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Disrupting the Ventral Premotor Cortex Interferes with the Contribution of Action Observation to Use-dependent Plasticity

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Abstract

■ Action observation (AO), observing another individual perform an action, has been implicated in several higher cognitive processes including forming basic motor memories. Previous work has shown that physical practice (PP) results in cortical motor representational changes, referred to as use-dependent plasticity (UDP), and that AO combined with PP potentiates UDP in both healthy adults and stroke patients. In humans, AO results in activation of the ventral premotor cortex (PMv), however, whether PMv activation has a functional contribution to UDP, is not known. Here, we studied the effects disruption of PMv has on UDP when subjects performed PP combined with AO (PP + AO). Subjects participated in two randomized crossover sessions measuring the amount of UDP resulting from PP + AO while receiving disruptive (1 Hz) TMS over the fMRI-activated PMv or over OFC (Sham). We found that, unlike the sham session, disruptive TMS over PMv reduced the beneficial contribution of AO to UDP. To ensure that disruption of PMv was specifically interfering with the contribution of AO and not PP, subjects completed two more control sessions where they performed only PP while receiving disruptive TMS over PMv or OFC. We found that the magnitude of UDP for both control sessions was similar to PP + AO with TMS over PMv. These findings suggest that the fMRI activation found in PMv during AO studies is functionally relevant to task performance, at least for the beneficial effects that AO exerts over motor training. ■

INTRODUCTION

Action observation (AO), defined as observing another individual perform a task, has recently been implicated in a number of higher cognitive processes like understanding the actions and intentions of others (Iacoboni et al., 2005), imitation learning (Iacoboni et al., 1999), and motor learning (Mattar & Gribble, 2005), as well as disorders like autism (Cattaneo & Rizzolatti, 2009). In the motor domain, the mere observation of skill training can lead to performance improvements (Mattar & Gribble, 2005; Heyes & Foster, 2002; Vinter & Perruchet, 2002; Brass, Bekkering, & Prinz, 2001).

Human studies have shown that simple repetitive movements can elicit cortical motor representational changes referred to as use-dependent plasticity (UDP; Celnik, Webster, Glasser, & Cohen, 2008; Stefan, Classen, Celnik, & Cohen, 2008; Celnik et al., 2006; Stefan et al., 2005; Bütefisch, Khurana, Kopylev, & Cohen, 2004; Classen, Liepert, Wise, Hallet, & Cohen, 1998). Given that this form of plasticity encodes the specific kinematic aspects of the recently practiced movement, UDP has been interpreted as being indicative of a formation of a motor memory and possibly one of the initial steps in skill development (Classen et al., 1998). Interestingly, observing another individual perform the same repetitive training (i.e., AO) elicits similar corticomotor representational changes or memory formation (Stefan et al., 2005). Furthermore, when this AO is combined with physical practice (PP), the training effects are quantitatively enhanced beyond what either intervention alone can do in young healthy adults (Stefan et al., 2008), older healthy adults (Celnik et al., 2006), and stroke patients (Celnik et al., 2008).

Human imaging studies have indirectly shown an increased activation in the rostral part of the inferior parietal lobe (IPL) and the ventral premotor cortex (PMv) in association with AO (Iacoboni et al., 2005; Buccino, Lui, et al., 2004; Buccino, Vogt, et al., 2004; Buccino et al., 2001; Iacoboni et al., 1999). Physiological studies in humans have also demonstrated that AO results in increased excitability of the cortical representation in the primary motor cortex (M1) of the muscles participating in the observed training (Edwards, Humphreys, & Castello, 2003; Nishitani & Hari, 2000; Hari et al., 1998; Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995). Furthermore, recent studies using TMS have shown that AO changes the excitability of connections between PMv and M1 (Koch et al., 2010; Lago et al., 2010). These investigations have proposed that, during AO, connections from PMv map the observed movement onto the same neuronal substrate that is involved for generating the movements (Cattaneo & Rizzolatti, 2009), and this drives the performance improvements associated

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with AO. However, whether the activation observed in the human PMv area is crucial to the behavioral or physiological effects of AO or is a mere epiphenomenon has not been determined. In this study, we investigated the functional relevance of PMv activation resulting from AO using fMRI-guided TMS. We hypothesized that disruptive TMS over PMv while healthy individuals perform PP combined with AO would disrupt the additive effect AO has on UDP. Thus, we predicted that disruptive PMv stimulation would result in less UDP changes as a consequence of PP + AO and would have a similar magnitude as performing PP alone.

METHODS

Ten healthy, right-handed subjects (four men and six women, ranging from ages 19 to 27 years) with no history of neurological disorders participated in this study. All subjects gave informed consent approved by the Johns Hopkins School of Medicine Institutional Review Board and in accordance to the Declaration of Helsinki.

Recording Procedures

Participants sat comfortably with the right forearm supported in a semipronated position in a molded arm cast that allowed the thumb to move unrestrained. EMG activity was recorded with disposable surface electrodes placed over the right extensor pollicis brevis and right flexor pollicis brevis muscles. Signals were sampled at 2 kHz, visually displayed on-line, and analyzed off-line using MATLAB (The Mathworks Inc., Natick, MA).

