Brain Research Bulletin 131 (2017) 47–54

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

High-gamma oscillations in the motor cortex during visuo-motor coordination: A tACS interferential study

E. Santarnecchi[a,b,c,*,1], A. Biasella[a,1], E. Tatti[a], A. Rossi[a], D. Prattichizzo[c], S. Rossi[a,d]

[a] Department of Medicine, Surgery and Neuroscience, Neurology and Clinical Neurophysiology Section, Brain Investigation & Neuromodulation Lab. (Si-BIN Lab), University of Siena, Italy
[b] Berenson-Allen Center for Non-Invasive Brain Stimulation, Beth Israel Medical Center, Harvard Medical School, Boston, MA, USA
[c] Siena Robotics and Systems Lab. (SIRS-Lab), Engineering and Mathematics Department, University of Siena, Italy
[d] Department of Medicine, Surgery and Neuroscience, Human Physiology Section, University of Siena, Italy

ARTICLE INFO

Article history:
Received 2 October 2015
Received in revised form 3 December 2016
Accepted 15 March 2017

Keywords:
Visuo-motor coordination
Gamma oscillations
Motor control
tACS
Motor cortex
Entrainment

ABSTRACT

Background: While the role of beta (~20 Hz), theta (~5 Hz) and alpha (~10 Hz) oscillations in the motor areas have been repeatedly associated with defined properties of motor performance, the investigation of gamma (~40–90 Hz) oscillatory activity is a more recent and still not fully understood component of motor control physiology, despite its potential clinical relevance for motor disorders.
Objective/hypothesis: We have implemented an online neuromodulation paradigm based on transcranial alternating current stimulation (tACS) of the dominant motor cortex during a visuo-motor coordination task. This approach would allow a better understanding of the role of gamma activity, as well as that of other oscillatory bands, and their chronometry throughout the task.
Methods: We tested the effects of 5 Hz, 20 Hz, 60 Hz (mid-gamma) 80 Hz (high-gamma) and sham tACS on the performance of a sample of right-handed healthy volunteers, during a custom-made unimanual tracking task addressing several randomly occurring components of visuo-motor coordination (i.e., constant velocity or acceleration pursuits, turns, loops).
Results: Data showed a significant enhancement of motor performance during high-gamma stimulation – as well as a trending effect for mid-gamma – with the effect being prominent between 200 and 500 ms after rapid changes in tracking trajectory. No other effects during acceleration or steady pursuit were found.
Conclusions: Our findings posit a role for high-frequency motor cortex gamma oscillations during complex visuo-motor tasks involving the sudden rearrangement of motor plan/execution. Such a "prokinetic" effect of high-gamma stimulation might be worth to be tested in motor disorders, like Parkinson's disease, where the switching between different motor programs is impaired.

1. Introduction

Oscillatory rhythmic brain activity of human primary motor cortex plays a key role on motor control and represents a fundamental mechanism for sensory-motor information processing (Mackay, 1997). Neurophysiological underpinnings of motor behavior suggest the existence of two patterns of movement-related oscillatory activity in the human motor system: (i) an "antikinetic" one, relying on activity within the beta (β) band (15–30 Hz), and (ii) a "prokinetic" one, mostly associated with activity within the gamma (~30–100 Hz, γ hereafter) and the theta (3–6 Hz, θ hereafter) bands, the latter having a significant role in control of fine movements especially when coupled with high-level cognitive tasks (Brown, 2003; Brucke et al., 2013). More specifically, activity in the β range appears to be prominent during the ongoing phase of tonic contractions, attenuated prior to and during voluntary ballistic movements (Baker, 2007; Bressler, 2009; Kristeva et al., 2007), as well as increased when movement has to be resisted or voluntarily suppressed (Androulidakis et al., 2007; Kuhn et al., 2004). This evidence suggests that β activity promotes postural and tonic contractions, in parallel with an active increase of corticospinal excitability, probably due to a reduced selectivity of motoneuronal recruitment (Feurra et al., 2011).

* Corresponding author at: Policlinico "Le Scotte" Viale Bracci, 2, Siena, Brain Investigation & Neuromodulation Lab., University of Siena, Italy, Siena, 53100, Italy.
E-mail address: esantarn@bidmc.harvard.edu (E. Santarnecchi).
[1] Both authors contribute equally to the study.

http://dx.doi.org/10.1016/j.brainresbull.2017.03.006
0361-9230/

In contrast, the nature of γ oscillations in the brain is less clear, with data supporting its functional role in brain processing (Crone et al., 2006), contrasting others suggesting γ activity as a mere epiphenomenon of brain oscillatory dynamics in general (Fries et al., 2007, 2008). Recent investigations using magnetoencephalography (MEG) helped to elucidate a series of specific properties of γ oscillations by investigating their occurrence during complex visuo-motor tracking tasks: for instance, (i) γ oscillations were selectively localized in the primary motor cortex and reached their peak amplitude 137 ms after the electromyographic movement onset; (ii) they were not differently affected by whether movements were cued or self-paced; (iii) they were found to be stronger for larger movements while absent during the sustained phase of isometric movements (Muthukumaraswamy, 2010, 2011). Additionally, increase of γ oscillations (60–90 Hz) peaking between 125 and 375 ms after the onset of brisk self-paced movements has been also reported (Waldert et al., 2008). These results are also consistent with γ oscillations playing some role in a relatively late stage of motor control, encoding information related to limb movement rather than to muscle contraction itself, like in the case of isometric force. However, due to the intrinsic correlational nature of these studies, no causal inferences can be drawn. Overall, this provides some ground for non-invasive brain stimulation (NIBS) interventions aiming at visuomotor enhancement via manipulation of γ activity.

In order to evaluate the role of γ oscillations during visuo-motor coordination, as well as those played by alpha (α), θ and β regional activity, we adopted a perturbation-based approach based on transcranial electrical stimulation (tES) (Santarnecchi et al., 2015; Filmer et al., 2014): tES includes a group of NIBS techniques able to induce transient modification of brain cortical excitability (Santarnecchi et al., 2014), signal-to-noise ratio (Terney et al., 2008) or endogenous oscillatory patterns (Santarnecchi et al., 2016; Kanai et al., 2008). With the aim of modulating brain activity in a frequency-specific manner, we applied transcranial Alternating Current Stimulation (tACS): that is, a tES approach with experimentally supported efficacy in the modulation of brain oscillations through resonance phenomena with endogenous brain rhythms (Schmidt et al., 2014; Reato et al., 2010), with also possible cascade effects at the behavioural level both in the motor and cognitive domains (Kanai et al., 2008; Feurra et al., 2013; Polania et al., 2012; Santarnecchi et al., 2013). Based on previous correlational electrophysiological studies (Muthukumaraswamy, 2010, 2011), it is reasonable to assume that motor cortex stimulation with tACS in the γ range may lead to performance improvement when applied during the execution of a complex motor task characterized by randomly generated motor sequences and broader movements recruiting a wide network of neurons. The occurrence of a positive modulatory effect of γ-tACS in specific time windows during motor performance may support the functional role of γ oscillations in the motor cortex, thereby having functional implications and potential clinical relevance related to movement disorders. According to the abovementioned correlational evidences, experimental predictions were that β and γ oscillations would produce divergent effects on a visuo-motor tracking tasks, and that γ tACS might improve task components where a sudden online rearrangement of the motor plan is required.

