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Enhanced action performance following TMS manipulation of associative plasticity in ventral premotor-motor pathway

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A B S T R A C T

Skillful goal-directed manual actions such as grasping and manipulating objects are supported by a large sensorimotor network. Within this network, the ventral premotor cortex (PMv) transforms visual information about objects into motor commands that are conveyed to the primary motor cortex (M1), allowing fine control of finger movements. However, it is unknown whether transcranial magnetic stimulation (TMS) of this PMv-to-M1 hierarchical pathway improves action performance. To fill in this gap, here, we used cortico-cortical paired associative stimulation (ccPAS) with the aim of manipulating synaptic efficacy in the human PMv-to-M1 pathway. We found that repeatedly pairing TMS of pre-and post-synaptic nodes of the PMv-to-M1 pathway (i.e., PMv-to-M1 ccPAS) increased motor excitability and enhanced performance on the 9-Hole Peg Test (9-HPT), which taps into PMv-M1 functioning. These effects were specific to the ccPAS protocol consistent with the direction of the PMv-to-M1 hierarchy, as no effects were observed when reversing the order of the paired TMS pulses (i.e., following a M1-to-PMv ccPAS) or when administering sham ccPAS. Additionally, the effect of PMv-to-M1 ccPAS appeared functionally specific, as no behavioral enhancement was observed in a visuomotor control task. We therefore provide novel causal evidence that the PMv-to-M1 pathway, which is instrumental to object-oriented hand actions, is sensitive to TMS manipulations of associative plasticity. Our study highlights the causal role of the PMv-to-M1 pathway in controlling skillful object-oriented hand actions and suggests that ccPAS might be a useful tool for investigating the functional relevance of directional connectivity in humans. These findings may have implications for designing novel therapeutic strategies based on the manipulation of associative plasticity in cortico-cortical networks.

1. Introduction

Goal-directed manual actions such as grasping, manipulating and moving objects are the result of complex interactions within dorsal occipito-parieto-frontal streams involved in sensorimotor transformations (Jeannerod et al., 1995; Castiello, 2005; Grol et al., 2007; Cavina-Pratesi et al., 2010; Davare et al., 2011). At least part of this process is thought to occur in a serial, hierarchical fashion: monkey studies have suggested that, within a dorsolateral stream, the ventral premotor cortex (PMv) transforms visual information about object properties (e.g., their shape, size, etc.) into appropriate motor commands; these commands are conveyed to the primary motor cortex (M1), allowing fine control of individual finger movements (Muir and Lemon, 1983; Murata et al., 1997; Fagg and Arbib, 1998; Fogassi et al., 2001; Lang and Schieber, 2004; Raos et al., 2006). Although alternative/parallel pathways also exist (e.g., Dum and Strick, 1991; He et al., 1993), these monkey studies point to a pivotal role of the PMv-to-M1 hierarchy in performing skilled, visually guided, object-oriented manual actions such as grasping observed objects (Prabhu et al., 2009; Rizzolatti et al., 2014; Borra et al., 2017; Gerbella et al., 2017).

Neuroimaging and transcranial magnetic stimulation (TMS) studies suggest that the human brain is endowed with neural systems for goal-directed actions analogous to those of monkeys (Castiello, 2005; Cavina-Pratesi et al., 2007; Króliczak et al., 2007; Tunik et al., 2007; Davare et al., 2008, 2009, 2010). These studies have shown that visually guided, object-oriented manual actions are at least partly underpinned by neural interactions within the dorsolateral stream (e.g., Davare et al., 2010, 2011; for further involvement of dorsomedial areas see Vesia et al., 2017). For example, Grol and colleagues reported increased connectivity between occipito-parieto-frontal nodes of the dorsolateral stream (i.e., V3A, AIP and PMv) during precision grasping (Grol et al., 2007). In addition, Davare and colleagues have shown that, during grasp preparation, short-latency PMv-to-M1 connections are facilitated in a muscle-specific manner (i.e., grasp-related facilitation is specific to those circuits controlling the muscles involved in the upcoming grasp; see Davare et al., 2008, 2009, 2010). These studies converge with monkey findings and support the notion of a human PMv-to-M1 hierarchy in fine motor control of object-oriented manual actions.

A variety of experiences ranging from learning new motor skills to experiencing a stroke in motor areas have been associated with

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neuroplastic changes in premotor and motor areas and the connection between them (Nelles et al., 2001; Sun et al., 2007; Albert et al., 2009; Taubert et al., 2011; Wiestler and Diedrichsen, 2013; Horn et al., 2016). For example, training in a fine motor task involving grasping and moving pegs and marbles strengthened functional connectivity between PMv and primary sensorimotor representations of the hand (Hamzei et al., 2012). Increased functional connectivity between PMv and sensorimotor cortex was also found following training in a precision drawing task (Philip and Frey, 2016). Moreover, performing skillful hand actions after extensive training was associated with increased premotor-motor connectivity (Dayan and Cohen, 2011). However, these previous studies used a correlational approach that does not address the critical question of whether direct strengthening of premotor-motor connectivity (e.g., via exogenous brain manipulation) would *cause* an enhancement in hand motor functions. Answering this outstanding question is the goal of the present study.

Recent advances in TMS allow us to directly address this question through a protocol called cortico-cortical paired associative stimulation (ccPAS) (Rizzo et al., 2009, 2011; Koganemaru et al., 2009; Arai et al., 2011; Buch et al., 2011; Lu et al., 2012; Koch et al., 2013; Veniero et al., 2013; Johnen et al., 2015; Romei et al., 2016a; Casula et al., 2016; Chiappini et al., 2018). This protocol consists of repeated paired stimulation of two interconnected brain areas with the aim of mimicking patterns of neuronal stimulation shown to induce spike-timing-dependent plasticity (STDP) – a form of synaptic plasticity meeting the Hebbian principle that synapses are potentiated if the presynaptic neuron fires immediately before the postsynaptic neuron in a coherent and repeated manner (Jackson et al., 2006; Caporale and Dan, 2008; Markram et al., 2011). In the ccPAS protocol, pre- and post-synaptic coupling is achieved by repeatedly administering pairs of TMS pulses. In each pair, a first pulse over a target area is followed by a second pulse over an interconnected target area with an inter-stimulus interval (ISI) consistent with the activation of short-latency connections between the two areas. In a relevant study, Buch et al. (2011) administered a ccPAS protocol by delivering the first pulse in each pair over PMv and the second over M1 using an ISI of 8 ms, i.e., the critical ISI at which the PMv exerts a short-latency physiological effect on the excitability of the ipsilateral M1 (see dual-site TMS studies of Davare et al., 2008, 2009, 2010; Bäumer et al., 2009 pointing to an ISI of 6–8 ms). Thus, with this protocol, the cortico-cortical volley elicited by PMv stimulation (first pulse) would reach M1 slightly before/at the same time as the exogenous M1 stimulation (second pulse), resulting in convergent M1 activation. This repeated stimulation of the PMv-to-M1 pathway enhanced the physiological effect of PMv conditioning over M1 excitability, and the time-course of the long-term potentiation (LTP)-like effect resembled that of STDP effects observed in animal studies (Buch et al., 2011). In a further study, the PMv-to-M1 ccPAS protocol was found to increase the functional connectivity of the stimulated pathway, as measured by functional magnetic resonance imaging (fMRI). Increased connectivity was anatomically specific and did not occur in non-stimulated parallel motor pathways (Johnen et al., 2015).

These physiological studies provided direct evidence that ccPAS can transiently strengthen PMv-to-M1 connections by increasing synaptic efficiency in a hierarchical motor pathway involved in visually guided object grasping and manipulation. However, these studies did not answer the critical question of whether exogenous enhancement of PMv-to-M1 synaptic efficiency also causes an improvement in performing object-oriented manual actions.

In the present study, we sought to investigate the malleability and behavioral relevance of PMv-to-M1 connectivity by combining a ccPAS PMv-to-M1 protocol with two behavioral tasks. Based on the notion that the PMv is a key region for visually guided, object-oriented manual actions (Binkofski et al., 1999; Ehrsson et al., 2000; Kuhtz-Buschbeck et al., 2001; Horn et al., 2016) and the PMv-to-M1 hierarchy is involved in the implementation of such actions (Prabhu et al., 2009; Rizzolatti et al., 2014; Borra et al., 2017; Gerbella et al., 2017), we hypothesized that administering a ccPAS protocol aimed at enhancing PMv-to-M1 connectivity would improve performance on the Nine-Hole Peg Test (9-HPT; Mathiowetz et al., 1985; Grice et al., 2003), a well-established manual dexterity task tapping into the ability to grasp and manipulate small objects.

