Title

Contribution of the right temporoparietal junction and ventromedial prefrontal cortex to theory of mind in autism: A randomized, sham-controlled tDCS study

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List of abbreviations

tDCS = transcranial direct current stimulation
ASD = Autism spectrum disorder
ToM = Theory of Mind
TOMT = Theory of Mind Test
r-TPJ = right temporoparietal cortex
vmPFC = ventromedial prefrontal cortex
dlPFC = dorsolateral prefrontal cortex
PFC = prefrontal cortex

Abstract

Theory of Mind (ToM) is the ability to attribute subjective mental states to oneself and others and is significantly impaired in Autism Spectrum Disorder (ASD). A frontal-posterior network of regions including the ventromedial prefrontal cortex (vmPFC) and temporoparietal junction (TPJ) is involved in ToM. Previous studies show an underactivation of these regions in ASD. Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation method for causally investigating brain-behavior relationships via induction of cortical excitability alterations. tDCS, mostly over the dorsolateral prefrontal cortex, has been increasingly applied for improving behavioral problems in ASD leaving other potentially interesting regions untouched. Here we investigated the contribution of the vmPFC and right TPJ in ToM abilities of ASD children via tDCS in a pilot study. Sixteen children with ASD (mean age = 10.7±1.9) underwent three tDCS sessions (1 mA, 20 min) in a randomized, double-blind, sham-controlled design. Stimulation protocols included: i) anodal vmPFC tDCS, ii) anodal r-TPJ tDCS, and iii) sham tDCS. ToM abilities were explored during tDCS using the Theory of Mind Test (TOMT). Our results show that activation of the vmPFC with anodal tDCS significantly improved ToM in children with ASD compared to both, r-TPJ tDCS and sham stimulation. Specifically, precursors of ToM (e.g. emotion recognition, perception and imitation) and elementary ToM skills (e.g. first-order mental state reasoning) were significantly improved by anodal vmPFC tDCS. Based on these results, the vmPFC could be a potential target region for the reduction of ASD symptoms via non-invasive brain stimulation, which should be examined in larger detail in future studies.

Keywords: Theory of Mind (ToM); Autism Spectrum disorder; Ventromedial prefrontal cortex; Temporoparietal junction; Non-invasive brain stimulation; Transcranial direct current stimulation.

Lay abstract

Theory of mind (ToM) is the ability to infer mental states of oneself and others, which is impaired in autism. Brain imaging studies have shown the involvement of two brain regions in ToM (ventromedial prefrontal cortex, temporoparietal junction) which are underactivated in autism. We increased activation of these regions via non-invasive brain stimulation in this experiment to see how it would affect ToM abilities in autism. We found that increased activation of the ventromedial prefrontal cortex improved ToM abilities in children with autism.

1. Introduction

Autism spectrum disorder (ASD) is an early-appearing neurodevelopmental disorder characterized by core impairments in social interactions and the presence of repetitive, restrictive and stereotyped patterns of interests and behaviors (American Psychiatric Association, 2013). Impaired social cognition is a core deficit in ASD (Baron-Cohen, Golan, Chakrabarti, & Belmonte, 2008; Lord, Elsabbagh, Baird, & Veenstra-Vanderweele, 2018). Social cognition broadly refers to cognitive processes used to understand and store information about the self, other individuals, and interpersonal norms for effectively interacting in society. To do this, reading others' thoughts and beliefs in order to understand their mental state, an ability commonly known as Theory of Mind (ToM) or mentalizing (Van Overwalle, 2009), is required. ToM depends on several cognitive processes, including self-referential processing and the ability to handle mental representations of both, the self and other people (Sellaro, Nitsche, & Colzato, 2016). More accurately, ToM includes two separate systems that are involved in processing inferences about others' beliefs and intentions (i.e., cognitive ToM) and inferences about other people's emotions and feelings (i,e., affective ToM) (Shamay-Tsoory & Aharon-Peretz, 2007). Pervasive impairment of social interaction in ASD is suggested to be related to poor ToM abilities. Patients with ASD have difficulties in understanding or extracting motives and intentions of others, which impairs interaction with others (Bottema-Beutel, 2017).

ToM deficits in ASD are indeed related to a predominately executive dysfunction system and related brain regions (Salehinejad, Ghanavati, Rashid, & Nitsche, 2021). Neuroimaging studies have identified a frontal-posterior network activated during ToM tasks, including the medial prefrontal cortex (e.g. ventromedial prefrontal cortex-vmPFC, posterior cingulate cortex, and bilateral temporoparietal junction-TPJ) (Gallagher et al., 2000; Hiser & Koenigs, 2018; Van

Overwalle, 2009). More specifically, a distinction is also suggested for the cognitive vs affective ToM. Here, the vmPFC is more related to affective ToM while broader regions of the prefrontal cortex (PFC) are associated with cognitive ToM (Kalbe et al., 2010; Poletti, Enrici, & Adenzato, 2012; Shamay-Tsoory & Aharon-Peretz, 2007). Disruption of activity in these regions impairs ToM learning (Lev-Ran, Shamay-Tsoory, Zangen, & Levkovitz, 2012). In ASD, which is marked by poor ToM ability, neuroimaging studies have shown altered activation and connectivity of this frontal-posterior network, and specifically the vmPFC and TPJ (Kana et al., 2015; Nijhof, Bardi, Brass, & Wiersema, 2018; Yuk, Anagnostou, & Taylor, 2020). These regions, which are part of a prefrontal-cingular network, are highly engaged in ASD reciprocity abilities (Salehinejad et al., 2021). Such alternation of activity is in general accordance with the broader pathophysiology of ASD that results from early altered brain development and neural reorganization (Lord et al., 2018). Modulating activity of these regions might open up novel treatment approaches for improving behavioral and social deficits in ASD. With technological advances in cognitive neuroscience, it is possible to study brain-behavior relationships in ASD.

Non-invasive brain stimulation (NIBS) techniques are introduced as a novel approach for studying and modifying brain-behavior relationships (Polania, Nitsche, & Ruff, 2018), and improving behavioral and cognitive deficits rooted in functional brain abnormalities (e.g., Kuo, Paulus, & Nitsche, 2014; Alizadehgoradel et al., 2020; Molavi et al., 2020; Salehinejad, Ghanavai, Rostami, & Nejati, 2017). Transcranial direct current stimulation (tDCS) is a NIBS technique suited for modulating cortical excitability in target brain regions (Nitsche & Paulus, 2000). In tDCS, a weak electrical current is applied on the scalp and depending on the polarity of stimulation, it increases (i.e., anodal stimulation), or decreases cortical excitability (i.e., cathodal stimulation) with standard protocols (Nitsche & Paulus, 2000; Nitsche & Paulus, 2001). Accordingly, tDCS

has been increasingly used for modulating cortical excitability of the healthy brain, or in patients with cognitive and behavioral dysfunctions, to reveal its effect on psychological, and behavioral processes (Lefaucheur et al., 2017; Polania et al., 2018). TDCS has been also increasingly applied in neurodevelopmental disorders, including attention-deficit hyperactivity disorders (ADHD) (Salehinejad, Nejati, Mosayebi-Samani, et al., 2020; Salehinejad, Wischnewski, Nejati, Vicario, & Nitsche, 2019) and ASD (García-González et al., 2021). In ASD, most available studies applied repetitive tDCS sessions for improving behavioral symptoms and overall functioning with promising results. In these studies that aimed for improving behavioral and overall functioning in ASD, tDCS targeted primarily the dorsolateral prefrontal cortex (dlPFC), or frontocentral areas (Amatachaya et al., 2015; Costanzo et al., 2015; D'Urso et al., 2015; Hadoush, Nazzal, Almasri, Khalil, & Alafeef, 2020; Osório & Brunoni, 2019). However, tDCS has also the potential to study and modulate the physiological foundation of social cognition (Sellaro et al., 2016), and can be used as an exploratory method for studying the contribution of potentially interesting cortical regions relevant for respective ASD deficits, such as ToM. For social cognition and ToM, which are rarely explored in current tDCS studies in ASD, however, regions other than the dlPFC are relevant.

A pilot tDCS study paired with social skills interventions report promising effects for application of tDCS over the r-TPJ on cognitive abilities required for social and emotional skills of 6 adults with ASD (Wilson, Trumbo, Wilson, & Tesche, 2018). Participants (18–58 years) received active (2 mA, 30 min) and sham tDCS over the r-TPJ with the reference electrode placed over the ipsilateral deltoid. Social skill treatment, which included exposure to social interaction via videos, was combined with online stimulation. Participants performed a social skills questionnaire and a verbal fluency task after stimulation. The vmPFC is another region involved

in social cognition and ToM, which has not been targeted in the available tDCS studies. Importantly, targeting both vmPFC and r-TPJ has not been investigated so far in children with ASD. Such studies allow to understand the relative contribution of these two key regions in ToM and social cognition in ASD and come to specific conclusions about promising target areas to improve these functions. In this line, the purpose of the present study was to investigate the impact of activity modulation of the vmPFC and r-TPJ, as two major brain regions involved in ToM in ASD, on ToM ability in children with ASD. Both vmPFC and r-TPJ have been suggested as codependent regions in ToM in previous neuroimaging studies (Gallagher et al., 2000; Hiser & Koenigs, 2018; Van Overwalle, 2009) characterized by underactivation in ASD (Kana et al., 2015; Lombardo, Chakrabarti, Bullmore, & Baron-Cohen, 2011). Here, we specifically investigated the impact of increasing activity of the vmPFC and r-TPJ with anodal tDCS during performance of a ToM task. Based on previous neuroimaging studies showing underactivation of these brain regions, we hypothesized that the intervention would enhance ToM during anodal tDCS over both, the vmPFC and r-TPJ, compared to sham stimulation.