2. Material and methods

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Le Scotte Hospital of Siena. Subjects were invited for participation by word-of-mouth within the Siena University and Le Scotte Hospital communities. Fourteen healthy, right-handed adult participants (7 males, mean age 28, SD 3; 7 females, mean age 25, SD 4) were recruited for the experiment. They all met safety criteria for tES studies (Nitsche et al., 2003; Nitsche and Paulus, 2011) and gave written informed consent for the study. Inclusion required a full right handedness (tested using the Oldfield test), normal neurological (including vision) and psychiatric medical history and examination. Subjects with personal and family history of epilepsy were excluded, as well as subjects with unstable medical conditions, taking psychoactive or central nervous system-active medication, and those reporting recent migraine attacks.

2.1. Tracking task

In order to capture different subcomponents of visuo-motor-coordination, a continuous visually-guided pursuit task was created with the Psychophysics Toolbox package for MATLAB (Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States). Participants were required to pursue a target (white square size 0.3 ° of the visual field) with a mouse held in their right hand, by constantly keeping the cursor (a green frame) perfectly adherent to the target (Fig. 1). The target was moving following a pseudo-randomly generated pattern (for each trial), consisting of different subtasks addressing fine visuo-motor coordination skills: (1) constant velocity pursuit (PURS hereafter), (2) pursuit during acceleration (ACC), (3) 90° turns (TURNS), (4) pursuit with a repetitive pattern composed by circle (LOOP). Each subtask was equally presented during the task, for a total number of 48 subtasks on each stimulation block (i.e. 12 trials for each aforementioned task type). Each LOOP event consisted of a minimum of 3 consecutive circles, in order to compensate for participants' frequent errors (i.e. increase in displacement) observed at the onset of the first LOOP. Subtask events were pseudo-randomized, with each subtask appearing only for a maximum of three consecutive times before switching to another subtask (e.g. ACC, LOOP*3, TURN, TURN, ACC, PURS, TURN, TURN, LOOP*3, ACC, PURS). The frequency of appearance of each subtask was equally distributed across the entire screen. The target was moving at 60 pixels/s during PURSUIT and linearly increasing velocity during ACC (60–100 pixels/s within one linear segment). It always remained within the screen boundaries, changing direction (i.e. summoning a TURN event) in case the right-left-up-down margin of the screen was reached. The average length of each trial was 6.5 min; the total experimental time was ∼1.30 h. To measure participants' performance, the deviation between the template and participant's trace was computed off-line at screen pixel resolution (see Fig. 1D and E).

2.2. Experimental setup

Participants sat comfortably in a chair in front of an LED PC-monitor, with their right elbow and forearm constantly placed on the table in order to avoid fatigue during the experimental trials. The arm was not constrained in order to render the task as ecological as possible. However, subjects were instructed to move only the hand, keeping their forearm resting on the table and avoiding proximal movements of the arm: this task was easily accomplished after few learning trials. The study took place in a quiet room with a temperature between 20 °C and 24 °C. Subjects were instructed about the main experiment's features, timing, stimulation protocol and potential issues related to tACS stimulation. They also underwent a training session with the tracking task, with one of the investigator constantly monitoring their performance, correcting and preventing unwanted arm movements, and clarifying any doubt about task instructions. Finally, all the participants were also familiarized with tACS through the application of a brief stimulation (∼30 s, 1000 μA) in order to reduce anxiety, any effects due to novelty and to become

