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Research Report

Primary motor cortex crucial for action prediction: A tDCS study

Riccardo Paracampo [a,b], Mirella Montemurro [a], Manuel de Vega [c] and Alessio Avenanti [a,b,*]

[a] Fondazione Santa Lucia, IRCCS, Rome, Italy
[b] Dipartimento di Psicologia and Centro studi e ricerche in Neuroscienze Cognitive, Università di Bologna, Campus di Cesena, Cesena, Italy
[c] Departamento de Psicología Cognitiva, Universidad de La Laguna, La Laguna, Tenerife, Spain

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ABSTRACT

The neural network underlying action observation – i.e., the action observation network – forms an anticipatory representation of observed actions. Although correlational studies suggest that the motor cortex (M1) might be involved in this anticipatory coding, it is unclear whether M1 is also causally essential for making accurate predictions about observed actions. To test the functional relevance of M1 to action prediction, we used offline monopolar transcranial direct current stimulation (tDCS). In four tDCS groups of healthy participants, we administered 15 min of anodal or cathodal constant currents of 1 or 2 mA over the left M1 before participants performed two tasks requiring them to make predictions about the outcomes of reaching-grasping human actions (Action Prediction – AP) or non-human movements (Non-human Prediction – NP). In each group, participants received sham and active tDCS in two separate sessions. We found that 2 mA cathodal tDCS (c-tDCS$_{2mA}$) selectively impaired accuracy in the AP task, but not in the NP task. No change in performance was found following anodal or 1-mA tDCS protocols. Additionally, no change was found following c-tDCS$_{2mA}$ administered over a control site. These findings show task-, polarity-, intensity- and site-specific disruption of AP abilities following c-tDCS$_{2mA}$ over M1. Thus, our study establishes specific tDCS parameters for effective M1 stimulation in AP and highlights the functional relevance of the motor system to making accurate predictions about the outcomes of human actions.

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* *Corresponding author*. Department of Psychology and Center for Studies and Research in Cognitive Neuroscience, University of Bologna, Cesena Campus, Viale Europa 980, 47521 Cesena, Italy.
E-mail address: alessio.avenanti@unibo.it (A. Avenanti).

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1. Introduction

Seeing the actions of others activates an action observation network (AON). The AON encompasses high-order visual regions encoding biological motion, i.e., the superior temporal sulcus (STS) (Jellema & Perrett, 2003; Keysers & Perrett, 2004; Perrett, Xiao, Barraclough, Keysers, & Oram, 2009), and parieto-frontal regions involved in controlling and sensing body actions (Caspers, Zilles, Laird, & Eickhoff, 2010; Gazzola & Keysers, 2009; Grafton, 2009; Rizzolatti, Cattaneo, Fabbri-Destro, & Rozzi, 2014; Urgesi, Candidi, & Avenanti, 2014; Valchev, Gazzola, Avenanti, & Keysers, 2016; van Overwalle & Baetens, 2009). Premotor and parietal regions have been classically considered key nodes of the AON, as they implement a mirror mechanism coupling action perception with execution (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Fogassi et al., 2005; Gallese, Fadiga, Fogassi, & Rizzolatti, 1996; Rizzolatti & Sinigaglia, 2010; Bonini, 2017). Moreover, causal evidence indicates that both transient stimulation and stable lesions of premotor and parietal regions affect action recognition in humans (Avenanti, Candidi, & Urgesi, 2013b; Avenanti & Urgesi, 2011; Cattaneo, 2010; Cattaneo, Sandrini, & Schwarzbach, 2010; Fazio et al., 2009; Jacquet & Avenanti, 2015; Michael et al., 2014; Moro et al., 2008; Paracampo, Pirruccio, Costa, Borgomaneri, & Avenanti, 2018, Paracampo, Tidoni, Borgomaneri, di Pellegrino, & Avenanti, 2017; Pobric & Hamilton, 2006; Tidoni, Borgomaneri, di Pellegrino, & Avenanti, 2013; Urgesi et al., 2014). Mounting evidence suggests that the primary motor cortex (M1) might also implement a mirror mechanism (Dushanova & Donoghue, 2010; Tkach, Reimer, & Hatsopoulos, 2007; Vigneswaran, Philipp, Lemon, & Kraskov, 2013). However, M1 is not classically considered a key node of the AON (Caspers et al., 2010; Keysers & Gazzola, 2009). Whether M1 is causally essential for perceiving the actions of others remains unclear, as previous studies using causal methods have provided mixed results (Avenanti, Bolognini, Maravita, & Aglioti, 2007; Borgomaneri, Gazzola, & Avenanti, 2015; Cattaneo, 2010; Naish, Barnes, & Obhi, 2016; Palmer, Bunday, Davare, & Kilner, 2016; Valchev, Tidoni, Hamilton, Gazzola, & Avenanti, 2017; Borgomaneri, Vitale, & Avenanti, 2017).

Previous studies have reported that online transcranial magnetic stimulation (TMS) administered over M1 at a supra-threshold intensity (i.e., >100% of the threshold for evoking visible movements or motor-evoked potentials – MEPs) during action observation disrupted effector recognition (Naish et al., 2016) and body posture recognition (Borgomaneri et al., 2015). On the other hand, online/offline TMS administered at sub-threshold or near-threshold stimulation intensities did not consistently affect processing of others' actions (Avenanti et al., 2007; Cattaneo, 2010; Palmer et al., 2016; Valchev et al., 2017). In particular, two recent studies used sub-threshold offline continuous theta burst stimulation (cTBS) (Palmer et al., 2016; Valchev et al., 2017) that avoids nonspecific, distracting effects of online supra-threshold TMS. Both studies reported variable behavioral responses following cTBS over M1, with no net changes in action perception. In particular, Palmer et al. (2016) showed that cTBS induced highly variable physiological responses across participants, leading to inhibition of M1 excitability in some participants and an increase in M1 excitability in others. Remarkably, the subsample of participants showing M1 inhibition also showed hindered action perception performance. In contrast, participants showing M1 facilitation did not show any change in action perception (Palmer et al., 2016), thus pointing to a specific relationship between M1 inhibition and disruption of action perception. In sum, while some studies have shown that M1 might be critical for processing some features of observed actions, the heterogeneous pattern of results reported in the literature might depend on at least two factors: the intensity of stimulation and its capability to induce inhibition in M1.

While these studies have focused on action recognition, another key function of the AON is processing observed actions in order to make predictions about their outcomes. Theoretical models suggest that the motor system acts as an anticipation device that humans can use to generate internal forward models when perceiving the action of others (Blakemore & Decety, 2001; Friston, Mattout, & Kilner, 2011; Grush, 2004; Kilner, Friston, & Frith, 2007; Prinz, 1997, 2006; Schütz-Bosbach & Prinz, 2007; Wilson & Knoblich, 2005; Wolpert, Doya, & Kawato, 2003). There is substantial correlational evidence indicating that the motor nodes of the AON form an anticipatory representation of observed actions and M1 activity reflects such anticipatory encoding (Abreu et al., 2012; Avenanti, Annella, Candidi, Urgesi, & Aglioti, 2013a; Balser et al., 2014; Kilner, Vargas, Duval, Blakemore, & Sirigu, 2004; Maranesi, Livi, Fogassi, Rizzolatti, & Bonini, 2014; Sebanz, Bekkering, & Knoblich, 2006; Urgesi et al., 2010). Moreover, TMS perturbation of M1 affected the execution of predictive eye movements during action observation (Elsner, D'Ausilio, Gredebäck, Falck-Ytter, & Fadiga, 2013). However, whether M1 is also causally essential for making explicit predictions about others' actions remains unclear, and answering this question is the main goal of the present study.

