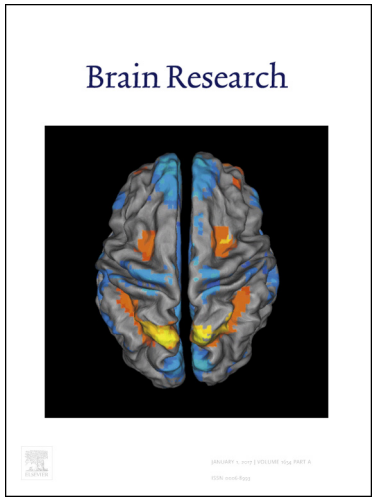
Effects of repetitive transcranial magnetic stimulation (rTMS) on attribution of movement to ambiguous stimuli and EEG mu suppression

Accepted Manuscript



Research report

Effects of repetitive transcranial magnetic stimulation (rTMS) on attribution of movement to ambiguous stimuli and EEG mu suppression

Agata Ando', Jaime A. Pineda, Luciano Giromini, Gregory Soghoyan, QunYang, Miranda Bohm, Daniel Maryanovsky, Alessandro Zennaro

PII: S0006-8993(17)30541-3
DOI: https://doi.org/10.1016/j.brainres.2017.12.007
Reference: BRES 45585

To appear in: *Brain Research*

Received Date: 7 July 2017
Revised Date: 23 November 2017
Accepted Date: 8 December 2017

Please cite this article as: A. Ando', J.A. Pineda, L. Giromini, G. Soghoyan, QunYang, M. Bohm, D. Maryanovsky, A. Zennaro, Effects of repetitive transcranial magnetic stimulation (rTMS) on attribution of movement to ambiguous stimuli and EEG mu suppression, *Brain Research* (2017), doi: https://doi.org/10.1016/j.brainres.2017.12.007

Effects of repetitive transcranial magnetic stimulation (rTMS) on attribution of movement to ambiguous stimuli and EEG mu suppression

Agata Ando' [a], Jaime A. Pineda [b], Luciano Giromini [a, *], Gregory Soghoyan [b], QunYang [c], Miranda Bohm [b], Daniel Maryanovsky [b] and Alessandro Zennaro [a]

[a] Department of Psychology, University of Turin, Italy

[b] Department of Cognitive Science, University of California San Diego, CA, USA

[c] Department of the Medical Psychology, Fourth Military Medical University, Xi'an, China

*** Corresponding Author**:

Luciano Giromini

Department of Psychology, University of Turin

Via Po 14, 10123 Torino (TO) – Italy

Luciano.giromini@unito.it

Fax number 0118146231

ABSTRACT

Recent research suggests that attributing human movement to ambiguous and static Rorschach stimuli (M responses) is associated with EEG mu suppression, and that disrupting the left inferior gyrus (LIFG; a putative area implicated in mirroring activity) decreases the tendency to see human movement when exposed to the Rorschach ambiguous stimuli. The current study aimed to test whether disrupting the LIFG via repetitive transcranial stimulation (rTMS) would decrease both the number of human movement attributions and EEG mu suppression. Each participant was exposed to the Rorschach stimuli twice, i.e., during a baseline condition (without rTMS but with EEG recording) and soon after rTMS (TMS condition with EEG recording). Experimental group (N = 15) was stimulated over the LIFG, while the control group (N = 13) was stimulated over the Vertex. As expected, disrupting the LIFG but not Vertex, decreased the number of M attributions provided by the participants exposed to the Rorschach stimuli, with a significant interaction effect. Unexpectedly, however, rTMS did not significantly influence EEG mu suppression.

Keywords:

Human Movement

Electrophysiological activity

Left Inferior Frontal Gyrus

Mirror Neuron System

Rorschach Test

1. Introduction

Mirror neurons are a particular class of cortical cells, originally discovered in area F5 of the monkey premotor cortex, which fire both when the monkey performs an action and when it observes another individual performing the same action (di Pellegrino et al., 1992, Gallese et al., 1996, Rizzolatti et al., 1996). A similar neurological network likely exists in humans, although there is some debate as to where the mirror neurons are located (Molenberghs et al., 2009, 2012). In general, the evidence supporting the presence of a human mirror neuron system (MNS) is mainly indirect (Dinstein et al., 2007; Hickok, 2009; Lingnau et al., 2009; Rizzolatti & Craighero, 2004). Functionally, the MNS is hypothesized to represent the neurological substrate of a mirror-matching mechanism that allows individuals to quickly and pre-rationally understand the actions performed by others (for a review, see Pineda, 2005; Rizzolatti & Craighero 2004, Gallese et al., 2011). Previous neurophysiological experiments have shown that when individuals observe an action performed by another agent their motor cortex becomes active, in the absence of any visible motor activity, as a function of learning (Heyes, 2011). In fact, studies by Fadiga and coworkers (1995), and Strafella and Paus (2000) demonstrated that observation of complex actions, such as grasping and writing, provided changes in corticospinal excitability. Furthermore, when observing other acting individuals and their expressive and intense meaning, an embodied interpersonal link automatically and implicitly occurs (Gallese, 2009). Therefore, some researchers (Gallese, et al., 2004; Rizzolatti & Craighero, 2004; Oberman & Ramachandran, 2007; Iacoboni, 2009; Rizzolatti et al., 2001) have suggested that the MNS may provide the neurophysiological substrate for higher cognitive human functions such as action understanding, perspective taking, and empathy.

Although several studies on MNS properties investigated the specificity of the changes in motor corticospinal excitability during the observation of an action by using video

clips with overt actions, the motor simulation process can likewise be induced by the observation of static images of actions, e.g., in works of art (Proverbio et al., 2009; Sbriscia-Fioretti et al., 2013; Yao et al., 2007). An event related potential (ERP) study reported a direct relation between the observation of static images and the activation of the cortical motor system (Proverbio et al., 2009). More specifically, during the presentation of pictures representing human actions with different degrees of dynamism, a higher motor cortical activation occurred for observation of pictures with more dynamic actions than for observation of pictures representing less dynamic actions. Along the same lines, an EEG study (Yao et al., 2007) evaluated the extent to which mirroring activity occurred during the observation of modern abstract artwork by Lucio Fontana, using event-related sensory-motor alpha desynchronization (ERD) as index of motor simulation. Consistent with Proverbio et al.'s (2009) findings, when compared to the observation of the other type of images, the observation of Fontana artworks was associated with a significantly stronger ERD.

Furthermore, by using single and paired-pulse TMS, Battaglia and colleagues (2011) reported that (a) the observation of an action in an artistic representation activated the corticomotor system and (b) the mental rehearsal of observation of a painting induced a similar degree of corticomotor activation. Likewise, a recent study on implied motion (Concerto et al., 2015) showed that observation of a static image pulled out from a video depicting movement increased Motor Evoked Potentials (MEP) size and decreased the short-interval intracortical inhibition (SICI), which suggests that observation of highly dynamic images may implicate an overall facilitation in primary motor cortex. Also in line with these findings, Sbriscia- Fioretti and colleagues (2013) reported that activation of sensorimotor cortical circuits during the exposure to the static works art by Franz Kline was related to an increased ERP mean amplitude. These results were interpreted by the authors as indicative of an embodied simulation of the artist's actions in the production of the artwork.

1.2 Mu suppression, ambiguous Rorschach stimuli, and movement attributions

Based on these previous studies indicating that the MNS might be engaged by exposure to static artworks or designs, it has been recently suggested that exposure to ambiguous Rorschach inkblots might be sufficient to activate MNS activity, even in the absence of evident, visual indicators of movement, as far as the observer experiences a "feeling of movement." More specifically, using EEG mu suppression as a proxy biomarker for mirror neuron activation, Giromini *et a*l. (2010), Pineda *et al*. (2011), and Porcelli *et a*l. (2013) showed that EEG mu suppression occurred concomitantly with the participants perceiving/feeling human movement while exposed to ambiguous, Rorschach inkblot stimuli. Interestingly, the presumed embodied simulation produced by the Rorschach cards seemed to occur very early, suggesting that the "feeling of movement" was induced at a preconscious level, before the participants had time to formulate their Rorschach attributions, i.e., prior to reporting that they saw human movement or M responses in the inkblot designs (Pineda et al., 2011). The authors interpreted these findings as indicative of the existence of a link between EEG mu suppression, embodied simulation, and attribution of human movement to ambiguous stimuli.

