The Effect of Transcranial Direct Current Stimulation on Theory of Mind and Metacognition in Adults with Autism Spectrum Disorder

Nicola Singleton

Supervisors: Dr. Anna Moffat (Flinders University) &

Dr. Michael Ridding (The University of Adelaide)

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Abstract

Individuals with Autism Spectrum Disorder experience difficulties with social skills, which can be attributed to deficits in Theory of Mind (ToM, the ability to infer others thoughts) and metacognitive abilities (thinking about your own thinking). Neuroimaging studies have revealed that the medial prefrontal cortex is part of the ToM and metacognitive networks, and is underactive in individuals with Autism. The purpose of this study was to investigate whether transcranial Direct Current Stimulation (tDCS) would temporarily improve ToM and metacognitive performance. In a double-blind, randomised design, 15 adults with Autism attended two research sessions, 1 week apart, where they received active (20 minutes of 1mA anodal tDCS to the medial prefrontal cortex) or sham tDCS before completing tasks that assessed their ToM and metacognitive ability. Paired-samples t-tests revealed that active tDCS did not improve ToM or metacognitive ability. However, further analysis revealed interactions between tDCS type and tDCS order, for some of the tasks. We cannot conclude whether anodal tDCS improves or worsens performance on ToM tasks but it seems likely that under some circumstances active tDCS can improve metacognitive ability. Future research is needed to further explore the impact of tDCS on the medial prefrontal cortex in order to aid the development of new therapies for individuals with Autism.

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Overview

In addition to restricted and repetitive patterns of behaviour, Autism Spectrum Disorder causes difficulties with communication and social interaction (American Psychiatric Association, 2013). These difficulties have been shown to increase social anxiety (Bellini, 2004), and individuals with Autism are more likely to experience fewer close friendships and intimate relationships, and lower employment rates, compared to neurotypical peers (Howlin, 2000). Though complex, it is thought that social and communication deficits can be partly attributed to an impairment of Theory of Mind (ToM) ability (Baron-Cohen, 1991). ToM is defined as the ability to comprehend and reflect on the mental state of others, allowing us to understand their thoughts, feelings, intentions, desires and potential behaviours (Baron-Cohen, 1991). Another factor thought to impact the social skills of those with Autism are impairments noted in metacognitive abilities (Sawyer, Williamson & Young, 2014). Metacognition is defined as the ability to monitor, review and judge one's own knowledge and performance (Sawyer, et al., 2014). It is important that therapeutic methods that can improve social functioning in Autism are continually explored, so that intervention options for individuals with Autism continue to evolve.

One possible mode by which social and communication deficits may be improved is non-invasive brain stimulation. Techniques using this approach are currently being used safely and effectively to treat psychiatric disorders such as Major Depressive Disorder (Bersani et al., 2013). The aim of the current study is to investigate whether non-invasive brain stimulation is effective at temporarily improving ToM and metacognitive abilities in individuals with Autism. This research

could potentially make a contribution to the development of therapeutic techniques to improve the social deficits in Autism.

Theory of Mind in Autism Spectrum Disorder

Theory of Mind (ToM) is a very broad concept encompassing many skills that enable an individual to infer the mental state of others. These skills range in complexity and begin with the simple ability to understand that another individual may hold different beliefs to oneself (Baron-Cohen, 2000). This ability is measured with 'first-order false belief tasks' such as the 'Sally-Anne Task' (Baron-Cohen, Leslie & Frith, 1985). Neurotypical children aged 4-6 can successfully pass these basic ToM tasks while children with Autism usually pass them by 9 – 11 years of age (Baron-Cohen, 2000). ToM skills progress to using eye gaze, body language, facial expression and tone of voice, to infer the thoughts and feelings of another (Baron-Cohen, 2000). Examples of tests that measure these abilities include the 'Reading the Mind in the Voice' task which requires inferring emotion from tone of voice (Rutherford, Baron-Cohen, & Wheelwright, 2002) and 'Tricky Triangles' which involves reading the 'body language' of animated triangles (Abell, Happe, & Frith, 2000). Individuals with Autism consistently perform more poorly on these tasks compared to their neurotypical peers (Baron-Cohen, 2000).

In addition to behavioural tasks, ToM studies have also considered neurophysiological markers relating to this skill. Neuroimaging studies on neurotypical individuals consistently find the temporal poles, the posterior superior temporal sulcus and the medial prefrontal cortex to be activated during ToM tasks (Singer, 2006). Of these areas, the medial prefrontal cortex is believed to play the most significant role in ToM ability (Singer, 2006). A study on children with Autism using Positron Emission Tomography found reduced regional cerebral blood flow in

the left medial prefrontal cortex (compared to controls), which was associated with impairments of communication and social interaction (Ohnishi et al., 2000). Two neuroimaging studies have found that the medial prefrontal cortex is less active in adults with Autism than neurotypicals during the 'Tricky Triangle' task (Castelli, Frith, Happé, & Frith, 2002; Kana, Keller, Cherkassky, Minshew, & Just, 2009). Differences in activation of the medial prefrontal cortex between individuals with Autism and neurotypical peers were also found during a task that required participants to answer questions on social stories (Happe et al., 1996). Lesion studies also lend support to the involvement of the medial prefrontal cortex in ToM. A study on individuals with legions of the ventral medial prefrontal cortex found that they performed significantly worse on a task that involved identifying social faux pas compared to controls (Shamay-Tsoory, Tomer, Berger, Goldsher, & Aharon-Peretz, 2005). Shamay-Tsoory and Aharon-Peretz (2007) recruited participants with medial frontal lobe lesions and had them complete a ToM task called the 'Yoni' task. The task involves using character's eye gaze and/or facial expression to work out what the character 'Yoni' is thinking about, wants, loves or hates. They performed more poorly on the task compared to controls.

As there is a large body of support for the involvement of the medial prefrontal cortex in ToM reasoning, as well as evidence for abnormal activation of this area in individuals with Autism, we targeted the medial prefrontal cortex in the present study. It should be noted that the medial prefrontal cortex is a small part of a complex ToM network (Singer, 2006). By studying brain areas within the ToM network independently from one another, we can gain a greater understanding of how each of the areas makes a contribution to ToM.

Metacognition in Autism Spectrum Disorder

In addition to abnormalities with ToM ability, Frith and Happé (1999) argued that individuals with Autism have deficits in their metacognitive abilities, and this argument has been supported in a number of studies since (e.g. Sawyer, et al., 2014). Metacognition can be divided into two processes: metacognitive monitoring and metacognitive control and both are significant in how able an individual is to regulate their social interactions. Metacognitive monitoring involves evaluating the accuracy of judgements, knowledge and performance whereas metacognitive control is the decision making process of how to behave in response to these subjective appraisals (Grainger, Williams, & Lind, 2014). 'Feeling-of-knowing' judgements require the participant to assess the prospective likelihood of successful recognition of studied stimuli and are therefore a measure of metacognitive monitoring. Grainger et al., (2014) investigated metamemory (metacognition specific to memory) and found that participants with Autism gave significantly less accurate 'feeling-of-knowing' judgements than their neurotypical peers, suggesting a metamemory monitoring impairment. A study investigating metacognititon in the context of memory for faces also found poorer metacognitive performance in individuals with Autism (Wilkinson, Best, Minshew, & Strauss, 2010). Participants were required to decide whether they had previously seen the face that was presented to them and then rate how certain they were about their decision. They found that participants with Autism's confidence ratings did not correctly reflect their performance. This demonstrates poorer metacognitive monitoring compared to neurotypical peers.

Contrary to these findings, Sawyer et al., (2014) did not find metacognitive monitoring difficulties in individuals with Autism, but they did find impairments in metacognitive control. In Sawyer et al. (2014) study, participants were asked to

correctly identify facial expressions from photographs and then rate how confident they were with their answer (meta-cognitive monitoring) and whether they wished to withhold or submit their answer (meta-cognitive control). They found that individuals with Autism were as good as their neurotypical peers at gauging their accuracy with confidence ratings (meta-cognitive monitoring) but they were not as good at using their confidence to decide when to submit or withhold their answers (meta-cognitive control). It has been suggested that these different findings may be due to varying metacognitive abilities depending upon the task (Sawyer, et al. 2014). While it is unclear whether individuals with Autism have specific metacognitive monitoring impairments, it is clear that they have poorer general metacognitive abilities than their peers.