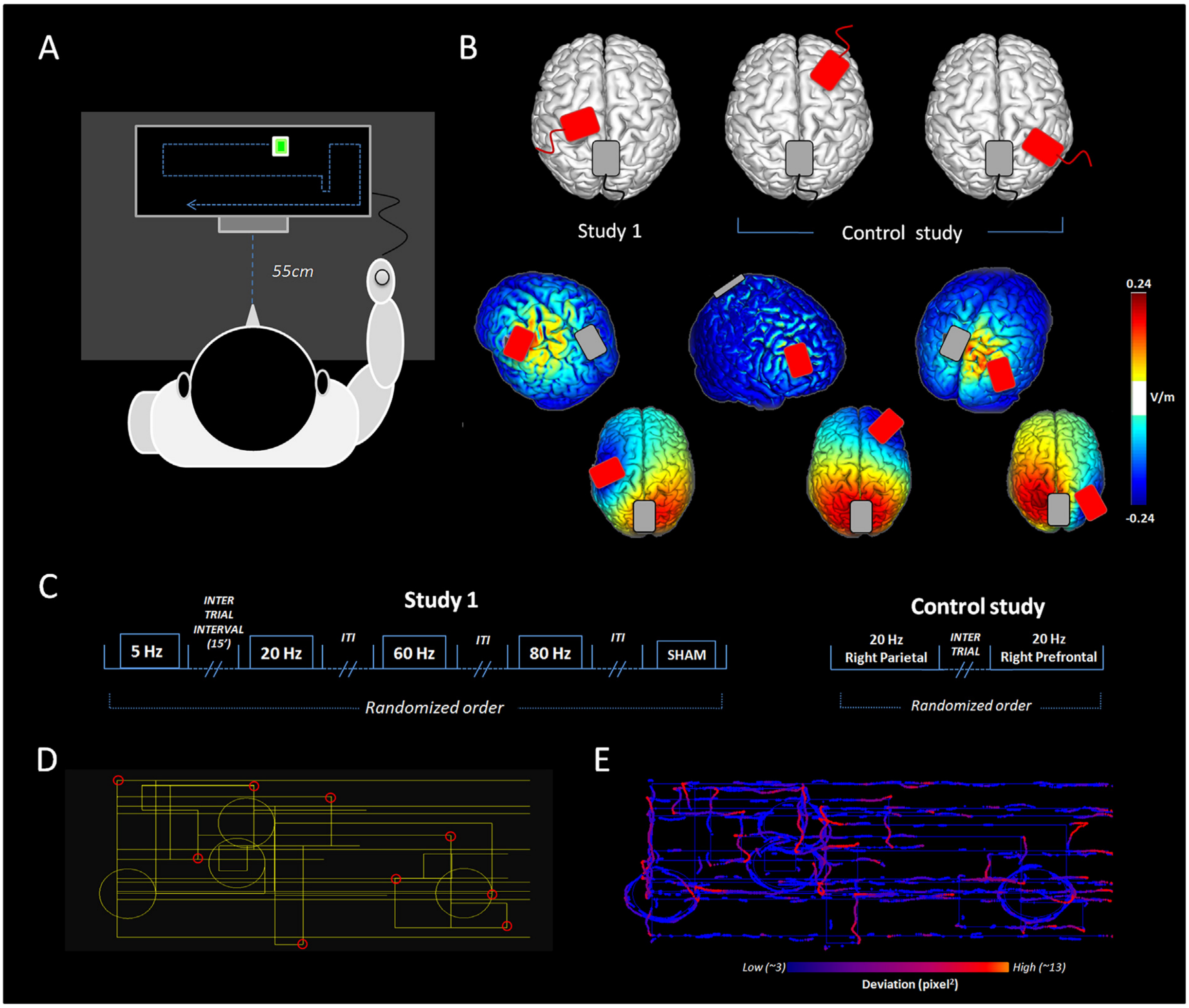

Fig. 1. Experimental paradigm, tES conditions and data analysis. Panel (A) reports a visual representation of the experimental setup, with subjects performing the visuo-motor coordination task using their dominant hand in front of a LCD monitor. (B) tACS was delivered using two rubber-electrodes placed on the TMS-located left motor cortex (C3, Experiment 1), (F4, Control experiment 1) and (P4, Control experiment 2). A model of the actual electrical field (top) and the normal component of tACS montages (bottom) is also reported, highlighting the specificity of current density on the primary motor cortex and right parietal lobe, while a less focal stimulation pattern was obtained for right prefrontal lobe stimulation (technical details are in the text). (C) Each participant underwent five different randomly administered tACS conditions spaced apart by a 20-min resting period, each stimulation block lasted for approximately 7 min. Control experiments for the effects of 20 Hz stimulation on phosphene perception were run on a subsample of participants on a separate day, delivering tACS on right parietal and prefrontal lobes. (D) An example of the randomly generated tracing task, composed by lLinear Pursuit, Accelerations, Turns and Loops. Red circles highlight examples of Turn events. (E) The resulting performance from a sample subject, with a blue-to-red color-code indicating the amount of displacement (mean squared deviation in pixel^2) with respect to the template trace. As is easily observable, Linear pursuit, Accelerations and Loops did not correspond to high deviation values during baseline evaluation (i.e. thin or thicker blue lines), while Turns represent the most challenging events (red lines). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

familiar with the slight cutaneous itching that occurs at stimulation onset and then vanishes.

2.3. Motor cortex localization

In order to localize the primary motor cortex in each individual, we used single-pulse transcranial magnetic stimulation (TMS) via a standard eight-shaped focal coil (diameter of each wing 70 mm) connected with a monophasic Bistim 200 stimulator with 2.2 T as maximal output (MagStim). The coil was positioned on the left hemiscalp, with the handle pointing backwards and at ~45° from the midline. The "hot spot" was the scalp location eliciting a motor evoked potential (MEP) of <50 μV with 50% probability in the right first dorsal interosseous muscle, corresponding to the resting motor threshold (Rossi et al., 2009).

2.4. Transcranial electrical stimulation protocol

tACS was delivered through a current stimulator (BrainStim, EMS, Italy). The stimulator was connected to conductive-rubber electrodes (size 5 × 5 cm) placed in sponges and applied on the scalp. To minimize skin sensation, the electrodes were constantly saturated with a saline solution, and impedances were kept below 10 kΩ throughout stimulation sessions. The "target" electrode was centered on the scalp overlying the hot spot of the left motor cortex as determined by TMS, whereas the other electrode ("return") was placed on Cz according to the International 10–20 EEG System (Fig. 1). Rubber strips around the head guaranteed stable electrode-scalp contact for the two electrodes. Several electrode montages have been suggested to make tACS more focal (Datta et al., 2009). The "return" electrode placement used here was based on previous studies in which clear regional and frequency-specific effects of tACS were found (Feurra et al., 2011, 2013; Kanai et al., 2010a). Even though defining a Target and a Return electrode could be misleading when applied to tACS, here we use the label "Target" electrode for those delivering tACS on the region of interest according to the task at hand, while the "Return" one is intended as the electrode targeting a silent (i.e. not relevant) brain region for the performance being monitored. A sinusoidal stimulation with no DC offset was delivered at an intensity of 1000 μA (peak-to-peak). The average current density at the stimulation electrode was 40 $\mu A/cm^2$. However, it should be noted that the peak current density that occurs near the edges of the electrodes is much higher, due to the "edge effect" (Miranda et al., 2006, 2009).

Five different conditions were run in a randomized fashion (see Fig. 1): a No Stimulation session (using placebo-SHAM tACS), tACS on the left motor cortex at 5 Hz (θ), 20 Hz (β), 60 Hz (mid-γ hereafter) and 80 Hz (high-γ hereafter). Each stimulation session lasted no more than 7 min, given the length of the motor task. Trials were spaced apart using an inter-trial interval (ITI) of 20 min to avoid potential carry-over effects of tACS. The full randomization of tACS conditions and the inclusion of "tACS condition order" as a covariate in the statistical model should have prevented the eventual long lasting effects of tACS delivered in previous blocks on the forthcoming block. However, it should be considered that these after-effects have not been documented yet – neither on the motor (Moliadze et al., 2010) or on the visual cortex (Antal et al., 2008; Kanai et al., 2010b) – for short stimulation time like the one applied in the current design. For Sham blocks, we applied 20 s of tACS using the frequency of stimulation applied in the previous or following tACS block. Due to the low and gentle rise of the intensity of stimulation, subjects did not feel any scalp sensation. At debriefing, subjects reported that they were blind to the frequency applied and that they were not aware of the location of the stimulation.

The intensity of stimulation was chosen following previous investigations using tACS on the motor cortex (Feurra et al., 2011, 2013). We also tried to avoid the perception of flickering lights usually reported by subjects with even lower stimulation intensities, especially during tACS within the β band (Kanai et al., 2008). Even though phosphenes are supposed to be originated at a retinal level, due to the electric fields volume conduction towards the scalp sites with lower resistance such as the orbit (Paulus, 2010), their perception could affect performance during the visuo-spatial task, which requires a precise perception of the moving target as well as the same amount of precision during the pursuit. Therefore, we carefully checked whether participants were reporting flickering in their visual field during β-tACS, identifying 5 participants reporting such experience. To disentangle the possible influence of phosphenes perceptions on performance during β-tACS, we ran a control experiment by comparing Sham-tACS with active stimulation at 20 Hz over the right parietal and prefrontal lobes (respectively corresponding to P4 and F4 electrodes positions in the 10–20 EEG system) (Fig. 1, panel B), in a randomized fashion on a subsample of participants ($n = 9$). If the effect of 20 Hz stimulation over C3 were linked to the presence of – possibly distracting-phosphenes in the visual field, similar behavioural effects could be expected after the same stimulation delivered on other non-motor regions.

2.5. *tES computational model*

To check for the degree of focality of tACS solution in the main experiment, as well as in the control experiment aimed at investigating the potential contribution of phosphenes on visuomotor performance, three separate models were simulated. All the electrode montages included a return electrode over the vertex (Cz), with different solutions for "active" electrodes placed on the left motor cortex (C3), right parietal (P4) and right prefrontal lobes (F4). Distribution of current and normal components of generated electrical fields is reported for each montage in Fig. 1 (panel B). A realistic head model based on T1-weighted and Proton Density weighted phantom MRI images of the single-subject template Colin27 was used to simulate the electric field distribution using the Stimweaver software (Neuroelectrics, Barcelona), as previously described (Paulus, 2010). Five different tissue types were distinguished. Isotropic conductivities were used as follows: 0.33 Siemens per meter (S/m) for the scalp and grey matter (GM), 0.008 S/m for the skull, 1.79 S/m for the cerebrospinal fluid (CSF) (including the ventricles) and 0.15 S/m for the white matter (WM). The plugs at the apexes of the orbits were given conductivity values equal to those of the scalp. In order to represent the conductivity of sponge electrodes soaked in saline solution, the electrodes were modeled with a high conductivity value of 2 S/m. Models suggested a more focal stimulation for tACS montages targeting C3 and P4, with a smaller in magnitude and more widespread stimulation area for F4 stimulation. This could possibly prevent from a reliable interpretation of the two control conditions in case one or both would lead to significant changes at the behavioural level during 20 Hz-tACS.