Kinematic measurements were made with a 2-D accelerometer (Kistler Instruments, Amherst, NY) mounted on the distal portion of the first thumb phalanx. Movement directions were calculated from the first peak acceleration vector composed of two components: acceleration in the vertical (extension–flexion) axis and in the horizontal (adduction–abduction) axis (Classen et al., 1998).

Transcranial Magnetic Stimulation

In all conditions, we applied TMS using a fan-cooled figure-of-eight coil connected to a super rapid magnetic stimulator (Magstim 200[2]). Using a frameless neuronavigation system (BrainSight, Rogue Research, Montreal, Quebec, Canada) we first coregistered the subjects' heads to their magnetic resonance images. We then identified and marked as "hot spot" the area of M1 that elicited isolated and directionally consistent thumb movements. In this location, we determined the resting motor threshold for the flexor pollicis brevis and extensor pollicis brevis as the minimum TMS intensity that evoked a motor-evoked potential (MEP) of 50 mV in at least 5 of 10 trials in the resting target muscle (Rossini et al., 1994). Muscle relaxation was monitored by visual feedback of the EMG recording.

Experimental Procedure

Each subject participated in two crossover counterbalance-ordered sessions designed to assess the amount of UDP changes as previously described (Celnik et al., 2008; Stefan et al., 2005, 2008; Classen et al., 1998). Briefly, at the beginning of each session (separated by at least 7 days), we determined the direction of 65 TMS-evoked thumb movements elicited at a frequency of 0.1 Hz over the hot spot (Figure 1). The mean direction of these 65 movements constituted the *baseline* TMS-evoked thumb movement direction. After this, subjects underwent one of two interventions:

PP + AO + TMS over PMv ($PP + AO + TMS_{PMv}$)

PP consisted of performance of voluntary thumb movements at 1 Hz in the opposite direction to the TMS-evoked baseline direction for 30 min (three blocks of 10 min each separated by a 2-min rest period). For example, if the principal baseline direction was extension and abduction, then the subject was instructed to perform repetitive flexion and adduction movements during training. We instructed the subjects to relax and let the thumb return to its original position after each movement. This was ensured by monitoring on-line EMG, acceleration signals, and providing verbal feedback when needed. AO consisted of watching a video displaying the hand of a volunteer performing the same motor training task at 1 Hz and in the same direction to the physically practiced. We instructed participants to synchronize and match the direction of their voluntary thumb movements with the movements observed in the video. Subjects also wore a pair of goggles with a cover below the eyes to ensure that subjects could not observe their own hand. Disruptive stimulation consisted of applying single TMS pulses over PMv triggered by the onset of each voluntary thumb movement. The onset of movement was determined by the thumb accelerometer and defined as a thumb movement acceleration of at least 0.65 cm/s^2 along the vertical axis. This resulted in stimulation being delivered at approximately 1-Hz frequency at the beginning of each voluntary thumb movement as previously done in earlier work (Bütefisch et al., 2004).

PP + AO + TMS over Frontal Cortex ($PP + AO + TMS_{FC}$)

This session was identical to the previously described except that TMS was delivered over the midline of the frontal cortex to the site corresponding to Fz in the 10–20 EEG coordinate system (Lagerlund et al., 1993). Fz is standard control site used in UDP studies because of its lack of involvement in motor memory formation (Cohen et al., 1997) and lack of fMRI activation during similar motor training (Morgen, Kadom, Sawaki, Tessitore, Ohayon,