We hypothesized this behavioral enhancement would be specific. No improvement was expected following a M1-to-PMv ccPAS protocol –controlling for the directionality of the stimulated pathway– or a sham ccPAS protocol –controlling for nonspecific effects of TMS. Additionally, we expected no ccPAS-induced changes in performance on a visual choice reaction time (cRT) task. Although both 9-HPT and cRT are visuomotor tasks, the latter does not tap into the ability to efficiently shape the hand to manipulate objects, and it was thus expected to be less sensitive to manipulation of PMv-M1 connectivity.

Lastly, based on prior work reporting that global measures of motor excitability predict the magnitude of LTP effects in the motor system (Müller-Dahlhaus et al., 2008) and TMS-induced behavioral effects (Kaminski et al., 2011), we expected to find a positive relationship between behavioral changes induced by PMv-to-M1 ccPAS and motor excitability as assessed before ccPAS.

2. Materials and methods

2.1. Participants

Fifty-four healthy participants (16 males, mean age 23.1 ± 3.3 years) took part in the study. All were right handed, based on the Edinburgh Handedness Inventory (Oldfield, 1971), had normal or corrected-to-normal vision and were naïve to the purpose of the experiment. All participants gave written informed consent prior to the study, and were screened to avoid adverse reactions to TMS (Rossi et al., 2009; Rossini et al., 2015). The experimental procedures were in accordance with the 1964 Declaration of Helsinki and approved by the Bioethics Committee of the University of Bologna (2.6/07.12.16). None of the participants reported adverse reactions or discomfort related to TMS.

2.2. General experimental design and procedures

To test the malleability and functional relevance of PMv-M1 connections, we administered ccPAS over the left PMv and the left M1, to repeatedly activate the neural pathways between them (Buch et al., 2011; Johnen et al., 2015). The participants were randomly assigned to 1 of 3 groups, according to the administered ccPAS protocol (see Table 1 and Fig. 1). In the experimental group ($Exp_{PMv \rightarrow M1}$; N = 18), we administered a PMv-to-M1 ccPAS protocol. In the active control group ($Ctrl_{M1 \rightarrow PMv}$; N = 18) we administered a M1-to-PMv ccPAS protocol, whereas in the sham control group ($Ctrl_{sham}$, N = 18) we administered a sham PMv-to-M1 ccPAS protocol. We used a double-blind procedure, as both the participants and the experimenter assessing behavioral performance were blind to participants' allocation.

Participants performed two behavioral visuomotor tasks (i.e., 9-HPT and cRT). After they were familiarized with the tasks for about 10 min (training), their performance was recorded in four experimental sessions (Fig. 1). Two sessions were recorded before the ccPAS (constituting the "Baseline" and "Pre" sessions) and two sessions were recorded after the ccPAS ("Post-0" and "Post-30"). Each session lasted ~5 min, during which the two tasks were administered in a counterbalanced order across participants. Behavioral performance was followed by ~25 min of rest (i.e., sessions were separated by 30 min each). TMS parameters and coil positions (see *ccPAS protocol* and *neuronavigation* paragraphs below) were identified in the rest periods before and after the Baseline session. Fifteen minutes after the beginning of the Pre session, the ccPAS protocol was administered for 15 min and performance was recorded immediately (Post-0) and 30 min (Post-30) after the end of the stimulation. Participants were invited to remain seated throughout the experiment and to keep their hands completely relaxed in the rest periods. The experiment lasted approximately 2.5 h.

Table 1

Demographic characteristics, TMS parameters and baseline performance of the three groups. A series of null hypothesis-testing analyses (one-way ANOVAs and χ^2) and their Bayesian implementations showed no differences between groups. **Notes:** [(a)] TMS intensity corresponding to 90% of the rMT as assessed with the coil of the monophasic stimulator over M1. [(b)] TMS intensity required to elicit a motor-evoked potential (MEP) of ~1-mV amplitude as assessed with the coil of the biphasic stimulator over M1; [(c)] In the sham group the biphasic stimulator was set at an intensity of 65% in all participants.

	$Exp_{PMv\rightarrow M1}$ (N = 18)	$Ctrl_{sham}$ (N = 18)	$Ctrl_{M1\rightarrow PMv}$ (N = 18)	Statistical comparison
Mean ± S.D. age (years)	22.9 ± 2.6	24.1 ± 4.3	22.7 ± 2.9	$F_{2,51} = .94$, $p = .40$; $\eta_p^2 = .04$; $BF_{01} = 3.5$
Gender (F/M)	13 F/5 M	12 F/6 M	13 F/5 M	$\chi^2 = .18$, $p = 1$; $\varphi = .06$; $BF_{01} = 5.0$
Mean ± S.D. PMv pulse intensity (% of maximal monophasic stimulator output)	37.9% ± 7.3[(a)]	38.8% ± 6.0[(a)]	36.8% ± 5.8[(a)]	$F_{2,51} = .47$, $p = .63$; $\eta_p^2 = .02$; $BF_{01} = 4.9$
Mean ± S.D. M1 pulse intensity (% of maximal biphasic stimulator output)	68.8% ± 11.6[(b)]	not assessed[(c)]	68.6% ± 9.5[(b)]	$F_{1,34} < .01$, $p = .96$; $\eta_p^2 < .01$; $BF_{01} = 3.1$
Mean ± S.D. 9-HPT performance at baseline (s)	20.8 ± 2.1	20.6 ± 1.8	21.2 ± 1.5	$F_{2,51} = .47$, $p = .63$; $\eta_p^2 = .02$; $BF_{01} = 4.9$
Mean ± S.D. cRT performance at baseline (ms)	397 ± 29	421 ± 59	425 ± 43	$F_{2,51} = 1.97$, $p = .15$; $\eta_p^2 = .07$; $BF_{01} = 1.7$
Mean ± S.D. cRT performance at baseline (%Corr)	96% ± 3	95% ± 5	96% ± 4	$F_{2,51} = .83$, $p = .44$; $\eta_p^2 = .03$; $BF_{01} = 4.5$

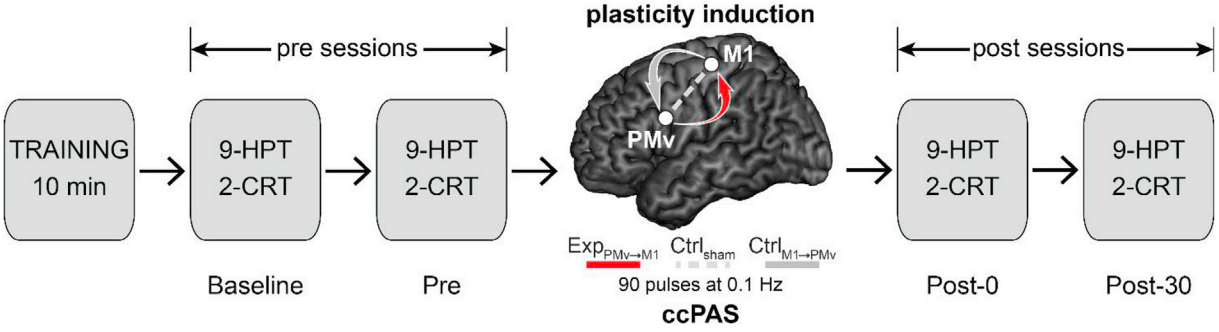

Fig. 1. Schematic representation of the experimental procedure.

2.3. ccPAS protocol

The ccPAS pulses were administered by means of two 50-mm figure-of-eight branding iron coils. These small focal coils are designed with the handle pointing perpendicular to the plane of the wings, and could be positioned near to each other without interference from the handles. One coil was placed over the left PMv and connected to a Magstim 200[2] monophasic stimulator; the other coil was placed over the left M1 and connected to a Magstim Rapid[2] biphasic stimulator (The Magstim Company, Carmarthenshire, Wales, UK). Ninety pairs of TMS pulses were delivered continuously at a rate of 0.1 Hz for 15 min (Rizzo et al., 2009, 2011; Buch et al., 2011; Johnen et al., 2015; Romei et al., 2016a; Chiappini et al., 2018). In each pair, PMv and M1 were stimulated with an ISI of 8 ms (Buch et al., 2011; Johnen et al., 2015) to activate short-latency connections between the two regions (e.g., Davare et al., 2009).