Frith and Happé (1999) suggested that metacognitive deficits in Autism might be attributable to impairment in the same underlying mechanism used for ToM. As previously discussed, the medial prefrontal cortex is involved in ToM processes. There are brain legion and neuroimaging studies demonstrating that the medial prefrontal cortex is also involved in metacognition, which lends support to this theory. For example, a study using functional magnetic resonance imaging (fMRI) presented personality traits to participants and asked them to make yes/no judgments as to whether the traits applied to themselves (self-evaluation condition), and significant others (significant-other condition). The authors found that the medial prefrontal cortex was active when making self-evaluation judgments as well as significant other related judgments but was not active in a non-referential control condition (Schmitz, Kawahara-Baccus, & Johnson, 2004). Following from this, Beer, Lombardo and Bhanji (2010) used an ‘on-line self-evaluation’ protocol which investigated metacognition more specifically. An on-line protocol refers to evaluations being made

about current performance on a task (as opposed to general self-evaluations). Participants were asked to answer multiple-choice general-knowledge type questions, and then asked to rate how confident they were about their answer. Using fMRI the authors concluded that the medial prefrontal cortex is involved in on-line self-evaluation – a construct synonymous with metacognitive monitoring. Additionally, a study found that participants with focal damage to the medial prefrontal cortex had less accurate 'feeling-of-knowing' judgments than controls (Modirrousta & Fellows, 2008). This again demonstrates the involvement of the medial prefrontal cortex in metacognitive monitoring.

For individuals with Autism, a deficit in metacognitive abilities could contribute to social impairments. Sawyer et al. (2014) suggest that when individuals with Autism correctly identify an emotion from a facial expression, they may not realise they are correct and so may not act accordingly. Furthermore, a deficit in the ability to monitor whether they remember a face could result in awkward social situations (Wilkinson, Best, Minshew, & Strauss, 2010). The present study therefore investigates whether brain stimulation of the medial prefrontal cortex can improve metacognitive monitoring and/or control.

Non-Invasive Brain Stimulation

Transcranial Direct Current Stimulation (tDCS) and Repetitive Transcranial Magnetic Stimulation (rTMS) are both types of non-invasive brain stimulation, which work by modulating neural activity in the brain. Repetitive Transcranial Magnetic Stimulation delivers rapidly changing magnetic fields to initiate action potentials (Paulus, Peterchev, & Ridding, 2013). tDCS involves passing a mild electrical current through two electrodes placed on the scalp. It has been theorised that although this current is too mild to produce action potentials, it can polarise neuronal membranes.

The positive electrode known as the anode, generally leads to excitation due to the depolarisation of neuronal membranes - meaning the neurons are primed ready to fire. The negative electrode, known as the cathode, generally leads to inhibition due to the hyperpolarisation of neuronal membranes – the likelihood of action potentials is therefore reduced (Vallence & Ridding, 2014). Scientists have studied this effect by stimulating the motor cortex and measuring motor evoked potentials. Anodal tDCS increases the occurrence of motor evoked potentials during and after stimulation (depending on stimulation duration), while cathodal tDCS inhibits the occurrence of motor evoked potentials (Nitsche & Paulus, 2001). tDCS and rTMS can affect the simultaneous processing of afferent synaptic inputs as well as affecting synaptic plasticity (a strengthening or weakening of synapses) and with these neurological changes comes the potential for changes in cognition (Vallence & Ridding, 2014). tDCS has been found to accelerate learning and enhance performance on a range of cognitive tasks (Parasuraman & McKinley, 2014). A study combing cognitive training with tDCS found anodal tDCS resulted in superior cognitive performance than sham tDCS at a four week follow-up (Martin et al., 2013). This study highlights the potential of tDCS in facilitating long-term learning.

Non-invasive brain stimulation has been successfully used to manipulate the activation of the medial prefrontal cortex in neurotypical individuals, resulting in behavioural changes. For example, rTMS of the ventral medial prefrontal cortex significantly impaired performance on the ‘Yoni’ task (Lev-Ran, Shamay-Tsoory, Zangen, & Levkovitz, 2012) and rTMS of the posterior medial prefrontal cortex affected participants ability in a false-belief task (Schuwerk, Langguth, & Sommer, 2014). While metacognition has not been directly studied with non-invasive brain stimulation to the medial prefrontal cortex, a study stimulating the medial prefrontal

cortex with tDCS found it improved error monitoring (Bellaïche, Asthana, Ehlis, Polak, & Herrmann, 2013). Although a different construct to metacognition, it suggests stimulation of the medial prefrontal cortex has the potential to manipulate metacognitive abilities.

rTMS and tDCS have both been discovered to provide clinical benefits for a variety of disorders including: Major Depressive Disorder, Schizophrenia, Alzheimer's disease, Parkinson's disease, Multiple Sclerosis, and Aphasia, and with no major side effects (Bersani et al., 2013; Fregni et al., 2014). Research into non-invasive brain stimulation as a therapeutic tool for symptoms of Autism is in its infancy. Preliminary findings suggest rTMS is able to temporarily improve repetitive-ritualistic behaviours and cognitive skills such as error-monitoring and naming skills in individuals with Autism (Oberman, Rotenberg, & Pascual-Leone, 2013). As of yet only two studies have explored non-invasive brain stimulation as a therapeutic intervention for social deficits in Autism. A study using tDCS to stimulate the dorsolateral prefrontal cortex in children with Autism found a significant reduction in Autistic behaviours, which included a significant reduction in social symptoms (Amatachaya et al., 2014). A study using rTMS to stimulate the bilateral dorsal medial prefrontal cortex of adults with Autism found mixed results (Enticott et al., 2014). Social relating significantly improved post stimulation compared to the sham group and there was a near significant improvement on self-reported anxiety in difficult social and emotional situations. However, no improvements were found on ToM tasks (Reading the Mind in the Eyes: Baron−Cohen, Jolliffe, Mortimore & Robertson, 1997, and 'Tricky Triangles': Abell, et al., 2000). There are two explanations for this result. First, it is possible that the stimulation did not improve ToM ability and instead improved a different mechanism that is responsible for the

improvement in social relating. Second, it is possible that Enticott et al. (2014)'s mixed findings were due to a lack of sensitivity of the ToM tests used, meaning they were unable to pick up on subtle changes to ToM ability.

The Present Study

In the present study we stimulated the medial prefrontal cortex of adults with Autism using tDCS instead of rTMS because tDCS is a much more practical device. tDCS is significantly cheaper than rTMS, and is a smaller, more portable device that could be used in a home environment (Priori et al., 2009). These factors are incredibly important when investigating a potentially therapeutic technique. As Enticott et al. (2014) suggested, we included reaction time in our ToM measures in an attempt to improve sensitivity. Additionally, metacognitive measures were included in order to investigate whether stimulation of the medial prefrontal cortex could improve metacognitive functioning in individuals with Autism.

In summary, the present study stimulated the bilateral medial prefrontal cortex of individuals with high functioning Autism with tDCS, in order to investigate whether the stimulation would improve ToM and metacognitive monitoring and control. A battery of ToM tests was used in order to explore the effect of tDCS on ToM accuracy and speed. The findings of this study could influence future therapeutic approaches for improving social abilities in those with Autism.

The following was hypothesised:

1) There will be higher accuracy scores on ToM tasks after receiving active tDCS than sham tDCS
2) Reaction times on ToM tasks will be faster after receiving active tDCS than sham tDCS

3) There will be higher confidence ratings for correct items and lower confidence ratings for incorrect items on the metacognitive monitoring measure after active tDCS than sham tDCS
4) Submit and withhold decisions will be more accurate on the metacognitive control measure after receiving active tDCS than sham tDCS

Method

Participants

15 adults (10 male, 5 females) aged 24 - 64, with a mean age of 41, and a diagnosis of High Functioning Autism or Asperger's Syndrome were recruited. All participants confirmed that they had received a diagnosis of Autism Spectrum Disorder from a professional accredited by Autism SA and had never been diagnosed with an intellectual disability. Participants were recruited from the Flinders University Adult Autism Spectrum Disorder contact list via an email of invitation, and an advertisement was posted on the Flinders Autism Research Group Facebook and Twitter page. Additionally, an advertisement was placed on the Autism SA website and in their newsletter, and posters were displayed in a small number of private practises in South Australia. The advertisements invited potential participants to contact a researcher if they wanted more information or wished to participate. When a participant made contact with a researcher, they were screened and not permitted to participate if any of the following exclusion criteria applied to them: they had ever experienced an adverse reaction to tDCS, ever had a seizure, stroke or serious brain injury, have metal in their head (outside the mouth), have any implanted devices, experienced frequent or severe headaches, or may be pregnant. This exclusion criteria was a safety precaution for the use of tDCS and is consistent with studies using a

similar protocol (e.g. Sellaro et al., 2015). No medications were excluded but all medications were documented. 4 participants were taking psychotropic medications (1 Dexamphetamine, 1 Quetiapine Fumarate, 1 Risperadone and Fluvoxamine, and 1 Escitalopram). The Southern Adelaide Clinical Human Research Ethics Committee approved this study.