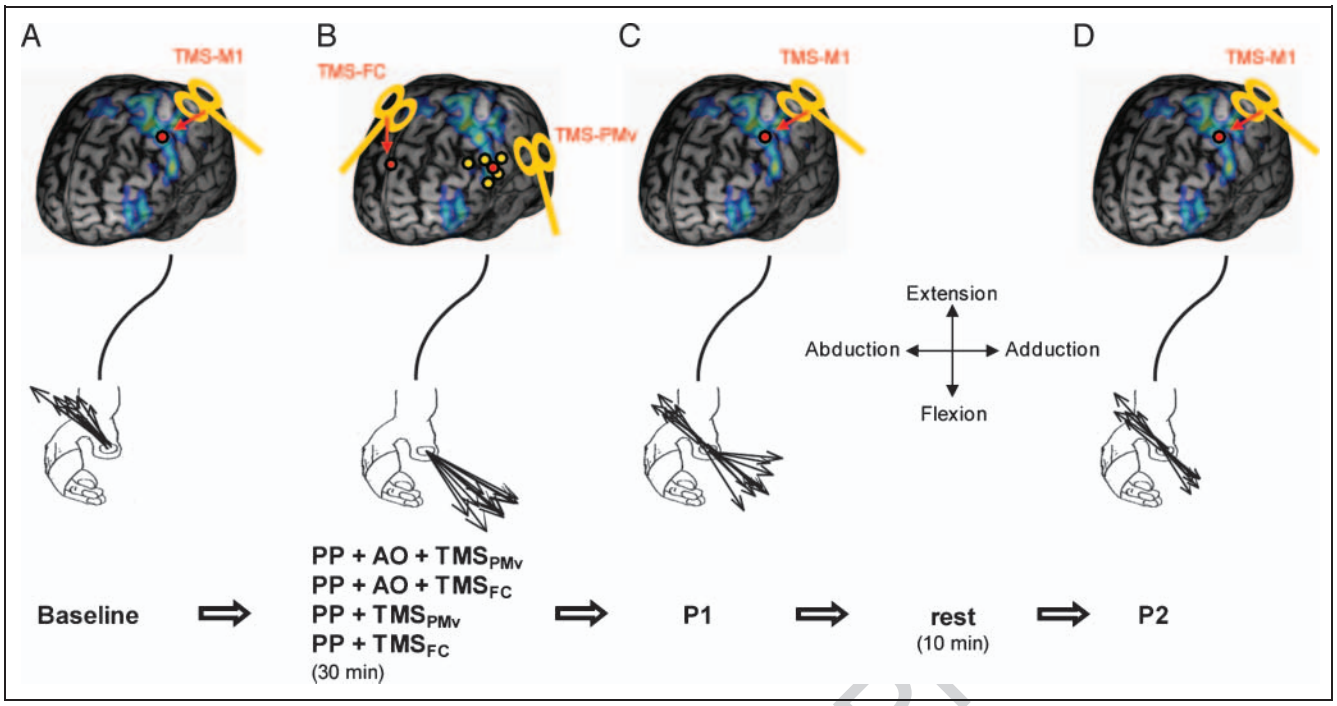

Figure 1. Experimental design. (A) Baseline. At the beginning, TMS-evoked thumb movements were elicited, and their directions were calculated from the first-peak acceleration along the two major axes of the movement, extension–flexion, and adduction–abduction using an accelerometer. Black lines depict the direction of the individual TMS-evoked thumb movements in this example extension and abduction. (B) Interventions. Immediately following baseline subjects underwent four interventions in separate sessions. (1) PP combined with AO plus disruptive TMS over PMv (PP + AO + TMS_{PMv}): Subjects performed voluntary thumb movements in the opposite direction to baseline for 30 min, in this case flexion and adduction. The PP was combined with observing a video displaying thumb movements in the same direction (AO). During this training, single TMS pulses were delivered over PMv. (2) PP combined with AO plus disruptive TMS over frontal cortex (PP + AO + TMS_{FC}): This session is identical to that previously described except that TMS was delivered over the midfrontal cortex (Fz in the 10–20 EEG–coordinate system). (3) PP with disruptive TMS over PMv (PP + TMS_{PMv}): This session is similar to the PP + AO + TMS_{PMv} condition except that subjects did not perform AO. (4) PP with disruptive TMS over OFC (PP + TMS_{FC}): Subjects performed PP without AO, as in PP + TMS_{PMv}, but the TMS was applied over OFC as done during the PP + AO + TMS_{FC} session. (C) P1. The direction of TMS-evoked thumb movements was determined identically as done at baseline. (D) P2. Following a break of 10 min, the direction of TMS-evoked thumb movements was determined again. Red dots represent the TMS location sites for each study phase. Yellow dots represent previously established fMRI-activated coordinates in PMv during AO studies (see Methods).

Frank, et al., 2004; Morgen, Kadom, Sawaki, Tessitore, Ohayon, McFarland, et al., 2004).

Following the interventions in each of the sessions, we reassessed the TMS-evoked thumb movement directions as done during baseline (Post 1 [P1]). A change in the TMS-evoked thumb movement direction is referred to as UDP and interpreted as a reflection of motor memory formation. After this, subjects rested for 10 min followed by another 65 TMS pulses applied over the M1 hot spot (Post 2 [P2]) to assess the longevity of the effects. At the end of each session, subjects reported their alertness, attention, and perceived pain of TMS using a self-scored visual analogue scale (Stefan et al., 2005).

Controls

Upon completion of the initial experiment, all subjects returned and completed two more subsequent control sessions in a randomized crossover design. Testing and recording procedures were identical to the previous sessions.

PP + TMS over PMv (PP + TMS_{PMv})

Similar to the PP + AO + TMS_{PMv} conditions, subjects performed PP and received TMS over PMv synchronized to each thumb movement, but they did not perform video observation. Here, subjects observed a blue dot blinking at 1 Hz to cue the subjects to perform the voluntary movements. This control was designed to determine whether TMS over PMv alone had a disruptive effect on UDP induced by PP alone.

PP + TMS over Frontal Cortex (PP + TMS_{FC})

In this session, subjects only performed PP without AO, as in PP + TMS_{PMv}, but the TMS was applied over the frontal cortex as done during the PP + AO + TMS_{FC} session.

These controls were necessary to ensure that any reduction in UDP changes with disruptive stimulation over PMv was because of the elimination of the contribution of AO and not because of TMS stimulation affecting the contribution of PP.

PMv Stimulation Site Determination

To determine the PMv stimulation location for each individual subject, we first performed a functional MRI study in all participants. Subjects laid in the scanner with an fMRI compatible accelerometer (Kistler Instruments, Amherst, NY) attached to their right thumb. The *movement period* of the task involved subjects flexing their thumb while observing a video displaying congruent thumb movements. The video showed a thumb of a volunteer flexing at a rate of 1 Hz. Participants were instructed to move their thumb at the same rate and time as in the video. The *rest period* involved subjects remaining motionless while observing a still picture of a thumb. The task lasted for 5 min with the movement and rest periods alternating every 30 sec.