The $Exp_{PMv\rightarrow M1}$ group received a PMv-to-M1 ccPAS with the PMv pulse always administered before the M1 pulse. The $Ctrl_{M1\rightarrow PMv}$ group received the pulses in the reverse order, i.e., with the M1 pulse prior to the PMv pulse, to control for the direction of stimulation. The $Ctrl_{sham}$ group received PMv-to-M1 ccPAS, but the coils were held perpendicularly to the scalp so that no current was induced in the brain. The pulses were triggered remotely using MATLAB (MathWorks, Natick, USA) to control both stimulators.

The coil position for targeting the PMv was determined by means of a neuronavigation system (see next paragraph), while M1 was localized functionally as the optimal scalp position for inducing motor-evoked potentials (MEPs) of maximal amplitude in the right first dorsal interosseous (FDI) (Rossini et al., 2015). During active ccPAS (i.e., in the $Exp_{PMv\rightarrow M1}$ and $Ctrl_{M1\rightarrow PMv}$ groups), coils were oriented to induce current flows consistent with previous dual-site TMS and ccPAS studies targeting PMv and M1 (e.g., Davare et al., 2008; Bäumer et al., 2009; Buch et al., 2011; see Fig. 2A and B). The left PMv was targeted using the monophasic stimulator and the coil was placed tangentially to the scalp, inducing a posterior-to-anterior and lateral-to-medial current flow in the brain. The left M1 was targeted using the biphasic stimulator with the coil placed tangentially to the scalp and oriented at a ~45° angle to the midline. In this way, the second and most effective component of the biphasic waveform induced a current flowing in an anterior direction in the brain, optimal for M1 stimulation (e.g., Kammer et al., 2001; Di Lazzaro et al., 2004).

Table 1 reports the intensity of PMv and M1 stimulations in the three groups. For both PMv and M1, TMS intensities were set based on MEPs induced by single pulse stimulation of the left M1. MEPs were recorded from the right FDI by means of surface Ag/AgCl electrodes placed in a belly-tendon montage, with the ground electrode placed on the right wrist. EMG signals were acquired by means of a Biopac MP-35 (Biopac, USA) electromyograph, band-pass filtered (30–500 Hz) and digitized at a sampling rate of 5 kHz. The intensity of PMv stimulation was individually adjusted to 90% of each participant's resting motor threshold (rMT), which was assessed by placing the coil of the monophasic stimulator tangentially to the scalp over the left M1, at a ~45° angle to the midline, inducing a posterior-anterior current direction in the brain (Kammer et al., 2001; Di Lazzaro et al., 2004). The rMT was defined as the minimum stimulator output intensity that induced a MEP with >50 μV amplitude in 5 out of 10 consecutive trials (Rossini et al., 2015). Although previous ccPAS studies focusing on PMv-to-M1 interactions have used higher intensities for targeting PMv (i.e., 110% of rMT; Buch et al., 2011; Johnen et al., 2015), subthreshold stimulation minimizes potential discomfort associated with inferior frontal sites. Importantly, the effectiveness of subthreshold conditioning has been demonstrated in other ccPAS studies (e.g. Koch et al., 2013; Veniero et al., 2013) and finds

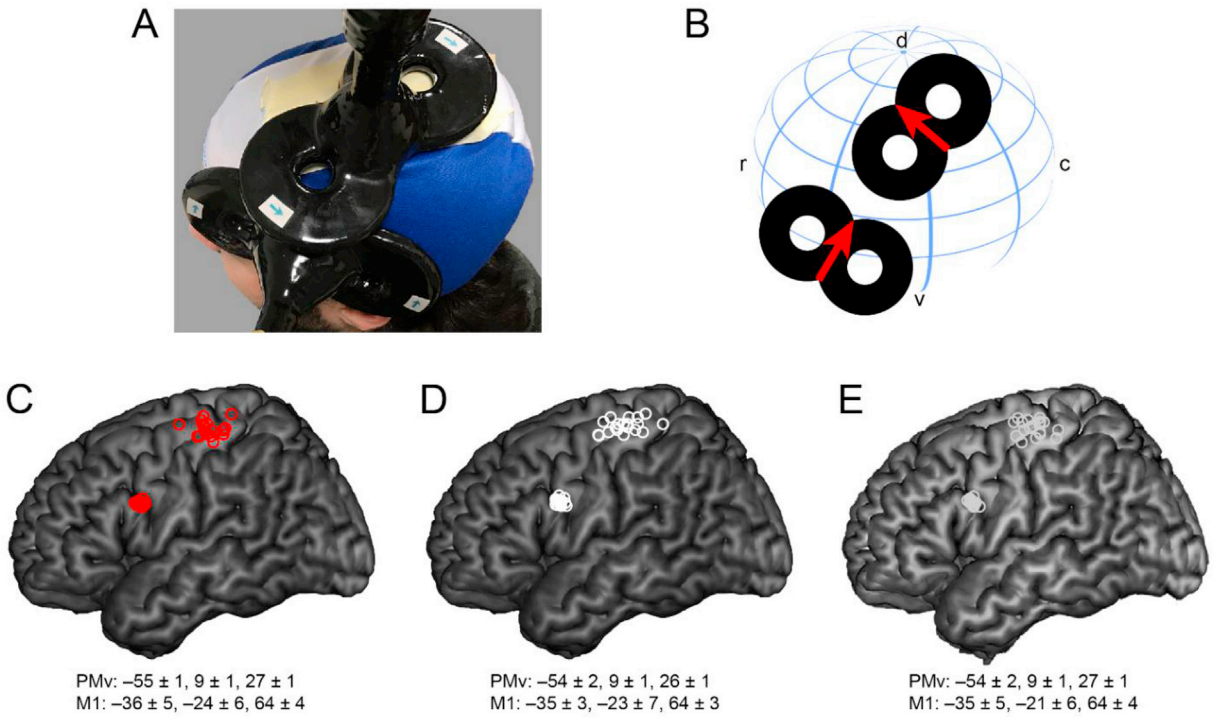

Fig. 2. Targeted sites and coil placement. (A) Coil positions during ccPAS on a representative participant and (B) schematic representation of the currents induced in the brain. For M1 stimulation, the arrow indicates the direction of the most effective phase of the biphasic pulse (see Methods). (C–E) Individual subjects' targeted sites reconstructed on a standard template using MRIcron software (MRIcron/NPM/dcm2nii) after conversion to MNI space, and corresponding mean ± S.D. coordinates. (C) $Exp_{PMv\rightarrow M1}$, (D) $Ctrl_{sham}$ and (E) $Ctrl_{M1\rightarrow PMv}$ group.

specific support from dual-coil TMS studies testing early PMv-to-M1 interactions (e.g. Davare et al., 2008, 2009; 2010; Bäumer et al., 2009; Cattaneo and Barchiesi, 2011). To minimize discomfort and surprise, before starting the administration of the active ccPAS protocols, we made participants experience active stimulation of PMv, using 3–4 pulses of increasing intensity. All participants reported that the stimulation was tolerable. In the active ccPAS groups ($Exp_{PMv\rightarrow M1}$ and $Ctrl_{M1\rightarrow PMv}$), the intensity of M1 stimulation was adjusted to elicit MEPs of about 1 mV in amplitude following a single TMS pulse over the left M1 (Buch et al., 2011; Johnen et al., 2015). In the $Ctrl_{sham}$ group, M1 stimulation was set at 65% of maximal stimulator output in all participants. No between-group differences were found in the intensities of PMv and M1 stimulation (Table 1).

During the ccPAS protocol, participants remained relaxed with the eyes open, and EMG activity was constantly monitored from the right FDI to ensure that full muscle relaxation was maintained during the protocol. In a subsample of participants, we stored the EMG trace so that MEPs induced by the M1 pulse could be assessed during PMv-to-M1 and M1-to-PMv ccPAS protocols (see Supplementary material).

