1, delivered at 1 Hz (10 min intervention) and at similar intensity used for evoking MEPs at T0. The same intensity was used for testing MEP size throughout the experiment at 5 (T1), 15 (T2), 25 (T3), 35 (T4), 45 (T5), and 55 min (T6) after conditioning. Fourteen subjects participated in this experimental session. In a subgroup of 5 subjects, we collected MEPs also at 65 (T7), 75 (T8), 85 (T9), and 95 min (T10) after 1-Hz rTMS.

Experiment 3: Effect of V-PAS and rTMS Delivered at 0.25 Hz on MEP Amplitudes
In Experiment 3, to examine the effect of stimulation frequency on V-PAS-induced aftereffects, we randomly delivered 600 pulses of...
0.25-Hz rTMS alone (40 min intervention) or V-PAS with similar procedures used in Experiment 1 except that the frequency of visual stimuli and rTMS was reduced at 0.25 Hz (40 min intervention) and the ISIs were limited to 40, 100, and 140 ms. rTMS was given over the left M1 at similar intensity used for evoking MEPs at T0 and the same intensity was used for testing MEPs throughout the experiment at 5 (T1), 15 (T2), 25 (T3), 35 (T4), 45 (T5), and 55 min (T6) after conditioning. A subgroup of 6 subjects participated in this experimental session.
**Experiment 4: Effect of a Short V-PAS₁₀₀ Protocol at 0.25 Hz on MEP Amplitudes**

In Experiment 4, to investigate whether 0.25-Hz V-PAS induced a "dose effect," we delivered V-PAS₁₀₀ with the same procedure used in Experiment 3 except that the total number of stimuli was reduced from 600 to 150 (10 min intervention). TMS was given over the left M1 at similar intensity used for evoking MEPs at T0 and the same intensity was used for testing MEPs throughout the experiment at 5 (T1), 15 (T2), 25 (T3), 35 (T4), 45 (T5), and 55 min (T6) after conditioning. A subgroup of 6 subjects participated in this experimental session.

**Experiment 5: Effect of Contralateral V-PAS₁₀₀ on MEP Amplitudes**

In this experiment, V-PAS₁₀₀ was applied as described in Experiment 1 except that visual stimuli were delivered to the left visual hemifield with the left eye bandaged, thus activating the right temporal hemiretina, and in turn the right V1 (contralateral V-PAS₁₀₀). A subgroup of 6 subjects participated in this experimental session.