Recently, brain stimulation studies have provided causal evidence that targeting frontal premotor regions of the AON affects action prediction abilities (Avenanti, Paracampo, Annella, Tidoni, & Aglioti, 2018; Makris & Urgesi, 2015; Stadler et al., 2012). In particular, in a previous study, we administered monopolar transcranial direct current stimulation (tDCS) over the left inferior frontal cortex (IFC, in a position at the border between the ventral premotor cortex and the pars opercularis of the inferior frontal gyrus) to test its role in action prediction. Participants were tested in an Action Prediction (AP) task which required them to observe the initial phases of a reaching-to-grasp action and to predict its outcome (i.e., which of two objects would be grasped), which was blocked from view. Participants' AP performance was disrupted following 15 min of offline cathodal currents at 2 mA (c-$tDCS_{2mA}$). No disruptive effects were observed in a difficulty-matched control task requiring prediction of the outcome of a non-human movement (Non-human Prediction –NP) or following c-$tDCS_{2mA}$ over other visual (i.e., left STS) or motor nodes of the AON. On the other hand, targeting the left IFC with anodal currents (a-$tDCS_{2mA}$) enhanced AP task performance. These findings show that a classical frontal node of the AON – the left IFC – is critical for making predictions about human actions (Avenanti et al., 2018).

Here, we build on previous studies to address the outstanding question of whether M1 also plays a critical role in action prediction. In four groups of healthy participants (total N = 48), we targeted the left M1 with tDCS and investigated the stimulation parameters optimal for altering action prediction abilities. We used an extracephalic reference over the contralateral deltoid to compare our results with those of our previous study using the same montage (Avenanti et al., 2018), and because of modeling studies suggesting that this montage induces fewer diffused currents over bilateral premotor/prefrontal areas relative to the classical bicephalic (M1-contralateral supra-orbital) montage (Im, Park, Shim, Chang, & Kim, 2012; Mehta, Pogosyan, Brown, & Brittain, 2015; Noetscher, Yanamadala, Makarov, & Pascual-Leone, 2014). Moreover, the M1-contralateral shoulder montage is thought to induce higher current densities deeper in the central sulcus, with a higher vertical current density over the targeted M1 (Im et al., 2012; Mehta et al., 2015; Noetscher et al., 2014). Although the effects appear more focused over the targeted M1 region, physiological evidence suggests that an extracephalic montage requires greater stimulation intensities to achieve the effects induced by the classical bicephalic montage on M1 excitability (e.g., Moliadze, Antal, & Paulus, 2010). Because optimal tDCS parameters for altering M1 functioning in AP have not been established, in this study, we used different polarities (c-tDCS and a-tDCS) and intensities (1 mA and 2 mA) of stimulation, following a 2 × 2 between-subject design. Moreover, for each group, we implemented a 2 × 2 within-subject design: we assessed participants' abilities to make predictions about the future outcomes of human actions or non-human motion trajectories (i.e., using the AP and NP tasks from Avenanti et al., 2018). In two different counterbalanced sessions, performance in both tasks was assessed following active tDCS or sham tDCS, which provided a baseline for behavioral performance.

If M1 is functionally relevant to prediction of human actions, we expect that altering neural functioning with active tDCS over M1 would disrupt performance in the AP task but not in the control NP task. By using different polarities and intensities, we investigated the optimal stimulation parameters for altering M1 functioning in AP. It should be noted that the relationships between physiological and behavioral effects of tDCS are not fully understood (e.g., Bestmann, de Berker, & Bonaiuto, 2015; Bestmann & Walsh, 2017; Miniussi, Harris, & Ruzzoli, 2013). Yet, based on prior brain stimulation studies testing the effect of M1 perturbation on action perception (Avenanti et al., 2007; Borgomaneri et al., 2015; Cattaneo, 2010; Naish et al., 2016; Palmer et al., 2016; Valchev et al., 2017), one could expect that a greater intensity and/or inhibitory efficacy of M1 neurostimulation would increase the chance of disrupting action observation processing. We thus hypothesized that c-tDCS$_{2mA}$ would be particularly effective in hindering AP task performance. Indeed, cathodal currents over the M1 likely reduce M1 excitability, as evidenced by a reduction of TMS-induced MEPs reported in previous studies (Horvath et al., 2015; Nitsche and Paulus 2011; Nitsche et al., 2008; Stagg, Antal, & Nitsche, 2018). Moreover, taking into account our tDCS montage (see Materials and Methods), one might predict that a 2-mA current intensity would be more effective than a 1-mA current intensity. Confirming our hypotheses, we found that c-tDCS$_{2mA}$ disrupted performance in the AP (but not the NP) task. Performance disruption was not only task-specific, but also intensity- and polarity-specific, as only c-tDCS$_{2mA}$ reliably affected AP performance. We thus provided evidence for a critical role of M1 in action prediction and established optimal stimulation parameters to alter this function. By comparing the present results with those of Avenanti et al. (2018), we also provide evidence for site-specificity of M1 c-tDCS$_{2mA}$ aftereffects on action prediction.

2. Methods

2.1. Participants

Forty-eight healthy volunteers took part in the study. Participants were randomly assigned to one of four groups. Twelve participants were assigned to the 'c-tDCS$_{2mA}$' group testing the effect of c-tDCS at an intensity of 2 mA (6 females, mean age ± SD: 25.1 ± 3.34 years, range 21–30); 12 were assigned to the 'a-tDCS$_{2mA}$' group testing the effect of a-tDCS at an intensity of 2 mA (7 females, mean age 25.6 ± 3.12 years, range 21–30); 12 were assigned to the 'c-tDCS$_{1mA}$' group testing the effect of c-tDCS at an intensity of 1 mA (7 females, mean age 22.9 ± 1.7 years, range 20–25); and, 12 to the 'a-tDCS$_{1mA}$' group testing the effect of a-tDCS at an intensity of 1 mA (6 females, mean age 22.3 ± 1.7 years, range 20–25). All subjects were right-handed according to a standard handedness inventory (Briggs & Nebes, 1975) and had normal or corrected-to-normal vision. None had a history of neurological, psychiatric illness, or any contraindication to brain stimulation (Rossi, Hallett, Rossini, & Pascual-Leone, 2009, Rossi, Hallett, Rossini, & Pascual-Leone, 2011), and no participant was on medication at the time of the experiment. Participants provided written informed consent. The procedures were approved by the local ethics committee and were in accordance with the ethical standards of the 1964 Declaration of Helsinki. No part of the study procedures was pre-registered prior to the research being conducted.

No adverse effects were reported or noticed during or after tDCS. A mild tingling sensation on the head was reported by some participants at the beginning of tDCS, but it was well tolerated and comparable across sessions and groups (see discomfort data below).

Sample size was determined though a power analysis conducted using G*Power 3 (Faul, Erdfelder, Lang, & Buchner, 2007), with power $(1 - \beta) = .80$ and $\alpha = .05$, two-tailed. We expected a large effect size based on our previous study showing strong modulation of AP task performance due to active a-tDCS$_{2mA}$ and c-tDCS$_{2mA}$ over the IFC (mean Cohen's d = .96; Avenanti et al., 2018). The analysis yielded required sample sizes of 11 participants. We thus decided to have 12 participants in each group.

2.2. General design

We conducted a double-blind, sham-controlled tDCS study testing the role of M1 in predicting the outcomes of observed movements. In 4 parallel experiments run on separate groups of participants, we administered monopolar c-tDCS$_{2mA}$, a-tDCS$_{2mA}$, c-tDCS$_{1mA}$ or a-tDCS$_{1mA}$ to a scalp position overlying

the left M1 (Fig. 1). To avoid learning effects associated with repeating the same tasks across multiple sessions, we manipulated tDCS polarity and intensity in different groups of participants. Both the participant and the experimenter who collected the behavioral data were blind to the specific tDCS manipulation. In each tDCS group, participants performed AP and NP tasks (Fig. 2A) in 2 separate sessions that were carried out immediately after 15 min of either active (cathodal or anodal) or sham tDCS over the target region. The order of the sessions was counterbalanced across participants, and the 2 sessions were separated by 7 ± 3 days (Fig. 2B).