According to various Rorschach experts (e.g; Klopfer, 1942; Piotrowski, 1957; Rorschach, 1921), seeing human movement in the ambiguous stimuli (e.g., "this inkblot reminds me of two people playing paddy-cake" or "here I see a person dancing flamenco"), would rely on an ongoing identification mechanism. Other Rorschach determinants (i.e., form, color, and shading) may reflect distinct perceptual features recognized directly from the actual characteristics of the inkblots, while human movement responses (or M responses) are added to the stimulus field, presumably as a result of imagined activity (Exner, 2003). Several studies support the validity of the M responses as linked to an identification mechanism (e.g., Meyer, 2002; Viglione et al., 2012; Mihura et al., 2013). The inter-rater reliability of the M response also is excellent (Cicchetti, 1994), indicating that two independent raters, blind to each

other's evaluation, code for the presence or absence of M responses reliably, with intraclass correlation coefficients (ICC) ranging from .96 to .97 (Meyer et al., 2002; Mihura et al., 2013; Pignolo et al., 2017; Viglione & Taylor, 2003; Viglione et al.,2012).

Important to our goal, about two years ago, Ando' et al. (2015) reported that disrupting the left inferior frontal gyrus (LIFG), a putative area of the mirror neuron system (Keuken et al., 2011; Pobric & Hamilton, 2006), via repetitive Transcranial Magnetic Stimulation (rTMS) decreased significantly the number of human movement attributions (or M responses) to the Rorschach ambiguous stimuli. According to the authors, this finding would be in line with Giromini et al.'s (2010) findings that the LIFG plays a key role in MNS functioning. Also in line with the hypothesis that the LIFG and MNS are involved in the attribution of M responses to the ambiguous Rorschach stimuli, Giromini et al. (2017) recently reported on an fMRI study, in which M responses were associated with increased activity in a MNS region of interest that included a small portion of the LIFG.

1.3 The current study

Giromini and colleagues (Giromini et al., 2010; Pineda et al., 2011; Porcelli, et al., 2013) discussed their EEG findings as supportive of a link between mirroring activity and attribution of human movement to spontaneous Rorschach stimuli (M responses). Ando' et al. (2015) also proposed, based on rTMS results, a relationship between the LIFG, the MNS, and the production of M responses. However, none of these studies demonstrated that disrupting the LIFG would affect *both* the production of M responses to the Rorschach *and* its presumably associated biometric, mu rhythm suppression. Hence, the aforementioned inferences concerning the existence of a link between MNS-related areas (such as the LIFG), mu suppression, and attribution of human movement to ambiguous stimuli, currently remain indirect.

The present study aimed at filling this gap in the literature, by testing the effects of rTMS over LIFG on Rorschach responses and EEG mu suppression. More specifically, we administered a subset of Rorschach inkblot stimuli to a student population twice, i.e., during a baseline condition and soon after rTMS. As for the rTMS condition, the experimental group was stimulated over the LIFG, the control group was stimulated over the Vertex. Compared to Ando' et al.'s (2015) study, our investigation also overcomes a technical limitation, i.e., while the earlier study did not implement a neuronavigation system (p. 138), the current study did.

2. Results

2.1 rTMS effects on human movement responses

As reported in Table 1 and Figure 1, disrupting the LIFG, but not Vertex, decreased the number of M codes produced by the participants during exposure to the Rorschach stimuli. In fact, a mixed 2 (between-subjects factor, site: LIFG vs. vertex) by 2 (within-subjects factor, condition: baseline vs. rTMS) ANOVA reported a statistically significant interaction effect, [F (*1*, *26*) = 24.60, $p < .001$, *Partial* η^2 = .486]. Importantly, within the control group, the baseline and rTMS (vertex) conditions provided a strikingly similar number of M codes [t (12)= -.693, p =.502, d = -.12]; but, within the experimental group, the number of M codes after rTMS (LIFG) was significantly lower than at the baseline condition [t (14) =5.77, $p < .001$., d = 1.47].

2.1.1 Additional analyses

Given that the LIFG includes Broca's area (BA 44, corresponding to F7 of the 10-20 EEG system) (Nishitani & Hari, 2000), we examined whether the rTMS effects had reduced general verbal production following disruption of LIFG. Indeed, the LIFG plays an important role in language at different stages of object naming acting as a top-down regulator system

(e.g., Kan and Thompson-Schill, 2004; Nozari and Thompson-Schill, 2013), and is associated with the various mechanisms involved at different representational levels, i.e., phonological, semantic, and syntactic (Novick et al., 2005; Hamilton and Martin, 2005, 2007). Furthermore, anodal tDCS or high frequency TMS over the LIFG could speed up responses while cathodal tDCS or low frequency TMS could reduce the verbal production and can slow the language processing, so the possible effects of phonological information, and thereby of phonological facilitation[1] too (Antal et al., 2004; Pisoni et a., 2017), needed to be addressed in our study. We thus inspected the effects of rTMS over the LIFG on general verbal production (i.e., the number of spontaneous attributions to the Rorschach stimuli), regardless of what types of responses were reported by the participants.

Our results, however, showed that the number of verbal responses did not change across conditions[2]: in fact, we also tested a 2 x 2 mixed ANOVA with site (LIFG, Vertex) as between-subjects factor and condition (baseline vs. rTMS) as within-subjects factor, and the total number of responses as the dependent variable. These additional results showed that the experimental and control groups produced a strikingly similar total number of responses. Indeed, the interaction effect was not statistically significant [F (*1, 26*) =.977, p = .332, Partial η^2 =.036]. Thus, the reduction in the number of M responses after rTMS over LIFG canùnot be explained by a general reduction of verbal production.

2.2 rTMS effects on Mu rhythm

A mixed, 2 (between-subject: vertex vs. LIFG) by 2 (within-subject: baseline vs. rTMS) ANOVA was performed to inspect the effects of rTMS on EEG mu suppression. Different from our hypothesis, neither the interaction effect [F (*1, 24)* = .020, p= .888, Partial

[1] Phonological facilitation effect is characterized by shorter picture naming latencies when both stimuli are phonologically related than when they are not related.
[2] The control group reported a mean value of 7.53 (*SD* = 2.18) in baseline condition and a mean value of 7.76 *(SD* = 1.65) in TMS condition. The experimental group reported a mean value of 7.80 (*SD* = 1.74) in baseline condition and a mean value of 7.20 (*SD* = 1.65) in TMS condition.

$\eta^2 = .001$] nor the main effects, F (*1,24*) =.580, *p* =.454, Partial $\eta^2 = .024$, were statistically significant. Examination of Figure 2 and Table 2 trends, however, reveals that while mu suppression occurred during the baseline condition, no mu suppression at all was observed for the rTMS conditions (neither for the LIFG nor for the Vertex conditions). Furthermore, it should be noted that these data were highly dispersed, as shown in Figure 2.

3. Discussion

Past research suggests that attributing human movement (M) to ambiguous stimuli associates with EEG mu suppression (Giromini et al., 2010; Pineda et al., 2011; Porcelli et al., 2013), and that disrupting the left inferior gyrus (LIFG) diminishes the tendency to see human movement when exposed to ambiguous, Rorschach stimuli (Ando' et al., 2015). The current study sought to test whether disrupting the LIFG via repetitive transcranial stimulation (rTMS) would decrease *both* the number of M responses produced while inspecting ambiguous stimuli, *and* EEG mu suppression. Our findings support our hypotheses only partially.

As expected, after disrupting the LIFG, but not vertex, a reduction in the number of M attributions provided by the participants exposed to the Rorschach stimuli occurred. Noteworthy, this result replicates – this time by using a neuronavigational system – the findings previously reported by Ando' et al. (2015). As such, one may reasonably conclude that the LIFG plays a key role in the attribution of human movement to ambiguous stimuli such as the Rorschach inkblot designs.

On the other hand, the hypothesis that disrupting the LIFG via rTMS would influence EEG mu suppression did not find support in our study. In fact, the disruption of the LIFG did not significantly affect the extent to which EEG mu suppression occurred. This finding deserves particular attention and a number of considerations should be discussed.

First, some technical issues reduced the power of our study, so that a type II error might be present. Indeed, because the after-effects of rTMS likely lasted 7-8 minutes only, very few data points were available for our EEG data analysis. Furthermore, the sample size (N = 26) was relatively small. Also, as shown in Figure 2, the available EEG data appeared to be highly variable. Therefore, future studies with larger sample sizes and additional EEG data points could in fact obtain different results from what was observed in this study.