**Experiment 6: Effect of V-PAS₁₀₀ on VEPs and Functional Connectivity Between PPC, PMd, PMv and M1**

In this experiment, we first recorded 100 VEPs and 20 MEPs at 1 mV intensity at baseline (T0a). Baseline recordings also included single and paired pulses designed to examine connections from PPC, PMd, and PMv to M1 (T0b) (Civardi et al. 2001; Koch et al. 2007a,b; Davare et al. 2008; Baümer et al. 2009). To localize the left PPC, PMd, and PMv, we used a Polaris Vicra optical measurement system (Northern Digital, Inc.) combined with the Softax Evolution navigator system (E.M.S., Bologna, Italy). This software uses a set of digitized skull landmarks (nasion, inion, and right and left preauricular points) and about 60 scalp points to provide a uniform scalp representation which is then adapted to a normalized reference volume of highly detailed T1-weighted magnetic resonance imaging (MRI) to obtain an estimated individualized MRI in the Talairach Space (Talairach and Tournoix 1988) for every subject. Previous studies demonstrated that the mean accuracy of the estimated MRIs is comparable to the spatial resolution of TMS (Herwig et al. 2001; Sandrini et al. 2008). PMd, PMv, and PPC were localized according to the following Talairach coordinates: PMd: (x, y, z) = (−27, −7, +62) (Oshio et al. 2010); PMv: (x, y, z) = (−52, −45, +39) (Davare et al. 2008); PPC: (x, y, z) = (−58, +13, +19) (Stoeckel et al. 2009). We used 2 monophasic Magstim 200 stimulators, each connected to a figure-of-eight coil. A first 9 cm-coil was placed over the left M1, whereas a second 5 cm-coil was placed over the left PPC, PMd, or PMv. To calculate the intensity of conditioning pulses, we also measured AMT and RMT with the 5 cm-coil placed over the left M1. We tested PPa-to-M1 facilitatory connections by delivering randomly 20 single pulses and 20 paired pulses at a 4 ms ISI, with conditioning pulses at 90% RMT (Koch et al. 2007a). PPa-to-M1 facilitatory and inhibitory connections were tested by delivering 20 single pulses and 20 paired pulses at a 6 ms ISI, with conditioning pulses at 120% or 90% AMT (Civardi et al. 2001; Koch et al. 2007b). Given a distance of 2.5–3 cm between the M1 hand representation and ipsilateral PMd (Civardi et al. 2001; Groppa et al. 2012), the 9-cm-coil placed over M1 was partly bent and adapted to the individual participant’s scalp position. In order to compensate for the suboptimal coil positioning, we slightly increased the stimulation intensity to elicit MEPs of about 1 mV at baseline. Finally, PMv-to-M1 facilitatory and inhibitory connections were tested by delivering 20 single pulses and 20 paired pulses at the 6 ms ISI, with conditioning pulses at 80% AMT or 90% RMT (Davare et al. 2008; Baümer et al. 2009). After completing our baseline recordings, we applied the V-PAS₁₀₀ technique with the same procedures used in Experiment 1. After V-PAS₁₀₀, we recorded again 100 VEPs (T1). To verify V-PAS₁₀₀-induced aftereffects we recorded 20 MEPs (T2). Finally, we delivered single and paired pulses (T3) with similar procedures used at T0 except that the intensity for single pulses was reduced to compensate for V-PAS₁₀₀-induced aftereffects. PPa-, PMd-, and PMv-to-M1 connections were randomly investigated, before and after V-PAS₁₀₀. We measured and compared MEP amplitudes elicited by single and paired pulses, before and after V-PAS₁₀₀. A subgroup of 6 subjects participated in this experimental session.

**Statistical Analysis**

Data collected in all experimental sessions were analyzed as absolute values (mV) by repeated measures analysis of variance (ANOVA).

We used separate 2-way ANOVAs with factors "Conditions" and "Time" as main factor of analysis in Experiments 1–6. The factor "Condition" consisted of V-PAS at the different ISIs for Experiment 1, 1-Hz rTMS and V-PAS₁₀₀ for Experiment 2, 0.25-Hz rTMS and V-PAS at 0.25 Hz at the different ISIs for Experiment 3, 600 and 150 pulses of V-PAS₁₀₀ at 0.25 Hz for Experiment 4, contralateral V-PAS₁₀₀ and V-PAS₁₀₀ or contralateral V-PAS₁₀₀ and 1-Hz rTMS for Experiment 5, and finally single and paired pulses designed to test PPa-, PMd-, and PMv-to-M1 connections for Experiment 6. The factor Time referred to T0 versus T1–T6 in Experiments 1–5, T0 versus T1–T10 in the subgroup of 5 subjects participating at Experiments 1–2, and finally T0a versus T2 and T0b versus T3 in Experiment 6. When we compared the 600 MEPs recorded during V-PAS at the various ISIs in Experiment 1, the factor Time referred to the 30 bins of 20 MEPs each. We also used separate 1-way ANOVAs with factors Time (T0 versus T1–T6, and also T7–T10 in the subgroup of 5 subjects) to test changes in MEP amplitudes after 1-Hz rTMS in Experiment 2, to compare VEP (baseline versus T1) and MEP amplitudes (T0a versus T2 and T0b versus T3) in Experiment 6. Finally, separate 1-way ANOVAs with factor Conditions were also used to compare the RMT, the intensity required to evoke MEPs at T0 and the VEP P100 latency and amplitude collected in each subject in all experimental sessions.

The Pearson correlation test was used to assess possible correlations in each subject between possible 1-Hz rTMS and V-PAS-induced changes in MEP amplitudes at each time point, VEP P100 latency and amplitude, RMT; and the intensity required for evoking MEPs of 1 mV in amplitude at T0.

Tukey honest significant difference test was used for all post hoc analyses. The Greenhouse-Geisser correction was used when necessary to correct for nonsphericity. A P-value <0.05 was considered significant for all statistical analyses.

