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Abstract

Influential theories suggest that humans predict others' upcoming actions by using their own motor system as an internal forward model. However, evidence that the motor system is causally essential for predicting others' actions is meager. Using transcranial direct current stimulation (tDCS), we tested the role of the inferior frontal cortex (IFC), in action prediction (AP). We devised a novel AP task where participants observed the initial phases of right-hand reaching-to-grasp actions and had to predict their outcome (i.e., the goal/object to be grasped). We found that suppression by cathodal (inhibitory) tDCS of the left IFC, but not the left superior temporal sulcus or the right IFC, selectively impaired performance on the AP task, but not on a difficulty-matched control task. Remarkably, anodal (excitatory) tDCS of the left IFC brought about a selective improvement in the AP task. These findings indicate that the left IFC is necessary for predicting the outcomes of observed human right-hand actions. Crucially, our study shows for the first time that down- and up-regulating excitability within the motor system can hinder and enhance AP abilities, respectively. These findings support predictive coding theories of action perception and have implications for enhancement of AP abilities.

Keywords: action prediction, inferior frontal cortex, transcranial direct current stimulation, action observation network, neuroenhancement

Introduction

The ability to predict the outcomes of observed actions is vital for social life, given its importance for both cooperative (e.g., joint actions) and competitive interactions (e.g., sport). Yet, the neural bases of this ability are poorly understood. There is widespread evidence that seeing the actions of others activates an action observation network (AON) that includes higher order visual regions involved in encoding biological motion (i.e., the superior temporal sulcus, STS) and parieto-frontal regions involved in controlling and sensing body actions (Keysers and Perrett 2004; Gazzola and Keysers 2009; Perrett et al. 2009; Caspers et al. 2010; Rizzolatti et al. 2014; Urgesi et al. 2014). In particular, the inferior frontal cortex (IFC), which includes the ventral premotor cortex and the posterior part of the inferior frontal gyrus, represents a key node of the AON involved in coupling action perception with execution. In the monkey IFC, a class of multimodal neurons—called mirror neurons—is directly involved in such coupling, which may be important for making sense of others' actions (di Pellegrino et al. 1992; Gallese et al. 1996; Rizzolatti et al. 2014).

Studies suggest that the motor node of the AON builds up an anticipatory representation of observed actions (Kilner et al. 2004; Sebanz et al. 2006; Urgesi et al. 2006, 2010; Aglioti et al. 2008; Avenanti et al. 2009, 2013a; Abreu et al. 2012; Balser et al. 2014; Ondobaka et al. 2014; Wurm et al. 2014; Makris and Urgesi 2015; Sacheli et al. 2015). This proposal echoes influential theoretical models positing that the motor system is designed to act as an anticipation device, and that one's own motor system can be used as an internal forward model when perceiving the actions of others (Prinz 1997; Blakemore and Decety 2001; Wolpert et al. 2003; Grush 2004; Wilson and Knoblich 2005; Kilner et al. 2007; Schütz-Bosbach and Prinz 2007; Friston et al. 2011). In this vein, predicting the outcomes of observed actions would critically rely on motor areas of the AON like the IFC. However, whether the IFC or other nodes of the AON are causally essential for predicting others' actions remains speculative, and establishing whether the IFC is critical for action prediction (AP) is the goal of the present study.

Human and monkey correlational studies indicate that: (1) activity in motor regions can occur prior to the observation of a predictable grasping movement (Umiltà et al. 2001; Kilner et al. 2004; Fogassi et al. 2005; Maranesi et al. 2014) and (2) there is a clear anticipatory bias in simulating the upcoming phases of observed reaching-grasping actions (Gangitano et al. 2004; Borroni et al. 2005; Urgesi et al. 2010; Avenanti et al. 2013a). These anticipatory motor activations appear to rely on the AON, as they are disrupted if the IFC is suppressed by low-frequency repetitive transcranial magnetic stimulation (TMS) (Avenanti et al. 2013b). Moreover, the IFC and other motor nodes of the AON are recruited during tasks requiring participants to predict the outcomes of observed actions (Abreu et al. 2012; Amoruso et al. 2014; Balser et al. 2014; Ondobaka et al. 2014; Wurm et al. 2014). An anticipatory bias in processing observed actions has also been shown in STS neurons (Perrett et al. 2009).

It is worth noting here that the notion of anticipatory bias is supported almost exclusively by indirect correlational evidence that leaves unsolved the fundamental question of whether motor and visual nodes of the AON are causally essential for behavior and, in particular, for the ability to make predictions about others' actions. Only 2 interferential studies on the anticipatory bias have been conducted thus far in humans. The first showed that, while low-frequency TMS suppression of the IFC disrupted anticipatory motor activations during observation of implied actions (see above), suppression of the STS had an opposite, enhancing effect on anticipatory motor activations, suggesting that motor simulation plays a compensatory role when visual input is degraded (Avenanti et al. 2013a). The second study showed that online repetitive TMS interference of the STS disrupted the ability of both novices and soccer players with great visual expertise (i.e., goalkeepers) to predict the direction of a ball after perceiving the initial phases of penalty kicks. In contrast, TMS interference with the dorsal premotor cortex impaired performance only in soccer players, whether outfield players or goalkeepers (Makris and Urgesi 2015). Although the lack of a control task for assessing nonspecific, distracting effects of online TMS makes any conclusion tentative, this study is in keeping with the idea that visual and motor nodes of the AON may play different roles in AP. Yet, the causal roles of the STS and the IFC in the ability to predict the outcomes of observed actions have not been established. Crucially, whether AP abilities can be enhanced by exogenous boosting of cortical excitability in the AON is a critical and entirely unexplored question.

Another fundamental, but thus far unresolved, theoretical issue is whether the IFC is critical for predicting event dynamics in general, or whether its involvement is specific to predicting human actions (Schubotz and von Cramon 2004; Schubotz 2007; Press and Cook 2015). Imaging evidence indicates that the IFC is active when predicting sequences of events, suggesting domain-general involvement (Schubotz and von Cramon 2004; Schubotz 2007). However, only causal methods can establish the domain-general versus domain-specific role of IFC in AP.

All these issues are dealt with in the present study, which used transcranial direct current stimulation (tDCS) to alter cortical excitability in the IFC and the STS before participants made predictions about human actions and nonhuman movements. tDCS is a valuable method of noninvasive cortical stimulation that allows researchers to induce polarity-dependent excitability changes in the underlying stimulated area. Using weak off-line cathodal or anodal DC currents, tDCS can induce cortical inhibition or excitation, respectively, and alter neural functioning for several minutes after the end of the stimulation (Nitsche 2003; Antal et al. 2004; Horvath et al. 2015). In 4 tDCS experiments, we applied 15 min of tDCS just before participants performed 2 novel tasks requiring them to predict the future end-states/outcomes of human actions (AP) or nonhuman movements (nonhuman prediction, NP) based on the initial phases of the movements. The tasks were calibrated and matched for difficulty in 3 behavioral studies that allowed us to select sets of AP and NP stimuli in which the outcome could be correctly predicted with ~75%

accuracy. With this accuracy criterion, we prevented ceiling and floor effects, thus providing the optimal behavioral conditions for revealing any potential detrimental or beneficial effects of tDCS.

In the tDCS experiments, task performance was assessed after active tDCS or a control sham tDCS condition that provided a baseline for behavioral performance. In Experiments 1 and 2, we applied cathodal tDCS (c-tDCS) to suppress neural functioning in the left IFC and the left STS, respectively. We tested whether these regions are specifically tuned to (and critical for) the prediction of human actions, or involved in event prediction in general. To test hemispheric specificity, in Experiment 3, we applied active and sham c-tDCS over the right IFC. Moreover, to test stimulation-polarity specificity, in Experiment 4, we applied anodal tDCS (a-tDCS) over the left IFC with the goal of increasing its excitability and thus enhancing its functioning.

Materials and Methods

Participants

A total of 142 healthy volunteers took part in the study. Fifty-two participants were tested in 1 of 4 tDCS experiments, and 90 participants were tested in 1 of 3 pilot studies. Thirteen different participants were assigned to each tDCS experiment (Experiment 1: 6 females, mean age ± standard deviation [SD] 23.4 ± 3.8 years, range 19–32; Experiment 2: 6 females, mean age 23.2 ± 1.5 years, range 21–31; Experiment 3: 6 females, mean age 24.3 ± 2.6 years, range 21–26; Experiment 4: 6 females, mean age 23.6 ± 3.6 years, range 19–30).