Second, examination of Figure 2 reveals that Mu suppression tended to be more evident during baseline than during the rTMS conditions, albeit not significantly so. The reduction of Mu suppression as a consequence of rTMS over the LIFG was expected. Conversely, one might wonder why rTMS over the Vertex affected Mu suppression as well. A possible explanation is that stimulating over the Vertex site did not preserve μ oscillations because the Vertex is located exactly where EEG mu suppression is typically recorded, i.e., Cz (Elfenbeinet al., 2007; Pineda, 2005; Keuken et al., 2011). Additional studies, with bigger sample sizes, different control sites, and more EEG data points might further investigate this hypothesis.

A third, possible, explanation for the lack of effect on EEG mu suppression is that the LIFG is just one of the multiple brain areas involved in mirroring processes, and therefore interfering with its activity only partially affects the MNS. Indeed, several brain regions outside the classic, fronto-parietal, MNS network seem to be involved in embodied simulation and mirroring processes (Molenberghs et al., 2009, 2012). For example, using single-cell recording, Mukamel et al. (2010) found that the medial temporal lobe includes several neurons with ‘mirror-like’ properties. Likewise, Tarhan, Watson, and Buxbaum (2015) observed that lesions in the posterior middle temporal gyrus associated with impairment of both action production and action recognition in a sample of left-hemisphere stroke patients. Perhaps more importantly, in a recent fMRI study conducted by Giromini et

al. (2017), Rorschach M responses associated with activity in various brain regions outside the classic, fronto-parietal MNS network, and the LIFG was only partially engaged by M responses. All in all, thus, it is possible that we did not observe an effect on EEG mu suppression simply because the MNS network goes far beyond the rTMS target of our study (i.e., the LIFG). Future studies, targeting brain regions other than the LIFG, should thus further test this hypothesis.

In terms of possible problems associated with our study, in addition to the limited amount of EEG data points available for the analysis of mu suppression, some other shortcomings of our research deserve mentioning. First, we could not investigate exactly the mu power when the subject was producing M responses vs other Rorschach determinants given that each participant was asked not to talk or move in order to minimize the risk of artifacts. Therefore, it was not possible to examine a possible direct link between the variation of the mu power and verbal production of M responses during the after effects of TMS. Second, only a subset of Rorschach cards was utilized for this study. Although this choice is consistent with previous studies (Ando' et al., 2015; Giromini et al., 2010), the ecological validity, vis-à-vis standard Rorschach procedures, might be questioned. Third, given that all participants were college students, the extent to which our findings might be generalized to other populations awaits additional research. Fourth, our use of inhibitory rTMS only may be questioned. Indeed, previous studies showed that anodal tDCS or high frequency TMS might interfere negatively with complex cognitive task involving various aspects of perception, recognition, working memory and verbal production such as the Rorschach test (e.g., Pascual-Leone et al.,1994; Hong et al., 2000; Koch et al., 2003). Hence, future studies might include a third condition, in which high frequency TMS is used, too.

3.1 *Conclusion and Final Remarks*

Our findings confirm that the LIFG plays a key role in the mental processes underlying the attribution of human movement to ambiguous or unstructured, visual stimuli. Importantly, our use of the Rorschach inkblot stimuli allowed us to focus on the cognitive processes underlying the "feeling of movement" in situations in which a person is asked to understand, elaborate, and/or attribute meaning to ambiguous stimuli. From this standpoint, our findings indicate that the LIFG plays a key role in the subjective interpretation of visual stimuli that may be perceived either as static or in motion.

On the other hand, our study fails to demonstrate that the LIFG also is important to the mu rhythms, although some technical details might have influenced this outcome. Because Giromini and colleagues (Giromini et al., 2010; Pineda et al., 2011; Porcelli et al., 2013) found an association between production of Rorschach M responses and EEG mu suppression, Keuken et al. (2011) found an association between disruption of LIFG and EEG mu suppression, and Ando' et al. (2015) found an association between disruption of LIFG and production of Rorschach M responses, the most intriguing challenge posed by our findings is to understand why in our study disrupting the LIFG reduced the number of M responses without influencing EEG mu suppression.

The degree to which mirror neurons are involved in the videos and tasks utilized by Keuken et al. (2011) is likely greater compared to attributing human movement to the Rorschach task. Indeed, mirroring activity tends to be more evident when the movement in the visual field is more explicit (Gallese, 2009; Strafella & Paus, 2000). As such, it is possible that our study failed to find an association between the disruption of the LIFG and EEG mu suppression simply because our baseline condition (i.e., in the absence of rTMS) did not involve a strong activation of mirror neurons. Said differently, it is possible that the extent to which EEG mu suppression occurred during the baseline (i.e., in the absence of rTMS) and soon after rTMS did not dramatically change simply because producing M responses to the

Rorschach activates the MNS only partially or to a moderate degree, whereas in Keuken et al.'s (2011) tasks the MNS was more evidently engaged. In this view, the lack of power of our design probably played a key role in determining our results, and future studies might in fact lead to different conclusions.

Alternatively, one could speculate that EEG mu suppression is only loosely associated with Rorschach M responses and with LIFG activity, whereas the LIFG is crucial to attributing a human movement to the ambiguous Rorschach stimuli. For example, it is possible that the LIFG gets involved when one tries to figure out what an ambiguous stimulus resembles, and that mentally describing a given scenario as involving human movement versus different, static scenarios requires additional LIFG activity. This hypothesis would explain why rTMS over the LIFG selectively reduced the number of M responses in our study, despite its effects on EEG mu suppression being small.

In our view, however, the most likely interpretation is that our study simply did not have enough power to detect medium-sized effects, so that the effects of rTMS over LIFG could be observed on Rorschach M responses and not on EEG mu suppression simply because the latter is characterized by a much smaller effect size. Regardless, the most noteworthy contribution of our study is that it replicated and extended Ando' et al.'s (2015) previous findings indicating that the LIFG plays a key role in the attribution of human movement to ambiguous stimuli such as the Rorschach inkblot designs.

4. Experimental Procedures

4.1 Participants

Thirty-two right-handed healthy students ranging in age from 18 to 31 years were recruited from the Departments of Cognitive Science and Psychology at the University of California, San Diego (UCSD) via an online recruitment system. Participants had no history of neurological or psychiatric illness and had never been administered the Rorschach test.

Handedness evaluation was based on the Edinburgh Handedness Inventory (EHI; Oldfiel, 1971). Potential participants were screened for inclusion/exclusion criteria based on recommended and safe use of TMS (Rossi, et al., 2009). Four individuals were excluded from the study due to missing data. The final sample comprised of twenty eight right-handed healthy individuals (8 males, 20 females), ranging in age from 18 to 31 years (*M=20.54; SD=3.08*). Two participants were excluded from the EEG data analysis portion of the study because of the presence of multiple artifacts during EEG recording, thus, the final sample for EEG analysis was comprised of 26 subjects. Participants gave their written informed consent to participate in the study, which was approved by the Institutional Review Board of the University of California, San Diego.

4.2 Procedure & Data Preparation

Each participant was exposed to a set of ambiguous Rorschach inkblot designs on a screen, twice, i.e., during a baseline condition (without rTMS but with EEG recording) and soon after rTMS (TMS condition with EEG recording) [3]. The time period between these two conditions was 4 weeks. During stimulus presentation, a black screen was shown for two seconds, and then the Rorschach stimuli were shown singularly. During the first 15 seconds of exposure to each Rorschach card, participants were instructed not to talk or move in order to record the electrophysiological activity appropriately.

The Experimental group (*N* = 15) received stimulation over the LIFG, while the Control group (*N* = 13) received stimulation over the Vertex [4] (see Table 3). Vertex was chosen as the control site since it is often used to test for non-specific rTMS effects (Foltys, et al., 2001; Nyffeler et al., 2006; Keuken et al., 2011). For both the Control and Experimental groups, 1 Hz rTMS at 90% of resting Motor Threshold (rMT) was applied for fifteen minutes

[3] Baseline and TMS conditions were counterbalanced. Therefore, half of the sample started with the baseline session followed by the stimulation session and the other half started with the stimulation session followed by the baseline session.
[4] Participants were randomly assigned to either the experimental or the control group.

(~900 pulses). Following stimulation, participants were exposed to the Rorschach cards on the computer monitor while EEG data were recorded.