2.6. *Statistical analysis*

Analyses were carried out using IBM SPSS Statistics (Version 21, release 21.0.0) and MATLAB (Release 2012b, The MathWorks, Inc., Natick, Massachusetts, United States). Experimental predictions were the following: (i) tACS within the γ bands would lead to a better performance than Sham, (ii) especially during TURN and/or any other transition between different motor plans (i.e. transitions between ACC and/or PURS and LOOP/TURN, and vice versa), (ii) tACS within the β band would result in a detrimental effect over motor performance, given the role of motor cortex β oscillations for tonic but not phasic motor control, as well as on the basis of previous tACS studies showing an impairment of motor performance during beta stimulation of the motor cortex (Joundi et al., 2012).

Therefore, repeated measure ANOVAs were performed to verify the occurrence of frequency-specific effects of tACS on PURS, ACC, LOOP and TURN components of the motor task during stimulation of the left motor cortex. While the analysis of motor performance during PURS was based on the average deviation ("mean squared deviation" expressed as pixel^2) during each segment, analysis of "transitions" was computed by looking at the deviation right after each event (PURS $\rightarrow$ LOOP; LOOP $\rightarrow$ ACC; etc) for a duration of 2000 ms, covering the maximum amount of time required by participants to address changes in the motor plan induced by new events. ANOVA models for each task included a five levels factor "STIMULATION" [Sham, tACS at θ, β, mid-γ and high-γ] and a four levels factor "TASK" [TURN, PURS, ACC, LOOP]. Greenhouse-Geisser correction was applied when necessary to compensate for the violation of the assumption of sphericity. In the presence of significant interactions, pairwise comparisons were performed by using Bonferroni correction for multiple comparisons. The level of significance was set at $p = 0.05$, two-tailed. The same repeated measures statistical models and parameters for multiple comparisons correction were applied for the analysis of the control experiment, including a three levels "STIMULATION" factor [tACS at β on P3 and F3, Sham tACS on C3] as well as the aforementioned factor "TASK". In the case of significant interaction values being detected, a high temporal resolution analysis of variance was run by dividing each 2000 ms-long segment in smaller time windows (100 ms long) starting at T0 (corresponding to the beginning of each event) and ending at 2000 ms after the event (T1). These $\cong$100 ms-long time windows represent the minimum resolution at which gamma activity has been detected after movement onset in previous studies (Muthukumaraswamy, 2010, 2011). All the statistical models included “tACS condition order”, “Age” and “Gender” as covariates.

3. Results

All participants tolerated the stimulation well. During experiment 1, 4 out of 14 participants reported slight flickering sensations in their peripheral left visual field at the beginning of stimulation. Two participants out of 14 reported mild transient headache after stimulation.

3.1. Experiment 1

Overall, subjects completed all the trials with no apparent difficulty, though showing differences in the performance at the different subtasks. As shown in Fig. 1E for an exemplar participant, the average displacement recorded for TURNS event during Sham condition (mean displacement = 13 pixel^2; SD = 2.4) was higher with respect to ACC (mean displacement = −1.5 pixel^2; SD = 0.3), PURS (mean displacement = 5 pixel^2; SD = 0.9) and LOOP (mean displacement = 11.7 pixel^2; SD = 2.1). A significant main effect of “STIMULATION” ($F_{(4,9)} = 7.362$, $p = 0.007$, Cohen’s $d = 0.82$) and “TASK” emerged ($F_{(3,10)} = 6.23$, MSE, $p = 0.013$, Cohen’s $d = 0.56$), with a significant interaction between the two factors ($F_{(12,2)} = 49.74$, $p = 0.034$, Cohen’s $d = 0.35$). A further decomposition of the effect using post-hoc analysis revealed a significant reduction of the deviation for TURNS during high-γ tACS compared to Sham ($t_{(13)} = -4.114$, $p = 0.012$, Cohen’s $d = 0.72$), with high-γ tACS also being significantly different from all the other experimental conditions except for mid-γ (versus θ, $p = 0.016$, Cohen’s $d = 0.58$; versus β, $p = 0.009$, Cohen’s $d = 0.82$; versus mid-γ, $p = 0.348$).

As anticipated in the previous paragraph, to further decompose the significant effect of 80 Hz-tACS on TURNS, a repeated measure ANCOVA was calculated including “STIMULATION” [Sham, tACS at mid-γ and high-γ] and “WINDOWS” [twenty 100 ms-long windows] as factors. Results identified the source of interaction in a decreased deviation taking place at specific time windows during high-γtACS (versus Sham): 200–300 ms ($t_{(12)} = 4.114$, $p = 0.012$, Cohen’s $d = 0.72$), 300–400 ms ($t_{(12)} = 3.982$, $p = 0.015$, Cohen’s $d = 0.70$), and 400–500 ms ($t_{(12)} = 3.353$, $p = 0.022$, Cohen’s $d = 0.64$) (Fig. 2A). A trending to significance result for mid-γ tACS was also present in the 200–500 ms time windows (p = *0.092*, Cohen’s $d = 0.34$) (Fig. 2A).

A trending to significance increase in deviation was also identified during PURS during β-tACS (versus Sham, $p = 0.082$, Cohen’s $d = 0.32$; versus θ, $p = 0.326$; versus mid-γ, $p = 0.210$; versus high-γ, $p = 0.194$) (Fig. 3A). All other pairwise comparisons were not significant versus Sham: ACC (θ, $p = 0{,}312$; β, $p = 0.451$; mid-γ, $p = 0.367$; high-γ, $p = 0.332$), LOOP (θ, $p = 0.355$; β, $p = 0.384$; mid-γ, $p = 0.419$; high-γ, $p = 0.435$) (Fig. 3B and C).

3.2. Control experiment

Four out of 8 participants reported flickering lights in their visual field during stimulation over F4, while 2 participants reported the same phenomenon while being stimulated over P4. This was possibly due to the increased proximity to the eyes during F4 stimulation. The repeated measure ANOVAs revealed no significant main effects of “STIMULATION” ($F_{(2,6)} = 2.12$, $p = 0.201$) and “TASK” ($F_{(2,5)} = 1.98$, p = 0.236), suggesting no modulation of visuo-motor performance induced by β stimulation over P4 or F4.