The results of the study showed a selective reduction in AP task performance following c-tDCS$_{2mA}$, but no change following the other tDCS protocols. To test the neuroanatomical specificity of c-tDCS$_{2mA}$ over M1, we compared the results of the c-tDCS$_{2mA}$ group in the present study with data from a published study from our laboratory (Avenanti et al., 2018). That study tested the effect of the same c-tDCS$_{2mA}$ monopolar protocol administered over the IFC and the STS, using the same behavioral tasks that we used here (Figs. 1 and 2).

2.3. *Tasks and stimuli*

In the Action Prediction (AP) task, participants observed 100 video-clips (640 × 480 pixels, 30 fps) depicting the initial phase of a reaching-grasping action. All stimuli subtended a 22.3° × 33.4° visual angle from the participant's viewing position. The videos started by showing two objects (left side of the screen) placed in front of a still right hand (right side of the screen; Fig. 2A). After a variable delay (1000–2200 msec), the hand started to reach towards and grasp one of the two objects. The final phases of the action were hidden from sight, and subjects had to guess which object was going to be grasped by the hand. Only 30–70% of the entire movement duration was shown, followed by a random-dot mask (150-msec duration) interrupting the video. Then a response screen showing the two objects lasted until a response was given. Participants provided their answer using two computer keys.

Video-clips in the AP task included 8 non-professional actors (4 females) reaching towards and grasping 8 different pairs of objects (i.e., lighter vs. glass; highlighter vs. corkscrew; deodorant spray vs. coffeepot; mug vs. book; clothespin vs. nutcracker; scoop vs. cup; little ball vs. soccer ball; fork vs. stapler; Fig. 2A). The two objects of each pair were close to each other and presented distinct affordances, thus implying slightly different hand trajectories and grips (i.e., from a power grip performed with the whole hand to precision grips performed with the index finger and the thumb). The percentage of the entire movement shown varied between trials (from 30% to 70%). The hand–object interaction was not visible in any of the videos. Thus, the AP task required processing of contextual (object location and affordance) and kinematic cues (hand trajectory and finger pre-shaping) during the initial reaching component of the action.

In the NP control task, subjects observed 100 similarly interrupted video-clips showing a non-biological geometrical shape approaching one of two targets (Fig. 2A). Participants had to guess which target was going to be approached by the geometrical shape. The NP videos (640 × 480 pixels, 30 fps) were animations created with Adobe Flash Professional software to roughly match key temporal and spatial features of the AP stimuli. Similarly to the AP task, NP stimuli showed incomplete movements (30–70%) of a geometrical shape which moved from the right side of the screen in order to reach and fit around with one of two different geometrical targets located on the opposite side of the screen. The "reaching" motion in the NP videos was designed to match the reaching motion in the AP videos (i.e., across frames, the position of the shape in a given NP video matched that of the hand in the source AP video). Then, the resulting movements were smoothed in order to get a more linear path trajectory. As in the AP task, the two targets were located in different spatial positions and presented different geometrical properties. Similarly to the pre-shaping of the fingers in the AP task, the configuration of the moving geometrical shape changed over time during the reaching phase in order to optimally fit with one of the two targets. For the NP video clips, we created eight different pairs of geometrical targets and eight moving geometrical shapes. A random-dot image was used for masking (Fig. 2A).

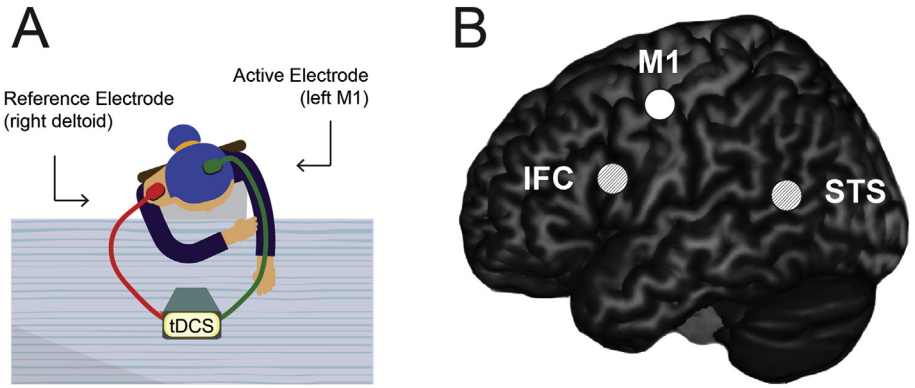

Fig. 1 – (A) tDCS montage showing the positions of the active and reference electrodes. (B) Stimulation sites for M1 (present study), IFC and STS (from Avenanti et al., 2018) reconstructed on a standard template using MRIcron (http://www.mccauslandcenter.sc.edu/mricro/mricron/). Mean surface MNI coordinates ± s.e.m. for the left M1 site (average of the 4 tDCS groups) were: x = −53.2 ± .9; y = −8.3 ± 1.0; z = 48.0 ± 1.2. Coordinates for the left IFC were: x = −53.6 ± .5; y = 10.0 ± .3; z = 24.0 ± .2. Coordinates for the left STS were: x = −55.1 ± .5; y = −53.6 ± .2; z = 9.3 ± .3.