2. Methods

2.1. Participants

16 right-handed children (8 boys, mean age=10.07±1.9) diagnosed with autism were recruited from the Neurodevelopmental Clinic at Shahid Beheshti University. The required sample size was calculated a-priori based on a medium critical effect size suggested for tDCS studies (Minarik et al., 2016) (f=0.4, α=0.05, *power*=0.90, N=15). Two participants did not complete one experimental session due to intolerability of the burning sensation and the final analysis was conducted based on 14 participants (Fig. 1). All patients were clinically interviewed based on the

DSM-5 diagnostic criteria. Demographic information is shown in Table 1. The inclusion criteria were: (1) diagnosis of autism by a professional child psychiatrist and moderate to severe scores on the Gilliam Autism Rating Scale (GARS) (> 70), (2) no current or past history of epilepsy, seizures, and head injury, (3) 8-12 years old, (4) not being on risperidone and other CNS-active medications, and (3) no comorbidity with other neurodevelopmental disorders.

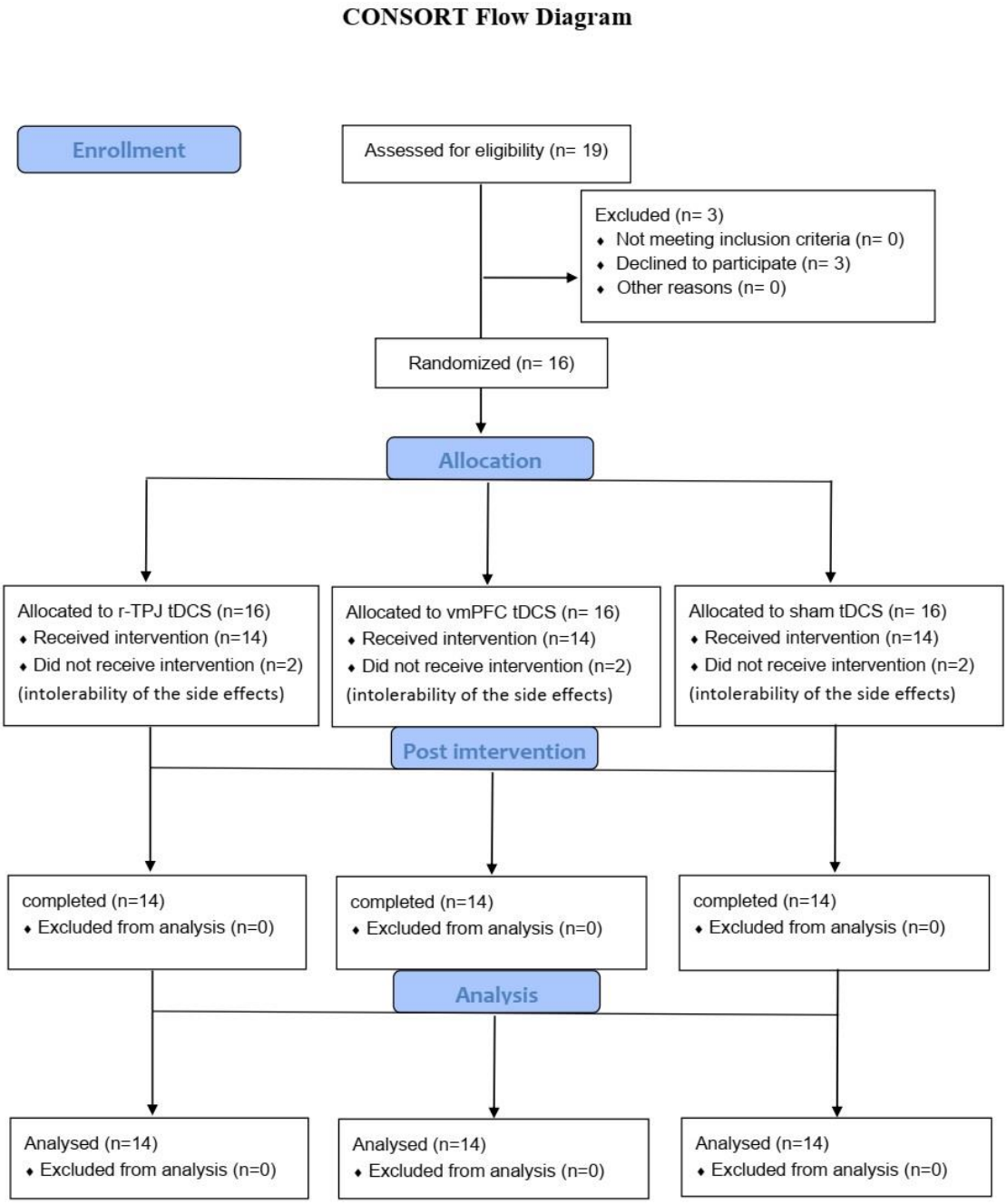

Fig 1. Flowchart of study inclusion

The study was performed according to the latest version of the Declaration of Helsinki ethical standards and approved by the Institutional Review Board of the Shahid Beheshti University. All patients' parents were instructed about the experimental procedures and gave their informed consent before participation.

2.2. Measures

Theory of Mind Test (TOMT): The TOMT is a 38-item test of ToM and is a reliable and valid test for measuring different aspects of ToM in children between 5-12 years. The test consists of vignettes, stories, and drawings about which the child has to answer a number of questions. TOMT conducted in an interview-like manner and has three subscales measuring 3 aspects of ToM: (1) precursors of ToM (e.g. perception and imitation, emotion recognition, pretence and physical-reality distinction) measured with 20 items, (b) first manifestations of ToM or elementary ToM (including first-order belief reasoning, understanding of false belief) measured by 13 items, and (c) more advanced aspects of ToM such as second-order belief, understanding of humour measured by 5 items (Muris et al., 1999). It is of note that the original version of the test has 72 items and here we used the 38-item version (Steerneman & Meesters, 2009) to fit online stimulation duration. The test includes several pictures and stories presented to children followed by a question with a 0 (failed) or 1 (passed) score and a higher total score indicates greater ToM knowledge. Score range of subscales 1 to 3, is 0-20, 0-13, and 0-5 respectively and the total score range is 0-38. Children are asked to look at a picture and/or listen to a story and answer the corresponding question. For example, "*Do as if you are scared*?" and "*How can I see that you are scared*?" represent ToM 1 and 3 respectively. A ToM 3 example is that the children is asked "*Why does the man say: "Wow, we have nice weather today!*" after being read a short story. The test

items start mostly with level 1 difficulty reaching higher levels. Cronbach's alpha of the original test version for the total scale, ToM 1, ToM 2, and ToM 3 is 0.92, 0.84, 0.86, and 0.85 respectively. We used a native language version of the test with Cronbach's alpha of 0.86 for the whole test, and 0.72, 0.80 and 0.81 for the three subscales respectively (Ghamarani, Alborzi, & Khayer, 2006). The concurrent validity of this version with a parallel test (e.g. Dolls House Task) is 0.89. The test performance takes about 15-20 min.

Side effect survey: At the end of each experimental session, the experimenter asked participants to rate potential tDCS side effects during stimulation. Given that realizing and rating tDCS common side effects (itching, tingling, burning, and pain) was not possible for children with ASD, we had to ask them to rate the overall "pleasantness" of the session on a 1-5 Likert-type scale with 1 indicating " quite pleasant" and 5" indicating "quite unpleasant".

2.3. tDCS

Direct current was delivered by an electrical stimulator (ActivaDose II Iontophoresis Delivery Unit, USA) with a 9-volt battery as current source. Electrical current was applied through a pair of saline-soaked (NaCl 0.9%) sponge electrodes with a size of 25 cm^2 (5 × 5cm) for 20 min with 30 s ramping up and 30 s ramping down. The current intensity was 1 mA. Electrode size was selected based on the smaller head size of children, as compared to adults. Three stimulation protocols were applied: (i) anodal stimulation over the r-TPJ (CP6), (ii) anodal stimulation over the vmPFC (Fpz), and (iii) sham stimulation. In all stimulation conditions, the reference electrode was positioned extracranially on the left shoulder, to selectively modulate target regions (i.e., r-TPJ, vmPFC) and avoid modulation of other cerebral regions (e.g. supraorbital area), which could interfere with cognitive and emotional processing. Electrode positions (CP6, Fpz) were chosen

according to the 10-20 International EEG System. In the sham condition, electrode placement resembled the vmPFC protocol (i.e., anodal Fpz, cathodal left shoulder). For sham stimulation, electrical current was ramped up for 30 s, followed by 15 s stimulation with the target intensity, then ramped down for 30 s, and switched off without the participants' knowledge to generate the same sensation as in the active condition (Gandiga, Hummel, & Cohen, 2006). The experimenter who set up tDCS was not blind to tDCS conditions (active or sham), but the ToM test was conducted by another independent experimenter who was not informed about the stimulation condition (active or sham).