4. Discussion

While several studies have attempted to elucidate the role of γ oscillations in the visual system by systematically varying stimuli across dimensions such as contrast (Hall et al., 2005), spatial frequency (Muthukumaraswamy and Singh, 2008, 2009) and motion (Siegel et al., 2007; Swettenham et al., 2009), there are just a handful of correlational studies addressing the underpinnings of movement subcomponents in respect to motor cortex γ oscillations, leaving their nature and role in motor control up to debate. In the current study, we used an electrical perturbation design to test the role of mid and high γ frequency oscillations in the motor cortex during fine visually-guided unimanual movements. We tested the effect of tACS on the execution of a visuo-motor tracking task in a group of healthy, right-handed subjects, by contrasting the effect of different oscillatory patterns against a Sham, i.e. placebo stimulation. tACS in the high-γ range (80 Hz) produced an enhancement in the performance of specific subcomponents of the task with a definite chronometry, while stimulation in the θ band did not significantly enhance motor performance and β stimulation tended to worsen it. Our results, that exclude an effect due to phosphenes perception during tACS, suggest a causal role for fast oscillatory activity of the motor cortex in motor control physiology, corroborating previous EEG and MEG reports identifying bursts of γ activity during visuo-motor coordination

However, the only motor pattern that was affected by γ-tACS was the 90° turn, while constant velocity or accelaration pursuit, as well as repetitive loops, remained unaffected by γ stimulation. While the latter tasks (acceleration and loops) might have been relatively easy to carry out according to a poorly demanding visuo-motor coordination strategy – thereby resilient to tACS perturbation –, it is intriguing that only the 90° turn required a sudden and brisk change of movement direction, rather than a constant adjustment of the linear pursuit trajectory. Thus, it could be hypothesized that γ oscillations play a role only when the visuo-motor plan requires an online update followed by engagement of an immediate decision-making process (i.e., change according to an unpredicted new trajectory).

Gamma oscillations in the motor cortex have been extensively documented. Increase in the γ band activity has been observed during phasic movements (Muthukumaraswamy, 2010, 2011; Gonzalez Andino et al., 2005), with such oscillatory activity usually being (i) evoked contralaterally to the body part engaged in the movement and (ii) most prevalent during specific subcomponent of evoked activity (i.e. movement onset or post-movement phases). Furthermore, it has been also suggested that γ oscillations reflect proprioceptive reafference following motor activity (Szurhaj et al., 2006), although they could play a more active role in motor control (Cheyne et al., 2008) or even in motor component of decision-making processes (Donner et al., 2009). More generally, and in line with current findings, it has been suggested that fast sensorimotor oscillations may be a mechanism for sampling peripheral data to properly guide subsequent motor acts (Mackay, 1997), by providing an high temporal resolution “updating system” for online motor control (Fries et al., 2007, 2008; Engel and Singer, 2001). These online inputs are crucial to allow the sudden rearrangement of the motor plan, as in case of unpredictable 90° turns, which represent the most sudden and brief events among those sampled in our task.

Our data support a causal role of γ oscillations in these processes by demonstrating how external perturbation in the form of imperceptible transcranial currents – known to resonate with endogenous activity (Helfrich et al., 2014) – were able to enhance motor performance with a definite timing reflecting previous neurophysiological evidence about γ oscillations in the motor system. Interestingly, in a set of MEG experiments using a complex visuo-

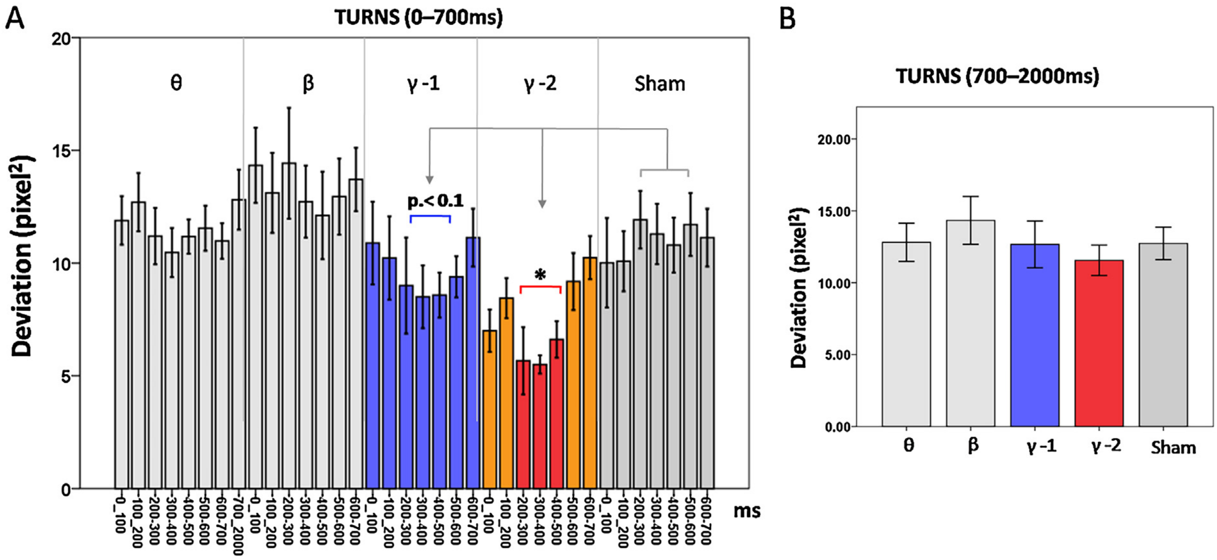

Fig. 2. Significant effect on TURN for high frequency tACS. The analysis of the average deviation during each event revealed a significant effect of tACS at 80 Hz during TURN (red bars), as well as a trending effect for tACS at 60 Hz (blue bars) (A). The effect was specifically captured for the motor performance happening between 200 and 500 ms after each TURN, while it was not present after this specific time window (i.e. 700–2000 ms) (B). Note: *=p < 0.01; θ = theta, 5 Hz; β = beta, 20 Hz; γ-1 = mid gamma, 60 Hz; γ-2 = high gamma, 80 Hz. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

motor tracking task, Muthukumaraswamy and colleagues have reported an increase in the high-γ band (~90 Hz) – along with a decrease of α oscillations- at a relatively late stage of motor control, suggesting such activity as an expression of the encoding of information related to limb movement rather than muscle contraction per se (as in isometric contraction). A similar timing of emerging γ activity in contralateral motor areas has been described also for brisk self-paced movements (Waldert et al., 2008). Interestingly, even though it did not reach statistical significance, the effect was also present during mid-γ stimulation at 60 Hz, suggesting the idea of tACS in the γ range as a modulator of "global" motor coordination, with a stronger effect observable for stimulation close to the γ activity detected using MEG (i.e. 90 Hz) (Muthukumaraswamy, 2010, 2011), and fitting the γ activity (60–80 Hz) accompanying post-onset epochs of self-paced movements (Waldert et al., 2008). Additionally, the present findings provide some insight about the relationship between the amount of γ oscillations in the motor system and the goodness of motor performance (Muthukumaraswamy, 2010, 2011). Even though estimating the optimal amount of γ activity "required" during a given motor task and concurrently deliver tACS in a closed-loop fashion is extremely challenging with current technology, estimating whether increased γ activity might be beneficial or detrimental for a task at hand represents a fundamental question. Interestingly, Polania et al. have shown how the effect of a transcranial electrical stimulation protocol aimed at improving motor performance is indeed tightly related to increased spectral power in the γ range (i.e. 60–90 Hz) induced by electrical stimulation (i.e. tDCS) (Polania et al., 2011). Further studies including EEG monitoring right before and after tACS, as well as studies exploring the after-effect of tACS delivered before the task is performed (therefore allowing for EEG monitoring during performance) will be needed in order to test such hypothesis.