Sample size was determined though a power analysis conducted using G*Power 3 (Faul et al. 2007), with power (1 – β) set at 0.80 and α = 0.05, two tailed. We expected a large effect size based on 3 recent transcranial stimulation experiments from our laboratory (exp2 and exp3 in Tidoni et al. 2013; Paracampo et al. 2016). In these studies, we targeted the left IFC to test its role in action perception, and used similar design and task requirements (i.e., participants had to discriminate between 2 observed actions and their performance was compared during active and sham stimulation), indices of task performance (d′), and task validation procedures (all stimuli were selected to be recognized with 75% accuracy) as in the present study (see below). We conducted 2 power analyses, one using the mean effect size across the 3 experiments (Cohen's d = 0.94), and the other using the effect size obtained by pooling data across the experiments (Cohen's d = 0.89). These analyses yielded required sample sizes of 11 and 12 participants, respectively. We thus decided to have 13 participants in each group.

All participants were right-handed and had normal or corrected-to-normal vision. Participants were screened for any general contraindications to noninvasive brain stimulation (Brunoni et al. 2011) using the questionnaire developed by Rossi et al. (2009, 2011) for TMS. No participant was on

medication at the time of the experiment or reported a history of neurological or psychiatric disorders. Participants provided written informed consent. Experimental procedures were approved by the ethics committee at the Psychology Department of Bologna University, and were performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. All participants were naïve to the purposes of the study. Information about the experimental hypothesis was provided only after the experimental tests were completed. No discomfort or adverse effects during tDCS were reported or noticed.

General Design

In 4 tDCS experiments, we tested the roles of the IFC and the STS in predicting the outcomes of observed movements. In Experiments 1, 2, and 3, we applied c-tDCS over the left IFC, the left STS, and the right IFC, respectively. In Experiment 4, we applied a-tDCS over the left IFC. In each experiment, participants were tested in 2 separate sessions that were carried out immediately after 15 min of 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.

Tasks and Stimuli

In the AP task, participants observed 120 video clips (640 × 480 pixels, 30 fps) depicting actors who were individually filmed while reaching and grasping an object. All stimuli subtended a 22.3° × 33.4° visual angle from the participant's viewing position. Videos started by showing 2 objects (left side of the screen) located in front of a still right hand (right side of the screen; see Fig. 1A). The 2 objects were placed at a distance of ~45 cm from the actors' hand. One object was located to the left and the other to the right of the actor's hand (~15 to 20 cm from one another). After a variable delay (1000–2000 ms), the hand started to reach for and grasp 1 of the 2 objects (Supplementary Movie 1). The final phases of the action were occluded and the video interrupted. In these clips, only 30–70% of the entire movement duration was shown, followed by a random-dot mask (150 ms duration) that interrupted the video. Then, a response screen showing the 2 objects appeared and lasted until the response (Fig. 1B). The objects placed to the left and to the right of the actor were displayed on the left and right sides of the screen, respectively. Participants had to guess which of the 2 objects was going to be grasped by the actor's hand, and provided their answers using 2 computer keys. The left and right keys were used to select the left and right target objects, respectively.

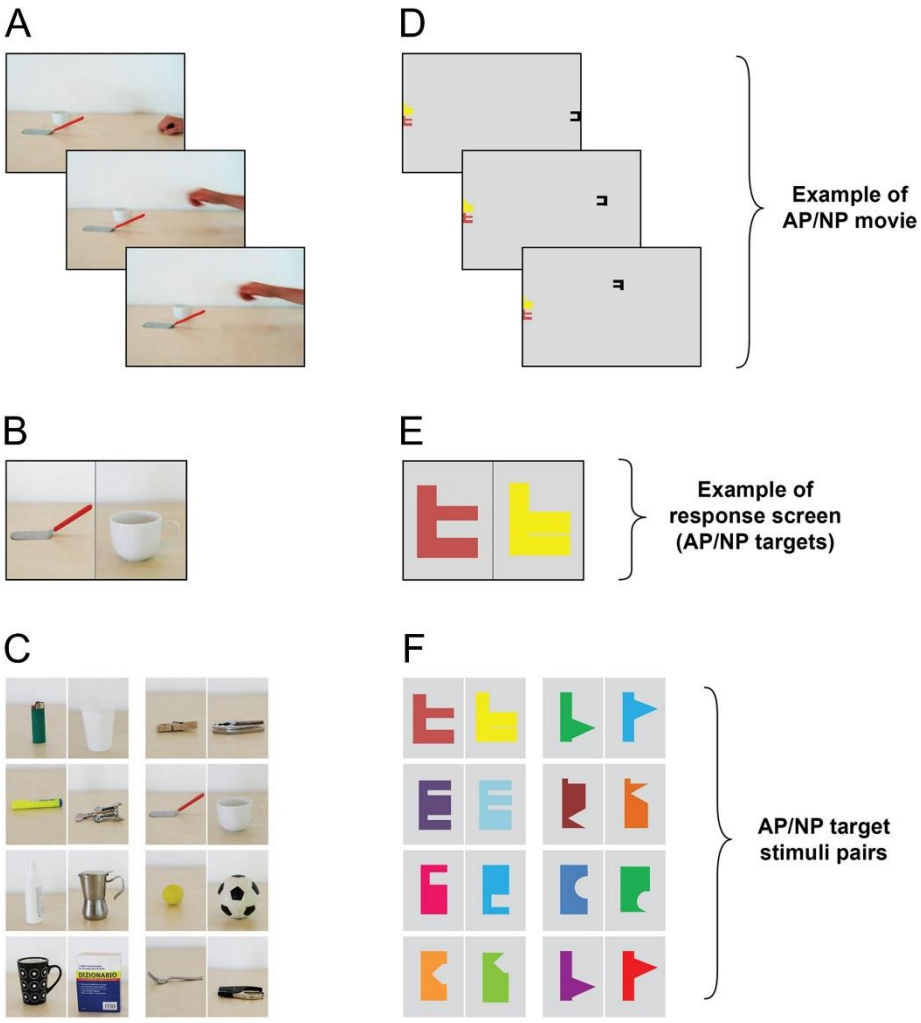

Figure 1. Trial example and stimuli. Example of AP task movie (A) and response screen (B). Target stimulus pairs in the AP task (C). Example of nonhuman prediction (NP) task movie (D) and response screen (E). Target stimulus pairs in the NP task (F). On each trial, a video-clip showed the initial movement of a hand (in the AP task) or a geometrical form (in the NP task) reaching and adapting to 1 of 2 targets. Participants were then presented with the 2 targets and had to guess which was selected by the hand/form.

Trial example and stimuli. Example of AP task movie (A) and response screen (B). Target stimulus pairs in the AP task (C). Example of nonhuman prediction (NP) task movie (D) and response screen (E). Target stimulus pairs in the NP task (F). On each trial, a video-clip showed the initial movement of a hand (in the AP task) or a geometrical form (in the NP task) reaching and adapting to 1 of 2 targets. Participants were then presented with the 2 targets and had to guess which was selected by the hand/form.

Video clips in the AP task included 8 nonprofessional actors (4 females; mean age ± SD; 23.6 years ± 1.06) reaching 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. 1C). The 2 objects in each pair were located near to each other in space, thus implying slightly different reaching trajectories of the grasping hand. The 2 objects in each pair also presented different affordances, thus implying different grips (i.e., from power grips performed with the whole hand to precision grips performed with the index finger and the thumb). The hand–object interaction was not visible in any of the videos. Thus, the AP task

required participants to process kinematic cues (i.e., hand trajectory and finger preshaping before grasping) signaling the upcoming grasping of 1 of the 2 objects.

In the NP control task, participants observed 120 video clips showing an articulated geometrical form approaching 1 of 2 targets (Fig. 1D). Participants had to guess which target was going to be approached by the geometrical form by pressing 1 of 2 keys during the presentation of the response screen (Fig. 1E). The NP videos (640 × 480 pixel, 30 fps) were animations created with Adobe Flash Professional software to grossly match temporal and spatial features of the AP stimuli. Similarly to the AP task, the NP stimuli showed incomplete movements (30–70% of the total duration) of a geometrical form which moved from the right side of the screen in order to reach and fit with 1 of 2 different geometrical targets placed on the opposite side (Supplementary Movie 2). The trajectories of the moving forms were designed to roughly match the hand movements in the AP task. As in the AP task, the 2 targets were located in different spatial positions and had different geometrical properties. Analogous to preshaping of the fingers in the AP task, the configuration of the moving geometrical form changed over time during the reaching phase in order to optimally fit with 1 of the 2 targets. Yet, the NP movement was clearly nonbiological. For the NP video clips, we created 8 different pairs of geometrical targets (Fig. 1F) and 8 moving geometrical forms, and random-dot images were used for masking.