**Results**

None of the subjects experienced any adverse effects during or after VEP recordings, rTMS and V-PAS in all the experiments. As in our data sample sphericity was never violated, we never applied the Greenhouse-Geisser correction.

**Experiment 1: Effect of V-PAS at Different ISIs on MEP Amplitudes**

In this experiment, we found that the V-PAS-induced MEP changes reflected the specific ISI used. Two-way ANOVA showed a significant interaction between factors Conditions and Time ($F_{(5, 90)} = 4.5; P < 0.01$) and a significant effect of factor Conditions ($F_{(5, 65)} = 7.4; P < 0.01$), whereas the factor Time was not significant ($F_{(6, 79)} = 0.9; P = 0.5$). Post hoc analysis showed that despite similar MEP amplitudes at T0 ($P > 0.05$ for all comparisons), V-PAS₁₀₀, V-PAS₁₀₀, and V-PAS₁₂₀ all induced changes in MEP amplitudes as shown by a significant effect of factor Time (V-PAS₁₀₀: $F_{(6, 73)} = 5.58; P < 0.01$; V-PAS₁₀₀: $F_{(6, 78)} = 7.71; P < 0.01$; V-PAS₁₂₀: $F_{(6, 78)} = 5.99; P < 0.01$). V-PAS₁₀₀ decreased MEPs significantly at T1–T6 ($P < 0.01$ for all comparisons), whereas V-PAS₁₀₀ and V-PAS₁₂₀ increased MEPs and both did so at T1–T6 ($P < 0.01$ for all comparisons). Post hoc analysis comparing MEPs, before and after V-PAS₁₀₀ at T1–T10, in the subgroup of 5 subjects, showed that the V-PAS₁₀₀-induced aftereffects lasted <65 min. ANOVA confirmed the significant effect of the factor Time (F₁₀,₄₀ = 4.04; P < 0.01) and demonstrated that, although V-PAS₁₀₀ increased MEPs at T1–T6 ($P < 0.05$ for all comparisons), at T7–T10 it did not ($P > 0.05$ for all comparisons). By contrast, ANOVA comparing MEP amplitudes before and after V-PAS₁₀₀, V-PAS₁₀₀, and V-PAS₁₄₀
showed that the factor Time was not significant (V-PAS60: $F_{6,78} = 0.51; P = 0.8$; V-PAS80: $F_{6,78} = 0.17; P = 0.9$; V-PAS140: $F_{6,78} = 1; P = 0.43$) demonstrating that V-PAS60, V-PAS80, and V-PAS140 left MEPs unchanged (Fig. 2).

When we compared the 50 bins of 20 MEPs each (600 MEPs in total) recorded during V-PAS at the various ISIs, 2-way ANOVA showed a nonsignificant effect of the factors Conditions ($F_{5,55} = 0.31; P = 0.9$) and Time ($F_{29,357} = 1.01; P = 0.45$) suggesting that during V-PAS, MEPs remained unchanged at all the ISIs examined.

**Experiment 2: Effect of 1-Hz rTMS on MEP Amplitudes**

One-way ANOVA showed a significant effect of the factor Time ($F_{6,78} = 11.99; P < 0.01$) and post hoc analysis showed that 1-Hz rTMS decreased MEP size significantly from T1 to T6 ($P < 0.01$ for all comparisons). In the subgroup of 5 subjects, ANOVA comparing MEPs before and after 1-Hz rTMS at T1–T10 showed that the 1-Hz rTMS-induced aftereffects lasted <65 min. The factor Time was significant ($F_{10,40} = 2.43; P < 0.05$) and ANOVA demonstrated that, although 1-Hz rTMS decreased MEPs at T1–T6 ($P < 0.05$ for all comparisons), at T7–T10 it did not ($P > 0.05$ for all comparisons) (Fig. 3).