2.4. Neuronavigation

The coil positions to target the left PMv and left M1 were identified using established methods. As reported above, the hand representation in the left M1 was identified functionally based on MEPs from the FDI muscle. The left PMv was identified using the SofTaxic Navigator System (Electro Medical System, Bologna, IT) as in previous studies (Avenanti et al., 2007, 2013; Tidoni et al., 2013; Paracampo et al., 2017). Skull landmarks (nasion, inion and 2 preauricular points) and ~80 points providing a uniform representation of the scalp were digitized by means of a Polaris Vicra digitizer (Northen Digital). An individual estimated magnetic resonance image (MRI) was obtained for each subject through a 3D warping procedure fitting a high-resolution MRI template to the participant's scalp model and craniometric points. This procedure has been proven to ensure a global localization accuracy of roughly 5 mm (Carducci and Brusco, 2012). To target the left PMv, the coil was placed over a scalp region overlying the Talairach coordinates: $x = -54$, $y = 10$, $z = 24$. These coordinates were obtained by averaging the coordinates reported in previous studies (Davare et al., 2006; Dafotakis et al., 2008; Avenanti et al., 2012a, 2018; Jacquet and Avenanti, 2015); these studies showed that stimulating this ventral frontal site (at the border between the anterior sector of the PMv and the posterior sector of the inferior frontal gyrus) affected planning, execution and perception of hand actions. These coordinates are also consistent with those used in previous ccPAS (Buch et al., 2011; Johnen et al., 2015) and dual-site TMS studies targeting PMv-to-M1 connections (Davare et al., 2008, 2009, 2010; Fiori et al., 2016, 2017).

The Talairach coordinates corresponding to the projections of the left PMv and left M1 scalp sites onto the brain surface were automatically estimated by the SofTaxic Navigator from the MRI-constructed stereotaxic template, and resulted in the following Talairach coordinates (mean ± S.D.) across the three experiments: left PMv: $x = -54 \pm 1$, $y = 10 \pm 1$, $z = 24 \pm 1$; left M1: $x = -35 \pm 4$, $y = -19 \pm 6$, $z = 60 \pm 3$. These coordinates are consistent with regions defined as human PMv and M1, respectively (Mayka et al., 2006). A series of ANOVAs ensured that PMv and M1 coordinates were comparable across the three groups (all $F \leq 1.96$, all $p \geq .15$). Fig. 2C–E shows individual targeted sites converted into MNI space for illustrative purposes.

2.5. Visuomotor tasks

The 9-HPT is a widely-used test to assess fine hand dexterity. It requires participants to finely shape their hand in order to grasp and manipulate small objects (Mathiowetz et al., 1985; Grice et al., 2003), an ability tapping into the activation of the dorsolateral stream (Grol et al., 2007; Davare et al., 2010; Hamzei et al., 2012; Philip and Frey, 2016). Performance on the 9-HPT was found to be sensitive to exogenous non-invasive manipulations of the motor system (Koch et al., 2008;

Avenanti et al., 2012b; Di Lazzaro et al., 2013) and correlate with the recruitment of sensorimotor areas including PMv and M1 (Hamzei et al., 2012). The 9-HPT apparatus (Fig. 3A) consisted of a plastic board with 9 small holes organized in a 3 x 3 matrix. The distance between holes was 3.2 cm, and pegs were placed in a tray of 8.5 x 10.4 × 2.3 cm fixed adjacent to the board. Upon receiving the start command, participants picked up the nine small pegs one by one with their right hand, put all of them into the nine holes and then removed them one by one, returning them to the box. Participants were required to execute the task as quickly as possible. The time taken to complete the task was recorded from the starting movement to the drop of the last peg into the tray by an experimenter blind to the ccPAS condition. In each session (Baseline, Pre, Post-0, Post-30), participants performed 5 repetitions of the task.

The cRT was used as a control task to assess visuomotor reaction times (Fig. 3B). We used a 2-choice version of the cRT to assess simple visuomotor mapping based on learned visuomotor associations. Although the cRT is sensitive to non-invasive brain stimulation of the motor system (Kobayashi et al., 2004; Mansur et al., 2005), this task does not involve dexterous hand shaping and object manipulation – as required by the 9-HPT– and relies less on the PMv-M1 circuit. Participants were instructed to respond by releasing the key pressed by the index or middle finger of the right hand according to the number '1' or '2' displayed on a monitor placed ~80 cm in front of them. Participants were instructed to perform the task as quickly and accurately as possible. The probability of appearance of each number was set to 50%. Each task consisted of 40 trials. The mean reaction times (RTs) and the accuracy (%Corr) of responses were collected.

2.6. Data analysis

ANOVAs and non-parametric tests (χ^2) were used to ensure that the three groups did not differ in age, gender, motor excitability or performance at Baseline (see Table 1).

ANOVAs were also used to test the effect of ccPAS on behavior. For the 9-HPT task, the mean execution time across the 5 repetitions was computed for each session, and data were entered into a two-way mixed factor ANOVA with ccPAS ($Exp_{PMv\rightarrow M1}$, $Ctrl_{M1\rightarrow PMv}$, $Ctrl_{sham}$) as the between-subjects factor and Session (Baseline, Pre, Post-0, Post-30) as the within-subjects factor. For the cRT task, we computed the mean RTs and % Corr from each session. RTs associated with incorrect responses or deviating more than 3 standard deviations from the mean RT in each session were excluded from analyses (<5% of trials, comparably distributed across groups and sessions). RTs and %Corr were analyzed through a ccPAS x Session ANOVA. MEPs recorded during ccPAS administration were also analyzed using an ANOVA (see Supplementary material for details). The Greenhouse-Geisser correction was applied when appropriate. Post-hoc analyses were performed using the Newman-Keuls test to correct for multiple comparisons. Partial η^2 (η_p^2) was computed as a measure of effect size for significant main effects and interactions, whereas repeated measures *Cohen's d* indices were computed for significant post-hoc comparisons. By convention, η_p^2 effect sizes of ~.01, ~.06, and ~.14 are considered small, medium, and large, respectively; *Cohen's d* effect sizes of ~.2, ~.5, and ~.8 are considered small, medium, and large, respectively (Cohen, 1992).

We computed one-tailed Pearson correlation coefficients to test whether inter-individual differences in the behavioral effect of ccPAS at Post-0 and Post-30 (i.e., reduction in 9-HPT execution time relative to Baseline, i.e., Post-0 minus Baseline and Post-30 minus Baseline) *positively* correlated with global indices of motor excitability (i.e., the rMT and the TMS intensity required to elicit a MEP of 1 mV amplitude, MEP_{1mV}).

All parametric and non-parametric analyses were conducted using STATISTICA version 12 and/or IBM SPSS Statistics version 25. Null hypothesis significance testing is the main statistical method in neuroscience, and for this reason we firstly used classical ANOVAs to show the effect of ccPAS on behavior. However, null hypothesis significance testing cannot assess whether observed data favor the null hypothesis in comparison to the alternative hypothesis (which is critical, for example, to ensure that our three groups of participants were comparable at baseline and that control ccPAS manipulations were ineffective in changing performance). Thus, ANOVAs were complemented by their Bayesian implementations using JASP v 0.8.4 (JASP Team, 2017). With Bayesian hypothesis testing, we could directly evaluate the relative strength of evidence for the null and alternative hypotheses, providing quantification of the degree to which the data support either hypothesis (Dienes, 2011; Wagenmakers et al., 2018). We used default priors in JASP (r scale fixed effects = 0.5; r scale random effects = 1). Following the current standards, we report subscripts on Bayes Factors to refer to the models compared. Accordingly, the Bayes Factor for the alternative relative to the null hypothesis is denoted BF_{10}, while the Bayes Factor for the null relative to the alternative hypothesis is denoted BF_{01}. We interpreted and labelled the sizes of *BFs* according to the recommendations of Raftery (1995) as referred to by Jarosz and Wiley (2014).

3. Results

All participants tolerated the ccPAS protocol well and no adverse effects were noted or reported.

3.1. Preliminary comparisons and physiological assessment

Table 1 shows that participants in the three ccPAS groups did not differ in age or gender. Moreover, they showed comparable 9-HPT and cRT performance at Baseline, and similar left M1 excitability.