4.2.1 Repetitive Transcranial Magnetic Stimulation (rTMS)

TMS is a noninvasive technique used to apply magnetic pulses to the brain (Rossi et al., 2009). The pulses are administered by passing currents through a coil placed upon the subject's scalp and induces electrical activity in the underlying cortical tissue. Induced electrical activity can result in neuronal depolarization or hyperpolarization depending on the stimulation parameters. When a train of pulses is delivered multiple times within one session, the stimulation method is referred to as "repetitive TMS" (rTMS). High frequency rTMS (> 5 hz) has excitatory or depolarizing effects. Conversely, low-frequency rTMS, meaning stimulus rates of 1 Hz or less produces inhibitory or hyperpolarizing effects.

In this study, rTMS was performed with a MagPro X100 Stimulator (MagVenture Co., 303 Perimeter Center North, Suite 300 Atlanta, GA 30346 USA) and a MCF-B65 Butterfly coil. To define the rMT, for each participant, the coil was positioned over the subject's left primary motor cortex (M1) at the optimum scalp position to elicit motor evoked potentials (MEPs) in the contralateral abductor pollicis brevis muscle (APB). rMT is defined as the minimum stimulus intensity that produces MEPs > 50 μV (peak-to-peak amplitude) in at least 5 out of 10 responses, and it is determined while the target test muscle is at rest. MEP responses to individual, successive stimuli when elicited in active muscles using threshold intensities may fluctuate in amplitude from 0 to about 1 mV. We started with a stimulus intensity of 35% of the maximal stimulator output (MSO) with the coil placed over the optimal site for stimulation. To determine rMT, stimulus intensity was gradually increased in steps of 5% MSO until TMS consistently evoked MEPs with peak-to-peak amplitudes of >50 μV in each trial. Thereafter, stimulus intensity was gradually lowered in steps of 1% MSO until there were less than 5 positive responses out of 10 trials (Groppa et al.2012). The motor

threshold (that varies in each subject) was detected only once in each subject before starting the stimulation.

In the Experimental group, 1 Hz rTMS was applied at 90% of rMT for fifteen minutes (900 pulses) over the LIFG prior to the task. The same stimulation and duration was used over the Vertex in the Control group. Offline low-frequency stimulation rTMS was applied with the aim of inducing a longer lasting suppression of neural activity (Rossini et al., 1994, 2015; Salatino et al., 2014). This approach (called *offline-TMS)* has the advantage of not requiring rTMS at the same time as task performance and of removing many of the non-specific concurrent effects of online TMS, such as nonspecific behavioral and attentional effects. In general, the duration of the rTMS hyperpolarization is expected to be about half of the total stimulation time, depending on the stimulation parameters and coil characteristics (Nowak et al., 2008). Hence, we expected that a stimulation duration of 15 minutes would provide a hyperpolarizing window of about 7-8 minutes (Mottaghy, et al., 2003; Hansenne et al., 2004) during which time subjects were asked to perform the Rorschach test and EEG recorded. We used the *Brainsight* targeting system to more accurately determine Vertex and LIFG sites. Brainsight TMS enables a TMS coil to be positioned over a specified target location based upon a subject's MRI image. Using and entering the coordinates of Vertex and LIFG, TMS sites were located using the Brainsight TMS –MRI coregistration system (Rogue Research). For LIFG, the following Talairach coordinates were used: - 42.5, 11.6, 19.9.[5] The Vertex was defined as a point midway between the inion and the nasion and equidistant from the left and right intertragal notches.

Next, we moved the coil close to the target while seeing the views in the continuously updated window showing the coil location. More in detail, the bull's eye view showed the distance from the target to the coil's projected axis. A value of zero indicated that the coil was

[5] We based on previous literature and focused on those studies including the use of rTMS (e.g., Amunts et al., 1999; Pitcher et al., 2008; Pobric et al., 2006) for choosing the coordinates for the LIFG.

pointing directly at the target. The target areas where localized before starting the TMS session.

The target location for rTMS in the left inferior frontal gyrus was the pars opercularis and the focal point was marked. The hand-held coil was securely positioned over the left temple, centered over this focal point, and oriented such that the maximal induced current flowed approximately in the anterolateral direction.

There were specific reasons for choosing the LIFG as the target site. The pars opercularis of the IFG is considered to be the human homolog of the monkey area F5, which is the region where mirror neurons were first discovered (Rizzolatti & Craighero, 2004). Furthermore, previous studies (Ando' et al., 2015; Elfenbein et al., 2007; Keuken et al., 2011; Pobric & Hamilton, 2006) have shown that rTMS over the LIFG conditioned the processes related to mirroring activity, and Pineda (see review, 2005) also hypothesized that the Mu rhythm is associated with IFG activity (Keuken et al., 2011). Therefore, several empirical observations suggest that the LIFG may be linked to embodied simulation and MNS activity (Hobson & Bishop, 2016; Molnar-Szakacs et al., 2006).

4.2.2 Electroencephalography (EEG)

EEG is a technique for recording and interpreting the electrical activity of the brain by multiple electrodes placed on the scalp. In this study, electrodes were applied to the face above and below the left eye to monitor the electrooculogram (EOG). Data were collected from 32 electrodes (i.e., FP1, FP2, F7, AF3, F3, FZ, F4, AF4, F8, T7, FC5, C3, FC1,Cz, FC2, C4, FC6, T8, P7, CP5, P3, CP1, PZ , CP2, P4, CP6, P8, PO3, O1, OZ, O2, PO4) embedded in a cap, using the international 10–20 system of electrode placement. EEG was recorded and analyzed using a Biosemi data acquisition system (band pass 0.1–30 Hz).

4.2.3 Rorschach Stimuli

The Rorschach test consists of ambiguous, inkblot designs, and in this study Rorschach stimuli were shown on a screen situated at a distance of 96 cm, at a size of 17-24 cm, similar to the original Rorschach cards. Participants were informed that they would see a series of inkblot designs with the task to think of what they might be. Additionally, consistent with Rorschach Performance Assessment System administration (R-PAS; Meyer et al., 2011), they were asked try to see two or three different things per each card. The three selected cards were shown one at a time. After 15 seconds of exposure to each of these Rorschach cards, participants were asked to describe what they saw in the card, and at that point the experimenter verbatim transcribed their responses.

Stimuli were presented using *Presentation* software and all event markers were sent to the EEG data acquisition system. The experiment took place in an appropriate and isolated room. Given that the offline effect of rTMS, as a rule, has duration about half of the total (i.e., 15 minutes) stimulation time (Mottaghy et al., 2003; Hansenne, et al., 2004), only 7-8 minutes were available for the rTMS condition. Thus, to maximize the variability in our dependent variables (i.e., the number of M responses) within that limited amount of time, we selected a small subset of stimuli from the entire set of Rorschach inkblots, to obtain the maximum number of allowed responses per card. Consistent with Giromini et al. (2010) and Ando' et al. (2015), we used the three Rorschach cards[6] that more frequently elicit spontaneous attribution of human movement (cards II, III, and VII; Exner & Erdberg, 2005). This choice was in order to avoid an unwanted floor effect (i.e., lack of variability due to absence of M responses). The Rorschach test was administered by an expert clinician and two independent raters coded for the presence vs. absence of M responses. The percentage of agreement was 95 %, the ICC was .94; statistics were computed on the overall number of M responses per respondent.

[6] The order of the Rorschach cards was randomized.

4.3 EEG Data Analysis

Before analysis, the data were evaluated manually and cleaned. All obviously malfunctioning channels were removed first, basing the more difficult decisions on moving kurtosis. Then, eye-blink and muscle artifacts were removed using frequency distribution, location, dipole distribution, etc. to make our decisions. Instead of removing segments of unusable data, we bandpassed the data into the mu range first, and ran Independent Component Analysis (ICA). Independent components were removed that corresponded to muscle and eye artifact based on outlying probability across channels, locality, shape of dipole-shaped scalp maps, magnitude, frequency distribution, (especially those components skewed towards extremely low and high frequencies) and relative kurtosis. With this framework, it was found that many components coded for very brief, topographically local, anomalous bursts of power. We erred on the side of leaving in, as long as the distributions across these features were smooth, and not likely to be noise. Because the analysis is based on mu suppression within-subject, any consistent patterns of noise (like attentional components, high-power saccades, or a chronic, low power twitch somewhere on the head) that otherwise resemble brain-related components were naturally corrected. For this reason, the focus was on anomalous noise.