When we compared MEP changes after 1-Hz rTMS and V-PAS at the different ISIs, 2-way ANOVA showed a significant interaction between factors Conditions and Time ($F_{36,468} = 5.18; P < 0.01$) and a significant effect of factor Conditions ($F_{6,78} = 7.01; P < 0.01$), whereas the factor Time was not significant ($F_{6,78} = 0.27; P = 0.95$). Post hoc analysis showed that although MEPs were similar in amplitude at T0 in the different conditions ($P > 0.05$ for all comparisons), after conditioning, MEPs differed significantly as demonstrated by a significant interaction between factors “Condition” and Time in the ANOVA comparing MEPs after 1-Hz rTMS and V-PAS60 ($F_{6,78} = 3.48; P < 0.01$), V-PAS80 ($F_{6,78} = 3.6; P < 0.01$), V-PAS100 ($F_{6,78} = 16.18; P < 0.01$), V-PAS120 ($F_{6,78} = 13.75; P < 0.01$), and V-PAS140 ($F_{6,78} = 2.41; P = 0.03$), but not V-PAS40 ($F_{6,78} = 0.62; P = 0.72$).

Finally, when we compared MEPs after 1-Hz rTMS and V-PAS100 in the subgroup of 5 subjects, 2-way ANOVA showed a significant interaction between factors Conditions and Time ($F_{10,40} = 8.54; P < 0.01$). Post hoc analysis confirmed that 1-Hz rTMS and V-PAS100 both induced changes in MEP amplitudes as demonstrated by the significant effect of the factor Time (1-Hz rTMS: $F_{10,40} = 2.43; P < 0.05$; V-PAS100: $F_{10,40} = 4.04; P < 0.01$). Although before 1-Hz rTMS and V-PAS100 MEP amplitudes were similar at T0 ($P > 0.05$), after 1-Hz rTMS and V-PAS100, they differed significantly at T1–T6 ($P < 0.05$ for all comparisons) but not at T7–T10 ($P > 0.05$ for all comparisons).

**Experiment 3: Effect of V-PAS and rTMS Delivered at 0.25 Hz on MEP Amplitudes**

In this experiment, 2-way ANOVA showed a significant interaction between factors Conditions and Time ($F_{10,90} = 6.43; P < 0.01$). When comparing MEPs after 0.25-Hz rTMS given alone and 0.25-Hz V-PAS100 ANOVA showed a significant interaction between factors Conditions and Time ($F_{6,30} = 6.13; P < 0.01$). Post hoc analysis showed that although MEPs were similar at T0 ($P > 0.05$) they differed at T1–T6 ($P < 0.05$ for all comparisons). After 0.25-Hz rTMS given alone, MEPs remained unchanged as shown by a nonsignificant effect of the factor Time ($F_{6,30} = 1.07; P = 0.4$), whereas after 0.25-Hz V-PAS100 MEP amplitudes increased ($F_{6,30} = 7.02; P < 0.01$) and did so at all time points ($P < 0.05$ for all comparisons) (Fig. 4).

When comparing MEPs after 0.25-Hz rTMS given alone and 0.25-Hz V-PAS40 ANOVA showed a significant interaction between factors Conditions and Time ($F_{6,30} = 5.76; P < 0.01$). Despite similar MEPs at T0 ($P > 0.05$), MEPs differed at T2–T3 and T4 ($P < 0.05$ for all comparisons). 0.25-Hz V-PAS40 inhibited MEPs ($F_{6,30} = 4.6; P < 0.01$) and did so at T2–T3 and T4 ($P < 0.05$ for all comparisons) (Fig. 4).

Finally, when comparing MEPs after 0.25-Hz rTMS given alone and 0.25-Hz V-PAS140 ANOVA showed a significant effect of the factor Time ($F_{6,30} = 2.3; P = 0.05$). Post hoc analysis showed that MEPs were comparable before and after conditioning ($P > 0.05$ for all comparisons); 0.25-Hz V-PAS140...
induced a nonsignificant trend for MEP facilitation ($F_{6.30} = 2.34; P = 0.07$) (Fig. 4).