Design

The effects of tDCS (active, sham) on ToM and metacognition task performance were examined in a double blind, within-subjects design. The order in which participants receive active and sham tDCS was randomised in order to control for practise effects.

Materials

A brief demographic questionnaire was administered to participants that asked for age, gender, handedness, and questions regarding how often participants play computer games and watch TV/movies. The Autism Quotient (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001) was administered to measure Autism symptom severity. It requires the participant to select how much they agree (definitely agree, slightly agree, slightly disagree, definitely disagree) with 50 statements. The AQ has good test-retest and interrater reliability and is able to discriminate between ASD and non-ASD samples (Baron-Cohen et al., 2001).

The National Adult Reading Test (Nelson & Willison, 1991) requires participants to read 50 words out-loud. It was administered in order to measure reading ability and provide an IQ estimate. The reading test has high inter-rater reliability and correlates highly with IQ scores (Crawford, Parker, Stewart, Besson, & De Lacey, 1989). These data were collected so that analysis could be carried out

to investigate whether demographical factors, Autism symptom severity, and reading ability may influence the affect of tDCS or task performance.

The 'Yoni' Task

The 'Yoni' task was adapted from Shamay-Tsoory and Aharon-Peretz (2007) task. On a computer screen a cartoon character's face 'Yoni' is surrounded by 4 objects, each of which has a characters face adjacent to it. In 'test item' trials participants have to make inferences about which objects 'Yoni' is thinking of, wants, loves, or hates using the eye gaze and/or simple facial expression of 'Yoni' and/or the other characters. 'Control item' trials require participants to identify which character has the same object as 'Yoni' and do not include eye direction or facial expression. Participants used the mouse to indicate which object/character they wish to give as their answer. The task was adapted in order to make it shorter and we did this by removing the 'first order' trials. The 'first order' trials are very easy and because the participants have high functioning ASD, we expected that they would perform at ceiling on these tasks and were therefore of limited use. Accuracy and reaction time was recorded for this task. The task duration is approximately 10 minutes. See Appendix A for an illustration.

Tricky Triangles

White, Coniston, Rogers, and Frith (2011) version of the 'Tricky Triangles' task was used. The task requires participants to watch 12 silent animations of 2 triangles interacting on the computer screen. After each animation, participants are required to categorize the animation by selecting one of three options displayed on the computer screen: ''no interaction'' (random),''physical interaction'' (goal-directed), or ''mental interaction''(ToM). If participants correctly identify a 'mental interaction' they are asked two more questions: "How do you think the *little* triangle feels at the

end of the clip?" and "How do you think the *big* triangle feels at the end of the clip?" The participant must answer by selecting one of 5 emotion adjectives presented on the screen. A new list of emotion adjectives is presented for each question. The definitions of the interaction types were provided on a card next to the computer so that participants could remind themselves of the definition throughout the task. This was done so that memory enhancement could be ruled out when analysing the effects of tDCS. Individuals with High Functioning Autism perform significantly worse than their neurotypical peers on this task (White et. al., 2011). This version of the task is as sensitive as the original task, which used open-ended questions rather than multiple-choice (Abell, et al., 2000). This version of the task was chosen due to it being much quicker and more objective than the original version (White et. al., 2011). Accuracy and reaction time was recorded for this task and it took approximately 15 minutes to complete. See Appendix B for details.

Reading the Mind in the Voice

The Reading the Mind in the Voice - Revised task, taken from Golan, Baron-Cohen, Hill, and Rutherford (2007), requires participants to listen to 25 short segments of speech (2-3 seconds each) and infer the emotion of the speaker from the tone of their voice. Participants make their choice by selecting one of four adjectives on the computer screen. A glossary of emotions was made available to the participants so that they could look up the meaning of the 4 emotion adjectives on the screen before playing the audio clip. This was to ensure that the task was testing ToM and not vocabulary. Accuracy and reaction time of answer selection (after participant had heard the audio clip) was recorded. The task has high discriminant validity as it is able to discriminate individuals with Autism from neurotypical individuals and test-retest reliability is high. Additionally, high accuracy on the task is correlated with low

Autism Quotient scores (Golan, et. al., 2007). See Appendix C for further details on the task.

Metacognitive Measure

After the participant selected their answer in the Reading the Mind in the Voice task, they were asked metacognitive questions. First participants were asked to rate how confident they were that their answer was correct using a sliding scale from 0 – 100% confidence (meta-cognitive monitoring). They then had to decide whether they would submit or withhold their answer in a hypothetical game show scenario (meta-cognitive control). This measure was based on Sawyer, Williamson and Young (2014). The Reading the Mind in the Voice Task with the metacognitive measure took approximately 20 minutes. See Appendix D for further details on the metacognitive measure.

Procedure

In order to achieve a randomised, double-blind design, a research assistant randomly assigned participants to receive active tDCS in session 1 or 2. The assistant then pre-programmed the tDCS machine to administer active or sham stimulation and provided the experimenter with a code to start the stimulation. The tDSC machine displayed faux information in the sham condition therefore the experimenter did not know when they were administering active or sham tDCS. After all of the experiments were complete, the research assistant revealed the tDCS conditions to the researcher.

Participants attended two participation sessions at least 1 week apart. In the first session, the demographic questionnaire, Autism Quotient and National Adult Reading Test were completed first and then participants received 20 minutes of tDCS (active or sham). After the tDCS was complete participants waited 5 minutes and then

completed the ToM test battery (including the metacognition measure) on the computer. The order in which the participants completed the tasks was randomised, as was the order in which individual test items were presented. This was done in order to prevent order-effects

In session two participants received 20 mins of tDCS (active or sham), waited 5 minutes, and then completed the ToM test battery again. Once participants had completed the ToM tests they were given a brief questionnaire asking for information on any side effects from the tDCS. Participants were then paid $30 for their time. Session one took approximately 1 hour and 40 minutes and session 2 took approximately 1 hour and 20 minutes.

tDCS Protocol

tDCS stimulation involves applying a small current through two pad electrodes on the scalp, which are held in place with a headband. As we were aiming to excite the neurons of the medial prefrontal cortex the anode (excitory electrode) will be referred to as the target electrode and the cathode (inhibitory electrode) will be referred to as the reference electrode. The placement of the reference electrode was carefully considered as it may have an inhibitory effect on the brain area beneath it (Bikson, Radman, & Datta, 2006). The occipital lobe was selected as the site for the reference electrode as it is involved in the processing of visual information (Bellaïche, et al., 2013) and there is no evidence that it has any involvement in ToM (Singer, 2006). Additionally, it has been found that by placing the electrodes far apart from one another on the scalp, it decreases the current shunted through the skull therefore increasing the current entering the brain (Miranda, Lomarev, & Hallett, 2006). Using the International 10-20 system for EEG electrode placement, the reference electrode was placed vertically on the occipital lobe over Oz, and the target electrode was

centred horizontally over the Fpz in order to stimulate the bilateral medial prefrontal cortex. 1mA of stimulation was delivered with a 'Neuroconn DC stimulator plus' via 5cm x 7cm surface sponge electrodes. The sponges were soaked in water and covered in electro-conductive gel prior to use. The stimulation lasted for 20 minutes with a linear fade in/fade out of 10 seconds. This protocol has been previously used safely and effectively to target the medial prefrontal cortex with no major complaints or discomfort reported (Sellaro et al., 2015). Bellaïche et al. (2013) whom used a very similar protocol (except that the reference electrode was placed between Oz and the inion) found that the visual system was not affected. No major side affects other than a tingling or itching sensation under the electrodes (which is commonly reported, see Fregni et al. 2014) were expected.