To determine the peak activation area of PMv for each participant, we compared activation during performance of PP + AO relative to rest (see *Supplementary Methods* for further details of the fMRI study). We then overlaid the individual peak activation of the PMv area to previously published PMv regions (Buccino, Vogt, et al., 2004; Grefkes, Weiss, Zilles, & Fink, 2002; Buccino et al., 2001; Ehrsson, Fagergren, & Forssberg, 2001; Kuhtz-Buschbeck, Ehrsson, & Forssber, 2001; Ehrsson et al., 2000; Binkofski et al., 1999). The coordinates from these studies were transferred from MNI coordinates to each subject's brain space using MRIcroN. The area of largest individual activation that approximated the previously described PMv coordinates was chosen as the target for TMS over PMv used in conditions 1 and 3 (Figure 1).

To determine whether the PMv stimulation site chosen with the above procedure was also activated during the PP alone session, we performed a second fMRI session similar to that previously described. The only difference was that in this session subjects performed the PP without video observation. Movements were cued as in the controls sessions (see above). The images were analyzed as previously described (see also Supplementary Methods).

Data Analysis

The primary outcome measure was the change in direction of TMS-evoked movements as a function of the different interventions. This was determined as the percentage of movements falling within the training target zone (TTZ), defined as a window of ±20° around the training direction (i.e., 180° opposite to the baseline TMS-evoked movements), before and after each intervention. The secondary outcome measures included (1) relative angular distance (RAD), defined as the mean TMS-evoked movement direction at baseline subtracted from the mean TMS-evoked movement direction after training, and (2) corticomuscular excitability, calculated by measuring MEP amplitudes in the agonist and antagonist muscles of the trained movement direction. To describe the net effects of training on corticomuscular excitability, we calculated the ratio between posttraining and pretraining MEP amplitudes for the agonist and antagonist muscles.

To assess the consistency of training across conditions, we measured the compound acceleration of the voluntary training movements, defined as the mean magnitude of the first peak acceleration in the extension–flexion direction regardless of direction. In addition, we calculated angular variability (Galea & Celnik, 2009; Stefan et al., 2008) that depicts the movement direction dispersion during training, radial distance, which indicates the mean length of each thumb movement, and the angular difference between TMS-evoked movement directions at baseline and training.

Statistical Analysis

We analyzed the primary and secondary outcome measures using separate polynomial nested repeated measures ANOVA ($ANOVA_{RM}$) with factors $TIME_{(baseline,\ postintervention\ 1,\ postintervention\ 2)}$ and $SESSION_{(PP + AO + TMS\text{-}FC,\ PP + AO + TMS\text{-}PMv,\ PP + TMS\text{-}FC,\ PP + TMS\text{-}FC)}$. When appropriate, we performed post hoc testing using paired *t* tests. To analyze general measurements of baseline corticomotor excitability (motor threshold, TMS stimulus intensity, $MEP_{agonist}$ and $MEP_{antagonist}$ amplitudes), attention, fatigue, and motor training kinematics (angular dispersion, compound acceleration, radial distance, and angular difference between TMS-evoked movement direction at baseline and during voluntary motor training), we employed separate $ANOVA_{RM}$ with factor $SESSION_{(PP + AO + TMS\text{-}FC,\ PP + AO + TMS\text{-}PMv,\ PP + TMS\text{-}FC,\ PP + TMS\text{-}FC)}$. To determine changes in the $MEP_{Postintervention/Baseline}$ ratio between the agonist and antagonist muscles we performed a preplanned paired *t* test analysis only in the conditions that showed significant difference for the primary outcome measure and corrected for multiple comparisons when appropriate. All data are presented as mean ± *SEM*.

To determine fMRI activation differences in the stimulated PMv site between sessions, we created a 6-mm ROI centered on the PMv trajectory and analyzed the activation intensity using a paired *t* test.

RESULTS

Summary

All subjects completed the study without adverse events. fMRI activation of the stimulated PMv site during the PP + AO condition was significantly higher than during the PP alone condition ($p = .03$). TMS-evoked movements at baseline and training kinematics were consistent across sessions. All interventions showed training-induced changes in TMS-evoked movement directions. However, this effect was most prominent in the PP + AO + TMS_{FC} session immediately after training (P1) and 20 min later (P2) relative to all other interventions. Training-induced

effects in PP + AO + TMS_{PMv}, PP + TMS_{FC}, and PP + TMS_{PMv} sessions were comparable.

Training Characteristics

Subjects' ratings of attention, fatigue, and discomfort were similar across all sessions (Table 1).

Motor training kinematics were comparable across all training interventions for compound acceleration, angular variance, radial distance, and the angular difference between TMS-evoked movement directions at baseline and training (Table 1). On average, the EMG activity duration of each thumb movement was 137.51 ± 15.38 msec and the TMS pulse was delivered at 36.78 ± 6.40 msec into the onset of the EMG activity.