When we compared MEP changes after 1-Hz V-PAS and 0.25-Hz V-PAS in the same group of subjects, the separate ANOVAs showed a significant effect of the factor "Condition" at the 40 and 100 ms ISIs (1-Hz V-PAS$_{40}$ vs. 0.25-Hz V-PAS$_{40}$: $F_{1.5} = 8.5; P < 0.05$; 1-Hz V-PAS$_{100}$ vs. 0.25-Hz V-PAS$_{100}$: $F_{1.5} = 9.52; P < 0.01$) but not at the 140 ms ISI (1-Hz V-PAS$_{140}$ vs. 0.25-Hz V-PAS$_{140}$: $F_{1.5} = 3.4; P > 0.05$). Post hoc analysis demonstrated similar MEP amplitudes at T0 in all conditions ($P > 0.05$ for all comparisons). MEPs were higher in amplitude after 0.25-Hz V-PAS than after 1-Hz V-PAS at the 40 and 100 ms ISIs; there was a trend of increased amplitude MEPs also at 140 ms ISI (Fig. 4).

**Experiment 4: Effect of a Short V-PAS$_{100}$ Protocol at 0.25 Hz on MEP Amplitudes**

In this experiment, 2-way ANOVA showed a significant interaction between factors Conditions and Time ($F_{6.30} = 7.01; P < 0.01$). Post hoc analysis showed that although MEPs were similar at T0 ($P > 0.05$), 600 pulses of 0.25-Hz V-PAS$_{100}$ increased MEP amplitudes, as demonstrated by the significant effect of the factor Time ($F_{6.30} = 7.02; P < 0.01$), whereas 150 pulses of 0.25-Hz V-PAS$_{100}$ did not ($F_{6.30} = 0.96; P = 0.47$) (Fig. 5).

**Experiment 5: Effect of Contralateral V-PAS$_{100}$ on MEP Amplitudes**

In this experiment, 2-way ANOVA showed a significant interaction between factors Conditions and Time ($F_{6.30} = 6.34; P < 0.01$). Post hoc analysis showed that both V-PAS$_{100}$ and contralateral V-PAS$_{100}$ induced changes in MEP amplitudes as demonstrated by the significant effect of the factor Time (V-PAS$_{100}$: $F_{6.30} = 3.50; P < 0.01$; contralateral V-PAS$_{100}$: $F_{6.30} = 7.18; P < 0.01$). Even though MEPs were similar at T0 ($P > 0.05$), they differed significantly at T1–T6 ($P < 0.05$ for all comparisons) (Fig. 6).

When we compared MEP changes after 1-Hz rTMS and contralateral V-PAS$_{100}$, 2-way ANOVA showed a significant effect of the factor Time ($F_{6.30} = 10.02; P < 0.01$), whereas the factor Conditions was not significant ($F_{1.5} = 0.57; P = 0.5$). rTMS (1-Hz) and contralateral V-PAS$_{100}$ induced similar MEP amplitude inhibition.

**Experiment 6: Effect of V-PAS$_{100}$ on VEPs and Functional Connectivity Between PPC, PMd, PMv, and M1**

One-way ANOVA comparing VEP amplitudes before and after V-PAS$_{100}$ showed a nonsignificant effect of the factor Time suggesting similar amplitude VEPs before and after V-PAS$_{100}$. Conversely, 1-way ANOVA comparing MEPs before and after V-PAS$_{100}$ showed that the factor Time was significant ($F_{1.5} = 29.66; P < 0.01$) confirming that after V-PAS$_{100}$ MEPs, as expected, increased in size at T2 compared with T0a.
When we compared MEPs elicited by single and paired pulses designed to examine PPC-, PMd- and PMv-to-M1 facilitatory connections, 2-way ANOVAs showed the significant effect of the factor “Condition” (PPC-to-M1: $F_{1,5} = 49.4; P < 0.01$; PMd-to-M1: $F_{1,5} = 13.5; P < 0.01$; PMv-to-M1: $F_{1,5} = 48.19; P < 0.01$) but a nonsignificant effect of the factor Time. Despite similar single pulse MEPs at T0b and T3, MEPs elicited by paired pulses increased significantly in amplitude at T3 after each protocol ($P < 0.05$ for all comparisons) and were similar before and after PAS100 (Fig. 7).

By contrast, when we examined PMd- and PMv-to-M1 inhibitory connections, 2-way ANOVAs showed a significant interaction between factors “Condition” and Time (PMd-to-M1: $F_{1,5} = 20.69; P < 0.01$; PMv-to-M1: $F_{1,5} = 14.04; P < 0.01$). Post hoc analysis showed similar single pulse MEP amplitudes at T0b and T3, whereas MEPs were inhibited by paired pulses before ($P < 0.05$ for all comparisons) but not after PAS100 (Fig. 7).