Pilot Studies and Task Validation

The final sets of 120 AP videos and 120 NP videos used in the main experiment were selected from an initial sample of ~1400 AP and ~1200 NP videos using a 2-step procedure. Initially, we selected 180 stimuli for each task based on the performance of 2 groups of participants. We presented the initial sample of AP stimuli to 30 participants (15 female, mean age: 24.5 years ± 2.4) and the sample of NP stimuli to 30 other participants (15 female, mean age: 24.2 years ± 2.6). In these 2 pilot studies, stimuli included movies showing 30–80% of the entire movement. We selected stimuli that were recognized with ~75% accuracy (range: 65–85%) in these 2 groups of participants. This resulted in about 350 stimuli per task, from which 180 stimuli per task were chosen (90 stimuli for the upper object/target and 90 stimuli for the lower object/target, with comparable representations of the different actors/forms). To assure that the 2 tasks were matched for difficulty, in a third pilot study, 30 additional participants (15 female, mean age: 23.9 years ± 2.9) were presented with the 180 AP and 180 NP stimuli selected in the first step. Each video was presented twice (720 trials in total).

The final set of stimuli included 120 AP stimuli and 120 NP stimuli whose outcome could be correctly predicted with ~75% accuracy (range: 65–85%). In both tasks, the hand/form reached both objects/targets with 50% probability. The percentage of the total movement shown in the 2

tasks was matched (AP: mean 45% of total movement, range 30–70%; NP: mean 45% of total movement, range 30–70%; $P > 0.99$). With this procedure we created 2 difficulty-matched tasks with an optimal accuracy level for avoiding floor and ceiling effects. Importantly, half of stimuli in the AP task (N = 60) showed only 30–40% of the total movement, with the hand remaining far from the target objects (not crossing the midline of the screen) and displaying only the initial phase of hand preshaping (well before the maximal grip aperture). In a control analysis, we used this subsample of AP stimuli to assure that tDCS acted on the ability to predict the outcomes of observed actions based on the processing of very early kinematic cues.

tDCS and Neuronavigation

tDCS was delivered using a battery-driven Eldith constant direct current stimulator (neuroConn GmbH). A pair of surface sponge electrodes was soaked in a standard saline solution (NaCl 0.9%) and held in place with elastic rubber bands. In Experiments 1–3, the cathodal electrode (25 cm^2) was applied over the target region (left IFC, left STS, or left IFC). In Experiment 4, the anodal electrode (25 cm^2) was applied over the left IFC. In all 4 experiments, the reference electrode (35 cm^2) was applied over the contralateral deltoid muscle (Priori et al. 2008; Bolognini et al. 2010). It is thought that extracephalic electrode montages allow more focal stimulation, and avoid the confounding effect of the reference electrode (Cogiamanian et al. 2007; Brunoni et al. 2011).

tDCS has been shown to elicit polarity-dependent excitability changes in the cortical area under the stimulation electrodes. Studies of the motor cortex showed that anodal tDCS increases motor excitability while cathodal tDCS decreases it (Nitsche and Paulus 2001; Nitsche 2003; Antal et al. 2004; Nitsche et al. 2008 see Horvath et al. 2015 for a recent quantitative meta-analysis), although many factors may contribute to the efficacy of the stimulation, including intensity, electrode size and disposition and duration of stimulation (Cogiamanian et al. 2007; Nitsche et al. 2008; Moliadze et al. 2010; Brunoni et al. 2011). Importantly, similar polarity-dependent effects can be reliably observed at the behavioral level, at least when testing perceptual/attentional cognitive functions (Jacobson et al. 2012), with anodal and cathodal tDCS being involved in the enhancement and inhibition of such functions, respectively.

Active tDCS was delivered with a constant current of 2 mA (current density ~0.08 mA/cm2), complying with current safety guidelines (Nitsche 2003; Poreisz et al. 2007). Stimulation lasted for 15 min, plus 20 s of ramp-up and ramp-down at the beginning and end of stimulation. Impedance was constantly monitored and kept below 8 kOhm. This protocol is known to affect cortical excitability for more than 30 min after the end of stimulation (Nitsche and Paulus 2001; Nitsche et al. 2008), thus covering the entire duration of the testing phase. For sham tDCS, the electrodes were placed on the same locations, but the current was turned on for only 30 s at the beginning of

the session, and then turned off in a ramp-shaped fashion (fade in/out: 20 s), 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 ensures successful blinding of participants (Gandiga et al. 2006; Ambrus et al. 2012). Although, the intensity used in our study (2 mA) may be less effective in ensuring blinding (O'Connell et al. 2012); but see (Loo et al. 2010, 2012), we used relatively small cephalic electrodes to reduce scalp sensations and make active and sham stimulation feel comparable (Turi et al. 2014; Fertonani et al. 2015; Tang et al. 2016).

Electrode positions were identified on each participant's scalp with the SoftTaxic Navigator system (Electro Medical Systems, Bologna, Italy), as in previous research (Avenanti et al. 2007, 2012; Bertini et al. 2010; Serino et al. 2011; Tidoni et al. 2013; Jacquet and Avenanti 2015; Sacheli et al. 2015). Skull landmarks (nasion, inion, and 2 preauricular points) and ~80 points providing a uniform representation of the scalp were digitized by means of a Polaris Vicra digitizer (Northern Digital, Inc.). An individual estimated magnetic resonance image (MRI) was obtained for each participant through a 3D warping procedure fitting a high-resolution MRI template with the participant's scalp model and craniometric points. This procedure has been proven to ensure a global localization accuracy of roughly 5 mm, a level of precision closer to that obtained using individual MRIs than can be achieved using other localization methods (Carducci and Brusco 2012). Talairach coordinates of target regions and corresponding scalp projections were automatically estimated by the SofTaxic Navigator from the MRI-constructed stereotaxic template. Figure 2 shows the stimulated sites. In Experiments 1, 3, and 4, the IFC was targeted over the pars opercularis of the inferior frontal gyrus at the border with the anterior-ventral aspect of the precentral gyrus, that is, the ventral premotor cortex (coordinates: x = ± 54, y = 10, z = 24, corresponding to Brodmann's area 6/44) (Mayka et al. 2006; Avenanti et al. 2007, 2012; Gazzola et al. 2007; van Overwalle and Baetens 2009; Caspers et al. 2010; Avenanti et al. 2013a). In Experiment 2, the STS was targeted in its posterior aspect (x = –52, y = –53, z = 9, corresponding to Brodmann's area 21) (van Overwalle and Baetens 2009; Caspers et al. 2010; Avenanti et al. 2013a). Talairach coordinates corresponding to the projections of the IFC and STS target sites on the brain surface were automatically estimated through the neuronavigation system. In Experiment 1, mean left IFC surface coordinates ± SD were: x = −53.6 ± 1.5; y = 10.0 ± 0.6; z = 24.0 ± 0.5. In Experiment 2, left STS coordinates were: x = −55.1 ± 1.9; y = −53.6 ± 0.8; z = 9.3 ± 1.0. In Experiment 3, right IFC coordinates were: x = 55.3 ± 1.7; y = 10 ± 0.6; z = 24.5 ± 0.8. In Experiment 4, left IFC coordinates were: x = −54.0 ± 1.5; y = 10.1 ± 0.7; z = 24.2 ± 0.4 (Fig. 2A).