2.4. Modeling of electrical current flow

A 3D model of the electric field (EF) distribution induced by tDCS was created via SimNIBS (version2.1.2) (Thielscher, Antunes, & Saturnino, 2015) to simulate distribution and amplitude of the electric field in the brain for the applied tDCS protocols (1.0 mA, 5×5 cm electrode size, anodal CP6 - cathodal left shoulder, anodal Fpz – cathodal left shoulder, 20 min). A realistic head model was created using T1- and T2-weighted average MRI templates of children with the mean age of 10 years, taken from the Neurodevelopmental MRI database (Richards, Sanchez, Phillips-Meek, & Xie, 2016). Two montages were simulated: a- one electrode over Fpz, according to 10-20 EEG system, and the return electrode over the left side of the neck to simulate the shoulder location; b- one electrode over CP6, with the return electrode placed at the same position as in the first montage. The calculated normEF numbers were converted to nii using the msh2nii command and exported to MATLAB (R2019a, version 9.6.0, The MathWorks Inc). Then, the average value of normEF strength was quantified in the two regions of interest, vmPFC and rTPJ (Mackey & Petrides, 2014; Mars et al., 2011).

2.5. Procedure

The experiment had a randomized, sham-controlled, cross-over design. Each subject participated in three tDCS sessions in between-subject randomized order with a 72 h interval between sessions. This between session interval was chosen based on previous studies (for a review see Salehinejad, Nejati, Mosayebi-Samani, et al., 2020) as well as findings from physiological studies showing that aftereffects of 1 mA tDCS in children do not last longer than several hours and the potential interference duration of aftereffects, if any, is up to 24 hr (Moliadze et al., 2015), while the interval in our study was at least 72 hours. Moreover, between-session intervals between 24 hr and 2 weeks do not appear to have a significant effect on observed cognitive effects (Dedoncker, Brunoni, Baeken, & Vanderhasselt, 2016). Children were instructed about the experimental procedures before the start of the experiment. After electrode placement, electrical stimulation began. Five minutes after the start of stimulation, participants started to conduct the TOMT while receiving electrical stimulation (online stimulation) (Fig. 2). TOMT took 5-10 min longer than stimulation. Thus, subjects conducted the task during, and after stimulation when stimulation aftereffects were still present. The reason for initiating tDCS 5 min before the start of the test was to accustom the children to sensations elicited by tDCS. During the first 5 min of stimulation in both, active and sham conditions, the children were watching an emotionally neutral animation video irrelevant to the task. The purpose to show the video during this 5 min was to keep the children relaxed and avoid movements, while they were sitting on a chair waiting for the ToM task to begin. Participants were blinded about the stimulation state, but not electrode position and the blinding efficacy (e.g., asking participants to guess whether they received real or sham stimulation) was not explored. In order to guarantee blindness of the experimenter, tDCS

setup was conducted by an independent care provider blinded to the study hypothesis. Another independent experimenter blinded to stimulation conditions then conducted the TOMT task.

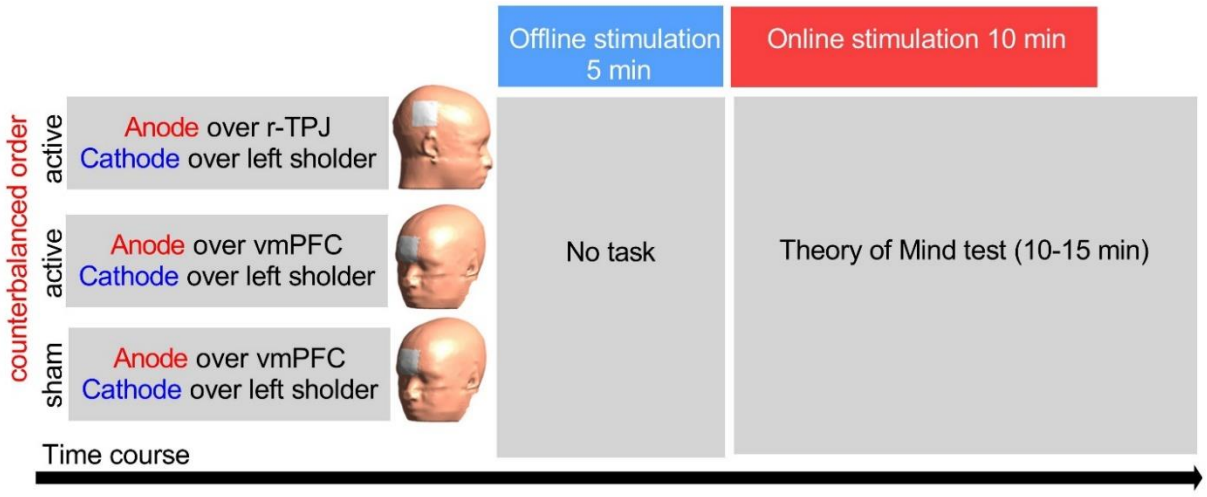

Fig. 2: The course of the study.

2.6.Statistical Analysis

All analyses were performed with IBM SPSS Statistics Version 26 for Windows. A 3×3 within-subject repeated-measures ANOVA was conducted for TOMT scores with stimulation (anodal r-TPJ, anodal vmPFC, sham) and ToM levels (subscale 1, subscale 2, subscale 3) as the within-subject factors. Additionally, a within-subject repeated-measures ANOVA was conducted for tDCS side effect evaluations with session (3 levels) as the within-subject factors. In order to examine the potential confounding effect of session order, we entered "*order*" as a covariate in separate analysis of covariances (ANCOVA) as well. The normality and homogeneity of variance of data collected at each time point were confirmed by Shapiro-Wilk and Levin tests, respectively. The Mauchly test was performed to test for sphericity violations, and the Greenhouse–Geisser correction was applied when necessary. Conditional on significant results of the ANOVAs, Bonferroni-corrected post *hoc* t-tests were performed for post *hoc* analysis. A critical *p*-value of < 0.05 was used for all statistical analyses.

3. Results

3.1. Data overview

Of the 16 patients initially recruited, two could not tolerate the burning sensation in one session and did not complete the study. The stimulation was well-tolerated in the remaining participants. Only mild adverse effects were reported during stimulation (Table 2). The results of the ANOVA showed no significant main effect of stimulation condition ($F_{1.80}$ = 2.16, p = 0.141) indicating that there was no significant difference in rating tolerability of stimulation across sessions (Table 2). The results of the ANCOVA of the order effect showed no significant effect of *session order* on ToM performance in the r-TPJ session (F_1 = 0.06, p = 0.800), anodal vmPFC session (F_1 = 3.03, p = 0.107), and sham stimulation session (F_1 = 1.05, p = 0.326). The mean ToM score measured with the TOMT is summarized in Table 2. The total ToM score was 18±5.88, 25.35±7.35, and 16.57±6.08 for the r-TPJ tDCS, vmPFC tDCS and sham tDCS. These scores can be compared with scores of typically developing 8-10 years old children measured with the same version of the test which ranged from 27.05-31±4.80-5.09 (Aliakbari et al., 2013).

3.2. Effects of tDCS on ToM

The results of the 3 × 3 ANOVA showed a significant interaction of tDCS × ToM ($F_{2.55}$ = 14.64, $p < 0.001$, ηp2 = 0.53) indicating that tDCS conditions had a discernable impact on different levels of ToM. Bonferroni corrected post *hoc* t-tests showed that anodal vmPFC tDCS significantly enhanced ToM 1 (t = 3.75, $p < 0.001$), and ToM 2 (t = 2.71, p = 0.011) but not ToM 3, compared to sham tDCS. Moreover, anodal vmPFC tDCS significantly enhanced ToM 1 (t = 2.68, p = 0.012) and ToM 2 (t = 2.74, p = 0.010) but not ToM 3, compared to anodal r-TPJ tDCS. Anodal r-TPJ tDCS did not significantly enhance ToM levels compared to sham tDCS (Fig. 3). The main effects

of ToM (F_2 = 193.67, p < 0.001, ηp2 = 0.93) and tDCS (F_2 = 40.38, p < 0.001, ηp2 = 0.75) were significant as well. Bonferroni-corrected post *hoc* t-tests for the main effect of ToM scores showed that children with ASD showed significantly larger ToM scores at level 1 compared to ToM 2 (t = 7.96, p < 0.001) and ToM 3 (t = 12.92, p < 0.001). ToM 2 was also significantly larger compared to ToM 3 (t = 5.09, p < 0.001). These data show that, regardless of stimulation condition, ToM was significantly better at levels 1 and 2 compared to level 3. For the main effect of tDCS, Bonferroni corrected post *hoc* t-tests showed that anodal tDCS over vmPFC significantly enhanced ToM total score (regardless of difficulty level) compared to both, r-TPJ tDCS (t = 2.92, p = 0.007) and sham stimulation (t = 3.44, p < 0.001).