One-way ANOVAs with factor “Conditions” showed comparable RMT, intensity required to evoke MEPs at T0, VEP P100 latency and amplitude in each subjects and in all experimental sessions.

The Pearson correlation test found no correlations in any subject between 1-Hz rTMS- and V-PAS-induced changes in MEP amplitudes, VEP P100 latency, and amplitude.

**Discussion**

In this study in healthy subjects, to investigate whether visuomotor integration processes induce LTP/LTD-like plasticity in M1, we designed a new PAS protocol coupling VEPs with rTMS. V-PAS induced changes in MEP amplitude that depended on the specific ISI used. V-PAS at 40 ms ISI induced MEP inhibition, whereas V-PAS at the 60, 80, and 140 ms ISIs left MEP sizes unchanged. By contrast, after we delivered V-PAS at the 100 and 120 ms ISIs, MEPs increased significantly. Conversely, as expected, 1-Hz rTMS given alone induced MEP inhibition. V-PAS delivered at 0.25 Hz elicited different changes in MEP amplitudes according to the ISI used, whereas 0.25-Hz rTMS given alone left MEPs unchanged. When we applied 0.25-Hz V-PAS for 10 min with a reduced number of stimuli (150 stimuli), we failed to elicit MEP changes. After a V-PAS protocol designed to record VEPs from the contralateral right V1, MEPs again decreased in size. In our final control experiment, we found comparable VEPs and facilitatory connections between PPC-, PMd-, and PMv-to-M1 before and after V-PAS. Differently, V-PAS reduced the PMd- and PMv-to-M1 inhibitory connections. Overall, these findings provide new information on the physiological mechanisms induced by early visuomotor integration processes in the human M1.

When we applied our V-PAS protocol, postintervention MEP amplitudes reflected the specific ISIs used. Given the similar baseline RMT, AMT, and MEP amplitudes, and the intensity used for V-PAS at all the ISIs tested, we can exclude the possibility that responses to the various V-PAS protocols depended on differences in baseline excitability. Because subjects randomly participated in the different experimental sessions and were blinded to the specific ISI used, we can also exclude differences in attention (Stefan et al. 2004). By allowing at least 1 week to elapse between the different sessions, we also excluded possible interference between the different experimental sessions (Ziemann et al. 2008). Finally, the similar VEP amplitude and latency in all subjects through the different sessions excludes confounding from differences related to baseline VEPs.

The observation that V-PAS induced different MEP changes according to the specific ISI used strongly suggests that, as others reported for the sensorimotor PAS protocol (Stefan et al. 2000), the V-PAS-induced aftereffects reflect mechanisms involving associative plasticity. Given that experimental protocols implying associative plasticity and characterized by temporally correlated pre- and postsynaptic activity are thought to induce spike timing-dependent plasticity (STDP), we suggest that the V-PAS-induced aftereffects reflect STDP in M1. The mechanisms underlying STDP may also explain why the V-PAS-induced aftereffects ranged from inhibition to facilitation. Cortical STDP depends on appropriately timed excitatory post-synaptic potentials in layer 2/3 apical dendrites in layer 5 cortico-spinal neurons, and back-propagating action potentials from the same neurons (Dan and Poo 2004, 2006; Caporale and Dan 2008). When presynaptic inputs precede postsynaptic back-propagating action potentials (“pre-post... 

![Figure 7](https://academic.oup.com/cercor/article-abstract/25/3/703/350047)
sequence), within a narrow time window, they presumably induce LTP, whereas the opposite temporal order (post–pre) commonly drives LTD (Dan and Poo 2004, 2006; Caporale and Dan 2008). According to the STDP model, our finding that V-PAS at the 40 ms ISI inhibited MEPs, whereas at 100–120 ms, ISIs it facilitated MEPs, might depend on the specific sequential order in pre- and postsynaptic activity. In theory, at the 40 ms ISI, TMS-induced postsynaptic activity would have consistently preceded the VEP-induced presynaptic activity (post–pre), thus leading to LTD, whereas at 100–120 ms ISIs, the opposite temporal order (pre–post) would have driven LTP, depending on the 60–80 ms required for VEP-induced visual afferent inputs to travel from V1 to M1. The lack of MEP changes observed at the 60–80 and 140 ms ISIs would reflect in-between cellular activity leading to no LTP/LTD (Fig. 8). Accordingly, because TMS is thought to activate layer 5 corticospinal neurons (Di Lazzaro et al. 2004) and assuming that projections coming from remote cortical regions reach M1 cortical layers 2/3 (Dum and Strick 2002, 2005; Dum et al. 2009), we speculate that the V-PAS-induced aftereffects reflected heterosynaptic STDP in M1 cortical layers 2/3.