Effects of Disruptive TMS on UDP Resulting from PP + AO

$ANOVA_{RM}$ revealed a significant effect of time ($F_{(2,\ 18)} = 8.09, p < .01$), session ($F_{(3,\ 27)} = 3.08, p < .05$), and time × session interaction ($F_{(6,\ 54)} = 3.62, p < .05$) for the percentage of movements falling within the TTZ, the primary outcome measure (Figure 2). Given the lack of significant differences in the percentage of movements falling in TTZ at baseline ($F_{(3,\ 27)} = 0.97, p = .41$), we evaluated the change in the percentage of movements falling with the TTZ relative to baseline (ΔTTZ). $ANOVA_{RM}$ showed a significant main effect for session for ΔTTZ ($F_{(3,\ 27)} = 7.23, p < .05$). Post hoc paired t tests revealed that at P1 ΔTTZ was significantly larger than 0 in all sessions ($PP + AO + TMS_{FC}\ t_{(9)} = 4.12, p < .01$; $PP + AO + TMS_{PMv}\ t_{(9)} = 1.93, p < .05$; $PP + TMS_{FC}\ t_{(9)} = 2.16, p < .05$; $PP + TMS_{PMv}\ t_{(9)} = 1.74, p < .05$). This effect was similar at P2 in all sessions except for PP + TMS_{FC} ($PP + AO + TMS_{FC}\ t_{(9)} = 3.94, p < .01$; $PP + AO + TMS_{PMv}\ t_{(9)} = 1.96, p < .05$; $PP + TMS_{PMv}\ t_{(9)} = 1.67, p = .07$). In addition, two-tailed paired t tests post hoc showed that PP + AO + TMS_{FC} effects on ΔTTZ were significantly larger in P1 and P2 relative to all other sessions (Table 2). Importantly, there were no significant differences in ΔTTZ between PP + AO + TMS_{PMv}, PP + TMS_{FC}, and PP + TMS_{PMv} for P1 or P2 (Table 2).

In summary, disruptive stimulation over PMv significantly reduced the percentage of movements falling within the TTZ after training, a parameter indicative of the magnitude of UDP. Specifically, ΔTTZ for the PP + AO + TMS_{FC} session was significantly larger in P1 and P2 relative to all other sessions, whereas there were no significant differences in ΔTTZ between PP + AO + TMS_{PMv}, PP + TMS_{FC}, and PP + TMS_{PMv} for P1 nor P2.

In addition, given that the angular difference between TMS-evoked movement directions at baseline and training were similar across conditions ($F_{(3,\ 27)} = 1.39, p = .27$), we determined the effects of the interventions on the angular difference between posttraining minus baseline. Unlike the TTZ analysis, the RAD gives continuous information about angular changes of the TMS-evoked movement directions. $ANOVA_{RM}$ revealed a significant main effect of session ($F_{(3,\ 27)} = 9.130, p < .01$) and time ($F_{(1,\ 9)} = 5.318, p < .05$) in RAD. Two-tailed paired t tests showed that RAD was significantly larger than 0 at P1 in all groups ($PP + AO + TMS_{FC}\ t_{(9)} = 6.88, p < .01$; $PP + AO + TMS_{PMv}\ t_{(9)} = 4.15, p < .01$; $PP + TMS_{FC}\ t_{(9)} = 4.05, p < .01$; $PP + TMS_{PMv}\ t_{(9)} = 3.03, p < .05$). This effect was also found at P2 ($PP + AO + TMS_{FC}\ t_{(9)} = 4.64, p < .01$; $PP + AO + TMS_{PMv}\ t_{(9)} = 9.53, p < .01$; $PP + TMS_{FC}\ t_{(9)} = 4.62$,

Table 1. Psychological Measures

Parameter	*PP + AO + TMS_{FC}*	*PP + AO + TMS_{PMv}*	*PP + TMS_{FC}*	*PP + TMS_{PMv}*	*$ANOVA_{RM}$*
Psychological measures					
Attention	5.44 ± 0.50	5.56 ± 0.23	5.80 ± 0.39	5.90 ± 0.66	$p = .75$
Fatigue	2.67 ± 0.52	3.00 ± 0.38	2.30 ± 0.33	2.00 ± 0.94	$p = .30$
Discomfort	1.00 ± 0.00	1.67 ± 0.32	1.20 ± 0.13	1.33 ± 0.60	$p = .21$
Training kinematics					
Compound acceleration (cm/s^2)	1.62 ± 0.15	1.84 ± 0.27	1.50 ± 0.14	1.67 ± 0.21	$p = .44$
Angualar variance (degrees)	14.01 ± 1.54	13.56 ± 1.60	11.44 ± 1.30	14.39 ± 0.2.21	$p = .36$
Distance from baseline (degrees)	138.12 ± 4.41	125.89 ± 5.73	127.05 ± 4.86	129.72 ± 6.27	$p = .38$
Radial distance (cm)	2.02 ± 0.18	2.32 ± 0.27	1.80 ± 0.16	2.08 ± 0.19	$p = .11$

Values represent subjects' reported alertness and attention using a self-scored Visual Analog Scale: 1 represents the *poorest attention, least fatigue, and least discomfort*, and 7 represents the *maximal attention, ad most fatigue, and most discomfort*. *Training kinematics*. Distance from baseline reflects the mean angular difference between movement directions at baseline and training. The compound acceleration describes the mean acceleration of each thumb movement during training regardless of direction. Angular variability depicts the movement direction dispersion during training. The radial distance indicates the mean length of each thumb movement during training. All psychological and kinematic measures were similar across sessions. Data are means ± *SEM*.