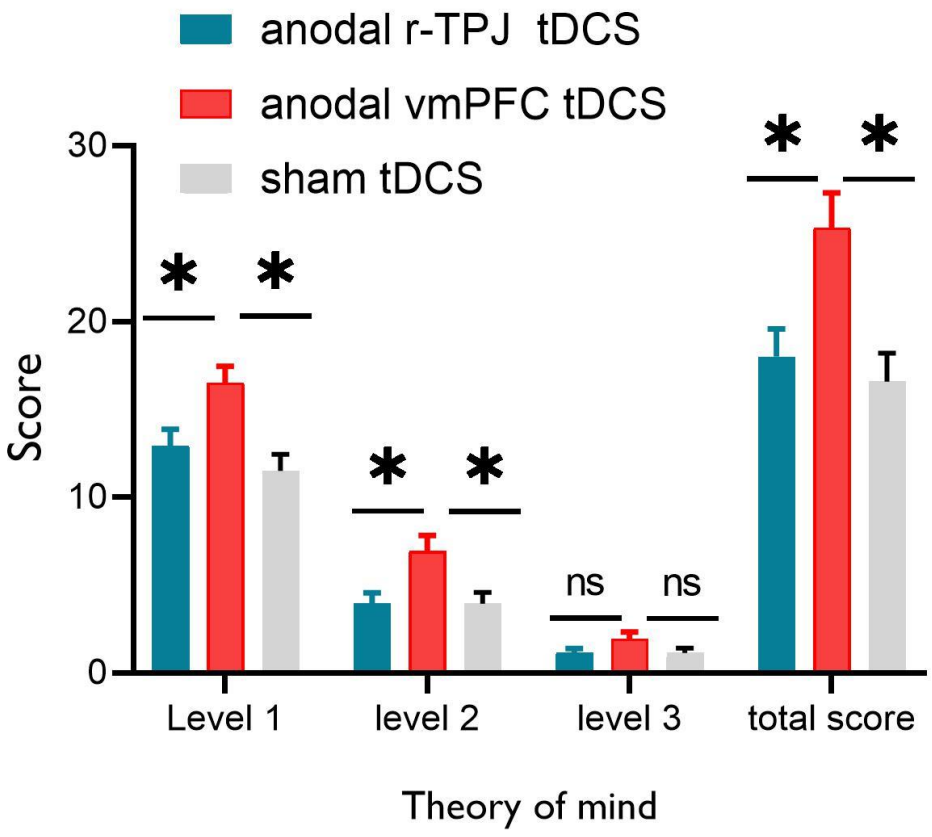

Fig. 3: ToM test performance for anodal r-TPJ tDCS, anodal vmPFC tDCS, and sham tDCS in children with autism (legend at the end).

3.3. *Modeling of the current flow in the head*

Results of modeling showed that the r-TPJ stimulation protocol (anodal CP6-cathodal neck) induced a mean electrical field of 0.3506 V/m within the r-TPJ, defined as Brodmann areas 5, 7, 39, and 40 (Fig. 4a,c). The vmPFC stimulation protocol (anodal FPz-cathodal neck) induced a mean electrical field of 0.2412 V/m within the vmPFC, defined as Brodmann areas 10, 11, and 32 (Fig. 4b,d). We also calculated the distribution of normEF in these regions when they were not the target regions. Anodal r-TPJ induced a mean electrical field of 0.0960 V/m within the vmPFC and anodal vmPFC stimulation induced a mean electrical field of 0.0789 V/m within the r-TPJ. These results indicate that maximum electrical field strength is induced in the target region of interest in each stimulation protocol, as compared to the respective target region of the other protocol. The results also show that an electrical field of 0.1883 and 0.1522 V/m was induced in the cerebellum and an electrical field of 0.2956 and 0.0951 V/m was induced in the inferior temporal cortex by the r-TPJ and vmPFC stimulation protocols, respectively.

[Figure 4 about here]

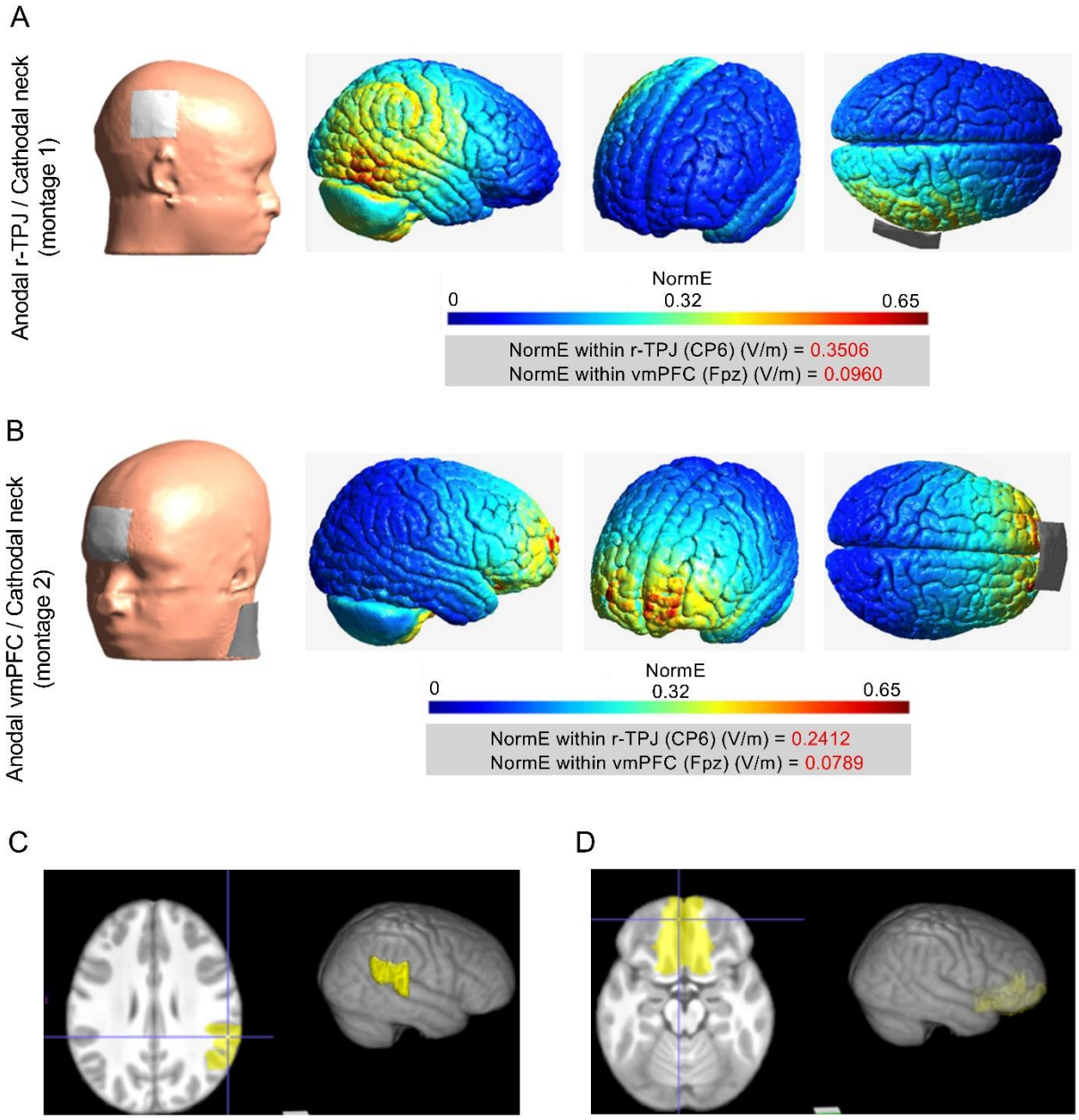

Fig. 4. Modeling of the electrical current flow in the head (legend at the end)

4. Discussion

Recent studies using tDCS in autism have found promising results in improving behavioral symptoms (García-González et al., 2021; Osório & Brunoni, 2019). ToM, as a core social deficit in autism, is a pivotal skill for effective social interactions and thus a promising target for treatment

purposes in autism which has not been explored with tDCS yet. The purpose of this study was to determine the impact of enhancing activity of the r-TPJ and vmPFC with anodal tDCS on ToM of children with autism. We found that anodal vmPFC tDCS significantly improved overall ToM in participants compared to both, anodal r-TPJ and sham stimulation. Specifically, ToM at levels 1 and 2 was significantly enhanced by activation of the vmPFC.