These possible mechanisms notwithstanding, the STDP model leaves the physiological mechanisms involved in the V-PAS-induced aftereffects partly unexplained. Our first control experiment (Experiment 2) showing that 1-Hz rTMS given alone decreased MEPs for about 60 min, in agreement with previous studies (Chen et al. 1997; Wassermann 1998; Fitzgerald et al. 2006) nevertheless provides further insights. Given that our M1-rTMS protocol was given at the same intensity used to evoke MEPs at baseline, we believe that the 1-Hz rTMS-induced aftereffects reflect homosynaptic LTD-like plasticity in M1 (Ziemann et al. 2008; Suppa and Berardelli 2012). Accordingly, the V-PAS-induced aftereffects we found in this study can be better explained by an “extended” STDP model which implies a physiological interaction between heterosynaptic STDP and homosynaptic LTD-like plasticity in M1. This hypothesis also agrees with the similar aftereffect time course we observed after 1-Hz rTMS and V-PAS at the 100 ms ISI.

In line with our hypothesis proposing an “extended” STDP model, in the experiment testing the effect of V-PAS at 0.25 Hz (Experiment 3), we found that although 0.25-Hz rTMS given alone elicited, as expected, no aftereffects on MEPs (Fitzgerald et al. 2006), 0.25-Hz V-PAS decreased, increased, or left MEPs unchanged according to the ISIs used and again in line with an STDP model (Stefan et al. 2000; Dan and Poo 2004, 2006; Caporale and Dan 2008). In addition, when we compared MEP amplitudes after delivering 0.25-Hz and 1-Hz V-PAS in the same group of subjects, we found increased MEPs at 40 and 100 ms ISIs and a trend of increased amplitude MEPs at 140 ms ISI. Overall these findings strongly support the hypothesis that the V-PAS-induced aftereffects observed in Experiment 1 reflect the physiological interaction between V-PAS-induced heterosynaptic STDP and 1-Hz rTMS-induced homosynaptic LTD-like plasticity in M1.

The physiological interaction underlying V-PAS-induced aftereffects between heterosynaptic STDP and homosynaptic LTD in the same M1 neuronal population might operate in line with homeostatic or nonhomeostatic metaplasticity rules. Homeostatic metaplastcity refers to the mechanisms needed to maintain overall excitability within a dynamic range, and prevent unrestricted plasticity (Bienenstock et al. 1982; Davis 2006). According to the Bienenstock–Cooper–Munro (BCM) theory of homeostatic metaplassticity (Bienenstock et al. 1982; Davis 2006) the threshold for inducing LTP and LTD varies as a function of the previous history of post-synaptic activity. Low activity levels increase the probability of inducing LTP, whereas high activity levels promote LTD (Bienenstock et al. 1982; Davis 2006). To discriminate homeostatic or nonhomeostatic metaplasticity mechanisms, we compared MEPs after V-PAS delivered at 1 and 0.25 Hz and at 40, 100, and 140 ms ISIs, in the same group of subjects. After 0.25-Hz V-PAS, we found larger amplitude MEPs at 40 and 100 ms ISIs and a non-significant trend for increased MEPs also at 140 ms ISI compared with MEPs elicited after 1-Hz V-PAS at the same ISIs. These findings fit in poorly with the BCM rules because it demonstrates a nonhomeostatic metaplasticity interaction in M1 between V-PAS-induced heterosynaptic STDP and 1-Hz rTMS-induced homosynaptic LTD-like plasticity.