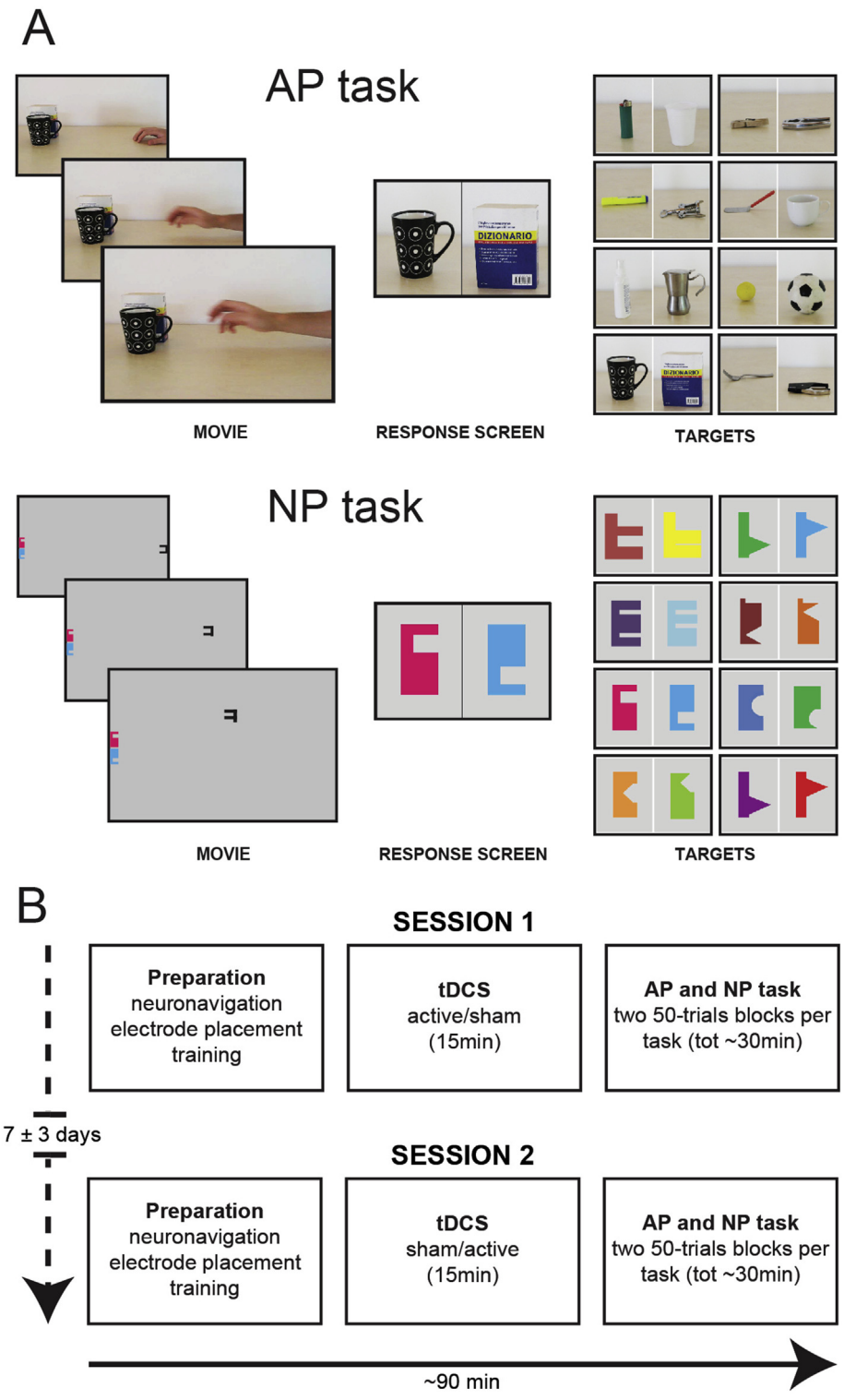

Fig. 2 – (A) Example trial and stimuli. Examples of a movie, response screen and targets in the Action Prediction (AP) task (above) and the Non-human Prediction (NP) task (below). On each trial, a short movie showed the initial movement of a hand (AP) or a geometrical form (NP) reaching towards and adapting to one of two targets. Participants were then presented with the two targets and had to guess which was selected by the hand/form. (B) Schematic representation of the experimental design. Participants took part in 2 sessions in which performance in the AP and NP tasks was tested immediately after 15 min of sham/active tDCS over a target brain region.

The two tasks were taken from our previous study (Avenanti et al., 2018) and were designed to be equally difficult (~75% accuracy, i.e., they were doable but not trivial) based on a series of three pilot studies (reported in Avenanti et al., 2018). In those pilot studies, participants were exposed to different movies showing 30–80% of the entire movement. We selected only stimuli that were recognized with ~75% accuracy (range: 65–85%). In the final sample, in both tasks, the hand/shape reached both objects/targets with 50% probability. The percentages of the total hand/shape movement shown in the two tasks were matched (AP: mean 45% of total movement, range 30–70%; NP: mean 45% of total movement, range 30–70%; $p > .99$).

2.4. *tDCS parameters*

tDCS was delivered using a battery-driven Eldith constant direct current stimulator (neuroConn GmbH, Ilmenau, Germany). A pair of surface sponge electrodes were soaked with a standard saline solution (NaCl .9%) and held in place by elastic rubber bands. To target M1 in all the tDCS groups, the active electrode (5 × 5 cm) was placed over the C3 electrode position of the 10–20 system. The reference electrode (5 × 7 cm) was placed over the contralateral deltoid muscle (Bolognini, Olgiati, Rossetti, & Maravita, 2010; Priori et al., 2008) (Fig. 1A). It is thought that extracephalic electrode montages avoid the confounding effect from the reference electrode (Brunoni et al., 2011; Cogiamanian, Marceglia, Ardolino, Barbieri, & Priori, 2007) and, as reported in the introduction, modeling studies confirm that the M1-contralateral shoulder montage results in a more focused involvement of M1, compared to the bicephalic (M1-contralateral supra-orbital) montage (Im et al., 2012; Mehta et al., 2015; Noetscher et al., 2014).

Active tDCS was delivered with a constant current of 2 mA (current density: ~.08 mA/cm^2) or 1 mA intensity (current density: ~.04 mA/cm^2), complying with current safety recommendations (Nitsche et al., 2008; Poreisz, Boros, Antal, & Paulus, 2007). Stimulation lasted for 15 min, not including 20 sec of ramp-up and ramp-down at the beginning and end of stimulation, respectively. Impedance was constantly monitored and kept below 5 kΩ.

For sham stimulation, the electrodes were placed on the same locations, but the current was turned on for only 30 sec at the beginning of the sham session and was then turned off in a ramp-shaped fashion (fade in/out: 20 sec). This was done so that participants experienced the sensations initially associated with the onset of stimulation (mild local tingling), without inducing any effective modulation of cortical excitability. This procedure ensured successful blinding of participants in previous research (Ambrus et al., 2012; Gandiga, Hummel, & Cohen, 2006). Although a previous study found inadequate blinding for tDCS at 2 mA (O'Connell et al., 2012), when we asked our participants to distinguish between active and sham stimulation at the end of the experiment, their responses were at chance in all groups.

It should be noted that the mechanism of tDCS action is not fully understood, and, generally speaking, it is hard to predict a behavioral effect based on expected physiological modulations (e.g., Bestmann & Walsh, 2017; Bestmann et al., 2015; Miniussi et al., 2013). However, based on a previous brain stimulation study that directly showed disruption of action perception when the stimulation inhibited M1, but no effect when the stimulation facilitated M1 (Palmer et al., 2016), we hypothesized that AP task disruption would be greater following c-tDCS rather than a-tDCS over M1, as the latter protocol typically increases M1 excitability (Brunoni et al., 2011; Cogiamanian et al., 2007; Jamil et al., 2017; Kidgell et al., 2013; Moliadze et al., 2010; Nitsche et al., 2008). On the other hand, the effects of c-tDCS are variable and non-linear (Batsikadze, Moliadze, Paulus, Kuo, & Nitsche, 2013; Stagg et al., 2018; Wiethoff, Hamada, & Rothwell, 2014). For example, while c-tDCS$_{1mA}$ inhibits M1, 20 min of c-tDCS$_{2mA}$ with a supra-orbital reference can be excitatory (Batsikadze et al., 2013). However, using a shorter stimulation period (Jamil et al., 2017; Kuo et al., 2013; Wiethoff et al., 2014) and an extracephalic montage (Brunoni et al., 2011; Cogiamanian et al., 2007; Moliadze et al., 2010; Nitsche et al., 2008) decreases the efficiency of the stimulation and can thus prevent excitatory mechanisms from occurring. Thus, we assumed that our c-tDCS protocols would mainly induce physiological M1 inhibition (or no effect) at the group level. Moreover, in view of the apparent intensity-dependence of brain stimulation effects found in previous studies on action perception (e.g., Borgomaneri et al., 2015; Cattaneo, 2010; Naish et al., 2016) and the proven efficacy of c-tDCS$_{2mA}$ in disrupting AP task performance when tDCS was administered with the same montage over another motor region (i.e., the IFC; Avenanti et al., 2018), we predicted that disruption of AP task performance would be maximal following c-tDCS$_{2mA}$ over M1.

2.5. *Neuronavigation*

After C3 localization over the scalp, Talairach coordinates corresponding to the target region were automatically estimated by the SofTaxic Navigator (Electro Medical Systems, Bologna, Italy) from a magnetic resonance imaging (MRI)-constructed stereotaxic template (Avenanti, Annela, & Serino, 2012a; Bertini, Leo, Avenanti, & Làdavas, 2010; Jacquet & Avenanti, 2015; Sacheli, Candidi, Era, & Aglioti, 2015; Serino, Canzoneri, & Avenanti, 2011). Skull landmarks (nasion, inion and two preauricular points) and ~100 points providing a uniform representation of the scalp were digitized by means of a Polaris Vicra digitizer (Northern Digital Inc, Ontario, Canada). An individual estimated MRI was obtained for each subject through a 3D warping procedure fitting a high-resolution MRI template with the participant's scalp model and craniometric points. Talairach coordinates corresponding to the projection of the targeted scalp sites on the brain surface were automatically estimated through the neuronavigation system and converted into MNI space (Fig. 1B). A series of one-way ANOVAs testing the effect of the tDCS protocol (4 levels: c-tDCS$_{2mA}$, a-tDCS$_{2mA}$, c-tDCS$_{1mA}$ and a-tDCS$_{1mA}$) on the x, y and z coordinates assured that coordinates were similar across groups (all $p > .4$).