These findings primarily suggest that vmPFC activation, compared to r-TPJ activation, seems to be more involved in reading mental states and social cognition in autism. Previous neuroimaging studies have repeatedly shown that a frontal-posterior network is activated during ToM and social cognition, including the medial PFC, posterior cingulate cortex and TPJ (Barch et al., 2013; Boccadoro et al., 2019; Gallagher et al., 2000; Salehinejad et al., 2021; Van Overwalle, 2009). In ASD, these regions are usually underactive compared to healthy controls (Kana et al., 2015) implicating that increasing their activity could enhance ToM ability. Our results support this assumption by showing that increased activation of the vmPFC, a core region of the medial PFC, significantly enhances the ToM task performance. Involvement of the vmPFC in ToM originates from its role in social cognition, which is specifically impaired in ASD. In accordance, vmPFC lesions are associated with deficits in empathy (Shamay-Tsoory, Aharon-Peretz, & Perry, 2009), impaired facial emotion recognition (Tsuchida & Fellows, 2012), and reduced visual attention to the eye region of the face (Wolf, Pujara, Baskaya, & Koenigs, 2016), which are also significantly impaired in ASD. Another reason for the significant impact of vmPFC activation on ToM in our sample might be its pivotal role in multiple aspects of mental health and psychological functioning. This includes emotion regulation, moral sensitivity, self-reflection / rumination, value-based decision making and fear response (Hiser & Koenigs, 2018; Marković, Vicario, Yavari, Salehinejad, & Nitsche, 2021) which are impaired in ASD as well. With these transdiagnostic

functional domains, the vmPFC is thus an interesting anatomical target for interventions, including tDCS (Hiser & Koenigs, 2018), and it is reasonable to assume that the observed effects are at least partially due to its multifolded role in the psychopathology of ASD.

While increased activation of the vmPFC improved ToM in children with ASD, anodal tDCS over the r-TPJ did not significantly enhance performance. This finding is similar to those of the study of Wilson et al. (2018), which describes that tDCS over the r-TPJ combined with social skills intervention did not improve scores on a social skills questionnaire, although verbal fluency was significantly improved after active stimulation. Several reasons might account for this observation. First, although both vmPFC and r-TPJ are involved in social cognition, they differ in specific functions. The vmPFC is engaged in a broad range of social-processing tasks, involving other- and self-referential processing (Northoff & Bermpohl, 2004; Salehinejad, Nejati, & Nitsche, 2020; Spreng & Andrews-Hanna, 2015) as well as emotional processing (Nejati, Majdi, Salehinejad, & Nitsche, 2021; Winecoff et al., 2013), while the r-TPJ has been suggested to play a particular role in reflecting on the beliefs of other people (Spreng & Andrews-Hanna, 2015). These broader functions of the vmPFC and medial prefrontal regions might explain the discernable effects of stimulation of the respective regions. Second, ToM consists of two separate systems of cognitive ToM and affective ToM, which are associated with activation of the extensive PFC vs vmPFC respectively (Shamay-Tsoory & Aharon-Peretz, 2007). On the other hand, the results of the electrical filed modeling showed that extensive regions in the PFC, beyond the vmPFC, were modulated as a result of vmPFC tDCS, which was not the case for the r-TPJ tDCS. It is thus very likely that both cognitive and affective elements of ToM are affected by the vmPFC tDCS, yielding significant behavioral results. Moreover, previous studies have shown that both, the right and left TPJ are underactivated in ASD (Kana et al., 2015). In this study, we only targeted the r-TPJ, which

might not sufficiently modulate the brain network involved in ToM. Moreover, r-TPJ stimulation could have had even an inhibitory effect on the left TPJ due to transcallosal inhibition. Although such transcallosal inhibition was not observed directly for tDCS-induced aftereffects in the primary motor cortex (Lang, Nitsche, Paulus, Rothwell, & Lemon, 2004; Tazoe, Endoh, Kitamura, & Ogata, 2014), this might differ for non-motor areas, especially in clinical conditions, as suggested in a recent ADHD study (Soltaninejad, Nejati, & Ekhtiari, 2015). Nevertheless, this hypothesis should be examined by systematically targeting unilateral and bilateral TPJ with both anodal and cathodal tDCS in future studies.

Finally, we need to consider the possibility that the stimulation protocol with an extracranial reference electrode applied in the present study might have been not optimal for modulation of the r-TPJ and thus the stimulation effects cannot be merely attributed to this specific region. The reason we picked an extracranial reference electrode was to selectively target the regions of interest, however, some studies have shown that remotely placed return electrodes can reduce stimulation efficacy (Moliadze, Antal, & Paulus, 2010) and induce different electrical fields compared to when the reference electrode is placed on a cortical region (Salehinejad, Nejati, Mosayebi-Samani, et al., 2020). Specifically, placing the reference electrode cranially or extracranially at specific positions will affect the direction of electrical fields which is important for modulation of excitability and tDCS after-effects (Rawji et al., 2018). This could partially explain the observed non-significant effects of r-TPJ stimulation in the present study. Appling more focal stimulation protocols (e.g., 4×1 ring-like electrode arrangements), or systematically comparing different electrode positions, as done for the motor cortex before (Nitsche & Paulus 2000) in future studies would allow identifying optimized r-TPJ stimulation protocols, as compared to the conventional protocol used in our study, and to explore if these protocols generate

stronger behavioral results. This is important because the results of our study should be considered with respect to widespread electrical fields in brain regions not limited to the location of the scalp electrodes over r-TPJ and vmPFC. These preliminary data, however, can be used for futures studies to investigate the impact of selective stimulation of these regions by using more optimized/focal stimulation protocols.

In this line, there are some aspects to be discussed about the results of the 3D modeling. First, each stimulation protocol induced cross-sectional electrical fields in the non-target regions (i.e., r-TPJ stimulation-induced electrical field in the vmPFC and vice versa), but importantly the maximum electrical field in each protocol was induced in the target region. Although the electrical field induced in the non-target region could have an effect, the size of the effects does not seem to be sufficient to explain our observed effects. Second, the results of modeling also suggest physiologically and behaviorally relevant electrical field strengths, although weaker as compared to the target regions, in other regions relevant for social cognition in autism. This shows that the stimulation effects cannot be attributed to the specific regions targeted in our study (i.e., VMPFC, r-TPJ). One such region was the cerebellum, for which a relevance for social cognition in addition to its traditional role in motor behavior has been recently described (Van Overwalle et al., 2020). It is known that specific cerebellar zones that are important for social interaction have sensitive-period disruptions in autism (Wang, Kloth, & Badura, 2014). Although model-based electrical field strength in the cerebellum showed a relatively similar strength in both protocols, stimulation with the vmPFC target electrode was more efficient, which would not fit well with a dominant cerebellar impact. Moreover, for the cerebellar activation, the placement of the return electrode over the neck – which does not align with the real intervention condition– might have resulted in artificially strong cerebellar electrical fields in the modeling. Another region that was affected by

the current protocol, based on modeling results especially by r-TPJ stimulation was the inferior temporal cortex. Considering that the inferior temporal cortex has an important role in face perception in autism and that the ToM task used in the present study was not based on facial expression, it is not likely that this activation had a significant effect on behavior, as shown in the results.

Our results also imply that improved ToM as a result of intervention depended on the difficulty level of the ToM task. The precursors of ToM include perception and imitation, emotion recognition, pretence and physical-reality distinction, and elementary ToM involves stories about first-order belief reasoning and false belief understanding. The advanced ToM, on the other hand, involves situations related to understanding of second-order belief and complex humor. Our results show that anodal vmPFC tDCS only improved precursors of ToM and elementary ToM, but had no significant improving effect on advanced ToM. Improved ToM total score after the vmPFC stimulation is close to average score of typically developing children with a similar age range (8-10 years old) on this test performance (Aliakbari et al., 2013). Specifically, ToM total score during vmPFC tDCS was around 3 units lower than the average of 8-8.5 years old healthy control and 6 units lower than 8.6-9.6 years old children. It is important to consider that these findings do not imply that ToM abilities are shaped directly as a result of single-session tDCS. Instead, ToM is a cognitive ability that depends on several processes related to social cognition (e.g., attention to social stimuli) and these cognitive processes are related to specific activities of brain regions. We hypothesize that modulating the activity of these regions affects ToM performance via an impact on more general cognitive performance parameters. No tDCS study is so far available testing its application and efficacy for improving ToM in ASD. These results, however, are comparable with

those of previous studies that applied ToM training (Begeer et al., 2011) in which ToM training improved conceptual ToM skills in children with ASD.