Experiment 4 demonstrated that the shorter protocol for 0.25-Hz V-PAS at 100 ms elicited no aftereffects on MEP amplitudes showing that the V-PAS-induced aftereffects depend crucially on the total number of stimuli delivered. These findings suggest that 0.25-Hz V-PAS induces a “dose-effect” as others proposed for the original PAS protocol (Stefan et al. 2000; Kang et al. 2011).

Important information on the physiological mechanisms underlying V-PAS also comes from our experiment testing responses to a modified V-PAS protocol consisting of VEPs recorded from the contralateral right V1. When we delivered our contralateral V-PAS at the 100 ms ISI, we found that MEPs decreased suggesting that contralateral V-PAS left 1-Hz rTMS-induced inhibition unaffected. Our findings suggest that the V-PAS activated ipsilateral functional visuomotor connectivity.

Our hypothesis that VEP-induced visual afferent inputs take at least 60 ms to travel from V1 to M1, thus explaining why
TMS and VEPs interact, agrees with experimental studies in animals and in humans investigating early neural responses from cortical motor areas after stimuli directly or indirectly activating ipsilateral cortical visual areas. With the intracranial extracellular recording technique in monkeys (Riehle 1991; Schroeder et al. 1998; Lamme and Roelfsema 2000) and humans (Clarke et al. 1995; Gaillard et al. 2009) several investigators have provided evidence that visual area activation elicits time-locked neural activity that progressively spreads from posterior to anterior cortical areas and reaches the ipsilateral frontal lobe within 150 ms. By using the concurrent TMS-EEG technique, Casali et al. (2010) have investigated whole brain responses to focal TMS over the left superior occipital gyrus area, and found that neural activity spreads from the stimulated area to ipsilateral frontal regions within 100 ms. Although methodological factors including different stimulating and recording techniques probably explain slight differences in the intervals reported (Schroeder et al. 1998; Lamme and Roelfsema 2000; Gaillard et al. 2009; Casali et al. 2010), overall the previous studies support the proposed early visuomotor connectivity.

Our final control experiment provided important information on the possible anatomical pathways responsible for the V-PAS-induced aftereffects. The similar amplitude VEPs before and after V-PAS100 excludes the hypothesis that the V-PAS-induced aftereffects simply reflect increased activity in V1. When we examined the functional connectivity between parietal or premotor areas and M1, we found that V-PAS reduced the inhibitory connections between PMd and PMv and M1, whereas the facilitatory connections between Ppc, PMd, and PMv and M1 remained unchanged. These findings suggest that the V-PAS100-induced aftereffects arise from increased premotor-to-motor functional connectivity that is known to play a role in visuomotor integration processes (Weinrich and Wise 1982; di Pellegrino and Wise 1993; Dum and Strick 2002, 2005; Cisek and Kalaska 2005; Chouinard and Paus 2006; O’Shea et al. 2007; Buch et al. 2010; Rizzolatti and Luppino 2001).

Neuropsychological evidence showing V-PAS100-induced changes in premotor-to-motor functional connectivity imply fast visual input transmission from occipital to frontal areas. Studies using autoradiographic axonal tracing techniques in monkeys, and diffusion tensor MRI in humans showed that the superior longitudinal fasciculus (SLF) strongly connects occipito-parietal regions with ipsilateral frontal cortical areas including the PMd and PMv (Makris et al. 2005; Schmahmann et al. 2007; Mars et al. 2011; Thiebaut de Schotten et al. 2012). Given that the SLF intervenes in numerous visuomotor integration processes including conveying sensory and visual information relevant for motor control over body-centered action (Rizzolatti and Luppino 2001; Petrides and Pandya 2006; Schmahmann et al. 2007), we suggest that the SLF might be a suitable candidate for transmitting visual inputs fast to the cortical motor areas responsible for early visuomotor integration processes activated by V-PAS.

In conclusion, early visuomotor integration contributes to motor control by inducing LTP/LTD-like plasticity in the human M1. STDP operates in M1 as a general neurophysiological principle regardless of the stimuli used. Finally, our V-PAS protocol might be useful for noninvasively exploring the mechanisms underlying early visuomotor integration processes in the human M1.

Notes
Conflict of Interest: None declared.

References