2.6. *Procedure*

Participants were tested in two sessions characterized by the administration of either active or sham tDCS. In each session, they sat in front of a computer screen located ~50 cm from their face in a dimly illuminated room. The session was divided into three phases (Fig. 2B). In the first preparatory phase, after tDCS electrode placement and neuronavigation,

participants received instructions and performed two training blocks (one for each task, 30 trials each) in order to familiarize themselves with the tasks. If a subject's accuracy was <60% in one of the tasks, the corresponding instructions and training block were repeated. In the second, stimulation phase, participants received 15 min of either active or sham tDCS over M1. Then, in the third, experimental phase, participants were tested in the two behavioral tasks. In both the training phase and the third phase, they were asked to respond as quickly and accurately as possible by pressing a button with the left hand (ipsilateral to tDCS sites). They performed four blocks of 50 trials (2 blocks for each task). Block order and trial order within each block were randomized. A 1-min break was allowed between blocks. Participants completed the four blocks within 30 min after tDCS, thus well within the temporal window of cortical modulation induced by active tDCS. Indeed, stimulation with a current density and duration comparable to those used in our study can alter neural activity for approximately 1 h (Antal, Nitsche, Kruse, Kincses, Hoffmann, & Paulus, 2014; Ardolino, Bossi, Barbieri, & Priori, 2005; Horvath, Forte, & Carter, 2015; Kuo et al., 2013; Nitsche and Paulus 2011).

To test whether sham and active tDCS induced different scalp sensations, after each session, we asked participants to evaluate the discomfort caused by the stimulation using a 5-point Likert scale with 1 indicating "not unpleasant at all" and 5 indicating "extremely unpleasant".

2.7. Data analysis

For each task, session and tDCS group we computed accuracy (% of correct responses) and median response times (RTs) associated with correct response – after removing trials with responses faster than 100 msec and slower than 1000 msec. Accuracy and RTs were analyzed by means of a three-way mixed factors analysis of variance (ANOVA) with Task (2 levels: AP and NP) and Session (2 levels: sham tDCS and active tDCS) as within-subjects factors and tDCS group (4 levels: c-tDCS$_{2mA}$, a-tDCS$_{2mA}$, c-tDCS$_{1mA}$ and a-tDCS$_{1mA}$) as the between-subjects factor. The subjective ratings of discomfort caused by tDCS collected at the end of each session were analyzed with a two-way mixed ANOVA with Session (2 levels: sham tDCS and active tDCS) as a within-subjects factor and tDCS group (4 levels: c-tDCS$_{2mA}$, a-tDCS$_{2mA}$, c-tDCS$_{1mA}$ and a-tDCS$_{1mA}$) as a between-subjects factor. In all the ANOVAs, post hoc comparisons were performed using Tukey tests. Statistical analyses were carried out using STATISTICA 12.0 software (StatSoft, Inc.).

3. Results

3.1. Cathodal tDCS at 2 mA over M1 selectively disrupts action prediction

The tDCS group × Task × Session ANOVA conducted on the accuracy index revealed a Task × Session interaction ($F_{1,44} = 6.88$, $p = .012$, $\eta_p^2 = .14$), and, most importantly, a three-way tDCS group × Task × Session interaction ($F_{3,44} = 3.26$ $p = .03$, $\eta_p^2 = .18$; see Fig. 3). This indicated that tDCS differentially affected accuracy in the two tasks, and this differential effect was dependent on the specific tDCS parameters used. No other effects were significant in the ANOVA (all $F < 1.21$, all

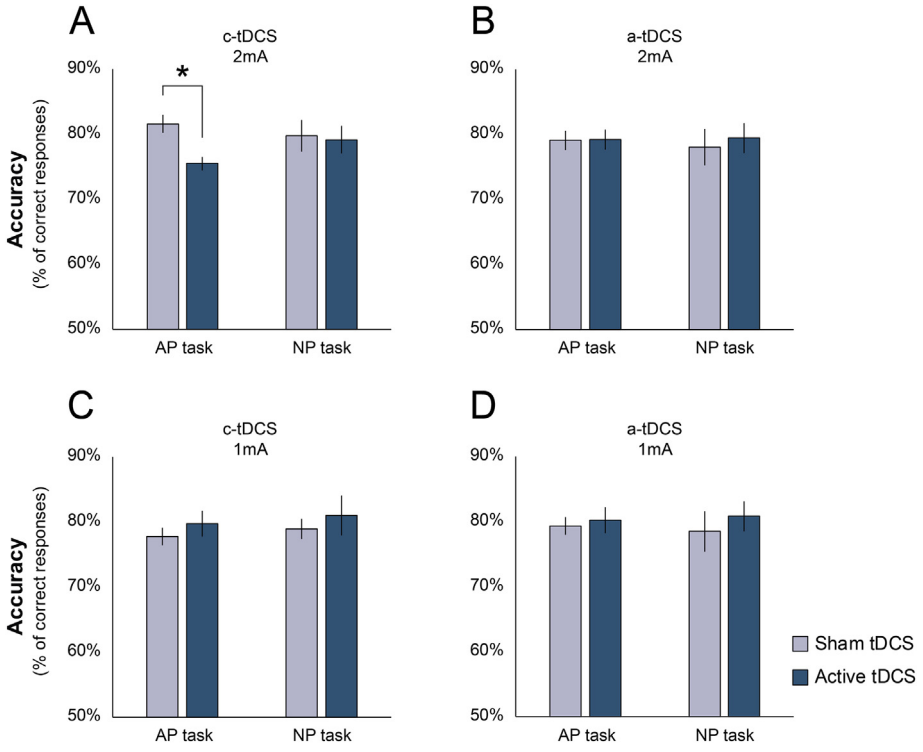

Fig. 3 – Percentage of correct responses in the c-tDCS$_{2mA}$ group (A), the a-tDCS$_{2mA}$ group (B), the c-tDCS$_{1mA}$ group (C) and the a-tDCS$_{1mA}$ group (D). Light gray and blue columns indicate sham and active tDCS conditions, respectively. Asterisks indicate significant post-hoc comparisons ($p < .05$). Error bars denote s.e.m.

$p > .31$). Post-hoc analysis (Tukey test) indicated that the three-way interaction was entirely accounted for by the modulatory effect of c-tDCS$_{2mA}$ on AP task performance (Fig. 3A): in the c-tDCS$_{2mA}$ group, accuracy in the AP task was consistently reduced in the active session (mean ± SD = 76% ± 4) relative to the sham session (82% ± 1; $p = .03$, *Cohen's d* = 1.93), whereas no significant difference was found between the active (80% ± 2) and sham sessions (83% ± 1; $p = .89$) for the NP task; moreover, accuracy in the AP and NP tasks was comparable in the sham sessions ($p > .99$), but differed in the active sessions ($p = .005$, *Cohen's d* = 1.12). No differences in performance were found between sham and active conditions in the other tDCS groups (all $p > .98$). Moreover, no differences in performance in the sham tDCS session were detected between any of the four tDCS groups (all $p > .99$).

The task-, polarity- and intensity-specific effect was further confirmed by splitting the main ANOVA into separate Task × Session ANOVAs, one for each tDCS group. Indeed, the Task × Session ANOVA conducted on accuracy in the c-tDCS$_{2mA}$ group showed a significant two-way interaction ($F_{1,11} = 24.19$, $p = .0005$, $\eta_p^2 = .69$), but no main effects (all $F < 3.50$, all $p > .09$), whereas no significant effects were found for the other tDCS groups, i.e., the a-tDCS$_{2mA}$ group (all $F < .28$, all $p > .60$; Fig. 3B), the c-tDCS$_{1mA}$ group (all $F < 1.21$, all $p > .29$; Fig. 3C) and the a-tDCS$_{1mA}$ group (all $F < 1.42$, all $p > .26$; Fig. 3D). This suggests that the selective drop in AP accuracy found in the c-tDCS$_{2mA}$ group might be specific for both the polarity (c-tDCS) and the intensity (2 mA) of the DC stimulation.

To directly compare the influence of different types of tDCS on task performance, we computed an index of change in accuracy (active tDCS − sham tDCS) for each tDCS group. A one-way ANOVA on the index of change in AP task accuracy was significant ($F_{3,44} = 4.93$, $p = .0005$, $\eta_p^2 = .25$; Fig. 4A). Index values were negative in the c-tDCS$_{2mA}$ group (−6% ± 3) indicating AP task interference due to c-tDCS$_{2mA}$. The index values were lower in the c-tDCS$_{2mA}$ group than in the other tDCS groups (range 0–2%; all $p < .05$; all *Cohen's d* > 1.35) which in turn did not differ from one another (all $p > .84$).