Fig. S1 shows MEP amplitudes induced by paired stimulation during the administration of the ccPAS protocol. MEP amplitudes were initially comparable in the $Exp_{PMv\rightarrow M1}$ and the $Ctrl_{M1\rightarrow PMv}$ group. Then, the $Exp_{PMv\rightarrow M1}$ group, but not the $Ctrl_{M1\rightarrow PMv}$ group, showed a consistent and gradual increase in MEP amplitudes throughout the protocol, indexing an enhancement of motor excitability.

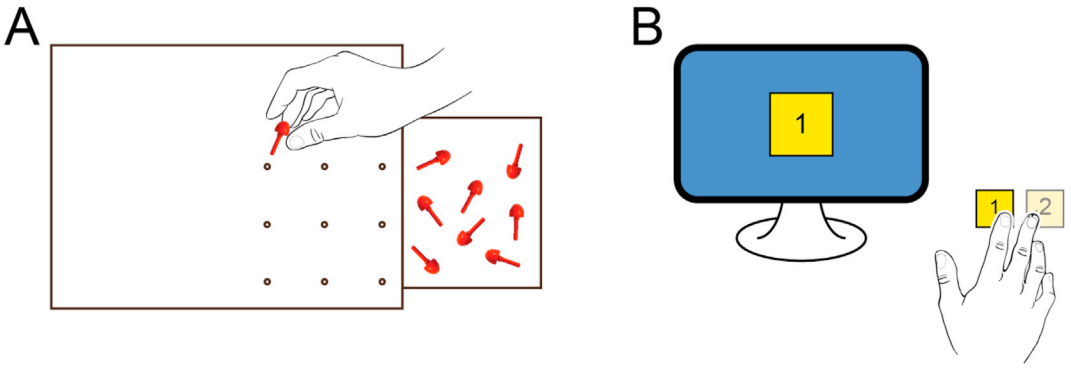

Fig. 3. Schematic representation of the tasks. (A) 9-HPT (B) cRT task.

3.2. Experimental task (9-HPT)

The ccPAS x Session ANOVA conducted on the mean execution time showed no main effect of ccPAS ($F_{2,51} = 2.80$, $p = .07$; $\eta_p^2 = 0.10$), but a main effect of Session ($F_{2.3,117.6} = 5.12$, $p = .005$; $\eta_p^2 = 0.09$) that was qualified by a ccPAS x Session interaction ($F_{4.6,117.6} = 3.31$, $p = .009$; $\eta_p^2 = 0.11$), indicating that changes in 9-HPT performance over time depended on the ccPAS protocol being administered (Fig. 4.; see also Fig. S2 for single participants' raw data).

Post-hoc analysis further clarified the ccPAS interaction. The $Exp_{PMv\rightarrow M1}$ group showed a reduction in the mean time necessary to complete the 9-HPT after ccPAS (Fig. 4A). In this group, execution time in the Baseline (mean ± S.D.: 20.8 s ± 2.1) and Pre (20.4 s ± 1.6) sessions were comparable ($p = .86$). At Post-0 (19.9 s ± 1.2), execution time appeared lower than at Baseline and Pre, although the relevant post-hoc comparisons were not significant (all $p \geq .19$; trends for reductions were detected with uncorrected planned comparisons: Post-0 vs. Baseline: $p = .02$, *Cohen's* $d = 0.59$; Post-0 vs. Pre: $p = .06$, *Cohen's* $d = 0.45$). Importantly, at Post-30 (18.9 s ± 1.3), mean execution time appeared strongly reduced relative to Baseline, Pre and Post-0 (all $p \leq .007$, all *Cohen's* $d \geq 1.14$).

No consistent changes in mean execution time were found in the $Ctrl_{M1\rightarrow PMv}$ (all $p \geq .35$) or the $Ctrl_{sham}$ groups (all $p \geq .60$) across time points; moreover, no differences were found between these two groups across time points (all $p \geq .83$).

The $Exp_{PMv\rightarrow M1}$ group showed comparable performance to the $Ctrl_{M1\rightarrow PMv}$ and $Ctrl_{sham}$ groups in Baseline and Pre sessions (all $p \geq .68$). At Post-0, the execution time of the $Exp_{PMv\rightarrow M1}$ group (19.9 s ± 1.3) started to appear shorter than the execution times of the $Ctrl_{M1\rightarrow PMv}$ (20.1 s ± .9) and the $Ctrl_{sham}$ groups (20.7 s ± 1.2), although the relevant post-hoc comparisons were not significant (all $p \geq .59$; uncorrected planned comparisons detected a difference relative to the $Ctrl_{M1\rightarrow PMv}$ group, $p = .03$, *Cohen's* $d = 0.70$). In contrast, at Post-30, the execution time of the $Exp_{PMv\rightarrow M1}$ group (18.9 s ± 1.3) was significantly reduced relative to the $Ctrl_{M1\rightarrow PMv}$ (20.6 s ± 1.2; $p = .004$; *Cohen's* $d = 1.53$) and the $Ctrl_{sham}$ groups (20.5 s ± 1.3; $p = .009$; *Cohen's* $d = 1.27$).

These findings were further corroborated by a Bayesian ANOVA with factors ccPAS and Session. The models including the main effect of Session ($BF_{10} = 7.4$) and both main effects ($BF_{10} = 8.1$) showed positive evidence favoring the alternative hypothesis, but the model that outperformed the null model the most was the model which also included the interaction ($BF_{10} = 75.2$). Data were ~8.8 times more likely under

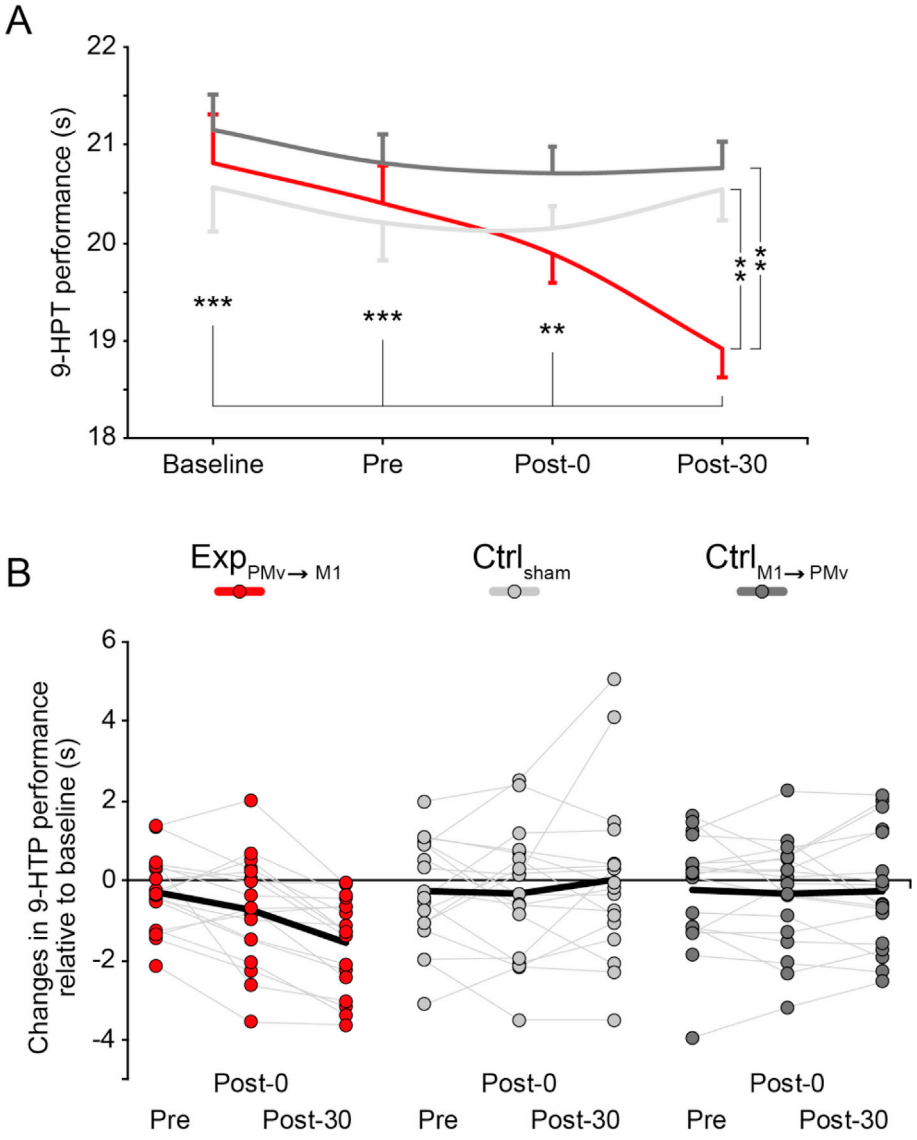

Fig. 4. Performance on the experimental task (9-HPT). A) ccPAS x Session interaction showing 9-HPT mean execution time (s) in the three groups across sessions. Error bars denote s. e.m. Asterisks indicate significant post-hoc comparisons, ** = $p \leq .01$, *** = $p \leq .001$. B) Individuals' changes in 9-HPT execution time relative to Baseline.