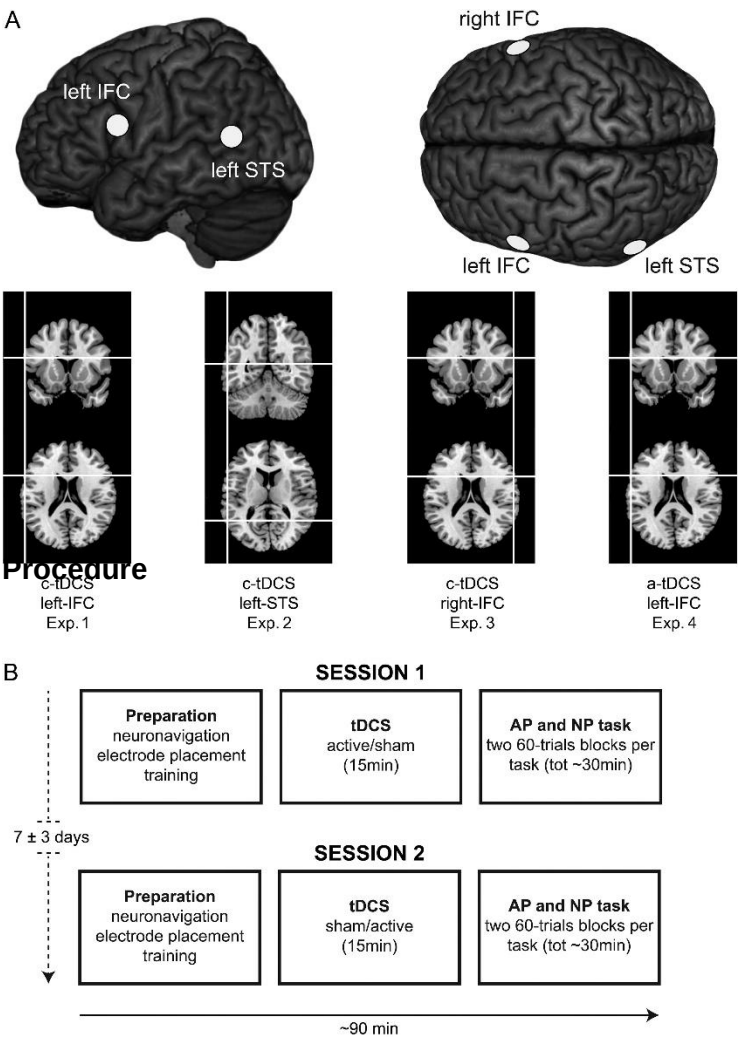

Figure 2. Brain stimulation sites and experimental design. (A) Brain areas targeted in Experiments 1–4. Stimulation sites are reconstructed on a standard template using MRIcron (http://www.mccauslandcenter.sc.edu/mricro/mricron/). (B) Schematic representation of the experimental design. Participants took part in 2 sessions in which performance in the 2 tasks was tested immediately after 15 min of sham/active tDCS over a target brain region.

Procedure

The experiments were programmed using Matlab software to control the video-clip sequence and acquire behavioral responses. Participants sat in front of a computer screen located ~50 cm from their head in a dimly illuminated room. After neuronavigation and tDCS electrode setup, participants received task instructions and performed 2 training blocks (one for each task, 30 trials each) in order to familiarize them with the tasks. They were asked to respond as quickly and accurately as possible by pressing 1 of 2 response buttons with the hand ipsilateral to the tDCS scalp site (the left hand in Experiments 1, 2 and 3, and the right hand in Experiment 4). Training trials were not included in the experimental blocks, but were similarly difficult (~75% accuracy). If a participant's accuracy was <60% in one of the tasks, the corresponding instructions and training block were repeated.

After training, participants received a 15-min session of active or sham tDCS over the target site (left IFC, left STS, or left IFC) and then performed 4 blocks of 60 trials (2 blocks for each task). Block order and the order of trials within each block were randomized. A 1-min break was allowed between blocks. All participants completed the 4 blocks within 35 min after tDCS (mean ± SD across experiments: 30 min ± 2), well within the temporal window of cortical modulation induced by

active tDCS (Fig. 2B). Indeed, tDCS with a current density and duration comparable to those used in our study can alter neural activity for ~1 h (Nitsche and Paulus 2001; Nitsche 2003; Antal et al. 2004; Ardolino et al. 2005; Kuo et al. 2013; Horvath et al. 2015).

To test whether sham or active tDCS-induced different scalp sensations, at the end of 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.”

Data Analysis

Behavioral data were processed off-line. For each task (AP, NP), tDCS condition (sham, active), and Experiments (1–4), we calculated measures of sensitivity (d′) and response bias (β) in accordance with signal detection theory (Macmillan and Creelman 1991; Stanislaw and Todorov 1999). For both tasks, the target objects/forms located in the left/bottom and right/upper parts of the scene were considered targets 1 and 2, respectively. Two types of responses were scored as correct: a “target 1” response to target 1 (hit), and a “target 2” response to target 2 (correct rejection). Two responses were scored as incorrect: a “target 2” response to target 1 (miss), and “target 1” response to target 2 (false alarm). A 3-way mixed analysis of variance (ANOVA) was performed on d′ and β with Task (2 levels: AP and NP) and Stimulation (2 levels: sham tDCS and active tDCS) as within-subjects factors and Experiment (4 levels: Exp. 1, Exp. 2, Exp. 3, and Exp. 4) as the between-subjects factor.

Response times (RTs) were extracted for each trial associated with a correct answer. RTs longer than 2 s were removed from the analysis (<1%). For each task and tDCS condition, we computed the median RTs as this measure is less sensitive to outlier values than the mean. RTs were analyzed with a Task × Stimulation × Experiment ANOVA.

The tDCS discomfort ratings collected at the end of each session were analyzed with a 2-way mixed ANOVA with Stimulation as a within-subjects factor and Experiment as a between-subjects factor.

In all the ANOVAs, post hoc comparisons were performed using Newman–Keuls tests to correct for multiple comparisons. Partial η^2 was computed as a measure of effect size for the main effects and interactions, whereas repeated measures Cohen's d was computed for post hoc comparisons. The normal distribution assumption was checked for each dependent variable using Shapiro–Wilk tests. In all the ANOVAs, we checked for participants with outlier values deviating >3 SD from the group mean. When outliers were detected, we assured that the results of the ANOVA were not due to such participants by replicating the ANOVA effects after removal of these participants. When violations of normality were detected, we also computed Wilcoxon matched pair tests to confirm

critical comparisons using nonparametric analyses. Statistical analyses were carried out using STATISTICA 8.0 software (StatSoft, Inc.).

Results

Task Sensitivity (d′)

The Experiment × Task × Stimulation ANOVA conducted on d′ values revealed a significant 3-way interaction ($F_{3,48}$ = 3.83 P = 0.02, Partial η^2 = 0.19) indicating that sensitivity in the 2 tasks was differentially modulated by active tDCS across the 4 experiments. No other effects were detected in the analysis (all F < 2.11, all P > 0.11). To identify the source of the triple interaction, 2 separate Experiment × Stimulation ANOVAs were performed, one for each task.

The Experiment × Stimulation ANOVA conducted on d′ values from the AP task (Fig. 3) showed a significant 2-way interaction ($F_{3,48}$ = 7.95, P < 0.001, Partial η^2 = 0.33) but no main effects (all F < 0.93, all P > 0.34). Post hoc analysis showed that, relative to sham c-tDCS (mean d′ ± SD: 1.64 ± 0.42), active c-tDCS of the left IFC in Experiment 1 robustly reduced AP sensitivity (1.31 ± 0.59; P = 0.04, Cohen's d = 0.85). No similar effects were found in Experiments 2 and 3, suggesting that suppression of the left STS and the right IFC did not change AP sensitivity (all P > 0.42). In contrast, relative to sham a-tDCS (1.47 ± 0.72), active a-tDCS of the left IFC in Experiment 4 strongly increased AP sensitivity (1.85 ± 0.69; P = 0.006, Cohen's d = 1.07).

We directly compared the influence of different types of tDCS on AP task sensitivity by computing an index of change in d′ (active tDCS–sham tDCS) in each of the 4 experiments (Fig. 4A). Mean index values in Experiment 1 were negative (mean difference index ± SD: −0.33 ± 0.39), indicating task interference after active c-tDCS over left IFC (see Fig. 4B for individual index difference values). They were also lower than the difference indexes in Experiments 2, 3, and 4 (all difference indexes > 0.07 ± 0.44; all P < 0.009, all Cohen's d > 0.97). Mean index values in Experiment 4 were positive (0.38 ± 0.36), indicating task enhancement after active a-tDCS over left IFC (see Fig. 4C for individual values). They were also greater than the difference indexes in Experiments 1 and 2 (all difference indexes < 0.08 ± 0.30, all P < 0.05, all Cohen's d > 0.78). Indexes were comparable in Experiments 3 and 4 (P = 0.92). Thus, the reduction (Experiment 1) and increase (Experiment 4) in d′ values induced by active tDCS were large, as indicated by the effect sizes, and corresponded to changes of −20 and +26% relative to sham tDCS.