Fig. 4B shows the distribution of individuals' index values across the 4 tDCS groups. The effect of tDCS on AP task accuracy was variable across participants in all groups. In the c-tDCS$_{2mA}$ group, we observed negative values in all participants, indicating that accuracy in the AP task was consistently lower following active c-tDCS$_{2mA}$ relative to sham stimulation. Yet, the magnitude of the reduction was variable across participants, ranging from −2% to −12%. In the other tDCS groups, index values were more distributed around zero with no net change at the group level. Although the variability of index values appears numerically lower in the c-tDCS$_{2mA}$ group (SD: 3.1%) relative to the other tDCS groups (SD range: 5.6–6.8%) – corresponding to a reduction of 33–48% – there were no statistical differences in variance between tDCS groups (Bartlett test, $Chi^2 = 6.24$, $p = .10$).

3.2. Control analyses

We performed three series of control analyses. First, we wanted to further test the robustness of the main ANOVA using nonparametric tests. Despite the fact that accuracy values were normally distributed (Shapiro Wilk tests: all $p > .069$), our sample size, which was based on a preliminary power analysis, was relatively small. Confirming the results of the main ANOVA, Wilcoxon matched-pairs tests showed that, in the c-tDCS$_{2mA}$ group, the critical comparison between sham and active tDCS sessions was significant for AP task accuracy ($Z = 3.06$, $p = .002$), but not for NP task accuracy ($Z = 1.64$, $p = .1$). Additionally, the same comparisons were not significant for any of the tasks in the other tDCS groups (all $Z < 1.07$, all $p > .25$). Moreover, a series of Mann–Whitney *U* tests conducted on the index of change in accuracy (active tDCS − sham tDCS) showed that tDCS interference with the AP task was significantly higher in the c-tDCS$_{2mA}$ group compared to the other tDCS groups (all $Z > 2.54$, all $p < .011$).

Second, to ensure that the disruption of accuracy found in the c-tDCS$_{2mA}$ group was not due to a speed-accuracy trade-off, a Session × Task ANOVA was computed on participants' RTs. No main effects or interactions were found (all $F < 1.58$, all $p > .21$; see Table 1).

Third, we ensured that unpleasant sensations induced by c-tDCS$_{2mA}$ did not explain our results. Discomfort was very

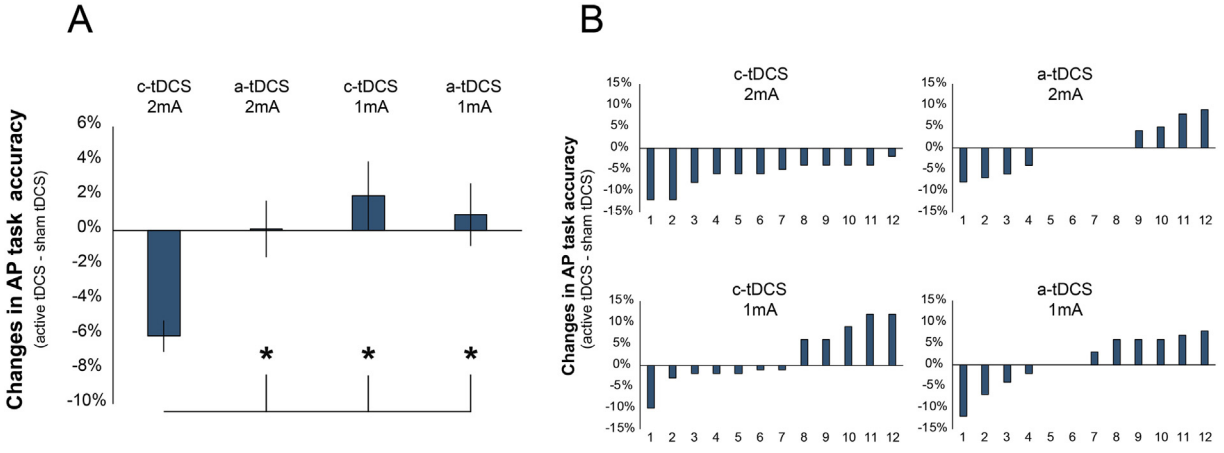

Fig. 4 – (A) Changes in AP task accuracy (active tDCS − sham tDCS) across tDCS groups. Active c-tDCS$_{2mA}$ reduced AP task accuracy, whereas the other active stimulation conditions did not. Asterisks indicate significant post-hoc comparisons ($p < .05$). Error bars denote s.e.m. (B) Distribution of individual participants' changes in AP accuracy.

low and comparable across tDCS sessions and groups, as suggested by the lack of main effects or an interaction in the tDCS group × Stimulation ANOVA on stimulation unpleasantness ratings (all $F < 2.31$, all $p > .09$; Table 2).

3.3. Comparing the effect of c-tDCS$_{2mA}$ across nodes of the AON

The main analyses reported above revealed that c-tDCS$_{2mA}$ over the left M1 selectively disrupted accuracy in the AP task. To test the neuroanatomical specificity of this finding, we compared the behavioral effect induced by 15 min of c-tDCS$_{2mA}$ over M1 with those induced by the same stimulation protocol administered over two different regions of the AON. This was done by comparing data in our c-tDCS$_{2mA}$ group with those of two previous experiments conducted in our laboratory (Avenanti et al., 2018) targeting the left IFC and the left STS. In those previous experiments, we found that, relative to sham, active c-tDCS$_{2mA}$ over the left IFC, but not over the STS, disrupted AP task performance (Table 3), suggesting a major role of motor rather than visual nodes of the AON in action prediction (Avenanti et al., 2018). We thus used a one-way ANOVA to compare the index of change in AP task accuracy (active tDCS − sham tDCS) due to c-tDCS$_{2mA}$ between M1, IFC and STS stimulation sites. The ANOVA was significant ($F_{2,35} = 10.75$, $p = .0002$, $\eta_p^2 = .38$; Fig. 5) and it was entirely accounted for by the greater negative index found after c-tDCS$_{2mA}$ over the left M1 (−6% ± 3) and the left IFC (−5% ± 6) relative to c-tDCS$_{2mA}$ over the left STS (2% ± 4, all $p < .003$, all *Cohen's d* > 1.29). Accuracy was comparable after c-tDCS$_{2mA}$ over the M1 and the IFC ($p = .71$), indicating similar disruptive effects of c-tDCS$_{2mA}$ over these two motor areas.

4. Discussion

In four different groups of healthy participants, we used tDCS to deliver polarity- and intensity-specific exogenous manipulations of the left M1 and thereby test its role in predicting the outcomes of human actions. Compared to sham tDCS, we found that active c-tDCS$_{2mA}$ impaired accuracy in the AP task, but not in the NP task. No changes were found in RTs, thus ruling out the possibility that the detrimental effects of c-tDCS$_{2mA}$ were due to a speed-accuracy trade-off. No changes in performance on either task were found in the other tDCS groups, thus indicating that only the administration of cathodal currents at 2 mA was effective at modulating action prediction. Direct comparison with data from a previous study (Avenanti et al., 2018) also indicates that c-tDCS$_{2mA}$ effects were site-specific and could be found when targeting M1 and IFC, but not STS. These findings establish specific tDCS parameters for effective M1 stimulation and provide, to our knowledge, the first causal evidence of the critical role of M1 in action prediction. Moreover, they suggest that action prediction critically relies on motor processes, more so than visual processes.