20 minutes of tDCS was decided upon because it appears to be the optimal protocol. The affect of tDCS increases with time, up until 20 minutes when the affect levels off (Gamboa, Antal, Moliadze, & Paulus, 2010). As the ToM tasks take about 45 minutes to complete, an offline protocol was used. This means that ToM tasks were administered after and not during the stimulation. The after-effect of thirteen minutes of anodal tDCS peaks after 5 minutes post-stimulation and lasts between 60-90 mins (Monte-Silva et al., 2013; Nitsche & Paulus, 2001). Participants were therefore required to wait 5 minutes post stimulation before beginning the computer tasks and it was estimated that the after-effects of 20 minutes of tDCS would last at least 60 minutes.

The sham tDCS protocol was the same as the active protocol apart from the fact that only 30 seconds of 1mA was administered. This procedure has been found to be an affective sham condition as it causes the same tingling sensation as the active tDCS (Priori, Hallett, & Rothwell, 2009). In the active condition this sensation

usually abates after roughly 30 seconds making it very difficult to notice any differences between the two conditions. Participants were instructed to relax during the stimulation. They were asked not to talk or engage in any activity, or fall asleep.

Results

Autism Traits and Reading ability

Scores on the Autism Quotient ranged from 11 – 42 with a mean score of 30.33 (*SD* = 7.74). 7 of our participants scored below 32, which suggests they do not demonstrate clinically significant levels of Autism traits (Baron-Cohen et. al., 2001). Error scores on the National Adult Reading Scale ranged from 4 – 18 with a mean score of 11.27 (*SD* = 5.05). This gives an estimate of a Full Scale IQ range of 108 – 126 with a mean IQ of 117 (Nelson & Willison, 1991). This demonstrates our participants had above average intelligence.

Theory of Mind Accuracy

In order to test the hypothesis that higher accuracy scores would be achieved on the ToM tests after active tDCS than sham tDCS, paired samples t-tests (and where necessary the non-parametric equivalent) were used. Descriptive statistics for the three ToM tests can be found in table 1 (higher scores indicate a more accurate performance on the ToM test). As multiple tests were being used to test the hypothesis, a Bonferroni correction was used to avoid type 1 errors ($\alpha = .017$).

Table 1

Theory of Mind Task Accuracy Scores

	Sham tDCS	Active tDCS
'Yoni' Task		
Median Test Item Score	33	32
Median Control Item Score	4	4
'Tricky Triangles' Task		
Mean Score (*SD*)	14.93 (3.75)	15.33 (3.46)
'Reading the Mind in the Voice' Task		
Mean Score (*SD*)	16.93 (2.46)	16.67 (3.04)
Total ToM Mean Score (*SD*)	64.73 (5.65)	63.33 (7.59)

Note. The 'Yoni' task has a total of 34 test items and 6 control items, the 'Tricky Triangles' task a total of 20 test items and the 'Reading the Mind in the Voice' task a total of 25 test items. Total ToM Mean score was calculated by summing the mean scores from the three ToM tasks (total ToM scores are therefore out of 79).

Yoni test item scores were positively skewed. This could not be corrected with a transformation and so a non-parametric test was used. A Wilcoxon Signed Rank Test revealed no significant difference between active and sham tDCS Yoni test item scores, $Z = -2.02$, $p = .043$, $r = -.37$. Yoni control item scores were also positively skewed. A Wilcoxon Signed Rank Test revealed no significant differences between sham tDCS and active tDCS control item scores, $Z = -1.56$, $p = .119$, $r = -0.28$ (median's are reported in Table 1). For the 'Tricky Triangles' task, paired samples t-

tests revealed no significant difference in scores between sham tDCS and active tDCS, $t(14) = -.462$, $p = .651$, $d = 0.11$. 'Reading the Mind in the Voice' task score data was positively skewed therefore the data was transformed with logarithm which resulted in a more normal distribution. The transformed data revealed no significant difference between sham and active scores, $t(14) = .089$, $p = .930$, $d = .03$. Total mean ToM scores were calculated by adding mean Reading the Mind in the Voice scores, mean Tricky Triangle scores, and mean Yoni test item scores. A paired-samples t-test revealed no significant difference in total ToM scores between active and sham tDCS, $t(14) = .913$, $p = .377$, $d = 0.21$. These data demonstrate the hypothesis is not supported; participants did not achieve higher accuracy scores after active tDCS compared to sham tDCS.

Theory of Mind Reaction Time

Paired samples t-tests (or non-parametric equivalent) were used to test the hypothesis that reaction times on ToM tasks would be faster after active tDCS than sham tDCS. Descriptive statistics can be found in Table 2. Again, a Bonferroni correction was used to avoid type 1 errors ($\alpha = .017$).

For the 'Yoni' task, paired samples t-tests revealed no significant difference between sham and active reaction time for test items, $t(14) = -.209$, $p = .838$, $d = .06$. Yoni control item reaction times were positively skewed and were therefore transformed with logarithm. There was no significant difference between active and sham control trial reaction time, $t(14) = -.441$, $p = .666$, $d = .09$.

Table 2

Theory of Mind Tasks: Reaction Time in Milliseconds

	Sham tDCS	Active tDCS
'Yoni' Task		
Mean Test Item Reaction Time (*SD*)	6,936 (2,483)	7,090 (2,568)
Mean Control Item Reaction Time (*SD*)	5,341 (3,339)	5,107 (3,083)
'Tricky Triangles' Task		
Median 'Type' Reaction Time	2,085	2,220
Mean 'Question' Reaction Time (*SD*)	12,399 (11,554)	9,615 (4,943)
'Reading the Mind in the Voice' Task		
Mean Reaction Time (*SD*)	9,870 (8,074)	9,520 (4,628)
ToM Total Mean Reaction Time (*SD*)	18,695 (6,115)	20,873 (6,725)

Note.' ToM Total Reaction Time' was calculated by summing the mean reaction times of the three ToM tests (not including Yoni control items).

Reaction times in the 'Tricky Triangles' task were analyzed separately for 'type' and 'question'. 'Type reaction time' refers to the time taken to choose from a multiple choice which type of interaction the animation demonstrated. If the participant correctly identified a 'mental interaction', they were asked two questions about how the triangles were feeling. 'Question reaction time' refers to the time taken to answer the feeling questions. They were analyses separately due to the largely differing reaction times. For 'type reaction time' there was an extreme outlier in the sham condition – this participant was excluded from the analysis. Despite removing this outlier, 'type reaction time' data was positively skewed. Transforming the data did not correct the issue and so Wilcoxon Signed Rank Test was used. There was no

significant difference between active and sham tDCS Tricky Triangle 'type reaction time' scores, Z = -.094, p = .925, r = -0.02. Tricky Triangle 'Question reaction time' data was also positively skewed. This was corrected by transforming the data with a logarithm. Paired-samples t-tests on the transformed data revealed no significant difference in 'question reaction times' between active and sham tDCS, $t(14)$ = -.457, p = .655, d = .31.

Reading the Mind in the Voice task reaction time data was positively skewed and therefore the data was transformed with a logarithm, which resulted in a more normal distribution. The transformed data revealed no significant difference between sham and active reaction times, $t(14)$ = .461, p = .652, d = 0.15.

Total mean ToM reaction times were calculated by adding Yoni test item, Reading the Mind in the Voice and Tricky Triangle mean reaction times. An extreme outlier was excluded from the analysis and this resulted in a normal distribution. Paired samples t-tests revealed no significant difference between active and sham tDCS total mean ToM reaction times, $t(13)$ = 1.219, p = .244, d = 0.34. The hypothesis was not supported, as reaction times were not faster after receiving active tDCS.

Metacognitive Monitoring

The hypothesis that there would be higher confidence ratings for correct items and lower confidence ratings for incorrect items after active tDCS was tested using paired sample t-tests (with the standard alpha level .05). Mean confidence ratings for correct and incorrect answers can be found in table 3. There was no significant difference between sham and active tDCS confidence ratings for correct answers, $t(14)$ = 1.95, p = .071, d = 0.27, or for incorrect answers, $t(14)$ = 1.05, p = .313, d = 0.21. The hypothesis was therefore not supported.