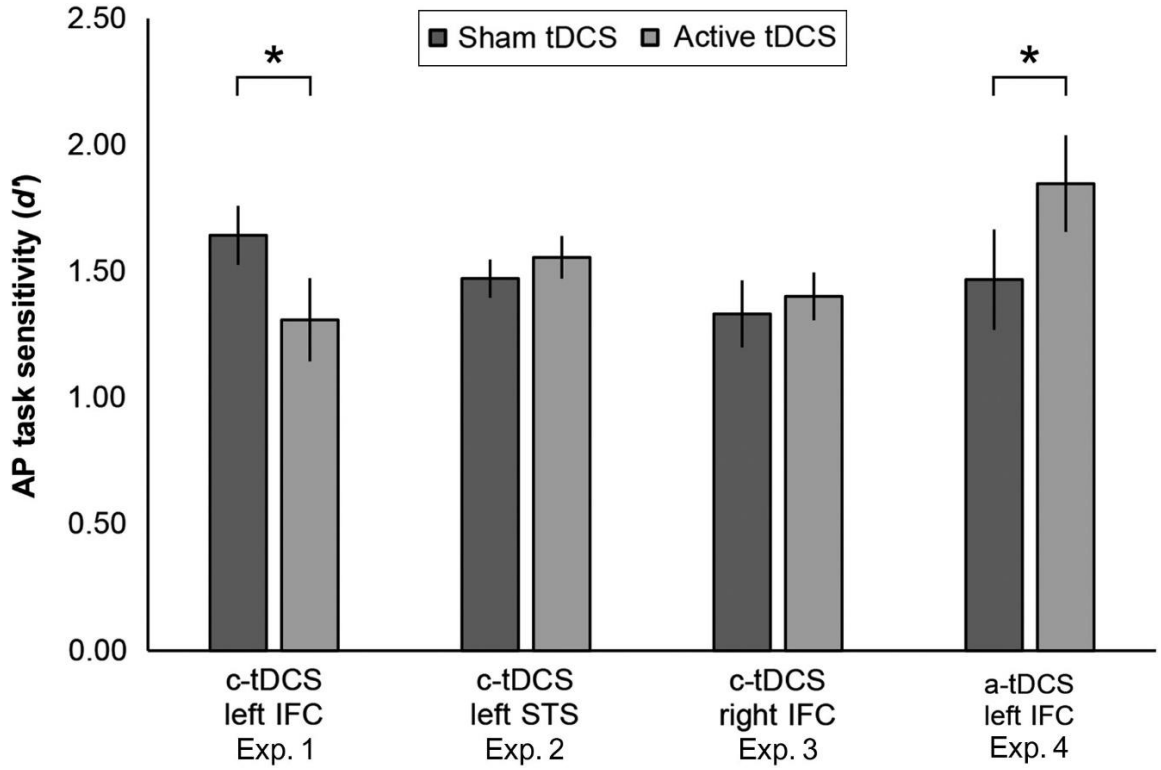

Figure 3. AP task sensitivity in Experiments 1–4. Dark gray and light gray columns indicate d' values in the sham and active tDCS conditions, respectively. Suppression (Experiment 1) and excitation (Experiment 4) of the left IFC disrupted and boosted task sensitivity, respectively. No change in AP task sensitivity was found after suppression of the left STS (Experiment 2) or the left IFC (Experiment 3). Asterisks indicate significant post hoc comparisons ($P < 0.05$). Error bars denote standard error of the mean (SEM).

In summary, the analysis of the differential indexes further demonstrates the selectivity and robustness of the bidirectional influence of left IFC tDCS on the ability to predict others' actions.

To ensure that the modulatory effects of tDCS found in Experiments 1 and 4 influenced the ability to predict the outcomes of observed actions based on the processing of early kinematic cues, we conducted an additional control analysis. For these 2 critical experiments, we computed a measure of AP task sensitivity (d′) on a subsample of 60 AP videos (i.e., half of the total number of videos in the AP task) that showed only the initial 30–40% of the entire movement (i.e., displaying the initial phase of hand preshaping, well before the maximal grip aperture). Planned t-tests showed that relative to sham c-tDCS (1.60 ± 0.46), active c-tDCS of the left IFC in Experiment one reduced AP sensitivity (1.20 ± 0.60; $P = 0.01$, Cohen's $d = 0.85$), whereas, relative to sham a-tDCS (1.46 ± 0.72), active a-tDCS of the left IFC in Experiment 4 increased AP sensitivity (1.92 ± 0.65; $P = 0.004$, Cohen's $d = 0.98$). These values corresponded to a d′ change of −25% in Experiment 1 and +31% in Experiment 4, suggesting reliable tDCS modulation of performance with this subsample of AP stimuli.

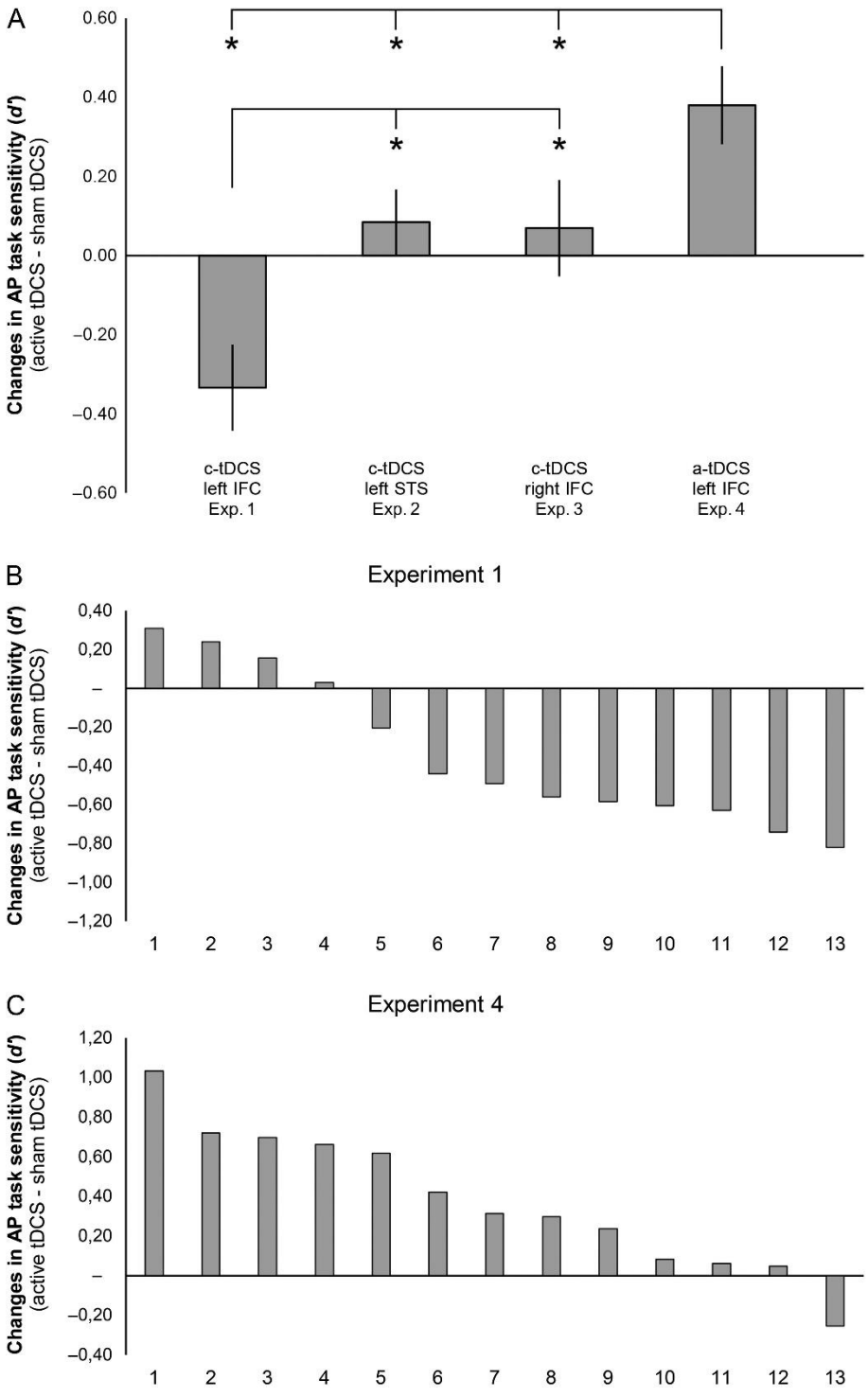

Figure 4. Changes in AP task sensitivity (active–sham tDCS). (A) Mean changes in Experiments 1–4. When applied over the left IFC, active c-tDCS (Experiment 1) and a-tDCS (Experiment 4) brought about a reduction and an increase in AP task sensitivity, respectively. Asterisks indicate significant post hoc comparisons ($P < 0.05$). Error bars denote SEM. (B) Changes in the AP task sensitivity of individual participants in Experiment 1. (C) Changes in the AP task sensitivity of individual participants in Experiment 4.

The Experiment × Stimulation ANOVA conducted on the d′ index for the NP task (Fig. 5) revealed no main effects or interactions (all $F < 0.64$, all $P > 0.59$), thus indicating that active tDCS specifically affected AP but not NP task sensitivity.