that model than under a null model including only the main effects, thus providing positive evidence indicating that 9-HPT performance changed over time depending on the type of the ccPAS protocol. Additionally, a series of Bayesian one-way ANOVAs with the factor Session provided very strong evidence supporting the alternative hypothesis for the $Exp_{PMv\rightarrow M1}$ group data ($BF_{10} = 7.6*10^4$), whereas they provided positive evidence supporting the null hypothesis of no change across sessions in the $Ctrl_{M1\rightarrow PMv}$ ($BF_{01} = 6.2$) and the $Ctrl_{sham}$ ($BF_{01} = 7.5$) groups.

3.3. Variability in the behavioral effect of ccPAS and its relation to motor excitability

Fig. 4B shows the distribution of individual changes in 9-HPT performance (relative to Baseline). In the $Exp_{PMv\rightarrow M1}$ group, the effect of ccPAS was variable at Post-0 with 13 participants showing a reduction and 5 showing an increase in 9-HPT execution time (range −4.2 to +2.3 s). At Post-30, all participants showed a reduction in 9-HPT execution time, although the magnitude of the reduction was still variable across participants, ranging from −130 ms to −4.3 s (corresponding to reductions of ~1%–~17% relative to Baseline execution time). The other two groups showed a more distributed performance centered at zero and no net change at the group level.

Based on prior work showing a relationship between motor excitability and TMS-induced effects (e.g., Müller-Dahlhaus et al., 2008; Kaminski et al., 2011), we explored whether reductions in 9-HPT execution time following PMv-to-M1 ccPAS (i.e., in the $Exp_{PMv\rightarrow M1}$ group) were positively associated with inter-individual differences in rMT and MEP_{1mV}. Fig. 5 shows that changes in 9-HPT execution time at Post-0 significantly correlated with rMT ($r = 0.42$, $p = .04$) and MEP_{1mV} ($r = 0.47$, $p = .02$); moreover, changes in 9-HPT execution time at Post-30 significantly correlated with rMT ($r = 0.41$, $p = .05$) and non-significantly with MEP_{1mV} ($r = 0.30$, $p = .12$). These findings indicate that $Exp_{PMv\rightarrow M1}$ individuals with more excitable motor systems tended to show greater 9-HPT improvements following PMv-to-M1 ccPAS administration.

No correlations were found between behavioral effects and changes in MEP amplitudes during ccPAS (see Supplementary material).

3.4. Control task (cRT)

The ccPAS x Session ANOVA conducted on the mean RTs showed no main effect of ccPAS ($F_{2,51} = 1.82$, $p = .17$), but a main effect of Session ($F_{2.6,132.4} = 15.67$, $p < .001$; $\eta_p^2 = 0.23$), showing that participants, regardless of the group to which they belonged (i.e., also in the $Ctrl_{sham}$ group), became faster as task repetitions increased (Fig. 6). Post-hoc analysis of the main effect of Session indicated that cRTs were comparable at Post-0 and Post-30 (398 ± 35 ms vs. 392 ± 30 ms; $p = .08$); however, cRTs in these sessions were lower than at Pre (405 ± 39 ms; all $p \leq .03$) and cRTs in the Pre, Post-0 and Post-30 sessions were lower than at Baseline (414 ± 46 ms; all $p \leq .01$). No significant ccPAS x Session interaction was revealed ($F_{5.2,132.3} = 0.71$ $p = .62$), suggesting similar trends across groups (Table 2).

These findings were further corroborated by a ccPAS x Session Bayesian ANOVA. The analysis provided very strong evidence supporting all the alternative models (all $BF_{10} > 105$) – with the exception of the model including the main effect of ccPAS, which showed weak evidence in favor of the null hypothesis ($BF_{01} = 1.3$). The model that outperformed the null model the most was the model including only the main effect of Session ($BF_{10} > 2.3*106$) which was ~20 times more likely than the model with the interaction. Thus, the reduction of RTs over sessions

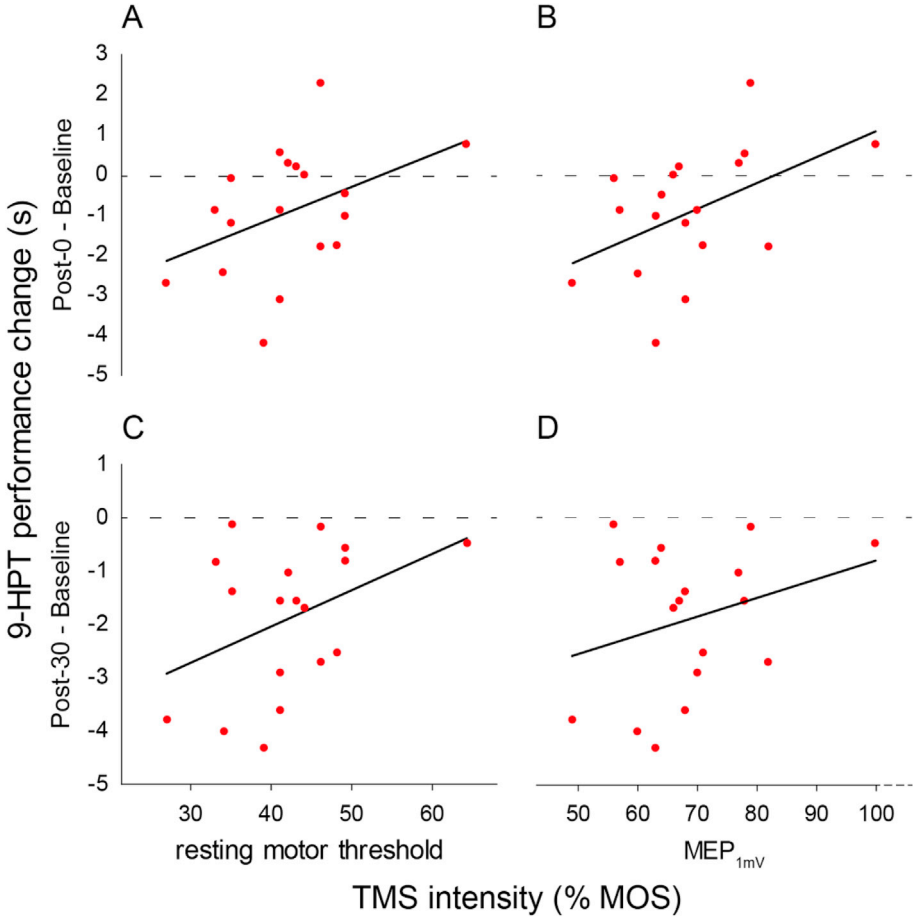

Fig. 5. Scatterplots showing the relationships between behavioral effects of PMv-to-M1 ccPAS and inter-individual differences in motor excitability. The y-axis displays the change in 9-HPT execution time at Post-0 (A, B) or at Post-30 (C,D) relative to Baseline (negative values indicate better performance). The x-axis reports individual values of rMT (A,C) and MEP_{1mV} (B,D) as assessed before ccPAS (lower values indicate greater motor excitability). Values are expressed as the percentage of maximal biphasic stimulator output (% MOS).