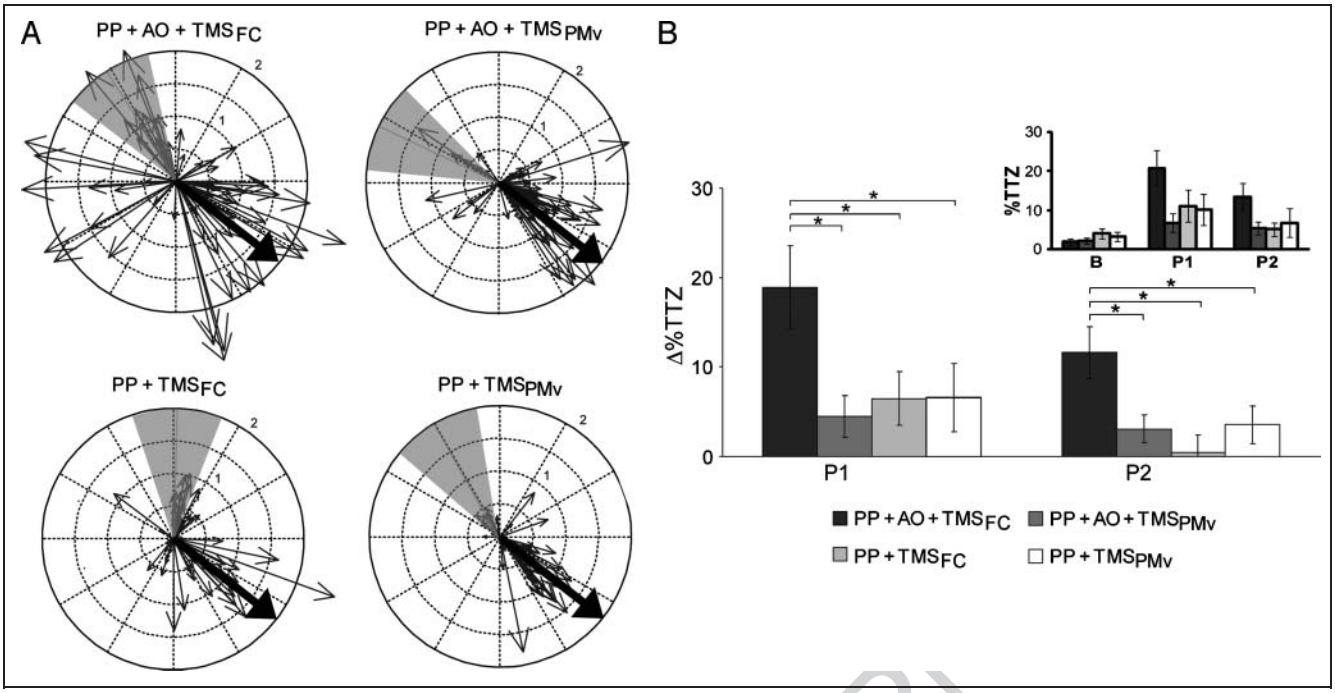

Figure 2. (A) Circle plot of a representative subject showing the distribution of TMS-evoked movement directions for P1 of each session. Each small arrow represents the direction of one movement and its radial distance (represented by its length in centimeters). The large arrow depicts the average direction of all TMS-evoked movement at baseline (size of the arrow is not to scale). Gray-shaded region represents the TTZ. (B) Percentage of TMS-evoked movements that fell within the TTZ after training relative to baseline (ΔTTZ). PP + AO + TMS_{FC} effects on ΔTTZ were significantly larger in P1 and P2 relative to all other sessions. There were no significant differences in ΔTTZ between PP + AO + TMS_{PMv}, PP + TMS_{FC}, and PP + TMS_{PMv} for P1 or P2. This suggests that disruptive stimulation over PMv significantly reduced UDP changes resulting from PP + AO. The small inset shows the percentage of TMS-evoked movements that fell within TTZ at baseline (B), P1, and P2. *$p < .05$. Data are means ± *SEM*.

$p < .01$; *PP + TMS*$_{PMv}$ $t_{(9)} = 4.76, p < .01$). Furthermore, two-tailed paired t tests showed that PP + AO + TMS_{FC} effects on RAD were significantly larger at both P1 and P2 relative to all other interventions (Table 2). Finally, two-tailed pairwise t tests for RAD were not significantly different between PP + AO + TMS_{PMv}, PP + TMS_{FC}, and PP + TMS_{PMv} at P1 or P2 (Table 2).

In summary, disruptive stimulation over PMv significantly reduced the change in RAD. Change in RAD is another parameter that reflects the amount of motor memory formation. RAD was significantly larger for PP + AO + TMS_{FC} relative to all other sessions for both P1 and P2, and was similar between PP + AO + TMS_{PMv}, PP + TMS_{FC}, and PP + TMS_{PMv} for P1 and P2.

Corticomuscular Excitability Changes Associated to Training

To determine changes in corticomotor excitability we assessed the MEP amplitude ratio (post/pre) for the agonist and antagonist muscles. A two-tailed pairwise t test revealed a significant difference between the increase in the $MEP_{agonist}$ ratio compared with the $MEP_{antagonist}$ ratio at P1 for the PP + AO + TMS_{FC} session ($t_{(9)} = 2.27, p < .05$; Figure 3). This effect was not present in the other conditions.

In summary, a differential corticomotor excitability change was seen only in the PP + AO + TMS_{FC} condition.