While the role of γ activity for higher-order cognition (Santarnecchi et al., 2013) and perception (Struber et al., 2014) has been recently investigated using a similar perturbation-based approach, no study has demonstrated yet the role of γ oscillations on complex visuo-motor tasks using externally driven oscillatory potentials. A previous study involving tACS over the left motor cortex (Joundi et al., 2012) tested participants while holding a grip force sensor with their right hand while performing a go/no-go task. By testing the effect of β-tACS and 70 Hz (roughly corresponding

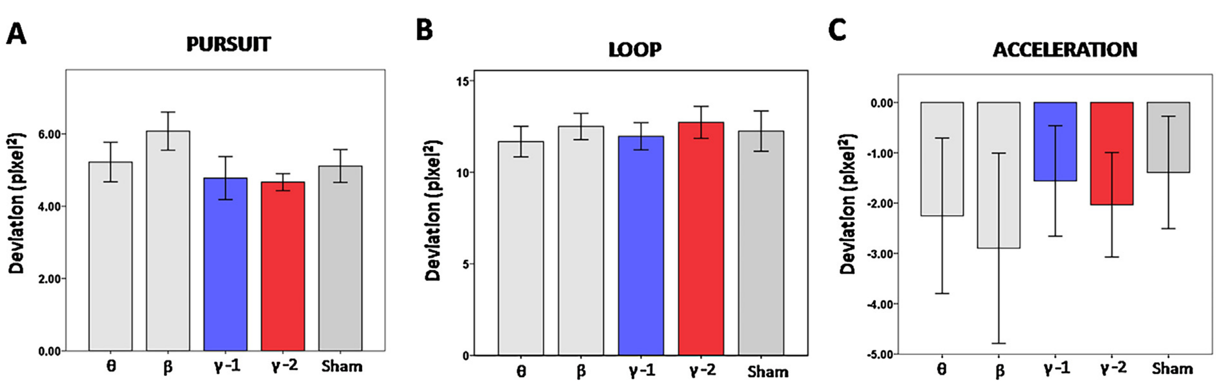

Fig. 3. Non-significant effects on performance during tACS at different frequencies. The analysis of the mean deviation during linear pursuit (PURS) when tACS was delivered at 20 Hz was trending to significance (A), suggesting a worsening of motor performance during β stimulation of the motor cortex. The analysis of data collected during LOOP (B) and ACCELERATION (C) events showed a non-significant effect of tACS regardless of the specific frequency applied. Note: θ = theta, 5 Hz; β = beta, 20 Hz; γ-1 = mid gamma, 60 Hz; γ-2 = high gamma, 80 Hz.

to high-γ tACS of the current study), the authors identified a significant modulation of initial grip force, but no changes in reaction times or peak force, selectively for "go" trails. Conversely, a significant effect of β stimulation (but no effect of 70 Hz tACS) was observed in the peak rate of force development in error trials ("no-go"), suggesting a detrimental effect of β stimulation on motor inhibition. In a different study, Pogosyan and colleagues focused on reaction times and speed components of a pursuit task to analyze the role of β and θ oscillations during voluntary movement, showing a detrimental effect of β-tACS and no effect of stimulation in the θ range (Pogosyan et al., 2009). Consistent with such an akinetic view of β activity, our data also show a trend for a negative effect of 20 Hz stimulation on the deviation during TURNS. Noteworthy, even though the two studies above show some similarities with the current one in terms of, respectively, the motor task and the tACS frequencies applied, they significantly differed in certain aspects of the experimental design. In the latter study (Pogosyan et al., 2009), the pursuit task included only one trajectory, with no additional features like sudden and unexpected turns, loops or accelerations. Differently, the task implemented in the former study (Joundi et al., 2012) is focused on grip force, while indirectly evaluating visuo-motor coordination performance only in the context of an executive function task. Moreover, both studies limited tACS to only two frequencies of stimulation. Finally, our results suggest a potential detrimental effect of β-tACS during steady pursuit; a finding in line with the inverse relationship between beta synchrony in the motor cortex and movement-related processing (Gaynor et al., 2008), as well as with the role of β oscillations in keeping tonic posture while not being functional during the emergence of a new movement (Muthukumaraswamy, 2010; Gilbertson et al., 2005). Noteworthy, such trending to significance result seems to reflect a more general trend for a worsening of motor performance also induced in other components of the visuo-motor task, where 20 Hz tACS always produces the largest – although not significant – reduction of performance.

Looking at emerging "prokinetic properties" of γ activity (Florin et al., 2013a,b), tACS in this frequency range may be of relevance for planning new rehabilitation strategies in motor disorders like Parkinson's disease (PD), where switching from one motor program to another one involving the same limb contributes to bradykinesia, one of the cardinal motor manifestations of the disease (Berardelli et al., 2001). A recent hypothesis posits γ activity in basal ganglia as a compensatory mechanism to counteract bradykinetic movements sustained by increased β activity in the cortico-striatal loop (Florin et al., 2013b), especially when patients are off medications (Williams et al., 2002). Moreover, bradykinesia scores of PD patients negatively correlate with the percentage of neurons oscillating at γ frequency in the subthalamic nucleus of patients who underwent deep brain stimulation (Sharott et al., 2014). Hence, there is theoretical ground to regard γ-tACS of the motor areas as a noninvasive way to increase endogenous γ activity in the dysfunctional cortico-thalamo-striatal loop of PD patients, thereby helping to alleviate bradykinesia. This hypothesis seems plausible also considering recent evidence of the effect of a similar neuromodulatory approach leading to transient positive effects on the amount of resting tremor in PD patients (Brittain et al., 2013).

With respect to potential confounding factors, the observed frequency-specific effect is likely not due to changes in cortical excitability induced by high frequency tACS: previous studies demonstrated how tACS was ineffective in this sense, when cortical excitability was tested either during (Feurra et al., 2011, 2013) or after (Moliadze et al., 2010) γ-tACS, thus supporting the idea of an effective entrainment of fast oscillatory dynamics. Moreover, any of the reported effects – specifically the detrimental impact of β-tACS – seem linked to phosphenes perception as demonstrated in the control experiment.

As for the limitations of the current investigation, a major issue is represented by the lack of electrophysiological monitoring throughout the experiment. Even though the effect of tACS on brain oscillatory potentials have been carefully modeled (Schmidt et al., 2014), a direct evidence of γ modulation would help understanding the rearrangement of cortical dynamics in response to tACS. Additionally, this would help investigating individual differences in the behavioral response to tACS, a well-known issue (Santarnecchi et al., 2016) which cannot be accounted for without monitoring brain online – and offline – response to external stimulation. Furthermore, it must be noticed that 90° turn also display the highest displacement during Sham stimulation, suggesting this component of the motor task as potentially the most difficult for our participants. This might raise a question about tACS being more effective on the specific task, with more room for improvement in respect to others where the average displacement was significantly smaller. However, LOOPS also displayed a similar displacement but no effective modulation by tACS. Future studies should investigate this issue by balancing the intrinsic difficulty of each subtask. Finally, the enhancement induced by γ-tACS should be explored in respect to age-related changes in γ activity (Crone et al., 2006; Fries et al., 2007, 2008), as well as in a motor-training/rehabilitation context, therefore going beyond single-session effects.