Table 3

Metacognitive Monitoring: Mean confidence ratings for correct and incorrect answers

	Sham tDCS	Active tDCS
Percent Confidence for Correct Answers	69.87 (17.79)	74.33 (15.47)
Percent Confidence for Incorrect Answers	62.20 (15.40)	65.33 (14.98)

Note. Confidence was measured on a scale from 0 – 100. Higher scores indicate higher confidence.

Metacognitive Control

It was hypothesised that submit and withhold decisions would be more accurate after receiving active tDCS than sham tDCS. This hypothesis was tested using paired samples t-tests (with the standard alpha level .05). Means for number of answers submitted and withheld can be found in table 4. There was no significant difference between active and sham tDCS for 'correct answers submitted', $t(14) = 1.07$, $p = .301$, $d = 0.29$, 'incorrect answers withheld', $t(14) = -1.74$, $p = .865$, $d = 0.03$, or 'total accuracy (which was calculated by summing mean number of correct answers submitted with mean number of incorrect answers withheld), $t(14) = 1.09$, $p = .292$, $d = 0.31$.

There was no significant different between active and sham tDCS for mean number of incorrect answers submitted $t(14) = 1.75$, $p = .863$, $d = 0.04$. Significantly less correct answers were withheld after active tDCS than sham tDCS $t(14) = 2.77$, $p = .039$. This was a small-medium effect ($d = .45$.) There was no significant difference between active and sham inaccuracy scores (which were calculated by summing mean

number of correct answers submitted with mean number of correct answers withheld), $t(14) = 1.28$, $p = .223$, $d = 0.37$. Although there was significantly less correct answers withheld after active tDCS than sham tDCS, the hypothesis was not supported because this did not improve overall accuracy.

Table 4

Metacognitive Control: Mean number of Correct and Incorrect answers submitted or withheld

	Sham tDCS	Active tDCS
Accuracy		
Mean no. Correct Answers Submitted	14.33 (3.52)	15.27 (2.96)
Mean no. Incorrect Answers Withheld	1.93 (2.46)	1.87 (2.38)
Total Accuracy Score	16.27 (2.92)	17.13 (2.70)
Inaccuracy		
Mean no. Incorrect Answers Submitted	6.40 (3.62)	6.53 (3.44)
Mean no. Correct Answers Withheld	2.60 (3.11)	1.40 (1.55)
Total Inaccuracy Score	9.00 (3.02)	7.93 (2.69)

Note. Total accuracy score was calculated by summing mean number of correct answers submitted with mean number of incorrect answers withheld. Total inaccuracy score was calculated by summing mean number of correct answers submitted with mean number of correct answers withheld.

Follow-up Analysis: Theory of Mind Interactions

We theorized that the order in which the active tDCS was received (in session 1 or in session 2) might have had an effect on performance. If active tDCS was received in session 1, this could increase learning and therefore have a carry-over effect for session 2. 2 (tDCS type: active, sham) x 2 (order group: active tDCS 1st, active tDCS 2nd) mixed-model ANOVA's were used to investigate whether there was an interaction between tDCS type and 'tDCS order' on ToM task performance.

As multiple tests were being used to test ToM accuracy and reaction time interactions, a Bonferroni correction was used to avoid type 1 errors ($\alpha = .017$). Mean ToM task scores and mean reaction times can be found in Table 5. For significant interactions pairwise comparison statistics are included.

For the 'Yoni' task, there was a significant interaction between tDCS type and 'tDCS order' on test item scores, $F(1,13) = 11.37$, $p = .005$, partial $\eta^2 = .467$. Pairwise comparisons revealed that for the 'active tDCS 1st' group, accuracy scores were lower after active tDCS than sham tDCS and this was a large affect. For the 'active tDCS 2nd' group, there was no significant difference between active tDCS and sham tDCS scores. These results suggest that active tDCS impairs performance on the Yoni test items, but practice effects counteract this effect. There was no interaction between tDCS type and tDCS order on control item scores, $F(1,13) = .72$, $p = .412$, partial $\eta^2 = .052$.

There was also a significant interaction between tDCS type and 'tDCS order' for Yoni test item reaction times, $F(1,13) = 19.77$, $p = .001$, partial $\eta^2 = .603$. Paired comparisons revealed that for the 'Active tDCS 1st' group there was no significant difference between active and sham tDCS reaction times. For the 'Active tDCS 2nd group' reaction times were significantly faster after active tDCS than sham tDCS.

However, for both groups reaction times were faster in session two and with similarly large effects. It is likely that these results are due to practice effects only. There was no interaction between tDCS type and order group on control item reaction time, $F(1,13) = 1.20$, $p = .294$, partial $\eta^2 = .084$.

There was no interaction between tDCS type and 'tDCS order' for Tricky Triangle accuracy, $F(1, 12) = .05$, $p = .835$, partial $\eta^2 = .003$, or for Tricky Triangles 'Type' reaction time, $F(1, 12) = 5.80$, $p = .033$, partial $\eta^2 = .326$. 'Type' reaction time data could not be corrected to satisfy assumptions and so this should be interpreted with caution. As for Tricky Triangle 'Question' reaction time, data transformed with a logarithm was used in order to satisfy assumptions. There was a significant interaction between tDCS type and order group, $F(1, 13) = 11.44$, $p = .005$, partial $\eta^2 = .468$. Paired comparisons revealed that for the 'active tDCS 1st' group, active tDCS reaction times were not significantly different from sham tDCS reaction times. For the 'active tDCS 2nd' group, active tDCS reaction times were significantly faster than sham tDCS reaction times and with a large effect. These results suggest that active tDCS in session 1 promoted learning (as demonstrated by increased speed) and this effect carried over into session 2.

For Reading the Mind in the voice, accuracy and reaction time data transformed with logarithm was used in order to satisfy assumptions. There was a significant interaction between tDCS type and 'tDCS order' for the Reading the Mind in the Voice accuracy scores, $F(1, 13) = 9.34$, $p = .009$, partial $\eta^2 = .418$. However pairwise comparisons did not reveal any significant differences between active and sham scores for either group. There was no interaction for Reading the Mind in the Voice reaction times, $F(1, 13) = 2.95$, $p = .596$, partial $\eta^2 = .022$.

Table 5

Theory of Mind Task Mean Scores (SD) and Mean Reaction Times (SD) for 'tDCS Order' groups. Pairwise comparisons are included for significant interactions.

	Active	Sham	$F(1, 13)$	p	d
Yoni Test Item Scores					
'Active tDCS 1st' Group	30.11 (3.48)	33.11 (1.36)	19.03	.001	1.14
'Active tDCS 2nd' Group	33.17 (1.17)	32.50 (1.64)	.63	.443	.47
Yoni Test Item Reaction Time					
'Active tDCS 1st' Group	7,941 (2,539)	6,339 (2,035)	6.58	.024	.70
'Active tDCS 2nd' Group	5,428 (1,552)	8,216 (3,049)	1.02	.003	1.15
Tricky Triangle Scores					
'Active tDCS 1st' Group	14.33 (4.06)	13.78 (4.35)	-	-	-
'Active tDCS 2nd' Group	16.83 (1.60)	16.67 (1.75)	-	-	-
Tricky Triangle 'Type' Reaction Time					
'Active tDCS 1st' Group	2,982 (1,572)	2,282 (757)	-	-	-
'Active tDCS 2nd' Group	1,996 (678)	4,334 (3,596)	-	-	-
Tricky Triangle 'Question' Reaction Time					
'Active tDCS 1st' Group	10,492 (5,695)	7,470 (4,972)	2.79	.119	.57
'Active tDCS 2nd' Group	8,299 (3,613)	19,794 (14,995)	9.01	.010	1.05

Reading the Mind in the Voice Score					
'Active tDCS 1st' Group	15.22 (2.99)	17.00 (2.83)	5.17	.041	.284
'Active tDCS 2nd' Group	18.83 (1.47)	16.83 (2.04)	4.36	.057	.251
Reading the Mind in the Voice Reaction Time					
'Active tDCS 1st' Group	8,167 (3,060)	8,529 (3,966)	-	-	-
'Active tDCS 2nd' Group	11,547 (6,063)	11,879 (12,218)	-	-	-

Note. The 'Yoni' task has a total of 34 test items and 6 control items, the 'Tricky Triangles' task a total of 20 test items and the 'Reading the Mind in the Voice' task a total of 25 test items. Total ToM Mean score was calculated by summing the mean scores from the three ToM tasks (total ToM scores are therefore out of 79).