DISCUSSION

The main finding of this study is that disruptive stimulation over PMv reduced the amount of UDP resulting from the combination of PP (PP) and AO. Moreover, the magnitude of plasticity changes found when disrupting PMv in the PP + AO condition was similar to performing the task without AO. This indicates that PMv activation during AO is functionally relevant to the behavioral performance and not a mere epiphenomenon.

Although skills are acquired through repetitive practice, it has been shown that the mere observation of PP, termed AO, can lead to improvement of performance and UDP (Stefan et al., 2005). It has been suggested that the beneficial effect of AO on performance is because of the merging of information from two anatomically different pathways onto M1 (Stefan et al., 2008; Celnik et al., 2006). One route provides input from the physical execution of movements via connections between dorsal premotor cortex and/or SMAs and M1 (*PP pathway*). The second route provides input from the observation of movements via connections between PMv and M1 (*AO pathway*). When these two pathways converge, they

Table 2. Statistical Measures

	ΔTTZ			RAD		
	PP + AO + TMS_{PMv}	*PP + TMS_{FC}*	*PP + TMS_{PMv}*	*PP + AO + TMS_{PMv}*	*PP + TMS_{FC}*	*PP + TMS_{PMv}*
P1						
PP + AO + TMS_{FC}	$t_{(9)} = 2.86, p < .05$	$t_{(9)} = 2.98, p < .05$	$t_{(9)} = 2.29, p < .05$	$t_{(9)} = 3.59, p < .01$	$t_{(9)} = 3.56, p < .01$	$t_{(9)} = 3.56, p < .01$
PP + AO + TMS_{PMv}	–	$t_{(9)} = -0.61, p = .56$	$t_{(9)} = -0.53, p = .61$	–	$t_{(9)} = 0.03, p = .98$	$t_{(9)} = 0.25, p = .81$
PP + TMS_{FC}	–	–	$t_{(9)} = 0.09, p = .93$	–	–	$t_{(9)} = -0.35, p = .73$
P2						
PP + AO + TMS_{FC}	$t_{(9)} = 2.75, p < .05$	$t_{(9)} = 3.80, p < .01$	$t_{(9)} = 2.88, p < .05$	$t_{(9)} = 2.73, p < .05$	$t_{(9)} = 2.58, p < .05$	$t_{(9)} = 2.83, p < .05$
PP + AO + TMS_{PMv}	–	$t_{(9)} = 0.94, p = .37$	$t_{(9)} = -0.16, p = .88$	–	$t_{(9)} = -0.01, p = .10$	$t_{(9)} = 0.03, p = .97$
PP + TMS_{FC}	–	–	$t_{(9)} = 1.13, p = .29$	–	–	$t_{(9)} = -0.04, p = .97$

p and *t* scores from post hoc paired *t* tests performed on the P1 and P2 values for the PP + AO + TMS_{FC}, PP + AO + TMS_{PMv}, PP + TMS_{FC}, and PP + TMS_{PMv} sessions for both the change in the percentage of movements falling within the TTZ (ΔTTZ) and the RAD between posttraining and baseline.

Figure 3. Corticomotor excitability changes as measured by MEP amplitude ratios (Post/Baseline) for the agonist (dark diamonds) and antagonist (light squares) muscles involved in training. After training, only the PP + AO + TMS_{FC} session resulted in a significant increase in the $MEP_{agonist}$ ratio compared with the $MEP_{antagonist}$ ratio at P1. Data are means ± *SEM*.

can potentially reinforce one another in inducing qualitatively similar UDP changes in M1 (Stefan et al., 2005), thus enhancing what either component could do alone. This hypothesis is in line with previous work showing that, when AO is combined with congruent PP, the training effects are quantitatively enhanced beyond what the linear summation of the effects of AO or PP alone can do for both healthy subjects (Stefan et al., 2008; Celnik et al., 2006) and stroke patients (Celnik et al., 2008). This hypothesis is further supported by prior evidence showing that the excitability of connections between PMv and M1 are influenced by AO (Koch et al., 2010; Lago et al., 2010).

In this study, we were interested in exploring whether activation of PMv is crucial for the contribution AO has on UDP changes in M1 that underlie motor memory formation. We speculate that disruption of PMv may be interrupting part of the AO pathway that allows AO to facilitate UDP occurring in M1. This information is important not only for motor exercises and rehabilitation but also because determining the functional role of PMv activation associated to AO can have significant implications in understanding the actions and intentions of others (Iacoboni et al., 2005), imitation learning (Iacoboni et al., 1999), and disorders like autism (Cattaneo & Rizzolatti, 2009).

Previous work has shown that repetitive TMS (rTMS) at 1 Hz can be used to transiently inactive different cortical areas to induce a "virtual lesion" (Chen et al., 1997). In addition, delivering TMS in synchrony with thumb movements over the involved M1 in a similar paradigm successfully interfered with motor memory formation (Bütefisch et al., 2004). Here, we used fMRI-guided rTMS to disrupt the activation of PMv during performance of PP and AO. We found that disruptive rTMS reduced the

change in angular direction and the percentage of movements following the training direction relative to performance of PP + AO with disruptive rTMS over a control site (OFC). This reduction in movement direction changes is interpreted as interference of UDP changes that underlie motor memory formation. In addition, we found that in both control groups where PP was performed without AO during disruptive stimulation over PMv or OFC, the effect of PP on UDP changes was similar to PP + AO with disruptive stimulation over PMv. These suggest that the contribution of AO to UDP was canceled out by disruptive rTMS over PMv resulting in similar gains as performing PP alone.