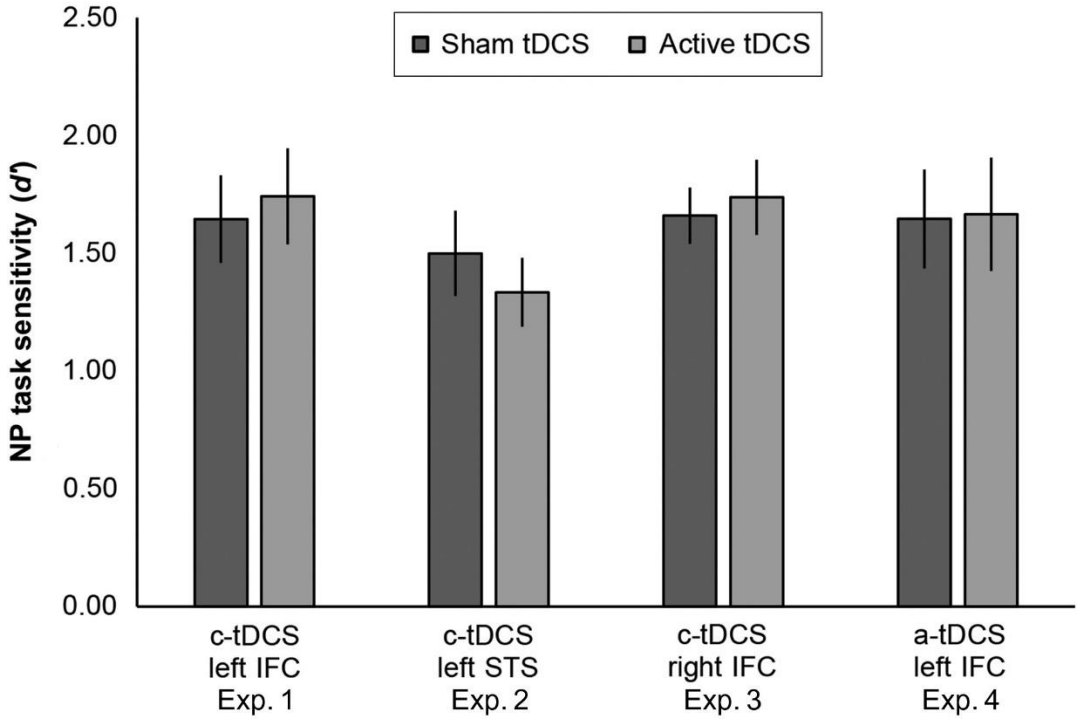

Figure 5. NP task Sensitivity in Experiments 1–4. Dark gray and light gray columns indicate d' values in the sham and active tDCS conditions, respectively. No effects on NP task sensitivity were found. Error bars denote SEM.

Note that the tDCS effects on AP task sensitivity and the lack thereof on the NP task sensitivity were not due to outlier participants, as no participant had d′ values (or a d′ difference index) deviating 3 SD or more from the group mean. We also checked whether our findings were due to tDCS acting mostly on some outlier trials by performing an item analysis. Thus, for each trial, we computed a difference in accuracy (% of correct answer) between the sham and active tDCS session across participants. This was done for each task and experiment separately. In both tasks, no trial deviated 3 SD or more from the mean group difference. In summary, although there was variability in the magnitude of c-tDCS (Fig. 4B) and a-tDCS effects (Fig. 4C) across participants, the results at the group level were strong, as shown by large effect sizes, and not driven by outlier participants or outlier trials.

Response Bias (β)

The Experiment × Task × Stimulation ANOVA conducted on the β index showed no significant main effects or interactions (all $F < 2.35$, all $P > 0.08$; Table 1). However, there were violations of normality in the distribution of β values (Shapiro–Wilk tests: $P < 0.05$). These were mostly due to one participant with β values deviating 3.15 SD from the group mean in one condition (active a-tDCS in the NP task) of Experiment 4. Removing this participant partially normalized the distribution of β values, but kept the results of the ANOVA nonsignificant (all $F < 3.11$, all $P > 0.08$).

Additionally, we used Wilcoxon matched pair tests on the entire sample to confirm that, relative to sham tDCS, active tDCS did not change response bias in the AP task (all P > 0.15) or the NP task (all P > 0.31) across experiments. In summary, manipulations of AON cortical excitability through active tDCS only affected task sensitivity, and did not change response bias.

	Exp. 1 c-tDCS left IFC		Exp. 2 c-tDCS left STS		Exp. 3 c-tDCS right IFC		Exp. 4 a-tDCS left IFC	
	Sham	Active	Sham	Active	Sham	Active	Sham	Active
AP task	0.97 ± 0.51	0.94 ± 0.54	1.55 ± 0.70	1.30 ± 0.54	1.06 ± 0.48	1.04 ± 0.43	0.87 ± 0.28	0.75 ± 0.45
NP task	0.94 ± 0.48	0.99 ± 0.65	0.97 ± 0.91	0.75 ± 0.45	1.11 ± 0.84	0.90 ± 0.60	0.90 ± 0.52	1.39 ± 1.91

Table 1. Response bias (β) index (mean ± SD)

Response Times

The Experiment × Task × Stimulation ANOVA conducted on RTs showed a significant Experiment × Stimulation interaction ($F_{3,48}$ = 2.99, P = 0.04, Partial η^2 = 0.16), but no other main effects or interactions (all F < 1.72, all P > 0.20; see Table 2). The 2-way interaction was accounted for by faster RTs in the active tDCS session (RTs ± SD: 376 ms ± 130) than in the sham tDCS session of Experiment 2 (470 ms ± 178; P = 0.014; Cohen's d = 0.71), indicating that c-tDCS over the left STS made participants respond faster in both the AP and NP tasks. No significant effects of active versus sham tDCS were found in the other experiments (all P > 0.24). It should be noted that the RT data in Experiment 3 (right IFC) slightly violated the normality assumption (Shapiro–Wilk test P < 0.05), possibly due to one participant with RTs deviating 3.03 SD from the group mean in one condition. Removing this participant corrected the violation of normality in that experiment (Shapiro–Wilk test, all P > 0.21), but did not change the Experiment × Stimulation interaction ($F_{3,47}$ = 2.93, P = 0.04, Partial η^2 = 0.16). In addition, the critical post hoc comparison between sham and active tDCS in Experiment 2 remained significant (P = 0.016), whereas the same comparisons were not significant in the other experiments (all P > 0.25), a pattern of results that was further replicated using Wilcoxon matched pair tests on the entire sample of participants (P = 0.05 and all P > 0.27, respectively).

	Exp. 1 c-tDCS left lFC		Exp. 2 c-tDCS left STS		Exp. 3 c-tDCS right lFC		Exp. 4 a-tDCS left lFC	
	Sham	Active	Sham	Active	Sham	Active	Sham	Active
AP task	462 ± 142	508 ± 222	470 ± 178	376 ± 130	433 ± 115	431 ± 139	452 ± 112	432 ± 103
NP task	440 ± 138	475 ± 151	460 ± 165	378 ± 174	445 ± 117	427 ± 126	457 ± 128	433 ± 130

Table 2. Response time (RTs) in ms (mean ± SD)

We also calculated an index of the RT difference in each experiment by subtracting the RT in the sham tDCS session from the RT in the active tDCS session. The RT difference found in Experiment 2 (mean RTs ± SD: −88 ms ± 124) was more negative than the RT difference found in Experiment 1 (+40 ms ± 120; P = 0.008; Cohen's d = 1.05) and nonsignificantly more negative than the RT differences in Experiments 3 (−10 ms ± 80; P = 0.09; Cohen's d = 0.77) and 4 (−22 ms ± 109; P = 0.13; Cohen's d = 0.56).

Discomfort Ratings

At the end of each session, we asked participants to rate the discomfort they felt during tDCS using a 5-point Likert scale. Discomfort ratings were very low, in keeping with the small size of the electrodes (Turi et al. 2014; Fertonani et al. 2015; Tang et al. 2016). Ratings were comparable across tDCS sessions and experiments, as suggested by the lack of any main effects or interactions in the Experiment × Stimulation ANOVA (all F < 2.14, all P > 0.11; Table 3).