In conclusion, our findings causally support the functional role of fast oscillatory activity in the motor cortex during visuo-motor tasks involving the sudden and unexpected rearrangement of motor plan/execution, also suggesting potential future scenarios involving high-frequency tACS interventions in patients with bradykinetic movement disorders.

Financial disclosures

All authors report no conflict of interest.

Acknowledgements

The present work has been partly supported within theEuropean Union Seventh Framework Programme FP7/2007-2013 under grant agreement no. 601165 of the project "WEARHAP – WEARable HAPtics for humans and robots". We would also like to thank Alexandra Emmendorfer for her help during the preparation of the manuscript.

References

Androulidakis, A.G., Doyle, L.M., Yarrow, K., Litvak, V., Gilbertson, T.P., Brown, P., 2007. Anticipatory changes in beta synchrony in the human corticospinal system and associated improvements in task performance. Eur. J. Neurosci. 25, 3758–3765.

Antal, A., Boros, K., Poreisz, C., Chaieb, L., Terney, D., Paulus, W., 2008. Comparatively weak after-effects of transcranial alternating current stimulation (tACS) on cortical excitability in humans. Brain Stimul. 1, 97–105.

Baker, S.N., 2007. Oscillatory interactions between sensorimotor cortex and the periphery. Curr. Opin. Neurobiol. 17, 649–655.

Berardelli, A., Rothwell, J.C., Thompson, P.D., Hallett, M., 2001. Pathophysiology of bradykinesia in Parkinson's disease. Brain 124, 2131–2146.

Bressler, S.L., 2009. The sensory component of tonic motor control. Clin. Neurophysiol. 120, 1035–1036.

Brittain, J.S., Probert-Smith, P., Aziz, T.Z., Brown, P., 2013. Tremor suppression by rhythmic transcranial current stimulation. Curr. Biol. 23, 436–440.

Brown, P., 2003. Oscillatory nature of human basal ganglia activity: relationship to the pathophysiology of Parkinson's disease. Mov. Disord. 18, 357–363.

Brucke, C., Bock, A., Huebl, J., Krauss, J.K., Schonecker, T., Schneider, G.H., Brown, P., Kuhn, A.A., 2013. Thalamic gamma oscillations correlate with reaction time in a Go/noGo task in patients with essential tremor. Neuroimage 75, 36–45.

Cheyne, D., Bells, S., Ferrari, P., Gaetz, W., Bostan, A.C., 2008. Self-paced movements induce high-frequency gamma oscillations in primary motor cortex. Neuroimage 42, 332–342.

Crone, N.E., Sinai, A., Korzeniewska, A., 2006. High-frequency gamma oscillations and human brain mapping with electrocorticography. Prog. Brain Res. 159, 275–295.

Datta, A., Bansal, V., Diaz, J., Patel, J., Reato, D., Bikson, M., 2009. Gyri-precise head model of transcranial direct current stimulation: improved spatial focality using a ring electrode versus conventional rectangular pad. Brain Stimul. 2, 201–207.

Donner, T.H., Siegel, M., Fries, P., Engel, A.K., 2009. Buildup of choice-predictive activity in human motor cortex during perceptual decision making. Curr. Biol. 19, 1581–1585.

Engel, A.K., Singer, W., 2001. Temporal binding and the neural correlates of sensory awareness. Trends Cogn. Sci. 5, 16–25.

Feurra, M., Bianco, G., Santarnecchi, E., Del Testa, M., Rossi, A., Rossi, S., 2011. Frequency-dependent tuning of the human motor system induced by transcranial oscillatory potentials. J. Neurosci. 31, 12165–12170.

Feurra, M., Pasqualetti, P., Bianco, G., Santarnecchi, E., Rossi, A., Rossi, S., 2013. State-dependent effects of transcranial oscillatory currents on the motor system: what you think matters. J. Neurosci. 33, 17483–17489.

Filmer, H.L., Dux, P.E., Mattingley, J.B., 2014. Applications of transcranial direct current stimulation for understanding brain function. Trends Neurosci. 37, 742–753.

Florin, E., Erasmi, R., Reck, C., Maarouf, M., Schnitzler, A., Fink, G.R., Timmermann, L., 2013a. Does increased gamma activity in patients suffering from Parkinson's disease counteract the movement inhibiting beta activity? Neuroscience 237, 42–50.

Florin, E., Erasmi, R., Reck, C., Maarouf, M., Schnitzler, A., Fink, G.R., Timmermann, L., 2013b. Does increased gamma activity in patients suffering from Parkinson's disease counteract the movement inhibiting beta activity? Neuroscience 237, 42–50.

Fries, P., Nikolic, D., Singer, W., 2007. The gamma cycle. Trends Neurosci. 30, 309–316.

Fries, P., Scheeringa, R., Oostenveld, R., 2008. Finding gamma. Neuron 58, 303–305.

Gaynor, L.M., Kuhn, A.A., Dileone, M., Litvak, V., Eusebio, A., Pogosyan, A., Androulidakis, A.G., Tisch, S., Limousin, P., Insola, A., et al., 2008. Suppression of beta oscillations in the subthalamic nucleus following cortical stimulation in humans. Eur. J. Neurosci. 28, 1686–1695.

Gilbertson, T., Lalo, E., Doyle, L., Di, L., Di Lazzaro, V., Cioni, C., Brown, P., 2005. Existing motor state is favored at the expense of new movement during 13–35 Hz oscillatory synchrony in the human corticospinal system. J. Neurosci. 25, 7771–7779.

Gonzalez Andino, S.L., Michel, C.M., Thut, G., Landis, T., de Grave, P.R., 2005. Prediction of response speed by anticipatory high-frequency (gamma band) oscillations in the human brain. Hum. Brain Mapp. 24, 50–58.

Hall, S.D., Holliday, I.E., Hillebrand, A., Singh, K.D., Furlong, P.L., Hadjipapas, A., Barnes, G.R., 2005. The missing link: analogous human and primate cortical gamma oscillations. Neuroimage 26, 13–17.

Helfrich, R.F., Schneider, T.R., Rach, S., Trautmann-Lengsfeld, S.A., Engel, A.K., Herrmann, C.S., 2014. Entrainment of brain oscillations by transcranial alternating current stimulation. Curr. Biol.

Joundi, R.A., Jenkinson, N., Brittain, J.S., Aziz, T.Z., Brown, P., 2012. Driving oscillatory activity in the human cortex enhances motor performance. Curr. Biol. 22, 403–407.

Kanai, R., Chaieb, L., Antal, A., Walsh, V., Paulus, W., 2008. Frequency-dependent electrical stimulation of the visual cortex. Curr. Biol. 18, 1839–1843.

Kanai, R., Paulus, W., Walsh, V., 2010a. Transcranial alternating current stimulation (tACS) modulates cortical excitability as assessed by TMS-induced phosphene thresholds. Clin. Neurophysiol. 121, 1551–1554.

Kanai, R., Paulus, W., Walsh, V., 2010b. Transcranial alternating current stimulation (tACS) modulates cortical excitability as assessed by TMS-induced phosphene thresholds. Clin. Neurophysiol. 121, 1551–1554.