Importantly, our controls showed that rTMS over PMv did not interfere with UDP induced by PP alone. We reasoned that if disruptive stimulation over PMv was interfering with the PP contribution to plasticity changes then the magnitude of memory formation during PP + TMS_{PMv} should be smaller than PP + TMS_{FC}. However, we found that this was not the case; in fact, the effects of training in both control sessions were similar to PP + AO + TMS_{PMV}.

Given that certain subgroups of PMv neurons have been shown to be involved in grasping and reaching movements (Hoshi & Tanji, 2007; Shadmehr & Wise, 2005; Kurata & Hoshi, 2002; Fogassi et al., 2001), one might have expected that disruption of PMv could have decreased UDP changes in M1 resulting from PP simply because of interference of motor performance. However, our behavioral task does not require reaching toward a target or grasping any object, which could explain why disruption of PMv had a similar effect on UDP induced by PP alone as disruption of OFC. Furthermore, it has been shown that, although premotor areas are activated during finger flexion or extension movements, UDP evoked by 30 min of voluntary thumb training is associated with fMRI activation changes in contralateral M1, S1, and IPL, but not with changes in activation of premotor cortex or inferior frontal gyrus (Morgen, Kadom, Sawaki, Tessitore, Ohayon, Frank, et al., 2004; Morgen, Kadom, Sawaki, Tessitore, Ohayon, McFarland, et al., 2004). Additionally, the same study showed that premotor activation during finger flexion and extension, as done in our study, was bilateral. This may be an alternative explanation as to why disrupting only one premotor area had relatively little effect on movement execution. In the current study, the PMv stimulated site chosen when subjects performed PP + AO was not similarly activated when the same subjects performed PP alone. Importantly, our repetitive stimulation over PMv during the PP did not affect the kinematics of the thumb movement training. Thus, although PMv has been shown to be involved in finger movements, it appears that this area is not critically responsible in eliciting UDP changes in M1, at least when assessed using our behavioral paradigm. This was supported by other studies showing that disruptive stimulation over other premotor areas such as the pars opercularis of the inferior fronta gyrus (i.e., Broca's area) resulted in an impairment in the imitation of a finger-movement task, but not in the mere execution of the same task (Heiser, Iacoboni, Maeda, Marcus, & Mazziotta, 2003). Alternatively, it may still be possible that TMS in this paradigm is not disruptive enough to override the PMv role during finger training. Therefore, we interpret that the reduction in UDP changes in M1 during the PP + AO + TMS_{PMv} session was because of a specific interference of the contribution of AO to UDP and not because of a disruption of the PP component.

Importantly, although here we found that disruption of PMv diminishes the contribution of AO to PP, other investigations have shown that interfering with M1 or the cerebellum can also affect the beneficial effects of AO to different behaviors (Brown, Wilson, & Gribble, 2009; Petrosini, 2007). This current investigation adds to these previous findings, showing here that disrupting PMv can also disturb the AO pathway in an upstream node to M1.

In addition, similar to previous studies, we also found that large UDP changes in M1 resulting from PP + AO with sham rTMS was associated with a specific change in excitability of the muscles involved in the observation and practice of the task (Stefan et al., 2008; Celnik et al., 2005; Bütefisch et al., 2004). Specifically, the excitability of the agonist muscle cortical representation of the observed/practiced movements increased whereas the antagonist muscle excitability decreased. This training-dependent plasticity, not observed in any of the other sessions, indicates a change in the distribution of neuronal network strength between cortical representations, a mechanism thought to represent the neurophysiological correlate of successful motor memory formation (Bütefisch et al., 2000). We did not find any significant changes in the $MEP_{agonist}$ excitability for the PP with sham rTMS, a finding inconsistent with previous results (Stefan et al., 2008; Bütefisch et al., 2000). This discrepancy may be attributed to the overall low percentage of TMS-evoked movement changes. This can be explained by an overall reduction of training effects when the practice is performed with TMS delivered to any part of the head (i.e., nonspecific TMS effects during training) or because, unlike previous studies, we did not exclude subjects for having low amounts of plasticity changes.

In the future, it would be important to explore the functional relevance of other cortical regions also believed to be associated with AO, such as the anterior IPL (Cattaneo & Rizzolatti, 2009; Buccino, Lui, et al., 2004; Buccino, Vogt, et al., 2004; Morgen, Kadom, Sawaki, Tessitore, Ohayon, Frank, et al., 2004). Furthermore, it would be interesting to figure out specifically which subset of neurons in the PMv are responsible for the contributions of AO; unfortunately, such a level of specificity cannot be tested using noninvasive brain stimulation techniques. However, using direct recordings of extracellular neural activity in patients (Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010) during AO in the same

regions explored in the current study could be used to asses more in depth the neurophysiological interactions described here.

In summary, our findings demonstrate that the fMRI-activated areas of PMv during AO are functionally relevant to task performance, at least for the beneficial effects that AO has over plasticity changes induced by PP. Importantly, our results open an opportunity to investigate the use of noninvasive brain stimulation techniques over PMv to enhance the effects of AO, a strategy that can potentially result in a therapeutic intervention for patients with neurological disease.

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