Exp. 1 c-tDCS left lFC		Exp. 2 c-tDCS left STS		Exp. 3 c-tDCS right lFC		Exp. 4 a-tDCS left lFC	
Sham	Active	Sham	Active	Sham	Active	Sham	Active
1.54 ± 0.66	1.62 ± 0.62	1.15 ± 0.38	1.77 ± 0.83	1.54 ± 0.66	1.46 ± 0.52	1.62 ± 0.65	1.77 ± 0.73

Table 3. Ratings of subjective tDCS unpleasantness (mean ± SD)

Discussion

In 4 different experiments, we used tDCS to induce polarity-dependent excitability changes (inhibitory for c-tDCS and excitatory for a-tDCS) (Nitsche and Paulus 2001; Antal et al. 2004; Ardolino et al. 2005; Nitsche et al. 2008; Kuo et al. 2013; Horvath et al. 2015) over 2 main nodes of the AON, namely, IFC and STS. We thus explored whether these regions play a causative role in AP, and whether any such role can be boosted or suppressed by exogenous manipulation of their functionality. In Experiment 1, we found that c-tDCS over the left IFC impaired AP task sensitivity (d′), compared with sham tDCS. No change in NP sensitivity was found. These results indicate that suppression of the left IFC selectively disrupted the ability to choose between possible goals/outcomes of a reaching-to-grasp action (i.e., which object was going to be grasped) that could be predicted based on kinematic cues (reaching direction and finger preshaping) shown in the initial phases of the observed action. No similar impairments in AP task sensitivity were observed in Experiments 2 and 3, which targeted the left STS and right IFC, respectively. Remarkably, in Experiment 4, an opposite behavioral effect—that is, enhanced sensitivity in the AP task—was obtained by a-tDCS excitation of the left IFC. No changes in the β index were found, indicating that tDCS-induced suppression and excitation of the IFC resulted in selective disruption and enhancement of AP task sensitivity, respectively. No significant changes in RTs were found in Experiment 1 or 4, thus ruling out that the observed effects were due to a speed-accuracy trade off. Finally, we found that disruption and enhancement of AP task sensitivity in Experiments 1 and 4 was detected even when testing performance with only those AP videos showing very early action kinematic cues (30–40% of the total movement).

From this complex set of results, we can draw 5 main conclusions: (1) the IFC is a crucial node of the AON involved in predicting the outcomes of observed hand actions based on early kinematic cues; (2) down- and up-regulation of left IFC excitability can hinder and boost AP abilities, respectively; (3) the critical involvement of the IFC in making predictions is specific for human actions, and does not extend to prediction of nonhuman movements; (4) prediction of right-hand actions relies on the left, not the right, IFC; and (5) motor (left IFC) more than visual (left STS) regions appear to be critical for AP.

Functional Relevance of Motor versus Visual Nodes of the AON for AP

We provide the first causal evidence that the IFC is involved not only in planning the execution of an upcoming action, but also in making predictions about the outcomes of observed actions. By optimally calibrating task difficulty through a series of behavioral pilot studies, we demonstrate that down-regulation (Experiment 1) and up-regulation (Experiment 4) of cortical excitability in the left IFC reduce and boost the ability to predict others' actions, respectively. These novel findings

provide strong support to theoretical models emphasizing that the IFC is a key node in the anticipatory neural network for the predictive coding of one's own and others actions (Prinz 1997; Blakemore and Decety 2001; Wolpert et al. 2003; Grush 2004; Wilson and Knoblich 2005; Kilner et al. 2007; Avenanti and Urgesi 2011; Brown et al. 2011; Avenanti et al. 2013a; Urgesi et al. 2014) and provide the first direct demonstration of the essential role of the IFC in making explicit predictions about others' actions.

Our findings complement previous causal evidence showing that brain lesions and noninvasive stimulation of the IFC can affect the ability: (1) to match/discriminate different actions/body postures (Urgesi et al. 2007; Pazzaglia et al. 2008; Cattaneo et al. 2010; Tidoni et al. 2013; Michael et al. 2014; Jacquet and Avenanti 2015; Paracampo et al. 2016); (2) to judge whether an observed action has been correctly performed (Pazzaglia et al. 2008; Nelissen et al. 2010); (3) to estimate the weight of a box seen being lifted (Pobric and Hamilton 2006); and (4) to perform/control the imitation of an observed action (Heiser et al. 2003; Catmur et al. 2009; Hogeveen et al. 2015). However, none of these previous studies tested whether the IFC (or the STS) is also critical for AP. Thus, our study goes beyond previous evidence by showing that the IFC is not only functionally relevant to recognition or imitation of others' actions, but also plays an essential causal role in AP.

Together with the recent study of Hogeveen et al. (2015) that addressed the neural bases of imitation control, our study is the first to show that off-line tDCS can affect the functioning of the AON. Hogeveen et al. (2015) found that a-tDCS over the right IFC (i.e., with anodal and cathodal electrodes over the FC6 and Cz scalp positions of the 10–20 system, respectively) improved performance in an imitation inhibition task and increased spontaneous imitation in a social interaction task. In contrast, a-tDCS did not change performance in a nonimitative inhibition task, suggesting that increasing excitability in the IFC selectively improves the control of imitation. Our study expands previous evidence by showing that: (1) c-tDCS and a-tDCS over the IFC can exert opposite behavioral influences; (2) tDCS can modulate not only motor (control of imitation) but also visual (AP) functions of the AON; and (3) stimulation of motor and visual nodes of the AON lead to a combination of anatomical and polarity specific effects, suggesting a division of labor within different AON regions during AP. It would also be worth considering that the use of relatively small active electrodes applied with an image-guided monocephalic montage might allow us to draw stronger neuroanatomical inferences about the causal role of the AON in behavior.

Although prior evidence suggested STS involvement in anticipatory action mechanisms (Perrett et al. 2009; Abreu et al. 2012; Makris and Urgesi 2015), we found no change in AP sensitivity after c-tDCS over this region (see Experiment 2). This suggests that the role of STS in AP is less crucial than that of the IFC. On the one hand, our AP task required participants to predict the goal of an action, and the IFC, more so than STS, may be critical for goal processing (di Pellegrino et al.

1992; Gallese et al. 1996; Cattaneo et al. 2010; Rizzolatti et al. 2014; Jacquet and Avenanti 2015). On the other hand, our findings may appear to contradict brain stimulation and neuropsychological evidence that both the IFC and the STS may be critical for action perception (Saygin 2007; Pazzaglia et al. 2008; Kalénine et al. 2010; Avenanti and Urgesi 2011; van Kemenade et al. 2012; Tidoni et al. 2013; Avenanti et al. 2013b; Urgesi et al. 2014; Jacquet and Avenanti 2015).

Our AP task clearly differs from previous action perception tasks, as it requires participants to extrapolate, from limited visual cues, the outcome of an observed action (i.e., its goal/the object to be grasped) that is blocked from view. According to predictive coding theories (Kilner et al. 2007; Friston et al. 2011), action perception requires constant feedforward and feedback interactions between visual (STS) and frontal (IFC) regions, with the latter being involved in generating predictions about observed actions, and the former being involved in comparing predicted actions with incoming sensory input, so as to adjust the initial prediction. However, such a continuous comparison in the STS may not be fully instantiated in our AP task because video interruption limited sensory inflow. This distinctive feature of the AP task could explain why task sensitivity (i.e., the d′ index) was more affected by exogenous manipulations of the IFC than the STS—at variance with previous studies that tested action perception in full vision and found comparable sensitivity of action perception to both STS and IFC manipulations (Saygin 2007; Pazzaglia et al. 2008; Kalénine et al. 2010; van Kemenade et al. 2012; Tidoni et al. 2013; Avenanti et al. 2013b; Urgesi et al. 2014).

Interestingly, active c-tDCS in Experiment 2 reduced RTs relative to the sham c-tDCS condition. This hints at a beneficial effect of c-tDCS over the STS, in keeping with studies showing that decreasing cortical excitability in visual regions evokes compensatory mechanisms that can improve task performance (Antal et al. 2004; Pirulli et al. 2014). The RT reduction was observed in both tasks, indicating nonspecific improvements. It is likely that this RT effect was not due to a local tDCS effect on the STS, a region that typically shows selectivity for biological movements (Press 2011; Lingnau and Downing 2015), but involved a spreading of the tDCS effect to nearby interconnected middle temporal regions (e.g., hMT+/V5) that represent dynamic information independently from the biological or nonbiological nature of the stimulus (Antal et al. 2004; Lingnau and Downing 2015). Indeed, the location of the reference electrode may have induced a spread of cathodal current in a ventral direction from the STS to hMT+, and this region may have contributed to the observed effects. The nonspecific RT changes found in Experiment 2 stand in contrast with the task-specific accuracy changes found in Experiments 1 and 4, further suggesting distinct roles of visual and motor AON nodes in AP (see also Avenanti et al. 2013a). Taken together, previous studies and our present data allow us to draw 2 preliminary conclusions. First, during classical action perception tasks where the entire action is visible, both the STS and the IFC are functionally relevant to task performance (Avenanti et al. 2013b; Rizzolatti et al. 2014; Urgesi et al. 2014). In contrast, the IFC, but not the STS, plays an essential role in making accurate predictions about an

action's outcome when, as in our AP task, limited information is provided. Second, brain stimulation over the STS may facilitate prediction of both human and nonhuman movements because of nonspecific effects, possibly involving visual motion-sensitive regions.