4.1. Functional relevance of M1 to action prediction

Classically, M1 has not been considered part of the AON, as functional imaging studies have not consistently detected M1 activation during action observation (Caspers et al., 2010; Gazzola & Keysers, 2009; Grafton, 2009; Molenberghs, Cunnington, & Mattingley, 2012; van Overwalle & Baetens, 2009; but see; Raos, Evangeliou, & Savaki, 2007) and initial studies on monkey mirror neurons did not find any evidence of these neurons in M1 (di Pellegrino et al., 1992; Gallese et al., 1996; see also; Maranesi et al., 2012). Therefore, it was assumed that M1 had little involvement in action perception. However, more recently, three single-cell studies have reported modulation of neuronal activity in M1 during action observation (Dushanova & Donoghue, 2010; Tkach et al., 2007; Vigneswaran et al., 2013). Moreover, neurophysiological studies in humans have consistently reported 'motor resonance' effects in M1: similarly to action execution, action observation modulated the power of beta electro/magnetoencephalographic rhythms with sources in M1 (Caetano, Jousmäki, & Hari, 2007; Hari et al., 1998; Koelewijn, van Schie, Bekkering, Oostenveld, & Jensen, 2008) and enhanced M1 corticospinal excitability in those muscles that would be involved in performing the observed action, as shown by TMS-induced MEPs (Alaerts et al., 2010; Borgomaneri, Gazzola, & Avenanti, 2012; Fadiga, Fogassi, Pavesi, & Rizzolatti, 1995; Naish, Houston-Price, Bremner, & Holmes, 2014; Schütz-Bosbach, Avenanti, Aglioti, & Haggard, 2009; Strafella & Paus, 2000; Valchev et al., 2015). Taken together, these findings have led scholars to propose that M1 might be considered an additional node of an extended AON (Alaerts, Swinnen, &

Table 1 – Mean RTs ± SD (in msec) in the four experimental groups.

	c-tDCS$_{2mA}$ M1		a-tDCS$_{2mA}$ M1		c-tDCS$_{1mA}$ M1		a-tDCS$_{1mA}$ M1	
	Sham	Active	Sham	Active	Sham	Active	Sham	Active
AP task	336 ± 101	329 ± 66	339 ± 112	341 ± 110	373 ± 132	346 ± 104	292 ± 74	288 ± 78
NP task	342 ± 75	308 ± 65	370 ± 136	373 ± 117	376 ± 126	341 ± 119	312 ± 112	305 ± 79

Table 2 – Mean ratings of subjective unpleasantness ± SD (range 1–5).

c-tDCS$_{2mA}$ M1		a-tDCS$_{2mA}$ M1		c-tDCS$_{1mA}$ M1		a-tDCS$_{1mA}$ M1	
Sham	Active	Sham	Active	Sham	Active	Sham	Active
1.75 ± .75	1.75 ± .75	1.33 ± .49	1.67 ± .78	1.25 ± .45	1.25 ± .45	1.25 ± .45	1.58 ± .67

Table 3 – Mean accuracy ± SD for experiments targeting left IFC and STS (from Avenanti et al., 2018).

	c-tDCS$_{2mA}$ left IFC		c-tDCS$_{2mA}$ left STS	
	Sham	Active	Sham	Active
AP task	78% ± 6%	73% ± 10%	75% ± 4%	77% ± 5%
NP task	77% ± 9%	78% ± 9%	73% ± 9%	71% ± 8%

A Task × Session ANOVA on the left IFC group (N = 13, 6 females, mean age ± SD 23.4 ± 3.8 years) revealed no main effects (all $F < 2.8$, all $p > .12$), but a significant two-way interaction ($F_{1,12} = 9.13$, $p = .011$, $\eta_p^2 = .43$): relative to sham, active c-tDCS$_{2mA}$ disrupted AP task accuracy ($p = .022$), whereas the same comparison was not significant for NP task accuracy ($p = .84$). The ANOVA on the left STS group (N = 13, 6 females, mean age 23.2 ± 1.5 years) showed no main effects or interactions (all $F < 2.50$, all $p > .14$).

Wenderoth, 2009b, Alaerts, Swinnen, & Wenderoth, 2009a, Alaerts, Swinnen, & Wenderoth, 2012; Kilner & Frith, 2007; Lepage, Lortie, & Champoux, 2008; Pineda, 2008). Our study supports this proposal by providing causal evidence that exogenous manipulation of M1 affects at least one key function of the AON, i.e., the ability to predict the actions of others.

Correlational evidence suggests that classical regions of the AON build up an anticipatory representation of others' actions, and M1 can reflect such anticipatory coding (Avenanti et al., 2013a; Gangitano, Mottaghy, & Pascual-Leone, 2004; Kilner et al., 2004; Urgesi et al., 2010), possibly via top-down influences from premotor areas, such as the IFC (Avenanti et al., 2013a, 2007; Catmur, Mars, Rushworth, & Heyes, 2011; Enticott et al., 2012; Koch et al., 2010; Nishitani, Avikainen, & Hari, 2004; Nishitani & Hari, 2000). For example, motor resonance in M1 (i.e., the muscle-specific increase in MEP amplitude induced by action observation) was found to reflect the

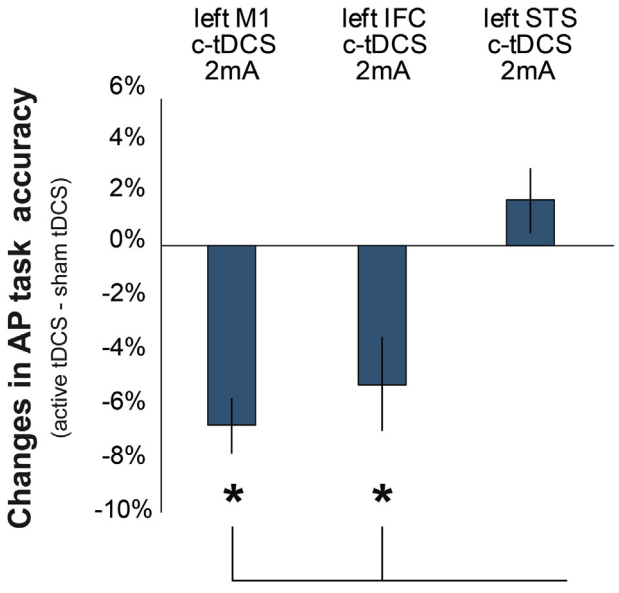

Fig. 5 – Changes in task accuracy (active tDCS – sham tDCS) associated with c-tDCS$_{2mA}$ over M1, IFC and STS. Active c-tDCS$_{2mA}$ reduced AP task accuracy when applied over the left M1 and the left IFC, but not over the left STS. Asterisks indicate significant post-hoc comparisons ($p < .05$). Error bars denote s.e.m.

encoding of future phases of observed actions (Borroni, Montagna, Cerri, & Baldissera, 2005; Gangitano et al., 2004; Urgesi et al., 2010) and inhibition of IFC by means of low-frequency repetitive TMS (rTMS) disrupted such anticipatory motor resonance in M1 (Avenanti et al., 2013a). However, while there is now causal evidence suggesting that IFC and other premotor areas might be critical for action prediction (Avenanti et al., 2018; Makris & Urgesi, 2015; Stadler et al., 2012), previous studies did not establish whether M1 activity is a mere epiphenomenon of the encoding of observed actions in IFC (i.e., a simple downstream consequence of the strong reciprocal cortico-cortical connections between IFC and M1; see Dum & Strick, 2005; Fiori et al., 2016; Prabhu et al., 2009; Rizzolatti & Luppino, 2001; Shimazu, Maier, Cerri, Kirkwood, & Lemon, 2004) or whether it plays a critical role in action prediction. By using exogenous manipulation of M1, we demonstrated that this region not only reflects an anticipatory representation of observed actions, but plays a causally essential role in making accurate predictions about the outcomes of observed actions.

4.2. *Biological tuning of M1 to the predicted outcomes of human actions*

The functional relevance of M1 appears specific for the prediction of human actions, as c-tDCS$_{2mA}$ did not alter performance in the NP task – which was designed as a difficulty-matched control to assess prediction of non-human motion. These results build upon our previous finding that c-tDCS$_{2mA}$ over IFC altered AP but not NP task performance (Avenanti et al., 2018). They are also in line with the notion that motor regions of the AON respond more to observed human movements than to non-human movements (Casile et al., 2010; Dayan et al., 2007; Press, 2011), including movements of geometrical stimuli (Engel, Burke, Fiehler, Bien, & Rosler, 2008; Kessler et al., 2006), inanimate objects (Costantini et al., 2005; Oberman et al., 2005), humanoid robots (Chaminade et al., 2010; Tai, Scherfler, Brooks, Sawamoto, & Castiello, 2004) and virtual hands (Perani et al., 2001), even when all movements are matched for kinematic profile.