Some limitations of this study should be taken into account. First, studies with larger sample sizes with follow-up measurements are needed to obtain information about the stability of outcomes. Second, measuring emotional recognition by other behavioral tasks, as well as other domains of cognitive deficits in ASD, would provide a more complete measure of abilities related to ToM. We could however not add more behavioral tasks in the present study because our purpose was to investigate the *online* effect of tDCS on cognitive performance. In this connection, TOMT is already a demanding and lengthy task. Adding more tasks to this protocol would have made the required stimulation duration relevantly longer, which might have induced non-linear effects of tDCS (Hassanzahraee, Nitsche, Zoghi, & Jaberzadeh, 2020; Monte-Silva et al., 2013), and thus compromised interpretability of the results. To clarify these above-mentioned relevant aspects, future studies with more objective and behavioral measures of social cognition would be required. Lastly, although we applied an extracranial reference electrode to prevent involvement of other cortical regions and used 25 cm^2 electrodes, which might induce less diffuse electrical fields over the target regions than conventional larger electrodes, electrical fields were not completely restricted to the vmPFC and r-TPJ. Accordingly, additional cortical and subcortical areas might have been affected, especially in our participants with relatively small head sizes as suggested by the results of the electrical field modeling. To address this, future studies are suggested to use stimulation protocols that allow more focal stimulation (e.g., 4×1 ring-like electrode arrangements). This highlights the importance of optimizing stimulation protocols in future studies. Examining blinding efficacy and also blinding of electrode placement that may generate different somatosensory feelings, are also highly recommended in future studies especially in high-

functional children with ASD. Nonetheless, as the same electrode placement was applied for the sham condition and vmPFC protocol, this potential confounding effect for the main important electrode position that induced significant results can be ruled out.

In sum, the results of the present study show that vmPFC activation, compared to r-TPJ, contributes to a larger degree to understanding and resolving ToM problems in ASD. One implication of this result is to consider vmPFC as a target region in NIBS studies in ASD. The majority of tDCS studies in ASD so far have targeted the dlPFC or frontocentral regions for improving behavioral problems in ASD. Based on the results of neuroimaging studies and the results of the present study, medial PFC regions, including the vmPFC, are interesting target regions in ASD. The missing effects of r-TPJ stimulation in the present study do not mean that this region is not important for ToM in ASD. It might be the case that activating both TPJs with an optimal stimulation protocol or concurrent activation of vmPFC and TPJ results in ToM improvement, which is an open question for future studies. Future randomized controlled studies, with larger sample size and application of repeated stimulation, are needed to evaluate the clinical efficacy of vmPFC tDCS in ASD.

Declarations

Ethics approval and consent to participate

All participants' parents were instructed about experimental procedures and gave their ***written*** consent to participate in the study. The protocol was conducted in accordance with the latest version of the Declaration of Helsinki and was approved by the Institutional Review Board and ethical committee at Shahid Beheshti University.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest

MAN is a member of the Scientific Advisory Board of Neuroelectrics and NeuroDevic. All other authors declare no competing interests.

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None

Authors' Credit contributions

MAS: Conceptualization, Formal analysis, Visualization, Writing - Original Draft, Writing - Review & Editing. **NP** & **AHH**: Investigation, Data Curation, Validation. **FY**: Visualization (3D modeling), Writing - Review & Editing. **MAN** & **CV**: Writing - Review & Editing. **VN**: Conceptualization, Methodology, Resources, Supervision, Project administration.

References

Aliakbari, M., Juibari, A. K., Amirabadi, F., Shaghaghi, F., Zare, N., & Khaleghi, F. (2013). The role of Theory of Mind and executive function in predicting empathy. *Advances in Cognitive Science*.

Alizadehgoradel, J., Nejati, V., Sadeghi Movahed, F., Imani, S., Taherifard, M., Mosayebi-Samani, M., . . . Salehinejad, M. A. (2020). Repeated stimulation of the dorsolateral-prefrontal cortex improves executive dysfunctions and craving in drug addiction: A randomized, double-blind, parallel-group study. *Brain Stimulation, 13*(3), 582-593. doi:https://doi.org/10.1016/j.brs.2019.12.028

Amatachaya, A., Jensen, M. P., Patjanasoontorn, N., Auvichayapat, N., Suphakunpinyo, C., Janjarasjitt, S., . . . Auvichayapat, P. (2015). The short-term effects of transcranial direct current stimulation on electroencephalography in children with autism: a randomized crossover controlled trial. *Behavioural Neurology, 2015*.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (DSM-5®)*: American Psychiatric Pub.

Barch, D. M., Burgess, G. C., Harms, M. P., Petersen, S. E., Schlaggar, B. L., Corbetta, M., . . . Van Essen, D. C. (2013). Function in the human connectome: Task-fMRI and individual differences in behavior. *NeuroImage, 80*, 169-189. doi:https://doi.org/10.1016/j.neuroimage.2013.05.033

Baron-Cohen, S., Golan, O., Chakrabarti, B., & Belmonte, M. K. (2008). Social cognition and autism spectrum conditions *Social cognition and developmental psychopathology.* (pp. 29-56). New York, NY, US: Oxford University Press.

Begeer, S., Gevers, C., Clifford, P., Verhoeve, M., Kat, K., Hoddenbach, E., & Boer, F. (2011). Theory of Mind Training in Children with Autism: A Randomized Controlled Trial. *Journal of Autism and Developmental Disorders, 41*(8), 997-1006. doi:10.1007/s10803-010-1121-9

Boccadoro, S., Cracco, E., Hudson, A. R., Bardi, L., Nijhof, A. D., Wiersema, J. R., . . . Mueller, S. C. (2019). Defining the neural correlates of spontaneous theory of mind (ToM): An fMRI multi-study investigation. *NeuroImage, 203*, 116193. doi:https://doi.org/10.1016/j.neuroimage.2019.116193

Bottema-Beutel, K. (2017). Glimpses into the blind spot: Social interaction and autism. *Journal of Communication Disorders, 68*, 24-34. doi:https://doi.org/10.1016/j.jcomdis.2017.06.008

Costanzo, F., Menghini, D., Casula, L., Amendola, A., Mazzone, L., Valeri, G., & Vicari, S. (2015). Transcranial Direct Current Stimulation Treatment in an Adolescent with Autism and Drug-Resistant Catatonia. *Brain Stimulation, 8*(6), 1233-1235. doi:https://doi.org/10.1016/j.brs.2015.08.009

D'Urso, G., Bruzzese, D., Ferrucci, R., Priori, A., Pascotto, A., Galderisi, S., . . . Bravaccio, C. (2015). Transcranial direct current stimulation for hyperactivity and noncompliance in autistic disorder. *The World Journal of Biological Psychiatry, 16*(5), 361-366. doi:10.3109/15622975.2015.1014411

Dedoncker, J., Brunoni, A. R., Baeken, C., & Vanderhasselt, M.-A. (2016). The effect of the interval-between-sessions on prefrontal transcranial direct current stimulation (tDCS) on cognitive outcomes: a systematic review and meta-analysis. *Journal of Neural Transmission, 123*(10), 1159-1172. doi:10.1007/s00702-016-1558-x

Gallagher, H. L., Happé, F., Brunswick, N., Fletcher, P. C., Frith, U., & Frith, C. D. (2000). Reading the mind in cartoons and stories: an fMRI study of 'theory of mind' in verbal and nonverbal tasks. *Neuropsychologia, 38*(1), 11-21. doi:https://doi.org/10.1016/S0028-3932(99)00053-6

Gandiga, P. C., Hummel, F. C., & Cohen, L. G. (2006). Transcranial DC stimulation (tDCS): A tool for double-blind sham-controlled clinical studies in brain stimulation. *Clinical Neurophysiology, 117*(4), 845-850. doi:https://doi.org/10.1016/j.clinph.2005.12.003

Ganho-Ávila, A., Gonçalves, Ó. F., Guiomar, R., Boggio, P. S., Asthana, M. K., Krypotos, A.-M., & Almeida, J. (2019). The effect of cathodal tDCS on fear extinction: A cross-measures study. *PLoS ONE, 14*(9), e0221282. doi:10.1371/journal.pone.0221282

García-González, S., Lugo-Marín, J., Setien-Ramos, I., Gisbert-Gustemps, L., Arteaga-Henríquez, G., Díez-Villoria, E., & Ramos-Quiroga, J. A. (2021). Transcranial direct current stimulation in Autism Spectrum Disorder: A systematic review and meta-analysis. *European Neuropsychopharmacology*. doi:https://doi.org/10.1016/j.euroneuro.2021.02.017

Ghamarani, A., Alborzi, S., & Khayer, M. (2006). VALIDITY AND RELIABILITY OF THE THEORY OF MIND TEST (TOM TEST) FOR USE IN IRAN. *Journal of Psychology, 10*(2 (38)), -.