We computed a baseline for every subject by extracting base 10 mu power over every channel, from the half-second preceding the presentation of the first card, and integrated the power over that time period. The same integrated base 10 mu power was calculated for all 15 second periods following the presentations of the three cards. In total, three integrated base 10 mu power values were calculated for every channel; each of these values given by the 15 seconds following a corresponding card presentation, for a total of 45 seconds worth of data. Each of the power values was then divided by 30 to create an average power over .5 seconds, (15/30 = ½) so as to compare to the baseline condition (black screen, i.e., the first 500

milliseconds of the total exposure time; Pineda, et al., 2011). Hence, per session, we had 32 channels worth of baseline mu power, and 3 sets of 32 channels worth of card-condition mu power. Dividing each of the conditional sets by the baseline, and taking the base-ten log of each of the resulting values gave us our three mu suppression values for each channel (scalp locations C3, Cz and C4), for each session. Data were segmented into epochs of 1 s beginning at the start of the segment, and Fast Fourier Transforms were performed on the epoched data. As a common procedure for this type of study (Altschuler, et al., 2000; Pineda, 2005; Pineda & Hecht, 2009), a ratio was used to control for variability in absolute mu power as a result of individual differences such as scalp thickness, electrode placement, and impedance, as opposed to differences in brain activity. Since ratio data are typically non-normal, as a result of lower bounding, a log transform was used for analysis. A log ratio of less than zero indicates suppression, whereas a value of zero indicates no suppression and a value greater than zero indicates enhancement. Thus, we hypothesized that during baseline and rTMS over the Vertex condition mu suppression would occur; whereas we expected the mu suppression would not occur when the rTMS was applied over the LIFG. Previous studies (Giromini et al., 2010; Pineda et al., 2011; Porcelli et al., 2013; Porcelli & Kleiger, 2016;) suggested that Mu suppression tends to be even stronger at the beginning of the exposure time than at the end. In fact, mu wave suppression occurred very early, during the first seconds of exposition to the cards (before the verbal articulation of the M response), and remained so for the entire exposure time.

Acknowledgments

We would like to thank Prof. Marta Kutas and Dr. Thomas P. Urbach for their assistance, guidance and providing us with access to their lab and TMS equipment.

No source of support is declared.

The authors declare no conflict of interest.

Author Contributions

Agata Ando' conceived and coordinated the study, reviewed and collected rTMS and Rorschach data, performed data analysis and wrote the manuscript.

Jaime A. Pineda contributed to the research design and supervised the study.

Luciano Giromini contributed to the research design, performed data analysis and wrote the manuscript.

Gregory Soghoyan collected rTMS and helped analyzing EEG data.

Qun Yang collected rTMS data and EEG data.

Miranda Bohm analyzed EEG data.

Daniel Maryanovsky analyzed EEG data.

Alessandro Zennaro conceived and supervised the study.

References

Altschuler, L.E., Vankov, A., Hubbard, M.E., Roberts, E., Ramachandran, V.S., Pineda, J. A. (2000, November). *Mu wave blocking by observer of movement and its possible use as a tool to study theory of other minds.* Poster session presented at the 30th Annual Meeting of the Society for Neuroscience, New Orleans, LA.

Amunts, K., Schleicher, A., Burgel, U., Mohlberg, H., Uylings, H.B., Zilles, K., 1999. Broca's region revisited: cytoarchitecture and intersubject variability. *J. Comp. Neurol. 412*, 319–341.

Ando', A., Salatino, A., Giromini, L., Ricci, R., Pignolo, C., Cristofanelli, S., Ferro, L., Viglione, D. J., Zennaro, A., 2015. Embodied Simulation and Ambiguous. Stimuli: the Role of the Mirror Neuron System. *Brain Res, 1629*, 135–142. http://dx.doi.org/10.1016/j.brainres.2015.10.025

Antal, A., Nitsche, M.A, Kruse, W., Kincses, T.Z., Hoffmann, K.P., Paulus, W., 2004. Direct current stimulation over V5 enhances visuomotor coordination by improving motion perception in humans. *J Cogn Neurosci.16,* 521-527.

Battaglia, F., Lisanby, S.H., Freedberg, D., 2011. Corticomotor Excitability during Observation and Imagination of a Work of Art. *Front Hum Neurosci, 5* , 79 http://dx.doi.org/10.3389/fnhum.2011.0007

Cicchetti, D. V., 1994. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. *Psych Assess. 6*, 284 -290 http://dx.doi.org/10.1037/1040-3590.6.4.284.

Concerto, C., Al Sawah, M., Infortuna, C., Freedberg, D., Chusid, E., Aguglia, E., Battaglia, F., 2015. Neural circuits underlying motor facilitation during observation of implied motion. *Somatosens Mot Res. 32,* 207-210. http://dx.doi.org/10.3109/08990220.2015

Cook, R., Bird, G., Catmur, C., Press, C., Heyes, C., 2014. Mirror neurons: From origin to function. *Behav Brain Sci*, 37, 177 - 192. doi:10.1017/S0140525X13000903

di Pellegrino, G., Fadiga, L., Fogassi, L., Gallese, V., Rizzolatti, G.,1992. Understanding motor events: a neurophysiological study. *Exp Brain Res, 91*, 76 - 180.

Dinstein, I., Hasson, U., Rubin, N., Heeger, D. J., 2007. Brain areas selective for both observed and executed movements. *J Neurophysiol*, *98*, 1415 - 1427.

Elfenbein, H., Davis, J., Brang, D., Agmon, E., Pineda, J. A., 2007. TMS of the inferior frontal gyrus inhibits Mu rhythm suppression and decreases performance on social cognition tasks. *Society for Neuroscience Abstract*. 748.17/FFF12, 2007.

Exner, J. E., Erdberg, P., 2005. *The Rorschach: Advanced Interpretation* (3rd ed.). Hoboken: Wiley.

Exner, J. E., 2003. The Rorschach: A Comprehensive System. The Rorschach foundations and principles of interpretation. 4th ed. vol.1. Wiley, New York

Fadiga, L., Fogassi, L., Pavesi, G., Rizzolatti, G., 1995. Motor facilitation during action observation: a magnetic stimulation study. *J Neurophysiol*, *73*, 2608 - 11.

Foltys, H., Sparing, R., Boroojerdi, B., Krings, T., Meister, I., Mottaghy, F., Töpper, R., 2001. Motor control in simple bimanual movements: a transcranial magnetic stimulation and reaction time study. *Clin Neurophysiol*. *112*, 265 - 274. http://dx.doi.org/10.1016/s1388-2457(00)00539-3

Gallese, V., 2009. Mirror Neurons, Embodied Simulation, and the Neural Basis of Social Identification. *Psychoanal Dialogues*, *19*, 519 - 536. http://dx.doi.org/10.1080/10481880903231910

Gallese, V., Fadiga, L., Fogassi, L., Rizzolatti, G., 1996. Action Recognition in Premotor Cortex. *Brain*, *119*, 593-609.

Gallese, V., Gernsbacher, M. A., Heyes, C., Hickok, G., Iacoboni, M., 2011. Mirror neuron forum. *Perspectives on Psychological Science*, *6*, 369 - 407. http://dx.doi.org/10.1177/1745691611413392

Gallese, V., Keysers, C., Rizzolatti, G., 2004. A unifying view of the basis of social cognition. *Trends Cogn Sci*, *8*, 396 - 403. http://dx.doi.org/10.1016/j.tics.2004.07.002

Giromini, L., Porcelli, P., Viglione, D.J., Parolin, L., Pineda, J. A., 2010. The feeling of movement: EEG evidence for mirroring activity during the observations of static, ambiguous stimuli in the Rorschach cards. *Biol Psychol*, *85*, 233 - 241. http://dx.doi.org/10.1016/j.biopsycho.2010.07.008 http://doi.org/10.1016/j.psychres.2016.09.036

Giromini, L., Viglione, D.J., Brusadelli, E., Zennaro, A., Di Girolamo, M., Porcelli, P., 2016. The Effects of Neurological Priming on the Rorschach: A Pilot Experiment on the Human Movement Response. *Rorschachiana*, *37*, 58-73. https://doi.org/10.1027/1192-5604/a000077

Giromini, L., Viglione, D.J, Pineda, J.A., Porcelli, P., Hubbard, D., Zennaro, A., Cauda, F. 2017. Human movement responses to the Rorschach and mirroring activity: An fMRI study. *Assessment*, *Advance Online Publication.* http://journals.sagepub.com/doi/10.1177/1073191117731813

Giromini, L., Viglione, D.J, Zennaro, A., Cauda, F. 2017. Neural activity during production of Rorschach responses: An fMRI study. *Psychiat Res*, *262*, 25 - 31. http://dx.doi.org/10.1016/j.pscychresns.2017.02.001.

Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L.G., Mall, V., Kaelin-Lang, A., H.R. Siebner., 2012. A practical guide to diagnostic transcranial magnetic stimulation: Report of an IFCN committee. *Clin Neurophysiol*, *123*, 858 - 882. http://dx.doi.org/10.1016/j.clinph.2012.01.010.

Hamilton, A.C., Martin, R.C., 2005. Dissociations among tasks involving inhibition: a single-case study. *Cogn Affect Behav Neurosci*. *5*, 1-13.

Hamilton, A.C, Martin, R.C., 2007. Proactive interference in a semantic short-term memory deficit: role of semantic and phonological relatedness. *Cortex*, *43*, 112-123.

Hansenne, M., Laloyaux, O., Mardaga, S., Ansseau, M., 2004. Impact of low frequency transcranial magnetic stimulation on event-related brain potentials. *Biol Psychol*, *67*, 331 - 341.
http://dx.doi.org/10.1016/j.biopsycho.2004.01.004

Hickok, G., 2009. The functional neuroanatomy of language. *Phys Life Rev*. *6*, 121-143.
http://dx.doi.org/10.1016/j.plrev.2009.06.001

Heyes, C., 2011. Automatic imitation. *Psychol Bull*. *137*, 463 - 483.
http://dx.doi.org/10.1037/a0022288

Hobson, H.M., Bishop, D. V. (2016). Mu suppression - A good measure of the human mirror neuron system? *Cortex*, *82*, 290 - 310.
http://dx.doi.org/10.1016/j.cortex.2016.03.019

Hong, K. S., Lee, S. K., Kim, J. Y., Kim, K. K., Nam, H., 2000. Visual working memory revealed by repetitive transcranial magnetic stimulation. *J Neurol Sci*. 181, 50-55.

Iacoboni, M. (2009). Neurobiology of imitation. *Current Opinion in Neurobiology*, *19*, 661 - 665.
http://dx.doi.org/10.1016/j.conb.2009.09.008

Kan, I.P., Thompson-Schill, S.L., 2004. Selection from perceptual and conceptual representations. *Cogn. Affect. Behav. Neurosci. 4*, 466–482.

Keuken, M., Hardie, A., Dorn, B., Dev, S., Paulus, M., Jonas, K., Van Den Wildenberg W.P.M., Pineda, J.A., 2011. The role of the left inferior frontal gyrus in social perception: An rTMS study. *Brain Res. 1383*, 196 - 205.

http://dx.doi.org/10.1016/j.brainres.2011.01.073

Klopfer, B.,Kelley, D.M., 1942. The Rorschach technique: A Manual for a projective method of personality diagnosis. Word Books, Yonkers on Hudson, NY.

Koch G, Oliveri M, Torriero S, Caltagirone C., 2003. Underestimation of time perception after repetitive transcranial magnetic stimulation. *Neurology*, 60, 1844–6.

Lingnau, A., Gesiericha, B., Caramazza, A., 2009. Asymmetric fMRI adaptation reveals no evidence for mirror neurons in humans. *PNAS, 106*, 9925–30. http://dx.doi.org/ 10.1073/pnas.0902262106

Meyer, G. J., Hilsenroth, M.J., Baxter, D., Exner, J., Fowler, J.C., Piers, C.C., Resnick J., 2002. An examination of interrater reliability for scoring the Rorschach Comprehensive System in eight data sets. *J Pers Assess. 78*, 219 - 274. http://dx.doi.org/10.1207/S15327752JPA7802_03

Meyer, G. J, Viglione, D.J., Mihura, J. L., Erard., R. E., Erdberg, P., 2011. *A manual for the Rorschach Performance Assessment System*. (1st ed.) OH: R-PAS, Toledo.

Mihura, J. L, Meyer, G. J, Dumitrascu, N., Bombel, G., 2012. The Validity of Individual Rorschach Variables: Systematic Reviews and Meta-Analyses of the Comprehensive System. *Psychol Bull. 139*, 548 - 605. http://dx.doi.org/10.1037/a0029406

Molenberghs, P., Cunnington, R., Mattingley, J., 2009. Is the mirror neuron system involved in imitation? A short review and meta-analysis. *Neurosci & Biobehav Rev*. *33*, 975-980. http://dx.doi.org/10.1016/j.neubiorev.2009.03.010

Molenberghs, P., Cunnington, R., Mattingley, J., 2012. Brain regions with mirror properties: A meta-analysis of 125 human fMRI studies. *Neurosci & Biobehav Rev*. *36*, 341 - 349. http://dx.doi.org/10.1016/j.neubiorev.2011.07.004

Mottaghy, F., Gangitano, M., Horkan, C., Chen, Y., Pascual-Leone, A., Schlaug, G., 2003. Repetitive TMS temporarily alters brain diffusion. *Neurology*, *60*, 1539 - 1541. http://dx.doi.org/10.1212/01.wnl.0000058903.15205.46

Molnar-Szakacs, I., Kaplan, J.T., Greenfield, P.M., Iacoboni, M., 2006. Observing Complex Action Sequences: The Role of the Fronto-Parietal Mirror Neuron System. *NeuroImage*, *15*, 923 - 35. http://dx.doi.org/10.1016/j.neuroimage.2006.07.035.

Mukamel, R., Ekstrom, A.D., Kaplan, J., Iacoboni, M., Fried, I., 2010. Single neuron responses in humans during execution and observation of actions. *Curr Biol*. 20, 750–756. http://dx.doi.org/10.1016/j.cub.2010.02.045

Nishitani, N., Hari, R., 2000.Temporal dynamics of cortical representation for action. *PNAS*, *97*, 913 - 18.

Nowak, D., Grefkes, C., Dafotakis, M., Eickhoff, S., Küst, J., Karbe, H., Fink, G., 2008. Effects of Low-Frequency Repetitive Transcranial Magnetic Stimulation of the Contralesional Primary Motor Cortex on Movement Kinematics and Neural Activity in Subcortical Stroke. *Arch Neurol.* *65*, 741 - 747. http://dx.doi.org/10.1001/archneur.65.6.741

Novick, J.M, Trueswell, J.C, Thompson-Schill, S.L., 2005, Cognitive control and parsing: reexamining the role of Broca's area in sentence comprehension. *Cogn Affect Behav Neurosci. 5*, 263-281.

Nozari, N, Thompson-Schill. S.L., 2013. More attention when speaking: Does it help or does it hurt? *Neuropsychologia*, *51*, 2770-2780. http://dx.doi.org/10.1016/j.neuropsychologia.2013.08.019

Nyffeler, T., Wurtz, P., Pflugshaupt, T., Wartburg, R., Luthi, M., Hess, C., Muri, R., 2006. One-Hertz transcranial magnetic stimulation over the frontal eye field induces lasting inhibition of saccade triggering. *Neuroreport*, *17*, 273 - 275. http://dx.doi.org/10.1097/01.wnr.0000199468.39659.bf

Oberman, L.M., Hubbard, E.M., McCleery, J.P., Altschuler, E.L., Ramachandran, V.S., Pineda, J. A., 2005. EEG evidence for mirror neuron dysfunction in autism spectrum disorders. *Cognitive Brain Res. 24*, 190 - 198.

Pascual-Leone, A., Hallett, M., 1994. Induction of errors in a delayed response task by repetitive transcranial magnetic stimulation of the dorsolateral prefrontal cortex. *Neuroreport*, 5, 2517-20.

Pignolo, C., Giromini, L., Ando', A., Ghirardello, D., Di Girolamo, M., Ales, F., Zennaro, A., 2017. An Interrater Reliability Study of Rorschach Performance Assessment System (R–PAS) Raw and Complexity-Adjusted Scores. *J Pers Assess*. 1 - 7. http://dx.doi.org/10.1080/00223891.2017.1296844

Pineda, J. A., 2005. The functional significance of mu rhythms: Translating “seeing” and “hearing” into “doing”. *Brain Res Rev*, *50*, 57 - 68. http://dx.doi.org/10.1016/j.brainresrev.2005.04.005

Pineda, J. A., Hecht, E., 2009. Mirroring and mu rhythm involvement in social cognition: Are there dissociable subcomponents of theory of mind? *Biol Psychol*. *80*, 306 - 314. http://dx.doi.org/10.1016/j.biopsycho.2008.11.003

Pineda, J. A., Giromini, L., Porcelli, P., Parolin, L., Viglione, D. J, 2011. Mu suppression and human movement responses to the Rorschach test. *Neuroreport*, *22*, 223 - 226. http://dx.doi.org/10.1097/wnr.0b013e328344f45c

Piotrowski, Z., 1957. *Perceptanalysis* (1st ed.). New York: MacMillan.