Follow-up Analysis: Metacognitive Interactions

Metacognitive monitoring and control interactions where tested with mixed model ANOVA's with the standard alpha level (α =.05). There was a borderline significant interaction between tDCS type and tDCS order for correct answer confidence ratings, $F(1, 13) = 4.61$, $p = .051$, partial $\eta^2 =. 262$. Paired comparisons revealed that for the 'active tDCS 1st' group, active tDCS confidence rating were not significantly different from sham tDCS confidence ratings. For the 'active tDCS 2nd' group, active tDCS confidence rating were significantly higher than sham tDCS confidence ratings, with a medium effect. These results suggest that active tDCS in session 1 increased confidence for correct items and this effect carried over into session 2. There was no interaction between tDCS type and tDCS order for incorrect answer confidence ratings, $F(1, 13) = .922$ $p = .355$, partial $\eta^2 =.066$.

For metacognitive accuracy there was a significant interaction between tDCS type and order group, $F(1, 13) = 11.16$, $p = .005$, partial $\eta^2 =.462$. Paired comparisons

revealed that for the 'active tDCS 1st' group, active tDCS total accuracy scores were not significantly different from sham tDCS total accuracy scores. For the 'active tDCS 2nd' group, active tDCS total accuracy scores were significantly higher than sham tDCS total accuracy scores, with a large effect. These results suggest that active tDCS in session 1 increased metacognitive accuracy and this effect carried over into session 2.

Table 6

Mean (SD) Confidence Ratings for Correct and Incorrect items, and Mean (SD) Total Accuracy Scores for 'tDCD Order' groups. Pairwise comparison are included for significant interactions.

	Active	Sham	$F(1, 13)$	p	d
Confidence for Correct Items					
'Active tDCS 1st' Group	72.44 (15.27)	71.56 (18.23)	.114	.741	.05
'Active tDCS 2nd' Group	77.17 (16.76)	67.33 (18.48)	9.29	.009	.59
Confidence for Incorrect Items					
'Active tDCS 1st' Group	63.56 (12.7)	62.78 (14.29)	-	-	-
'Active tDCS 2nd' Group	68.00 (18.87)	61.33 (18.33)	-	-	-
Total Accuracy					
'Active tDCS 1st' Group	16.22 (2.64)	17.00 (3.16)	1.00	.336	.027
'Active tDCS 2nd' Group	18.50 (2.35)	15.17 (2.32)	12.23	.004	1.43

Side Effects

All 15 participants reported a 'tingling' sensation during stimulation. 5 participants additionally reported an itching sensation under the electrode. 2 participants reported tiredness but both stated this might be attributable to other

causes (i.e. lack of sleep, a cold). 1 participant reported a headache and 1 participant reported nausea but both participants again reported this might be attributable to other causes. 1 participant reported dry skin underneath the electrode and another participant experienced a visual disturbance during stimulation. They described this disturbance as a 'pulsing/flickering sensation when eyes were closed'. This sensation lasted 'a few moments' and was not experienced post stimulation. 1 participant stated that the stimulation caused a 'stinging sensation' for the first few minutes of stimulation.

Discussion

This study stimulated the medial prefrontal cortex of adults with Autism for 20 minutes with 1mA anodal tDCS (active tDCS) and 20 minutes of sham tDCS, in order to investigate if active tDCS affected performance on ToM and metacognitive measures. The order in which active tDCS was received was counterbalanced and there was a break of at least 1 week between sessions. The effects of this stimulation on ToM ability will be discussed first, followed by a discussion of the effects on metacognitive abilities. General limitations and implications will then be discussed along with suggestions for future research.

Theory of Mind Performance

Participants' performance on the ToM tasks was no more accurate nor was it faster after receiving active tDCS than sham tDCS. This suggests that the stimulation protocol was not effective at temporarily improving ToM ability. The medial prefrontal cortex is a small part of the ToM network, which also includes the temporal poles and the posterior superior temporal sulcus (Singer, 2006). Perhaps 20 minutes of 1mA anodal stimulation to only one of the brain areas involved in ToM is not

enough to elicit a temporary observable change in ToM performance. However, it is also possible that our study was underpowered due to a very small sample size meaning we were unable to observe small effects. Another issue we encountered was ceiling effects. Our sample performed very well on the Tricky Triangles task, regardless of tDCS type. Participants mean score was comparable to the scores of neurotypical participants in White et al. (2011). Performance on the Yoni task was also very close to ceiling. When baseline scores are at ceiling it is impossible to observe an improvement. However, we included reaction times incase ceiling effects occurred and there were no observable differences in speed between active and sham tDCS.

There were some interesting interactions between tDCS type and the order in which it was received. If active tDCS had no effect on ToM ability, we would expect no significant difference in accuracy or reaction time between active and sham tDCS for either of the groups (one group received active tDCS in session 1 and the other group received active tDCS in session 2). We might also expect that in some instances both groups would significantly improve on their performance from session 1 to session 2 (irrespective of which order they received the active tDCS) demonstrating practice effects. For a lot of the tasks we did find this pattern of results, however for some of the tasks a different pattern of results emerged.

For the group that received active tDCS in session 2, reaction times when answering questions on the Tricky Triangles task were faster after active tDCS than sham tDCS. In contrast, for the group that received active tDCS in session 1, there was no difference in reaction time after active or sham tDCS. There are two speculative theories than can explain this pattern of results. The first theory is that active tDCS is equal to the effects of practice. For the group that received active tDCS

in session 2, session 1 reaction times demonstrate a baseline and session 2 reaction times demonstrate a combined effect of active tDCS and practice. For the group that received active tDCS in session 1, session 1 reaction times demonstrate the effect of active tDCS and session 2 reaction times demonstrate the effects of practice. This results in reaction times being equal across session 1 and 2 demonstrating that the effect of active tDCS is equal to the effects of practice. In other words, receiving active tDCS before completing the Tricky Triangles task has the same effect as practicing the Tricky Triangles task once before.

The other speculative theory that can explain this interaction is that active tDCS facilities learning and this benefit is still present 1 week after active tDCS. We did not expect learning effects of tDCS to last a week or more because studies using anodal tDCS to stimulate the motor cortex found that the after-effects of 13 minutes of anodal stimulation last approximately 60 minutes (Monte-Silva et al., 2013). We would therefore not expect the after-effects of 20 minutes of active tDCS to last a week (or more) and carry over into session 2. However, in a study that used an 'online' protocol, participants engaged in cognitive training computer tasks whilst their dorsolateral prefrontal cortex was stimulated with anodal tDCS or sham tDCS, for 10 sessions (Martin et al., 2013). At a four week follow-up the group that received active tDCS demonstrated superior cognitive performance than those who had received sham stimulation. This study demonstrates that when tDCS is combined with cognitive training it can produce long-term effects. However, in our study we used an 'offline' protocol – participants began the ToM tasks after stimulaton. Is it possible that this protocol could still induce long-term learning? Anodal stimulation depolarizes the neuroanl membranes of the targetted area which can result in neural excition (Vallence & Ridding, 2014). When pariticpants engaged their medial

prefrontal cortex for the Tricky Triangle task this could have resulted in synaptic activity possibiliy causing 'long-term potentiation – like' changes (Vallence & Ridding, 2014). This means that there is a long-lasting increase in signal transmission between neurons and could explain why superior performance on the Tricky Triangles after active tDCS was still present one week later.

A combination of these two theories is also possible – practice effects could inflate the long-term learning effects of the active tDCS. We cannot conclude if long-term learning effects did occur or whether the result simply demonstates that active tDCS causes a short-lived immediate effect equivalent to the effect of practice. In order to prevent practice effects and therefore draw conclusions about long-term learning effects, future studies of a similar design should use a ToM test that is split into two parts with high test-retest reliability. This would mean that participants are presented with different stimuli in session 1 and session 2 and therefore practice effects are very unlikely. An example of a ToM test that could be used is 'The Awareness of Social Inference Test' which has strong test-retest reliability (McDonald, Bornhofen, Shum, Long, Saunders, & Neulinger, 2006). We did not use this test in our study due to the cost involved and administration time.