Hadoush, H., Nazzal, M., Almasri, N. A., Khalil, H., & Alafeef, M. (2020). Therapeutic Effects of Bilateral Anodal Transcranial Direct Current Stimulation on Prefrontal and Motor Cortical Areas in Children with Autism Spectrum Disorders: A Pilot Study. *Autism Research, 13*(5), 828-836. doi:10.1002/aur.2290

Hassanzahraee, M., Nitsche, M. A., Zoghi, M., & Jaberzadeh, S. (2020). Determination of anodal tDCS duration threshold for reversal of corticospinal excitability: An investigation for induction of counter-regulatory mechanisms. *Brain Stimulation, 13*(3), 832-839. doi:https://doi.org/10.1016/j.brs.2020.02.027

Hiser, J., & Koenigs, M. (2018). The Multifaceted Role of the Ventromedial Prefrontal Cortex in Emotion, Decision Making, Social Cognition, and Psychopathology. *Biological Psychiatry, 83*(8), 638-647. doi:10.1016/j.biopsych.2017.10.030

Kalbe, E., Schlegel, M., Sack, A. T., Nowak, D. A., Dafotakis, M., Bangard, C., . . . Kessler, J. (2010). Dissociating cognitive from affective theory of mind: A TMS study. *Cortex, 46*(6), 769-780. doi:https://doi.org/10.1016/j.cortex.2009.07.010

Kana, R. K., Maximo, J. O., Williams, D. L., Keller, T. A., Schipul, S. E., Cherkassky, V. L., . . . Just, M. A. (2015). Aberrant functioning of the theory-of-mind network in children and adolescents with autism. *Molecular Autism, 6*(1), 59. doi:10.1186/s13229-015-0052-x

Kuo, M.-F., Paulus, W., & Nitsche, M. A. (2014). Therapeutic effects of non-invasive brain stimulation with direct currents (tDCS) in neuropsychiatric diseases. *NeuroImage, 85*, 948-960. doi:https://doi.org/10.1016/j.neuroimage.2013.05.117

Lang, N., Nitsche, M. A., Paulus, W., Rothwell, J. C., & Lemon, R. N. (2004). Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. *Experimental Brain Research, 156*(4), 439-443. doi:10.1007/s00221-003-1800-2

Lefaucheur, J.-P., Antal, A., Ayache, S. S., Benninger, D. H., Brunelin, J., Cogiamanian, F., . . . Paulus, W. (2017). Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). *Clinical Neurophysiology, 128*(1), 56-92. doi:http://dx.doi.org/10.1016/j.clinph.2016.10.087

Lev-Ran, S., Shamay-Tsoory, S. G., Zangen, A., & Levkovitz, Y. (2012). Transcranial magnetic stimulation of the ventromedial prefrontal cortex impairs theory of mind learning. *European Psychiatry, 27*(4), 285-289. doi:https://doi.org/10.1016/j.eurpsy.2010.11.008

Lombardo, M. V., Chakrabarti, B., Bullmore, E. T., & Baron-Cohen, S. (2011). Specialization of right temporo-parietal junction for mentalizing and its relation to social impairments in autism. *NeuroImage, 56*(3), 1832-1838. doi:https://doi.org/10.1016/j.neuroimage.2011.02.067

Lord, C., Elsabbagh, M., Baird, G., & Veenstra-Vanderweele, J. (2018). Autism spectrum disorder. *The Lancet, 392*(10146), 508-520. doi:https://doi.org/10.1016/S0140-6736(18)31129-2

Mackey, S., & Petrides, M. (2014). Architecture and morphology of the human ventromedial prefrontal cortex. *European Journal of Neuroscience, 40*(5), 2777-2796. doi:10.1111/ejn.12654

Marković, V., Vicario, C. M., Yavari, F., Salehinejad, M. A., & Nitsche, M. A. (2021). A Systematic Review on the Effect of Transcranial Direct Current and Magnetic Stimulation on Fear Memory and Extinction. *Frontiers in Human Neuroscience, 15*(138), 1-26. doi:10.3389/fnhum.2021.655947

Mars, R. B., Sallet, J., Schüffelgen, U., Jbabdi, S., Toni, I., & Rushworth, M. F. S. (2011). Connectivity-Based Subdivisions of the Human Right "Temporoparietal Junction Area": Evidence for Different Areas Participating in Different Cortical Networks. *Cerebral Cortex, 22*(8), 1894-1903. doi:10.1093/cercor/bhr268

Minarik, T., Berger, B., Althaus, L., Bader, V., Biebl, B., Brotzeller, F., . . . Sauseng, P. (2016). The Importance of Sample Size for Reproducibility of tDCS Effects. *Frontiers in Human Neuroscience, 10*(453). doi:10.3389/fnhum.2016.00453

Molavi, P., Aziziaram, S., Basharpoor, S., Atadokht, A., Nitsche, M. A., & Salehinejad, M. A. (2020). Repeated transcranial direct current stimulation of dorsolateral-prefrontal cortex improves executive functions, cognitive reappraisal emotion regulation, and control over emotional processing in borderline personality disorder: A randomized, sham-controlled, parallel-group study. *Journal of Affective Disorders, 274*, 93-102. doi:https://doi.org/10.1016/j.jad.2020.05.007

Moliadze, V., Antal, A., & Paulus, W. (2010). Electrode-distance dependent after-effects of transcranial direct and random noise stimulation with extracephalic reference electrodes. *Clinical Neurophysiology, 121*(12), 2165-2171. doi:https://doi.org/10.1016/j.clinph.2010.04.033

Moliadze, V., Schmanke, T., Andreas, S., Lyzhko, E., Freitag, C. M., & Siniatchkin, M. (2015). Stimulation intensities of transcranial direct current stimulation have to be adjusted in children and adolescents. *Clinical Neurophysiology, 126*(7), 1392-1399. doi:https://doi.org/10.1016/j.clinph.2014.10.142

Monte-Silva, K., Kuo, M.-F., Hessenthaler, S., Fresnoza, S., Liebetanz, D., Paulus, W., & Nitsche, M. A. (2013). Induction of Late LTP-Like Plasticity in the Human Motor Cortex by Repeated Non-Invasive Brain Stimulation. *Brain Stimulation, 6*(3), 424-432. doi:https://doi.org/10.1016/j.brs.2012.04.011

Muris, P., Steerneman, P., Meesters, C., Merckelbach, H., Horselenberg, R., van den Hogen, T., & van Dongen, L. (1999). The TOM Test: A New Instrument For Assessing Theory of Mind in Normal Children and Children with Pervasive Developmental Disorders. *Journal of Autism and Developmental Disorders, 29*(1), 67-80. doi:10.1023/A:1025922717020

Nejati, V., Majdi, R., Salehinejad, M. A., & Nitsche, M. A. (2021). The role of dorsolateral and ventromedial prefrontal cortex in the processing of emotional dimensions. *Scientific Reports, 11*(1), 1971. doi:10.1038/s41598-021-81454-7

Nijhof, A. D., Bardi, L., Brass, M., & Wiersema, J. R. (2018). Brain activity for spontaneous and explicit mentalizing in adults with autism spectrum disorder: An fMRI study. *NeuroImage: Clinical, 18*, 475-484. doi:https://doi.org/10.1016/j.nicl.2018.02.016

Nitsche, M., & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of Physiology, 527*(3), 633-639. doi:10.1111/j.1469-7793.2000.t01-1-00633.x

Nitsche, M., & Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology, 57*(10), 1899-1901.

Northoff, G., & Bermpohl, F. (2004). Cortical midline structures and the self. *Trends in Cognitive Sciences, 8*(3), 102-107. doi:http://dx.doi.org/10.1016/j.tics.2004.01.004

Osório, A. A. C., & Brunoni, A. R. (2019). Transcranial direct current stimulation in children with autism spectrum disorder: a systematic scoping review. *Developmental Medicine and Child Neurology, 61*(3), 298-304. doi:10.1111/dmcn.14104

Polania, R., Nitsche, M. A., & Ruff, C. C. (2018). Studying and modifying brain function with non-invasive brain stimulation. *Nature Neuroscience, 21*(2), 174-187.

Poletti, M., Enrici, I., & Adenzato, M. (2012). Cognitive and affective Theory of Mind in neurodegenerative diseases: Neuropsychological, neuroanatomical and neurochemical levels. *Neuroscience & Biobehavioral Reviews, 36*(9), 2147-2164. doi:https://doi.org/10.1016/j.neubiorev.2012.07.004

Rawji, V., Ciocca, M., Zacharia, A., Soares, D., Truong, D., Bikson, M., . . . Bestmann, S. (2018). tDCS changes in motor excitability are specific to orientation of current flow. *Brain Stimulation, 11*(2), 289-298. doi:https://doi.org/10.1016/j.brs.2017.11.001

Richards, J. E., Sanchez, C., Phillips-Meek, M., & Xie, W. (2016). A database of age-appropriate average MRI templates. *NeuroImage, 124*, 1254-1259. doi:https://doi.org/10.1016/j.neuroimage.2015.04.055

Salehinejad, M. A., Ghanavai, E., Rostami, R., & Nejati, V. (2017). Cognitive control dysfunction in emotion dysregulation and psychopathology of major depression (MD): Evidence from transcranial brain stimulation of the dorsolateral prefrontal cortex (DLPFC). *Journal of Affective Disorders, 210*, 241-248. doi:http://dx.doi.org/10.1016/j.jad.2016.12.036

Salehinejad, M. A., Ghanavati, E., Rashid, M. H. A., & Nitsche, M. A. (2021). Hot and cold executive functions in the brain: A prefrontal-cingular network. *Brain and Neuroscience Advances, 5*, 1-19. doi:10.1177/23982128211007769