Pisoni, A., Cerciello, M., Cattaneo, Z., Papagno, C., 2017. Phonological facilitation in picture naming: When and where? A tDCS study. *Neuroscience*, 352, 106-121. http://dx.doi.org/10.1016/j.neuroscience.2017.03.043.

Pitcher, D., Garrido, L., Walsh, V., Duchaine, B.C., 2008. Transcranial Magnetic Stimulation Disrupts the Perception and Embodiment of Facial Expressions. *J Neurosci*. *28*, 8929-8933.
https://doi.org/10.1523/JNEUROSCI.1450-08.2008

Pobric, G., Hamilton, A. F., 2006. Action understanding requires the left inferior frontal cortex. *Curr Bio*, *16*, 524 - 529.
http://dx.doi.org/10.1016/j.cub.2006.01.033

Porcelli, P., Giromini, L., Parolin, L., Pineda, J. A., Viglione, D. J., 2013. Mirroring Activity in the Brain and Movement Determinant in the Rorschach Test. *J Pers Assess*, *95*, 444 - 456.
http://dx.doi.org/10.1080/00223891.2013.775136

Proverbio, A.M., Riva, F., Zani, A., 2009. Observation of Static Pictures of Dynamic Actions Enhances the Activity of Movement-Related Brain Areas. *Plos One*, *4*, e5389.
http://dx.doi.org/10.1371/journal.pone.0005389

Rizzolatti, G., Craighero L., 2004. The mirror-neuron system. *Annu Rev Neurosci*, *27*, 169 - 92.
http://dx.doi.org/10.1146/annurev.neuro.27.070203.144230

Rizzolatti, G., Fadiga, L., Gallese, V., Fogassi, L., 1996. Premotor cortex and recognition of motor action. *Cogn Brain Res*, *3*, 131 - 141.

Rizzolatti, G., Fogassi, L., Gallese, V., 2001. Neurophysiological mechanisms underlying the understanding and imitation of action. *Nat Rev Neurosci*. *2*, 661-670.
http://dx.doi.org/10.1038/35090060

Rorschach, H., 1921. *Psychodiagnostik* (1st ed.). Bern: Bircher.

Rossi, S., Hallett, M., Rossini, P., Pascual-Leone, A., 2009. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol*. *120*, 2008-2039. http://dx.doi.org/10.1016/j.clinph.2009.08.016

Rossini, P.M., Barker, A.T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., Tomberg, C., 1994. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroen clin neuro. 91*, 79 - 92.

Rossini, P.M, Burke, D., Cohen, L.G., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V., Ziemann., 2015. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clinical Neurophysiology, 6*, 1071 - 1107. http://dx.doi.org/10.1016/j.clinph.2015.02.001

Salatino, A., Momo, E., Nobili, M., Berti, A., Ricci, R., 2014. Awareness of symptoms amelioration following low-frequency repetitive transcranial magnetic stimulation in a patient with Tourette syndrome and comorbid obsessive-compulsive disorder. *Brain Stim.* 7, 341 - 3. http://dx.doi.org/10.1016/j.brs.2014.01.002.

Strafella, A., Paus, T., 2000. Modulation of cortical excitability during action observation: a transcranial magnetic stimulation study. *Neuroreport, 11*, 2289 - 92.

Sbriscia - Fioretti, B., Berchio, C., Freedberg, D., Gallese, V., Umiltà, M. A, 2013. ERP Modulation during Observation of Abstract Paintings by Franz Kline. *Plos One, 8* http://dx.doi.org/10.1371/journal.pone.0075241.

Tarhan, L.Y., Watson, C. E., Buxbaum, L.J, 2015. Shared and Distinct Neuroanatomic Regions Critical for Tool-related Action Production and Recognition: Evidence from 131 Left-hemisphere Stroke Patients. . Shared and Distinct Neuroanatomic Regions Critical for Tool-related Action Production and Recognition: Evidence from 131 Left-hemisphere Stroke Patients. *J Cogn Neurosci. 27*, 2491-2511. http://dx.doi.org/10.1162/jocn_a_00876

Yao, D., Wang, L., Arendt-Nielsen, L., Chen, A. C., 2007. The effect of reference choices on the spatio-temporal analysis of brain evoked potentials: the use of infinite reference. *Comput Biol Med. 37*, 1529 - 1538. http://dx.doi.org/10.1016/j.compbiomed.2007.02.002

Viglione, D. J., Blume-Marcovici, A. C, Miller, H. L., Giromini, L., Meyer, G. J., 2012. An Inter-Rater Reliability Study for the Rorschach Performance Assessment System. *J Pers Assess. 94*, 607 - 612. http://dx.doi.org/10.1080/00223891.2012.684118

Viglione, D. J., Taylor, N., 2003. Empirical Support for interrater reliability of Rorschach Comprehensive System coding. J *Clin Psychol*. *59*, 111 - 121. http://dx.doi.org/10.1002/jclp.10121

Table 1. Human Movement (M) responses by Group and Condition: Descriptive Statistics

	Control Group (Vertex)	Experimental Group (LIFG)
Baseline		
Range	2 – 5	2 – 5
M	3.61	3.60
Median	3.00	4.00
S.D.	1.04	.98
rTMS		
Range	2 – 6	0 – 5
M	3.76	1.80
Median	4.00	1.00
S.D.	1.42	1.47

Note. Descriptive statistics are reported for the number of human movement responses (M codes) produced by the participants included in the control and the experimental groups, at baseline and after repetitive transcranial magnetic stimulation (rTMS).

Table 2. Mu suppression by Group and Condition: Descriptive Statistics

	Control Group (Vertex)	Experimental Group (LIFG)
Baseline		
Range	-1.46 – 1.23	-.82 – 1.07
M	-.20	-.15
Median	-.27	-.20
S.D.	.83	.53
rTMS		
Range	-1.66 – 3.04	-1.48 – 1.62
M	.03	.01
Median	-25	.02
S.D.	1.13	.75

Note. Descriptive statistics are reported for Mu suppression in the control and the experimental groups, at baseline condition and after repetitive transcranial magnetic stimulation (rTMS).

Table 3. Demographic composition of control and experimental groups

	Control Group (Vertex)	Experimental Group (LIFG)
Gender (*phi* = .11 *p* =. 55)		
M	*n* = 3 (*76.90%*)	*n* = 5 (*33.3%)*
F	*n* = 10 (*23.10%*)	*n* = 10 (*66.7%)*
Race		
Caucasian	*n = 7 (53.80%)*	*n = 10 (66,70%)*
Other	*n* = 6 *(46.20 %)*	*n = 5(33,3%)*
Age *t* (26) = -.480, *p* = .64)		
Range	18 – 28	18 – 31
M	20.23	20.80
SD	2.80	3.38
Years of Education *t* (26) = -.184, *p* =.85)		
Range	12 – 21	12 – 18
M	14.38	14.53
SD	2.40	1.89

LIFG = Left Inferior Frontal Gyrus.

Figure Captions

Figure 1. M attributions to the Rorschach provided by the control and experimental groups, at baseline and after inhibitory repetitive transcranial stimulation (rTMS).

Disrupting the left inferior frontal gyrus (LIFG), but not Vertex, decreased the number of M responses in the ambiguous and static Rorschach stimuli.

Figure 2. Mu suppression values.

Mu suppression in the control and experimental groups, at baseline and after inhibitory repetitive transcranial stimulation (rTMS).