However, Schubotz and von Cramon (2004) reported that premotor regions within the AON are also active during predictions of abstract event sequences. This finding led scholars to propose that anticipatory motor coding is not limited to human actions, but extends to event prediction in general, and thus reflects domain-general processes (Press & Cook, 2015; Schubotz, 2007). However, we note that motor activations during non-human event prediction (e.g., Schubotz & von Cramon, 2004) may also reflect epiphenomenal activity that is not critical for making an accurate prediction. Causal methods are essential to establish the functional relevance of motor activations, and our findings that M1 (present study) and IFC perturbations (Avenanti et al., 2018) selectively affect AP task performance appear to support a domain-specific involvement of the human motor system in predicting the outcomes of human actions.

In a recent neuropsychological study, de Wit and Buxbaum (2017) used an established "temporal" version of the AP and NP tasks (e.g., Graf et al., 2007; Stadler et al., 2011, 2012) in which participants observed brief videos of human/non-human

movements that were transiently occluded; after each occlusion, participants had to judge whether the movement continued with coherent or incoherent timing. The authors reported that lesions of the left dorso-frontal, insular and inferior parietal cortices were associated with deficits in temporal prediction of both human actions and non-human movements (de Wit & Buxbaum, 2017) – a result that may appear in contrast with our task-specific findings that suggest a biological tuning of M1-IFC for predicting human actions. However, there are two important points worth considering. First, the findings of de Wit and Buxbaum refer to extensive fronto-insular-parietal lesions, and this opens up the possibility that impairments in non-motor (e.g., attentional) processes might have contributed to their findings. Second, the prediction task used by de With and Buxbaum is heavily based on the temporal features of observed movements (Springer, Parkinson, & Prinz, 2013), while our AP and NP tasks required participants to estimate future spatial trajectories and hand/shape configurations, with little or no need to process temporal information. Thus, in keeping with the notion that the motor system can contribute to time processing in a domain-general manner (e.g., Cook, Gaule, Aichelburg, & Press, 2014; Schubotz, Friederici, & von Cramon, 2000), we preliminarily conclude that the biological tuning of M1 (and other motor nodes of the AON, like the IFC) to human actions might be specific for predicting the spatial/configurational outcome of an action, rather than its temporal deployment. However, future studies should systematically test temporal and spatial/configurational versions of AP and NP tasks to directly test this possibility.

4.3. *Polarity- and intensity-specific modulations of task-relevant networks in M1*

Using a factorial design manipulating the polarity and the intensity of tDCS, we demonstrated highly specific behavioral aftereffects of M1 perturbation and established optimal stimulation parameters for interfering with AP task performance. Building on previous brain stimulation studies suggesting that relatively high neurostimulation intensities and inhibitory (but not excitatory) M1 modulations can impair processing of others' actions (Avenanti et al., 2007; Borgomaneri et al., 2015; Cattaneo, 2010; Palmer et al., 2016; Valchev et al., 2017), we hypothesized that c-tDCS$_{2mA}$ over M1 would affect AP performance. In line with our hypothesis, we found that c-tDCS$_{2mA}$ selectively disrupted AP (but not NP) task performance. Remarkably, effects of c-tDCS$_{2mA}$ were polarity- and intensity-specific, as we found no effect of tDCS in the other groups. This indicates that task-relevant M1 networks required for accurate AP are more sensitive to c-tDCS$_{2mA}$ than to other tDCS protocols involving lower current intensity and/or inverted polarity.

It should be noted that our study focused on behavioral effects of tDCS only. We did not monitor physiological effects of our c-tDCS and a-tDCS protocols, and this is a limitation of our study. Understanding the neural mechanisms underlying AP disruption therefore remains an important avenue for future research. Although we cannot draw any firm conclusions about such mechanisms, we find it interesting that alpha and beta oscillations – whose modulations are known to reflect the activity of the AON during action observation and prediction (i.e., motor resonance; Caetano et al., 2007; Hari et al., 1998; Kilner et al., 2004; Koelewijn et al., 2008; Sebastiani et al., 2014) – are more likely to be affected by brain stimulation protocols eliciting M1 inhibition rather than facilitation (e.g., Baxter, Edelman, Nesbitt, & He, 2016; Chen et al., 2003; McAllister et al., 2013; Pellicciari, Brignani, & Miniussi, 2013). That evidence, together with the present findings and the study of Palmer et al. (2016), supports the notion that inhibitory manipulations of M1 excitability (e.g., c-tDCS) can alter task-relevant AON networks for the (anticipatory) processing of observed actions, more so than facilitatory manipulations. However, further tDCS studies combining physiological and behavioral assessments are needed to directly test this proposal.

In sum, while previous studies have reported mixed results regarding the critical role of M1 in processing others' actions (Avenanti et al., 2007; Borgomaneri et al., 2015; Cattaneo, 2010; Palmer et al., 2016; Valchev et al., 2017; see also Vannuscorps & Caramazza, 2016), our study allows us to conclude that M1 is indeed functionally relevant to predicting the outcomes of observed actions. However, the likelihood of highlighting such a functional role might critically depend on methodological factors determining the effectiveness of M1 interference/inhibition.

4.4. *Neuroanatomical specificity*

To demonstrate the anatomical specificity of M1 stimulation, we directly compared our results to those of our previous tDCS study using the very same tDCS procedure (Avenanti et al., 2018). Our analysis shows that c-tDCS$_{2mA}$ affected AP performance when it was administered over the left M1 or the left IFC, but not when it was administered over the left STS. Moreover, disruption was similar following c-tDCS$_{2mA}$ of the two motor sites. Taken together, the present and previous findings highlight the site-specificity of c-tDCS$_{2mA}$ modulation. They further suggest that task-relevant networks for making predictions about the outcomes of observed actions are based more on motor processes than on visual processes, and are distributed across the M1 and the IFC.

Although the effects of c-tDCS$_{2mA}$ were site-specific, it is unlikely they were site-limited. Current flow modeling suggests that the montage we used primarily affects M1 and the surrounding cortex (Im et al., 2012; Mehta et al., 2015; Noetscher et al., 2014). However, tDCS can also affect the excitability of nearby and remote interconnected regions (Avenanti, Coccia, Ladavas, Provinciali, & Ceravolo, 2012b; Boros, Poreisz, Münchau, Paulus, & Nitsche, 2008; Nitsche et al., 2008). Thus, it is likely that somatosensory (Jacquet and Avenanti 2015; Valchev et al., 2017) or premotor (Makris & Urgesi, 2015; Stadler et al., 2012) regions of the AON may have been affected by tDCS and could have contributed to the observed effects. Yet, it should be noted that a-tDCS2mA over IFC (Avenanti et al., 2018), but not over M1 (present study), enhanced AP task performance. This provides further evidence of site-specificity and suggests that spreading to interconnected frontal regions may have occurred, but cannot entirely explain the present results.

5. Conclusions

All in all, our study demonstrates that monopolar offline c-tDCS$_{2mA}$ administered over the left M1 disrupts predictions about the outcomes of observed human actions, but not difficulty-matched predictions about the outcomes of non-human movements. No similar effects were found with a 1 mA current or when reversing the polarity of stimulation, thus indicating that only c-tDCS$_{2mA}$ perturbed task-relevant motor networks necessary for making accurate action predictions. These findings provide causal evidence that the motor system is functionally relevant to action prediction, and highlight the tDCS parameters optimal for interfering with the anticipatory coding of observed actions within the M1.

Open practices

The study in this article earned Open Materials and Open Data badges for transparent practices. Materials and data for the study are available at https://osf.io/d6hms/

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cortex.2018.09.019.

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