Salehinejad, M. A., Nejati, V., Mosayebi-Samani, M., Mohammadi, A., Wischnewski, M., Kuo, M.-F., . . . Nitsche, M. A. (2020). Transcranial Direct Current Stimulation in ADHD: A Systematic Review of Efficacy, Safety, and Protocol-induced Electrical Field Modeling Results. *Neuroscience Bulletin, 36*(10), 1191-1212. doi:10.1007/s12264-020-00501-x

Salehinejad, M. A., Nejati, V., & Nitsche, M. A. (2020). Neurocognitive correlates of self-esteem: From self-related attentional bias to involvement of the ventromedial prefrontal cortex. *Neuroscience Research, 161*, 33-43. doi:https://doi.org/10.1016/j.neures.2019.12.008

Salehinejad, M. A., Wischnewski, M., Nejati, V., Vicario, C. M., & Nitsche, M. A. (2019). Transcranial direct current stimulation in attention-deficit hyperactivity disorder: A meta-analysis of neuropsychological deficits. *PLoS ONE, 14*(4), e0215095. doi:10.1371/journal.pone.0215095

Sellaro, R., Nitsche, M. A., & Colzato, L. S. (2016). The stimulated social brain: effects of transcranial direct current stimulation on social cognition. *Annals of the New York Academy of Sciences, 1369*(1), 218-239. doi:10.1111/nyas.13098

Shamay-Tsoory, S. G., & Aharon-Peretz, J. (2007). Dissociable prefrontal networks for cognitive and affective theory of mind: A lesion study. *Neuropsychologia, 45*(13), 3054-3067. doi:https://doi.org/10.1016/j.neuropsychologia.2007.05.021

Shamay-Tsoory, S. G., Aharon-Peretz, J., & Perry, D. (2009). Two systems for empathy: a double dissociation between emotional and cognitive empathy in inferior frontal gyrus versus ventromedial prefrontal lesions. *Brain, 132*(3), 617-627. doi:10.1093/brain/awn279

Soltaninejad, Z., Nejati, V., & Ekhtiari, H. (2015). Effect of Anodal and Cathodal Transcranial Direct Current Stimulation on DLPFC on Modulation of Inhibitory Control in ADHD. *Journal of Attention Disorders*. doi:10.1177/1087054715618792

Spreng, R. N., & Andrews-Hanna, J. R. (2015). The Default Network and Social Cognition. In A. W. Toga (Ed.), *Brain Mapping* (pp. 165-169). Waltham: Academic Press.

Steerneman, P., & Meesters, C. (2009). ToM test-R: Handleiding.

Tazoe, T., Endoh, T., Kitamura, T., & Ogata, T. (2014). Polarity Specific Effects of Transcranial Direct Current Stimulation on Interhemispheric Inhibition. *PLoS ONE, 9*(12), e114244. doi:10.1371/journal.pone.0114244

Thielscher, A., Antunes, A., & Saturnino, G. B. (2015, 25-29 Aug. 2015). *Field modeling for transcranial magnetic stimulation: A useful tool to understand the physiological effects of TMS?* Paper

presented at the 2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC).

Tsuchida, A., & Fellows, L. K. (2012). Are You Upset? Distinct Roles for Orbitofrontal and Lateral Prefrontal Cortex in Detecting and Distinguishing Facial Expressions of Emotion. *Cerebral Cortex, 22*(12), 2904-2912. doi:10.1093/cercor/bhr370

Van Overwalle, F. (2009). Social cognition and the brain: A meta-analysis. *Human Brain Mapping, 30*(3), 829-858. doi:10.1002/hbm.20547

Van Overwalle, F., Manto, M., Cattaneo, Z., Clausi, S., Ferrari, C., Gabrieli, J. D. E., . . . Leggio, M. (2020). Consensus Paper: Cerebellum and Social Cognition. *The Cerebellum, 19*(6), 833-868. doi:10.1007/s12311-020-01155-1

Wang, Samuel S. H., Kloth, Alexander D., & Badura, A. (2014). The Cerebellum, Sensitive Periods, and Autism. *Neuron, 83*(3), 518-532. doi:https://doi.org/10.1016/j.neuron.2014.07.016

Wilson, J. E., Trumbo, M. C., Wilson, J. K., & Tesche, C. D. (2018). Transcranial direct current stimulation (tDCS) over right temporoparietal junction (rTPJ) for social cognition and social skills in adults with autism spectrum disorder (ASD). *Journal of Neural Transmission, 125*(12), 1857-1866. doi:10.1007/s00702-018-1938-5

Winecoff, A., Clithero, J. A., Carter, R. M., Bergman, S. R., Wang, L., & Huettel, S. A. (2013). Ventromedial Prefrontal Cortex Encodes Emotional Value. *The Journal of Neuroscience, 33*(27), 11032-11039. doi:10.1523/jneurosci.4317-12.2013

Wolf, R. C., Pujara, M., Baskaya, M. K., & Koenigs, M. (2016). Emotion recognition deficits associated with ventromedial prefrontal cortex lesions are improved by gaze manipulation. *Cortex, 82*, 255-262. doi:https://doi.org/10.1016/j.cortex.2016.06.017

Yuk, V., Anagnostou, E., & Taylor, M. J. (2020). Altered Connectivity During a False-Belief Task in Adults With Autism Spectrum Disorder. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging, 5*(9), 901-912. doi:https://doi.org/10.1016/j.bpsc.2020.04.007

Figure legends

Fig. 1: CONSORT flow diagram of study inclusion.

Fig. 2: The course of the study. After electrode placement, electrical stimulation was started 5 min before task performance (offline stimulation). Participants then conducted the ToM task while receiving electrical stimulation (online stimulation). Stimulation duration was 15 min and the ToM tasks took 5-10 minutes longer than the stimulation period, which means participants conducted the task during, and after stimulation when stimulation aftereffects were still present. The order of stimulation was randomized across participants.

Fig. 3: **ToM test performance for anodal r-TPJ tDCS, anodal vmPFC tDCS, and sham tDCS in children with autism**. **Level 1** refers to the precursors of ToM (e.g. perception and imitation, emotion recognition, pretence and physical-reality distinction); **level 2** refers to elementary ToM (e.g. first-order belief reasoning, false belief understanding); **level 3** refers to advanced ToM (e.g. understanding second-order belief, realizing humor). *Note*: tDCS = Transcranial direct current stimulation; r-TPJ = right temporoparietal junction; vmPFC = ventromedial prefrontal cortex. All pairwise comparisons were conducted with Bonferroni-corrected t-tests. All error bars represent Standard Error of Mean (s.e.m.). $n = 14$. Asterisks [*] represent statistically significant differences. ns = non-significant.

Fig. 4: **Modeling of the electrical current flow in the head**. 3D modeling results for electrical current flow in the head of a 10-year-old child (from the Neurodevelopmental MRI database) (Mackey & Petrides, 2014) induced by the anodal rTPJ-cathodal neck (**A**) and anodal vmPFC-cathodal neck (**B**) montages. The original position of the reference electrode in the applied montage was the left shoulder. For the simulation purpose, we chose the neck as the closest approximation to the shoulder based on previous studies (Ganho-Ávila et al., 2019). The results of the modeling show that the average normE strength in r-TPJ and vmPFC are 0.2664 and 0.2392 respectively. *Note*: r-TPJ = right temporoparietal cortex; vmPFC = ventromedial prefrontal cortex; NormE = norm/strength of the electrical field. *Note* that the simulated electrical field can differ from the induced electrical field under each mortgage because the return electrode is placed on the neck (for modeling feasibility purpose) while in reality, the return electrode was placed over the left shoulder. This may lead to a misplaced electrical field shown on target regions.

Table 1: demographic information

Variable	Category	Value
Gender	Male (female)	8 (6)
Age	Mean (SD)	10.7±1.9
Education year	Mean (SD)	3.64 (1.94)

Education year refers to the elementary school year (6 grades) where 1 is for grade 1 and so one up to 6. *Note*: SD = Standard Deviation.

Table 2: Means and SDs of the reported side effects during tDCS and ToM task performance

Task	**Outcome measures**	r-TPJ tDCS M (SD)	vmPFC tDCS M (SD)	Sham tDCS M (SD)
tDCS tolerability rating	Overall tolerability	2.28 (0.61)	2.42 (0.64)	2.00 (0.67)
ToM test	Level 1	12.93 (3.47)	16.50 (3.56)	11.50 (3.48)
	Level 2	3.93 (2.33)	6.69 (3.62)	3.93 (2.40)
	Level 3	1.14 (0.94)	1.93 (1.54)	1.14 (1.02)
	Total score	18 (5.88)	25.35 (7.35)	16.57 (6.28)

For side effect rating, children with ASD were asked to rate "tolerability" and "pleanasntness" of each stimulation session in a 1-5 scale. *Note*: M = means; SD = Standard Deviation; tDCS = transcranial direct current stimulation; r-TPJ = right temporoparietal cortex; vmPFC = ventromedial prefrontal cortex; ToM = theory of mind.