Results for the interaction of tDCS type and order were very different for the Yoni task. For the group that received active tDCS in session 2, there was no difference in accuracy scores between active and sham tDCS. For the group that received active tDCS in session one, accuracy was worse after active tDCS than sham tDCS. This result could demonstrate that active tDCS impaired performance on the Yoni task. For the group that received active tDCS in session two, the effects of practice counteracted this impairment. This finding was unexpected because the anode usually depolarizes neuronal membranes leading to excited neuronal activity,

which has a facilitatory effect (Vallence & Ridding, 2014). Three possible reasons for the inhibitory effect of the active tDCS on the Yoni task will be discussed: cathodal interference, baseline empathy levels, and anodal inhibition.

The cathode was placed over the occipital lobe and so we considered the possibility that this could have disrupted visual processing. The cathode usually hyperpolarizes neurons, which leads to the inhibition of neural activity (Vallence & Ridding, 2014). As the Yoni task is a visual task perhaps the cathode hindered performance. However, if this were the case it would be expected that performance on the control items of the Yoni task would also be impeded by active tDCS, but this was not the case. There was no difference in accuracy between active and sham tDCS on the Yoni control items in either of the groups. Additionally, the Tricky Triangles task is a visual task and so if the cathode were hindering performance on the Yoni task, we would expect it to also hinder performance on the Tricky Triangles task. We therefore do not believe the cathode is the cause of the impairment on the Yoni task.

Another theory that could explain why tDCS appeared to hinder Yoni task performance is the effect of baseline empathy. A study targeting the medial prefrontal cortex found that the effects of rTMS on 'Yoni' task performance were dependent on pre-existing levels of self-reported empathy (Krause, Enticott, Zangen, & Fitzgerald, 2012). For participants with high self-reported empathy the rTMS impeded performance on the Yoni task whereas for participants with low self-reported empathy it improved performance. On average, individuals with Autism have lower levels of empathy than neurotypical peers (Baron-Cohen, & Wheelwright, 2004) but perhaps our sample had higher levels of empathy than average. We did not measure empathy but we did measure Autism traits using the Autism Quotient. Individuals with Autism usually score 32 or above (Baron-Cohen et. al., 2001) but 7 of our participants scored

below 32 (it should be noted that this does not detract from their diagnosis of Autism). This may indicate that our sample was not representative of the Autistic population. It is therefore possible that they had higher than average empathy levels, which could explain why the anodal tDCS hindered Yoni performance instead of improving it.

Another theory that could explain why the tDCS made Yoni performance worse is that the anode actually inhibited neuronal activity. Studies that inform us that anodal tDCS has an excitory effect are usually studies on the motor cortex (e.g. Nitsche & Paulus, 2001). Studies using tDCS on the prefrontal cortex find mixed results with some reporting inhibitory effects of the anode (see Tremblay, Lepage, Latulipe-Loiselle, Fregni, Pascual-Leone, & Theoret, 2014, for a review). Early animal experiments found that anodal stimulation enhanced superficial cortical neurons but inhibited neurons situated deep in the cortical sulci. It was theorised that this was due to the neurons in the sulci being orientated differently to those on the surface (Creutzfeldt, Fromm, & Kapp, 1962). A highly speculative theory is that the Tricky Triangles task uses more superficial cortical areas while the Yoni task uses deeper cortical areas therefore resulting in the opposing effects of anodal stimulation on the two tasks. As we did not measure the physiological effects of the tDCS with neuroimaging or electroencephalograph (EEG), it is impossible for us to know exactly which brain areas were stimulated, and the exact brain areas used for each of the ToM tasks. Future studies could use a similar protocol as Maeoka, Matsuo, Hiyamizu, Morioka, and Ando (2012) and use tDCS with EEG power spectrum analysis. This would help us to understand how tDCS affects the cortical excitability of different areas of the medial prefrontal cortex.

Metacognitive Performance

There was no difference in confidence ratings for correct or incorrect answers between the tDCS conditions, demonstrating that metacognitive monitoring was no better after active tDCS than sham tDCS. Additionally, there was no difference in the number of incorrect answers submitted or withheld between tDCS type, but there was significantly less correct answers withheld after active tDCS than sham tDCS. This did not improve the overall accuracy of metacognitive control (total correct answers submitted and total incorrect answers withheld). Metacognitive control was the same across active and sham tDCS. This result implies that active tDCS causes participants metacognitive control to be more liberal in nature but not more accurate.

The reason active tDCS did not improve the accuracy of metacognitve monitoring or control may be due to the fact that we only stimulated a small part of the metacognitive network (as was also concluded with ToM performance), which also includes the dorsolateral and anterior prefrontal cortex and cingulate and insula cortices (Fleming & Dolan, 2012). As discussed with ToM outcomes, another possibility is that we cannot observe small effects due to the lack of stasticial power because of the small sample size.

There were some interesting interactions between tDCS type and the order in which it was received, on metacognitive performance. For the group that received active tDCS in session 2, there were higher confidence ratings for correct items and metacognitive control was more accurate after active tDCS than sham tDCS. In contrast, for the group that received active tDCS in session 1, there was no difference in metacognitive monitoring or control. Looking at the group that received active tDCS in session 2, it appears that active tDCS made participants more confident about their answers overall. Confidence not only increased for correct answers after active

tDCS, but also slightly increased for incorrect answers (although this increase was not significant). They were more accurately able to decide when to withhold and when to submit an answer demonstrating more accurate metacognitive control. It therefore appears that increased confidence resulted in higher accuracy. Perhaps without the active tDCS participants were too critical and were less confident about answers they got correct. This theory is consistent with the results between active and sham tDCS (regardless of order) as they indicated participant's metacognitive control was more liberal after active tDCS.

Why were there only differences in metacognitive monitoring and control between active and sham tDCS for the group that received active tDCS in session 2? It is unlikely that this result can be attributed to a combination of short-term affects of active tDCS and practice effects. The participants received no feedback after the first session as to how well they did so they could not adjust their metacognitive behaviour for session 2. Perhaps, as discussed for the Tricky Triangle result, when pariticpants engaged their medial prefrontal cortex for the metacognitive task this could have resulted in synaptic activity possibiliy causing 'long-term potentiation – like' changes in synaptic strength (Vallence & Ridding, 2014). This theory means that active tDCS facilitated metacognitive monitoring and control, and the improvement was still present one week later.

As the anode we used had a large surface area, it is very likely that the orbitofrontal cortex was stimulated as well as the medial prefrontal cortex. Changes in metacognitive ability could therefore not only be attributable to the medial prefrontal cortex, but also the orbitofrontal cortex. A study used neuroimaging to investigate the role of the medial prefrontal cortex and the orbitofrontal cortex in self-evaluation of task performance (Beer, et al., 2010). They found that the medial prefrontal cortex

was generally involved in evaluations but its activation was not specifically related to accurate or over-confident evaluation. However, a reduction in orbitofrontal cortex activity was associated with over-confident judgments. This evidence suggests that in our study the anodal stimulation may have inhibited the orbitofrontal cortex. Although it was expected that anodal stimulation would excite neurons, as previously mentioned, animal studies revealed that anodal stimulation can cause an inhibitory affects on some neurons (Creutzfeldt, et al., 1962). As we did not measure the physiological effects of the stimulation with neuroimaging or EEG, it is impossible to know whether the change in metacognition is due to inhibition or excitation of the medial prefrontal cortex or the orbitofrontal cortex, or whether it is a combination of effects. Future studies should use tDCS with EEG power spectrum analysis in order to understand how active tDCS increases metacognitive confidence and improves accuracy of metacognitive control.

General Limitations and Implications

Inter-subject variation is very high for tDCS because physiology, medication, age and gender can all influence its outcome (Tremblay et al., 2014). These individual differences could explain why no significant differences in ToM performance were found between active and sham tDCS (regardless of order). The theories proposed to explain the different outcome of active and sham tDCS on the two groups (active tDCS in session 1, active tDCS in session 2) have focused on the order in which active tDCS was received. It is possible though, that these differences were not due to order effects but individual differences within each group.

Individual physical characteristics such as hair thickness, skull thickness and cerebrospinal fluid density have an impact on how much electricity reaches the cortex (Horvath, Carter, & Forte, 2014). In our study we did not monitor brain activity and

therefore we do not know how much electricty reached the cortex for each individual. As previously suggested, future research could use EEG with tDCS in order to monitor brain activity. The intensity of tDCS could then be adjusted for each individual so that the amplitude of electricity that actually reaches the cortex is the same for all participants.