Figure 1

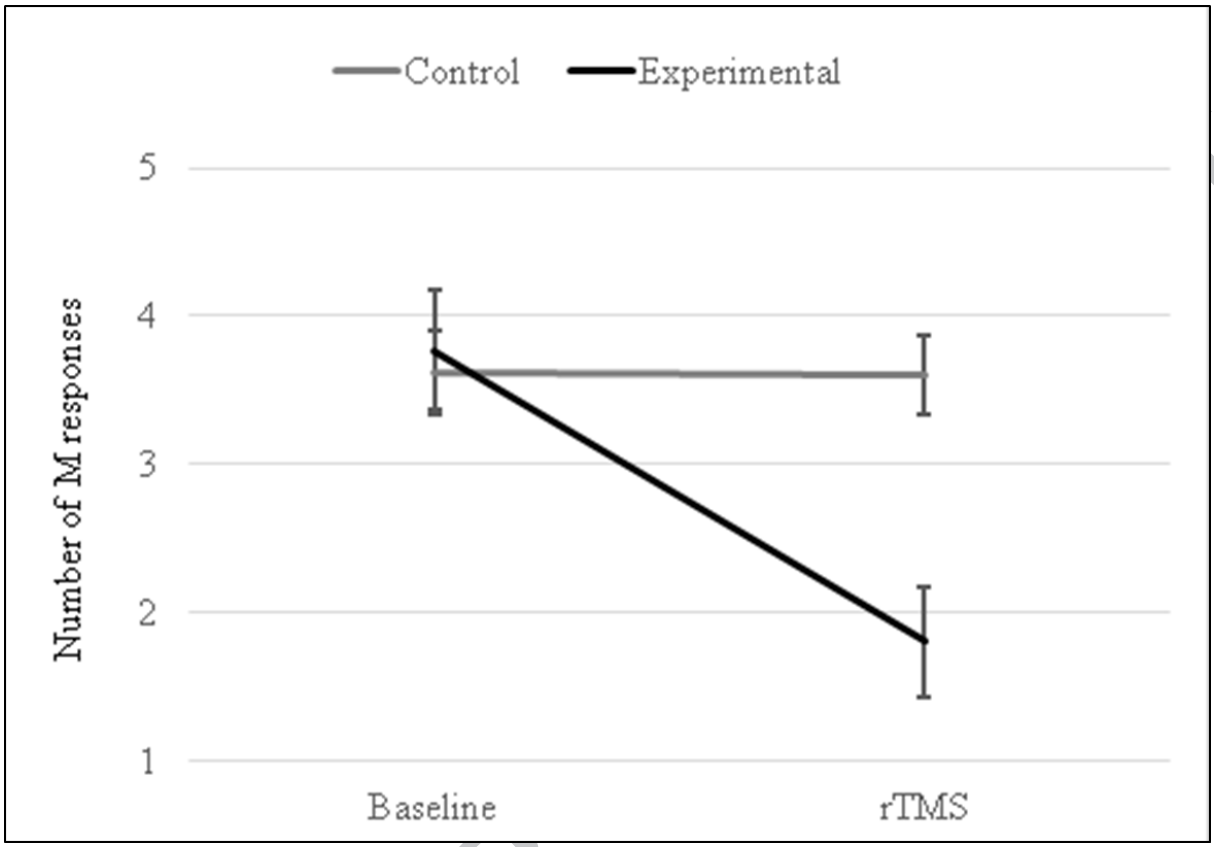
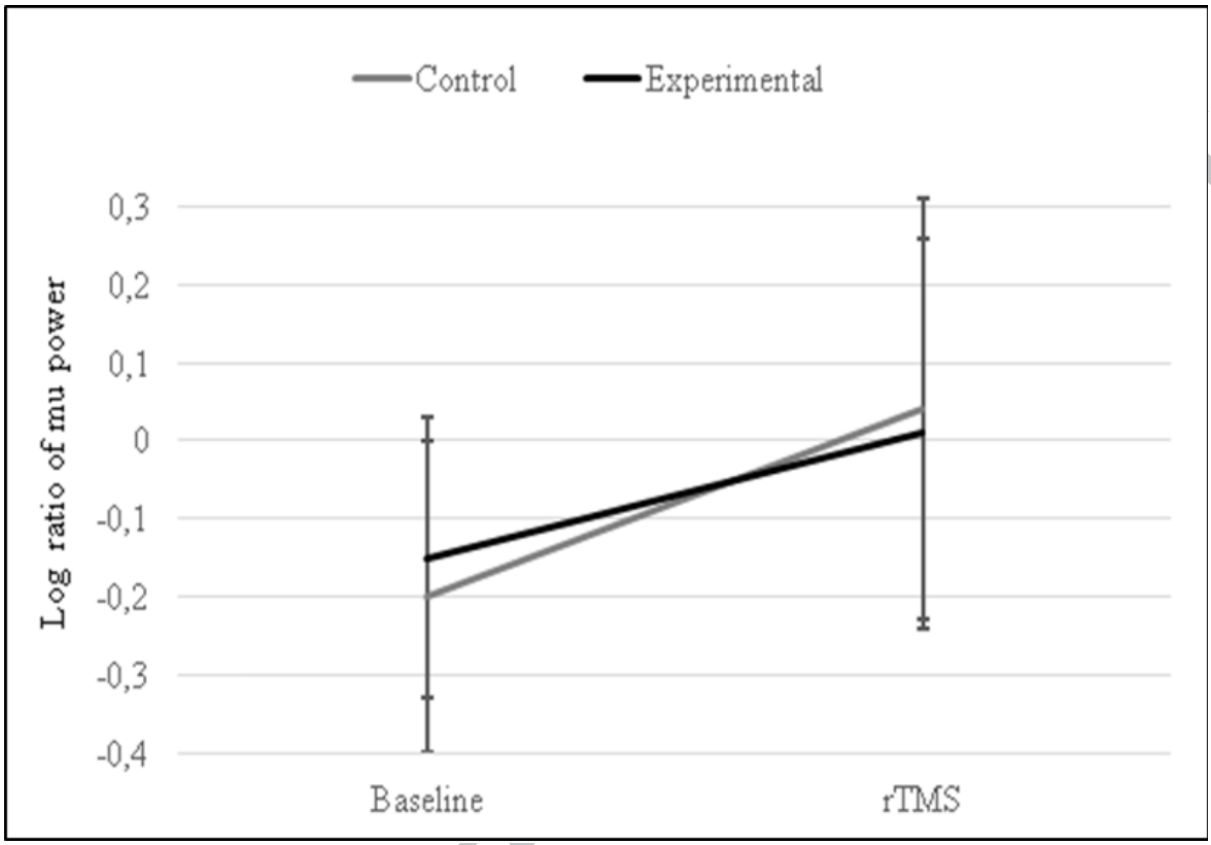


Figure 2



HIGHLIGHTS

We tested the effects of rTMS over the LIFG on Rorschach responses and EEG mu suppression.

Disrupting the LIFG decreased significantly the attribution of human movement to the Rorschach stimuli.

rTMS did not significantly influence EEG mu suppression.

Table 1. Human Movement (M) responses by Group and Condition: Descriptive Statistics

	Control Group (Vertex)	Experimental Group (LIFG)
Baseline		
Range	2 – 5	2 – 5
M	3.61	3.60
Median	3.00	4.00
S.D.	1.04	.98
rTMS		
Range	2 – 6	0 – 5
M	3.76	1.80
Median	4.00	1.00
S.D.	1.42	1.47

Note. Descriptive statistics are reported for the number of human movement responses (M codes) produced by the participants included in the control and the experimental groups, at baseline and after repetitive transcranial magnetic stimulation (rTMS).

Table 2. Mu suppression by Group and Condition: Descriptive Statistics

	Control Group (Vertex)	Experimental Group (LIFG)
Baseline		
Range	-1.46 – 1.23	-.82 – 1.07
M	-.20	-.15
Median	-.27	-.20
S.D.	.83	.53
rTMS		
Range	-1.66 – 3.04	-1.48 – 1.62
M	.03	.01
Median	-25	.02
S.D.	1.13	.75

Note. Descriptive statistics are reported for Mu suppression in the control and the experimental groups, at baseline condition and after repetitive transcranial magnetic stimulation (rTMS).

Table 3. Demographic composition of control and experimental groups

	Control Group (Vertex)	Experimental Group (LIFG)
Gender (*phi* = .11 *p* =. 55)		
M	*n* = 3 (*76.90%*)	*n* = 5 (*33.3%)*
F	*n* = 10 (*23.10%*)	*n* = 10 (*66.7%)*
Race		
Caucasian	*n = 7 (53.80%)*	*n = 10 (66,70%)*
Other	*n* = 6 *(46.20 %)*	*n = 5(33,3%)*
Age *t* (26) = -.480, *p* = .64)		
Range	18 – 28	18 – 31
M	20.23	20.80
SD	2.80	3.38
Years of Education *t* (26) = -.184, *p* =.85)		
Range	12 – 21	12 – 18
M	14.38	14.53
SD	2.40	1.89

LIFG = Left Inferior Frontal Gyrus.