Kristeva, R., Patino, L., Omlor, W., 2007. Beta-range cortical motor spectral power and corticomuscular coherence as a mechanism for effective corticospinal interaction during steady-state motor output. Neuroimage 36, 785–792.

Kuhn, A.A., Williams, D., Kupsch, A., Limousin, P., Hariz, M., Schneider, G.H., Yarrow, K., Brown, P., 2004. Event-related beta desynchronization in human subthalamic nucleus correlates with motor performance. Brain 127, 735–746.

Mackay, W.A., 1997. Synchronized neuronal oscillations and their role in motor processes. Trends Cogn. Sci. 1, 176–183.

Miranda, P.C., Lomarev, M., Hallett, M., 2006. Modeling the current distribution during transcranial direct current stimulation. Clin. Neurophysiol. 117, 1623–1629.

Miranda, P.C., Faria, P., Hallett, M., 2009. What does the ratio of injected current to electrode area tell us about current density in the brain during tDCS? Clin. Neurophysiol. 120, 1183–1187.

Moliadze, V., Antal, A., Paulus, W., 2010. Boosting brain excitability by transcranial high frequency stimulation in the ripple range. J. Physiol. 588, 4891–4904.

Muthukumaraswamy, S.D., Singh, K.D., 2008. Spatiotemporal frequency tuning of BOLD and gamma band MEG responses compared in primary visual cortex. Neuroimage 40, 1552–1560.

Muthukumaraswamy, S.D., Singh, K.D., 2009. Functional decoupling of BOLD and gamma-band amplitudes in human primary visual cortex. Hum. Brain Mapp. 30, 2000–2007.

Muthukumaraswamy, S.D., 2010. Functional properties of human primary motor cortex gamma oscillations. J. Neurophysiol. 104, 2873–2885.

Muthukumaraswamy, S.D., 2011. Temporal dynamics of primary motor cortex gamma oscillation amplitude and piper corticomuscular coherence changes during motor control. Exp. Brain Res. 212, 623–633.

Nitsche, M.A., Paulus, W., 2011. Transcranial direct current stimulation—update 2011. Restor. Neurol. Neurosci. 29, 463–492.

Nitsche, M.A., Liebetanz, D., Lang, N., Antal, A., Tergau, F., Paulus, W., 2003. Safety criteria for transcranial direct current stimulation (tDCS) in humans. Clin. Neurophysiol. 114, 2220–2222.

Paulus, W., 2010. On the difficulties of separating retinal from cortical origins of phosphenes when using transcranial alternating current stimulation (tACS). Clin. Neurophysiol. 121, 987–991.

Pogosyan, A., Gaynor, L.D., Eusebio, A., Brown, P., 2009. Boosting cortical activity at Beta-band frequencies slows movement in humans. Curr. Biol. 19, 1637–1641.

Polania, R., Nitsche, M.A., Paulus, W., 2011. Modulating functional connectivity patterns and topological functional organization of the human brain with transcranial direct current stimulation. Hum. Brain Mapp. 32, 1236–1249.

Polania, R., Nitsche, M.A., Korman, C., Batsikadze, G., Paulus, W., 2012. The importance of timing in segregated theta phase-coupling for cognitive performance. Curr. Biol. 22, 1314–1318.

Reato, D., Rahman, A., Bikson, M., Parra, L.C., 2010. Low-intensity electrical stimulation affects network dynamics by modulating population rate and spike timing. J. Neurosci. 30, 15067–15079.

Rossi, S., Hallett, M., Rossini, P.M., Pascual-Leone, A., 2009. Safety ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin. Neurophysiol. 120, 2008–2039.

Santarnecchi, E., Polizzotto, N.R., Godone, M., Giovannelli, F., Feurra, M., Matzen, L., Rossi, A., Rossi, S., 2013. Frequency-dependent enhancement of fluid intelligence induced by transcranial oscillatory potentials. Curr. Biol. 23, 1449–1453.

Santarnecchi, E., Feurra, M., Barneschi, F., Acampa, M., Bianco, G., Cioncoloni, D., Rossi, A., Rossi, S., 2014. Time course of corticospinal excitability and autonomic function interplay during and following monopolar tDCS. Front. Psychiatry 5, 86.

Santarnecchi, E., Ak, Brem, Levenbaum, E., Thompson, T., Cohen Kadosh, R., Pascual-Leone, A., 2015. Enhancing cognition using transcranial electricalstimulation. Curr. Opin. Behav. Sci. 4, 171–178.

Santarnecchi, E., Muller, T., Rossi, S., Sarkar, A., Polizzotto, N.R., Rossi, A., Cohen Kadosh, R., 2016. Individual differences and specificity of prefrontal gamma frequency-tACS on fluid intelligence capabilities. Cortex (in press).

Schmidt, S.L., Iyengar, A.K., Foulser, A.A., Boyle, M.R., Frohlich, F., 2014. Endogenous cortical oscillations constrain neuromodulation by weak electric fields. Brain Stimul. 7, 878–889.

Sharott, A., Gulberti, A., Zittel, S., Tudor Jones, A.A., Fickel, U., Munchau, A., Koppen, J.A., Gerloff, C., Westphal, M., Buhmann, C., et al., 2014. Activity parameters of subthalamic nucleus neurons selectively predict motor symptom severity in Parkinson's disease. J. Neurosci. 34, 6273–6285.

Siegel, M., Donner, T.H., Oostenveld, R., Fries, P., Engel, A.K., 2007. High-frequency activity in human visual cortex is modulated by visual motion strength. Cereb. Cortex 17, 732–741.

Struber, D., Rach, S., Trautmann-Lengsfeld, S.A., Engel, A.K., Herrmann, C.S., 2014. Antiphasic 40 Hz oscillatory current stimulation affects bistable motion perception. Brain Topogr. 27, 158–171.

Swettenham, J.B., Muthukumaraswamy, S.D., Singh, K.D., 2009. Spectral properties of induced and evoked gamma oscillations in human early visual cortex to moving and stationary stimuli. J. Neurophysiol. 102, 1241–1253.

Szurhaj, W., Labyt, E., Bourriez, J.L., Kahane, P., Chauvel, P., Mauguiere, F., Derambure, P., 2006. Relationship between intracerebral gamma oscillations and slow potentials in the human sensorimotor cortex. Eur. J. Neurosci. 24, 947–954.

Terney, D., Chaieb, L., Moliadze, V., Antal, A., Paulus, W., 2008. Increasing human brain excitability by transcranial high-frequency random noise stimulation. J. Neurosci. 28, 14147–14155.

Waldert, S., Preissl, H., Demandt, E., Braun, C., Birbaumer, N., Aertsen, A., Mehring, C., 2008. Hand movement direction decoded from MEG and EEG. J. Neurosci. 28, 1000–1008.

Williams, D., Tijssen, M., van Bruggen, G., Bosch, A., Insola, A., Di Lazzaro, V., Mazzone, P., Oliviero, A., Quartarone, A., Speelman, H., et al., 2002. Dopamine-dependent changes in the functional connectivity between basal ganglia and cerebral cortex in humans. Brain 125, 1558–1569.