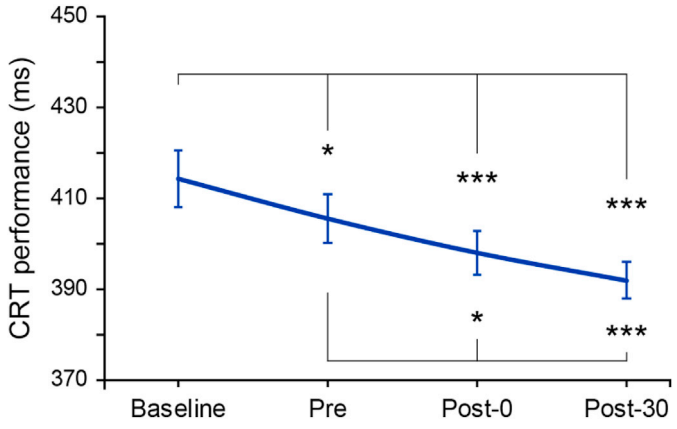

Fig. 6. Performance on the control task (cRT). Main effect of Session. Error bars denote s. e.m. Asterisks indicate significant post-hoc comparisons (* = $p \leq .05$, *** = $p \leq .001$).

likely reflected an effect of practice, as the Bayesian analysis provided evidence against an influence of ccPAS.

The ccPAS x Session ANOVA conducted on accuracy data (%Corr; Table 2) showed no main effects or interactions (all $F \leq 1.41$, all $p \geq .24$) and the corresponding Bayesian ANOVA showed positive evidence supporting the null hypothesis of no change in cRT accuracy (all alternative models with $BF_{01} \geq 4.2$).

4. Discussion

Seminal studies in animals have provided *in vitro* and *in vivo* evidence that repetitive paired stimulation of interconnected neurons, evoking sequential pre- and postsynaptic activity in such neurons, can induce STDP and elicit a transient (Hebbian) enhancement of the synaptic efficacy of those connections (Hebb, 1949; Markram et al., 1997, 2011; Antonov et al., 2003; Jackson et al., 2006; Caporale and Dan, 2008). Previous TMS studies in humans have shown that similar STDP-like synaptic strengthening can be induced in the motor system between two interconnected motor areas through ccPAS administered at an optimal ISI (Koganemaru et al., 2009; Rizzo et al. 2009, 2011; Arai et al., 2011; Buch et al., 2011; Lu et al., 2012; Koch et al., 2013; Veniero et al., 2013; Chao et al., 2015; Johnen et al., 2015). These studies showed that the ISI at which one targeted region (e.g., a premotor area) exerts a physiological effect on an anatomically connected second region (i.e., the M1) is also the ISI at which ccPAS can induce Hebbian-like cortico--cortical connection changes (e.g., ~8 ms for premotor-motor circuits; Davare et al., 2008; Buch et al., 2010, 2011; Arai et al., 2011, 2012). In particular, it has been demonstrated that the repeated paring of PMv and M1 stimulation (i.e., PMv-to-M1 ccPAS) with an ISI of 8 ms induces a transient enhancement of the effect of PMv stimulation on M1 excitability, thus providing direct evidence of increased PMv-to-M1 effective connectivity (Buch et al., 2011; see also Johnen et al., 2015).

Yet, these studies did not answer the critical question of whether plastic effects induced by PMv-to-M1 ccPAS are functionally relevant to behavior. To address this outstanding question, we combined a ccPAS protocol with a visuomotor task tapping into PMv-M1 interactions (i.e., the 9-HPT) and a control visuomotor task (i.e., the cRT). Based on prior neuroimaging studies suggesting that improved motor performance following training is associated with increased premotor-motor connectivity (Hamzei et al., 2012; Philip and Frey, 2016) and with evidence showing a hierarchy in PMv-M1 interactions underpinning skillful goal-oriented actions (Muir and Lemon, 1983; Murata et al., 1997; Fagg and Arbib, 1998; Fogassi et al., 2001; Lang and Schieber, 2004; Raos et al., 2006; Rizzolatti et al., 2014), here, we sought to examine whether exogenous manipulation of PMv-M1 connectivity through ccPAS can affect performance on the 9-HPT.

Our study provides the first evidence that PMv-to-M1 ccPAS meeting the physiological constraint of PMv-to-M1 short-latency connectivity (i.e., an 8-ms ISI) increases motor excitability and, remarkably, improves performance on the 9-HPT. Such a behavioral task requires dexterous control of grasping and manipulation of small objects (Mathiowetz et al., 1985; Grice et al., 2003), and hierarchical PMv-to-M1 interactions are thought to underpin this type of fine motor control (Grol et al., 2007; Davare et al., 2010). Critically, improvement on the 9-HPT was selectively found in the $Exp_{PMv \rightarrow M1}$ group that underwent a ccPAS protocol aimed at boosting synaptic efficiency in PMv-to-M1 connections. Moreover, by complementing classical hypothesis-testing with Bayesian analyses, we also show that no changes in 9-HPT performance were induced when reversing the order of the repeated PMv-M1 stimulation (i.e. in the $Ctrl_{M1 \rightarrow PMv}$ group that underwent active M1-to-PMv ccPAS) or when administering repeated PMv-to-M1 sham stimulation (in the $Ctrl_{sham}$ group). This allows us to rule out the possibility that mere repeated stimulation of PMv and M1, task practice or other nonspecific effects could explain the selective increase in 9-HPT performance. These findings indicate that hierarchical connections between frontal nodes of the network underlying motor control of object grasping and manipulation (Davare et al., 2008, 2009, 2010) are functionally malleable and sensitive to ccPAS.

In the $Exp_{PMv \rightarrow M1}$ group, we observed a gradual increase in MEP amplitudes during administration of the PMv-to-M1 ccPAS (see Fig. S1), indexing a rapidly growing facilitation of motor excitability that was already detectable in the second half of the protocol (i.e., before Post-0). On the other hand, behavioral enhancement in the 9-HPT was weak at Post-0 and increased at Post-30, i.e., 30 min after the end of the PMv-to-M1 ccPAS. This build-up of the plastic effects during stimulation and/or within the first minutes after stimulation offset is consistent with the time course of Hebbian plasticity (Bi and Poo, 2001; Caporale and Dan, 2008) and, more generally, with LTP-like effects induced in the human motor cortex (Stefan et al., 2000; Huang et al., 2005; Ziemann et al., 2008). Previous physiological studies administering ccPAS over premotor-motor areas have reported changes in motor excitability during (Arai et al., 2011) and/or immediately after the end of stimulation (Arai et al., 2011; Buch et al., 2011). Further studies have targeted other motor or visual regions and have reported behavioral effects immediately after the end of ccPAS (Koganemaru et al., 2009) or, in most cases, at later time points (Rizzo et al., 2009, 2011; Romei et al., 2016a; Chiappini et al., 2018). For example, we found a similar time course of behavioral gain in a previous study in which we administered ccPAS over extrastriate motion areas (V5) and primary visual cortex (V1) (Romei et al., 2016a). In that study, we found that ccPAS aimed at increasing V5-to-V1 (reentrant) connectivity significantly improved perceptual visual sensitivity after 30 min, whereas nonsignificant effects were observed immediately after ccPAS (Romei et al., 2016a). Based on previous physiological evidence (e.g., Buch et al., 2011), we would expect that behavioral improvements could

Table 2
Performance on the control task (cRTs). Mean RTs (in ms) ± S.D. and accuracy (% of correct responses) ± S.D. in the three groups across sessions.

	RTs (ms)				Accuracy (%Corr)			
	Baseline	Pre	Post-0	Post-30	Baseline	Pre	Post-0	Post-30
$Exp_{PMv \rightarrow M1}$	397 ± 29	393 ± 28	388 ± 25	382 ± 24	96 ± 3	96 ± 4	96 ± 3	96 ± 5
$Ctrl_{M1 \rightarrow PMv}$	425 ± 43	416 ± 40	402 ± 35	401 ± 28	96 ± 4	97 ± 4	96 ± 3	96 ± 3
$Ctrl_{sham}$	421 ± 59	407 ± 46	402 ± 43	393 ± 36	95 ± 3	97 ± 4	95 ± 3	95 ± 3

be detected at even later time points (e.g., at ~60 min after the end of ccPAS, based on Buch et al., 2011) – although future studies are needed to directly test this prediction.