Human Action Selectivity in the IFC

The modulatory effects found in Experiments 1 and 4 were specific for the prediction of human actions, as c-tDCS and a-tDCS over the left IFC did not alter performance in the NP task, which was designed as a difficulty-matched control to assess prediction of nonhuman motion. This selectivity is in line with the notion that the AON responds more to the observation of human movement than nonhuman movement (Press 2011). This tuning refers both to body form and kinematic profile. For example, reduced activation in the AON was found when participants saw humans moving with a nonhuman kinematics (Dayan et al. 2007; Casile et al. 2010). Moreover, interference with the IFC impairs perception (Candidi et al. 2008) and motor resonance with possible, but not biomechanically impossible, human body movements (Avenanti et al. 2007). Relevant to the present study, seeing human actions activates the anterior node of the AON more than seeing nonhuman movements—including movements of geometrical stimuli (Kessler et al. 2006; Engel et al. 2008), inanimate objects (Costantini et al. 2005; Oberman et al. 2005), humanoid robots (Tai et al. 2004; Chaminade et al. 2010), and virtual hands (Perani et al. 2001), even when all movements are matched for kinematic profile. While all the above studies indicate greater IFC sensitivity for human actions than for nonhuman movements, they cannot distinguish whether the IFC is only necessary for predicting human actions. Indeed, the same sector of the IFC that is involved in action perception is also recruited during predictions of abstract event sequences (Schubotz and von Cramon 2004). These studies suggest that the predictive properties of the IFC are not limited to human actions, but extend to event prediction in general, and thus reflect domain-general processes (Schubotz 2007; Press and Cook 2015).

Our study provides novel insight into this issue by showing that altering cortical excitability in the left IFC affects the ability to predict the outcomes of human actions, but not the outcomes of nonhuman movements. Importantly, during the NP task participants were required to predict movements of an articulated geometrical form with a spatial trajectory resembling that of the reaching hand in the AP task. Moreover, the form changed its geometrical configuration during the approaching phase in order to fit 1 of the 2 target objects, a process analogous to the finger preshaping in the AP clips. Yet, only the hand appeared to be and moved as a biological entity. Although it can be safely assumed that moving hands in the AP task were more familiar than geometrical forms in the NP task (Press and Cook 2015), it is worth noting that the 2 tasks were matched in difficulty based on a series of pilot studies with a large sample of participants. Thus, the fact that tDCS failed to induce changes in NP task sensitivity cannot be due to ceiling or floor

effects (see Pobric and Hamilton 2006; Tidoni et al. 2013). Our data provide causal evidence that the frontal node of the AON is tuned to human actions, and suggest that motor activations during nonhuman event prediction may reflect an outflow of neural activity into the motor system that is not essential for making an accurate prediction.

The AP task required participants to predict the goal of the action (i.e., which object would be grasped) on the basis of kinematic cues (reaching direction, finger preshaping) observed in the initial phase. Thus, our study does not clarify whether the IFC could rely on a prediction of the future trajectory of the movement (i.e., where the hand will end up) to identify a goal that is blocked from view. To shed light on this point, future studies could investigate whether IFC modulation affects the ability to predict the end-state of intransitive actions. Also, it remains unclear whether IFC modulation could affect processing of reaching direction, finger preshaping or both. Dorsal and ventral sectors of the premotor cortex play critical roles in motor control for reaching movements and grasping movements, respectively (Davare et al. 2006; Hoshi and Tanji 2007). Thus, future studies could orthogonally manipulate these 2 action components to test whether the left IFC and dorsal premotor cortices maintain similar divisions of labor during AP.

In principle, tDCS may have also affected visuo-spatial processing of targets, that is, processing of their location or their geometrical properties, which would suggest specific grips. However, target objects were shown in full view for the entire duration of every clip (i.e., 1500–3000 ms) and it is unlikely that tDCS of premotor regions would have affected perceptual processing of nonvisually degraded material (Avenanti et al. 2013b; Uithol et al. 2015). Moreover, spatial processing of targets was also required in the NP task, because the 2 targets were placed in distinct spatial locations and suggested different end-state configurations of the moving form. This suggests that tDCS mainly modulated prediction of (human) action-related information rather than visual processing of targets.

A Lateralization of AP in the IFC?

Another issue we addressed in our study deals with the differential roles of the left IFC and the right IFC in AP. We found that only left IFC manipulation (in Experiments 1 and 4) but not right IFC manipulation (in Experiment 3) affected task performance. These data may suggest a left hemisphere lateralization in AP. However, it should be noted that only right-hand actions were shown in the AP task, and our sample was limited to right-handers. Although AON activity is bilaterally distributed (van Overwalle and Baetens 2009; Borgomaneri et al. 2012, 2015; Grosbras et al. 2012), studies have shown a gradient of lateralization which depends on the laterality of the body part involved in the observed action, as well as the observers' hand preference. In particular, during observation of right-hand actions, AON activation of right-handers tends to be stronger

(Aziz-Zadeh et al. 2002; van Schie et al. 2004; Shmuelof and Zohary 2005; Gazzola and Keysers 2009; Cabinio et al. 2010; Caspers et al. 2010) and can be detected earlier (Ortigue et al. 2010) in the left, relative to the right, hemisphere. Such (partial) lateralization may account for the observed effects. Further studies will test whether suppression of activity in the left or the right IFC alters the ability to predict left hand actions both in right- and left-handers.

Because our AP task was optimized to show early kinematic cues of grasping (e.g., the preshaping of the right index finger and thumb), the AP stimuli depicted the mesial aspect of the actors' right arm, and the forward reaching movement of the actor went from the right to the left side of the screen, resulting in leftward visual motion for the viewer. Studies have suggested an asymmetry in the motor control of leftward versus rightward movements with fronto-parietal regions in the right hemisphere controlling leftward movements (Fujii et al. 1998; Mattingley et al. 1998; Neggers et al. 2007). Our results may appear in contrast with this asymmetry, as we found that stimulation of the left IFC but not the right IFC modulated performance in the AP task. However, the aforementioned asymmetry pertains to the direction of performed actions, whereas the leftward motion in our AP movies is only due to the viewer's perspective, while the actors actually moved their hand in a forward direction. However, future studies might use different actions and test additional movement directions to fully address the issue of IFC laterality in AP.

Although only the left IFC (but not the left STS or the right IFC) seems to be critical for our AP task, it is worth noting that tDCS can modulate the excitability of distant interconnected regions (Boros et al. 2008; Nitsche et al. 2008; Avenanti et al. 2012). Thus, it is entirely possible that other interconnected frontal (e.g., dorsal premotor cortex; see Stadler et al. 2012; Makris and Urgesi 2015) or parietal (e.g., inferior parietal or somatosensory; Caspers et al. 2010; Valchev et al. 2015, 2016) regions of the AON may have contributed to the observed effects. For example, Stadler et al. (2012) have implicated the dorsal premotor cortex in the ability to detect timing incongruities between predicted and observed actions.

Conclusions

Predictive coding theories posit that the brain is a machine evolved to reduce any discrepancy between what is expected and what actually happens (i.e., prediction error) when acting and interacting with others. In keeping with these theories, our current findings emphasize the active role of the frontal node of the AON in the predictive coding of others' actions. Our findings fit with recent evidence supporting predictive coding in frontal regions when processing action language (García and Ibáñez 2016), action intentionality (Koster-Hale and Saxe 2013; Hesse et al. 2016), and others' decisions (Koster-Hale and Saxe 2013; Ibañez et al. 2016; Melloni et al. 2016). Importantly, our experimental design allowed us to demonstrate that changes in the excitability of a

specific region within the AON bring about impairment or enhancement of the ability to predict the outcomes of human actions, depending on the polarity of stimulation. This result indicates that tDCS represents an important tool not only for disrupting human performance, but also for improving it.

It should be considered that we found a performance enhancement in healthy neurotypical participants. Atypical or patient populations may present different baseline levels of cortical excitability, and additional factors might interact with the efficacy and direction of stimulation effects (Krause and Cohen Kadosh 2014). Nevertheless, our study may have therapeutic value (e.g., in people with defective social prediction abilities, such as those with autism spectrum disorders or with impaired action perception due to a lesion affecting the AON), and implications for neuroenhancement (e.g., in healthy people who need to improve their prediction skills for professional reasons, like elite athletes of competitive and cooperative sports). Therefore, future studies should carefully assess clinical and applied potentialities of AON stimulation with tDCS.

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Authors' Contributions: A.A. came up with the study concept and designed the experiments; L.A., R.P., and E.T. performed the experiments; A.A., L.A., and R.P., analyzed the data; A.A., R.P., L.A., E.T., and S.M.A. wrote the manuscript.

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