Our study adds to previous physiological studies by showing that PMv-to-M1 ccPAS can improve motor performance in a functionally specific manner. Indeed, PMv-to-M1 ccPAS, but not the two control ccPAS protocols, improved motor functions tapping into PMv-to-M1 connectivity (i.e., 9-HTP performance), but no similarly selective effects were detected in the control visuomotor cRT task. In that task, we observed a linear increase in performance over time in all groups, irrespective of the ccPAS manipulation they underwent. Improvements were also detected in the Pre session relative to Baseline, clearly indicating a practice effect due to task repetition. Critically, these improvements were similar across the three groups – i.e., they were also found in the $Ctrl_{sham}$ group – suggesting they were not due to active ccPAS but merely reflected a practice effect.

It is worth noticing that improved behavioral performance was found in two previous studies following ccPAS over bilateral M1 (Koganemaru et al., 2009; Rizzo et al., 2009). These studies showed directional- and time-specific effects of ccPAS at a physiological level: for example, Koganemaru et al. (2009) administered right-to-left M1 ccPAS at an optimal ISI and found increased motor excitability in the left M1; no similar changes were observed following ccPAS protocols with suboptimal ISIs or when reversing the order of the ccPAS pulses (i.e., after left-to-right M1 ccPAS). Interestingly, in a separate behavioral experiment, better 9-HPT performance was observed after right-to-left M1 ccPAS. Similar findings were reported by Rizzo et al. (2009) using a left-to-right ccPAS protocol. However, in this latter study, improved motor performance was detected using a simple RT task. Although these previous studies did not use a sham ccPAS protocol to evaluate possible practice effects, the reported behavioral improvements on both the 9-HPT (Koganemaru et al., 2009) and the simple RT task (Rizzo et al., 2009) suggest that functional effects of M1-to-M1 ccPAS are more generic than those induced by PMv-to-M1. Similar generic improvements in hand function have been reported using brain stimulation protocols that increase motor excitability through excitatory stimulation of M1 (or inhibitory stimulation of its contralateral homolog; e.g., Avenanti et al. 2012b; Ayache et al., 2012; Di Lazzaro et al., 2013). Thus, behavioral improvements reported in previous studies might reflect a general role of bilateral M1 in controlling hand movements, whereas the PMv-to-M1 pathway appears more specifically involved in controlling object-oriented hand actions.

Thus, to date, at least three studies have detected motor improvements following ccPAS (present study; Koganemaru et al., 2009; Rizzo et al., 2009; see also Rizzo et al., 2011). It should be noted that in all these studies improvements were achieved by administering ccPAS over frontal motor areas (i.e., PMv-M1 or M1-M1) that directly control hand movements. On the other hand, a previous study by Chao et al. (2015) found that ccPAS over the posterior parietal cortex (specifically over electrode P3, which roughly corresponds to the left caudal intraparietal area, CIP) and the left M1 increased motor excitability, but failed to affect performance on a task that, similarly to the 9-HPT, is based on grasping and manipulating small objects (i.e., the Purdue pegboard test). These null findings are not surprising, as visually guided, object-oriented hand actions are not underpinned by direct CIP-to-M1 connections, but, rather, by a dorsolateral parieto-premotor-motor circuit connecting a more anterior parietal region, i.e., the anterior intraparietal area (AIP), to PMv, and then PMv to M1 (Prabhu et al., 2009; Davare et al., 2010, 2011; Rizzolatti et al., 2014; Borra et al., 2017; Gerbella et al., 2017). Thus, future studies are needed to understand the extent to which ccPAS strengthening of directional connections between posterior and anterior nodes of the dorsolateral stream can affect network functioning.

A growing literature shows that the effect of brain stimulation is highly variable across individuals (Ridding and Ziemann, 2010; Jones et al., 2016; Palmer et al., 2016; Avenanti et al., 2018; Valchev et al., 2016, 2017; Paracampo et al., 2018). Our data show that the behavioral effects of PMv-to-M1 ccPAS are highly variable at Post-0 and become more consistent at Post-30, with all 18 participants in the $Exp_{PMv \rightarrow M1}$ group showing a reduction in 9-HPT execution time. However, the effects were also variable at Post-30, ranging from a gain of ~1% to ~17% relative to baseline performance. As expected, the variability of this behavioral effect was partially accounted for by inter-individual differences in rMT and MEP_{1mV}. These parameters depend on the excitability of corticomotor neurons activated by the TMS pulse, as well as the excitability of synaptic connections at both cortical and spinal levels, providing reliable global measures of motor excitability (Paulus et al., 2008; Rossini et al., 2015). Our finding that individuals with more excitable motor systems display larger behavioral improvements following ccPAS confirm and expand previous work showing that inter-individual differences in MEP_{1mV} and/or rMT predict the magnitude of LTP effects in the motor system (Müller-Dahlhaus et al., 2008) and TMS-induced behavioral effects (Kaminski et al., 2011). A number of factors contribute to inter-individual differences in motor excitability (as indexed by rMT) including distance between the coil and the stimulated cortex, and cortical thickness and fiber coherence in the white matter beneath premotor and motor cortices (Kozel et al., 2000; McConnell et al., 2001; Klöppel et al., 2008; List et al., 2013). As microstructural properties of the corticospinal system contribute to the magnitude of LTP-like effects on M1 corticospinal neurons (e.g., List et al., 2013), similarly, individual differences in the microstructural properties of cortico-cortical pathways between PMv and M1 might represent a particularly relevant factor in determining individual sensitivity to PMv-to-M1 ccPAS, and may contribute to the observed relationship between behavioral improvements and motor excitability. Understanding the physiological and neural bases of TMS-induced variability is an important avenue for research, and future ccPAS studies could provide new insights by combining behavioral and neurophysiological, neuroimaging and/or genetic assessments (Cheeran et al., 2008, 2009; Ridding and Ziemann, 2010; Groppa et al., 2012; List et al., 2013).

A few limitations should be considered. First, PMv-to-M1 connections are modulated during object-oriented grasping (e.g. Davare et al., 2008), but also during response inhibition or action reprogramming (e.g., Buch et al., 2010; Neubert et al., 2010; Picazio et al., 2014; Bestmann and Duque, 2016). We focused on a motor task tapping into the ability to grasp and manipulate objects, and did not systematically evaluate the impact of PMv-to-M1 ccPAS on different domains of motor control. Moreover, while our data indicate functional specificity, performance in the control task tended to improve across sessions (independently of the ccPAS manipulation). Thus, future work could further test functional specificity by using experimental and control tasks with comparable learning rates over time. Lastly, although we assessed MEPs during the ccPAS protocols in a subsample of participants, we did not further assess the impact of ccPAS at a neural level. The effects of brain stimulation are known to spread along interconnected brain areas (Siebner et al., 2009a, 2009b; Dayan et al., 2013; Bortoletto et al., 2015; Valchev et al., 2015; Zanon et al., 2018). Although the behavioral effects of our PMv-to-M1 ccPAS protocol were directionally specific, it is likely that plastic effects were not limited to PMv-to-M1 hierarchical connections and may have extended to other components of the dorsolateral stream (e.g. as in Johnen et al., 2015) and/or nearby ventral and dorsal fronto-parietal areas involved in attention and higher-levels aspects of motor control (Vossel et al., 2014; Borra et al., 2017; Gerbella et al., 2017; Ptak et al., 2017). Thus, further research will be necessary to clarify how different components of these networks reconfigure following PMv-to-M1 ccPAS.

5. Conclusions

In conclusion, our study demonstrates that ccPAS aimed at strengthening the synaptic efficacy of PMv-to-M1 connections selectively enhances motor functions tapping into PMv-M1 networks. Plastic enhancement critically depended on the repeated pairing of pre- and post-synaptic nodes of the PMv-to-M1 pathway – meeting the

physiological constraint of the premotor-motor hierarchy – and showed a time course consistent with Hebbian effects. Moreover, the effect was functionally specific. These findings have important theoretical and methodological implications: they suggest that ccPAS might be a useful tool for targeting specific cortico-cortical pathways and they demonstrate a causal effect of directional connectivity on behavior (Romei et al., 2016a, 2016b; Chiappini et al., 2018). Moreover, our findings may have implications for designing novel therapeutic strategies based on associative brain stimulation of cortico-cortical pathways for the recovery of abilities that have been lost due to brain injury or neurodegenerative disease. Therefore, future studies should carefully assess the clinical and applied potentialities of ccPAS.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuroimage.2018.09.002.

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