Psychotropic medications can interact with active tDCS and alter its effects (Paulus et al., 2013). In our study 4 of the participants were on psychotropic medications, which could have influenced the effects of the active tDCS. This reduces the generalisability of our results. Future research could exclude all participants on psychotropic medications, however the rates of individuals with Autism who take psychotropic medications are quite high (Green, Pituch, Itchon, Choi, O'Reill, & Sigafoos, 2006) and so this would reduce the size of the participant pool.

Our study recruited participants from a very wide age bracket ranging from 24 to 64 with a mean age of 41. This poses a problem because age can interfere with the effect of tDCS. A recent study investigated the effects of anodal tDCS on cognitive performance in young and old adults (Fertonani, Brambilla, Cotelli, & Miniussi, 2014). They found that for young adults, offline (cognitive task performed after stimulation) and online (cognitive task performed during stimulation) protocols both improved performance on the task. For older adults, performance only improved for the online protocol. The authors believed this difference could be due to differences in synaptic connectivity in young and old brains. They propose that in young adults anodal stimulation can produce short-term plasticty whereas in older adults it cannot. As we used an 'offline' protocol, many of our participants may have been too old for the anodal stimulation to cause short-term plasticity. This could explain why we found no differences in ToM performance between active and sham tDCS (when not

considering order). Age differences may also be contributing to some of the differences between groups. The mean age of the 'Active tDCS 1st' group was 45 ranging from 24 -64. The mean age of the 'Active tDCS 2nd' group was 34, ranging from 24 -57. Future research should recruit participants within a smaller age bracket in order to reduce age effects. We recommend recruiting young participants due to the higher likelihood of tDCS causing short-term changes in plasticity.

Gender also influences the effect of tDCS. A study investigating the recognition of facial expressions found that anodal tDCS improved performance in women but worsened performance in men (Boggio, Rocha, da Silva, & Fregni, 2008). The authors argued that the effects of tDCS are dependent on baseline cortical activity, and at baseline women performed the task better than men. In our study gender effects were not the focus of our investigation and in order to increase sample size we recruited both genders. This poses a limitation to our study because if the tDCS had opposing effects on males and females this could have presented an illusion of no effect. Future studies that are not investigating gender effects should only recruite one gender. As there are more males with Autism than females (Williams, MacDermott, Ridley, Glasson, Wray, 2008), it would be logical to recruite males in the hopes of a larger sample.

It is possible that our sample may be suffering from selection bias. Many individuals with high functioning Autism have social anxiety (Bellini, 2004) and therefore it seems logical that many would avoid participating in an experiment such as this one which involves meeting new people in a new environment. Therefore our sample may not be representative of the high functioning Autism community. Future research could try to reduce this selection bias by conducting the experiment in the

participant's home environment in an attempt to reduce anxiety. This may also increase participant numbers due to less inconvenience.

Our study had a small sample of 15 participants. However, in Tremblay et al. (2014) review of studies using tDCS to stimulate the prefrontal cortex, the average sample size was 21 participants. As we have a clinical population, 15 participants is a respectable number. Nevertheless, future studies must endeavour to recruit a much higher number than this if we wish to uncover small effects and produce results than can be generalised to the Autistic population.

The montage we used for tDCS was carefully investigated in the hopes of achieving the most effective method of stimulation. However the possibilities are endless and future research should investigate different montages. Placement of the cathode can be adjusted to change the directional flow of electricity and the size of the electrodes used can be changed to alter the focality of the stimulation (Vallence & Ridding, 2014). Repeated intervals of stimulation could also be investigated as well as stimulation intensity.

Conclusion

In conclusion, this study's hypotheses were not supported. 20 minutes of 1mA anodal stimulation to the medial prefrontal cortex of adults with Autism did not temporarily improve accuracy or reaction time on ToM tasks, nor did it improve metacognitive monitoring or control. However, there was an interaction between tDCS type and order for some of the tasks. These interactions indicated that the tDCS may have increased speed on the Tricky Triangles task, reduced accuracy on the Yoni task, and increased metacognitive confidence and accuracy. These interactions could be explained by the following possibilities: active tDCS caused a temporary improvement/ hindrance equivalent to the effects of practice, active tDCS caused a

long-term learning effect, individual differences between the groups caused a different outcome for active tDCS, or a combination of all of these factors. From this study we cannot conclude whether anodal tDCS improves or worsens performance on ToM tasks but it seems likely that under some circumstances active tDCS can improve metacognitive ability.

This study highlights that future research with tDCS of the medial prefrontal cortex investigating ToM and metacognitive outcomes is warranted. To prevent practice effects from clouding future results, a ToM test that is split into two parts with high test-retest reliability could be used. EEG could be used with tDCS in order to gain a better insight into which brain areas are being reached with stimulation and understand how tDCS affects the physiological activity of the medial prefrontal cortex. Additionally, a much larger and more homogenous sample (e.g. smaller age bracket, males only) would increase the validity of results. It is important that research continues to investigate ways of improving ToM and metacognitive abilities because the outcomes of this research may assist with the development of new therapies for improving social skills in individuals with Autism.

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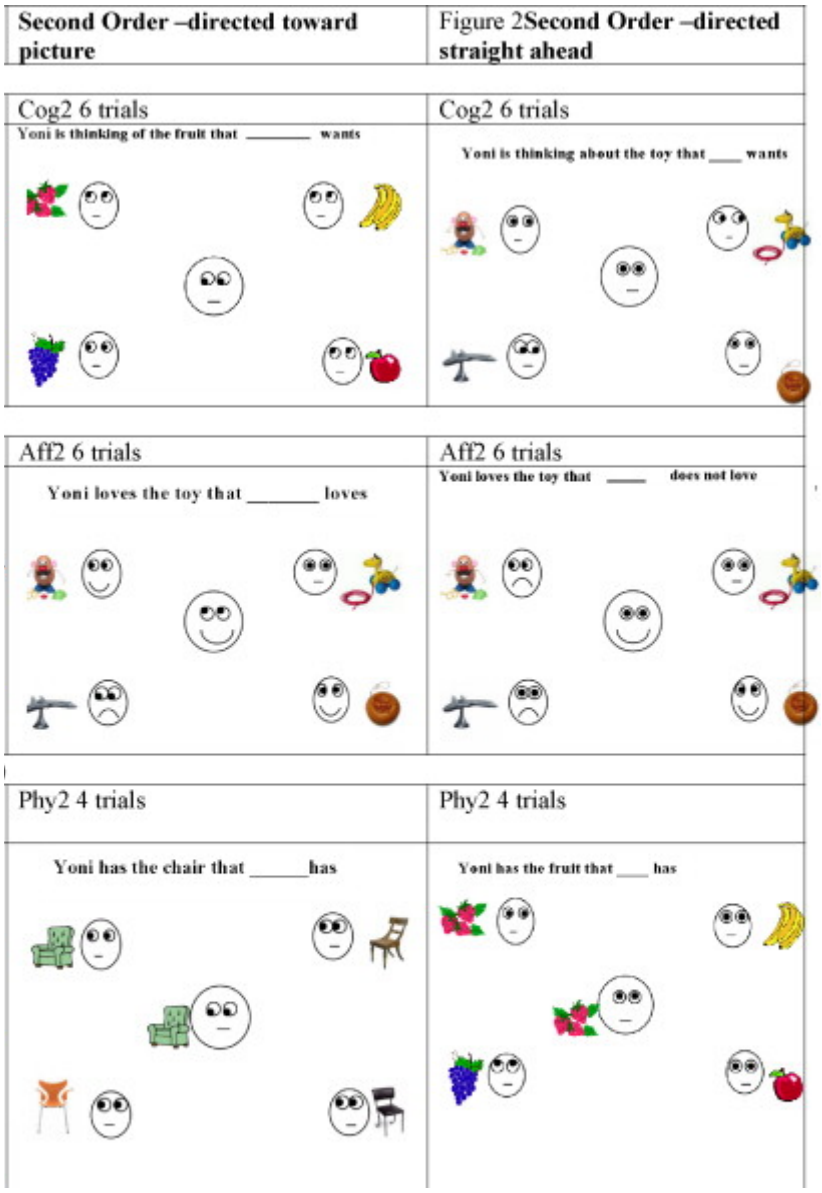
Appendix A: 'Yoni Task'

The figure to the right illustrates examples of the types of stimuli that were used in the 'Yoni' Task. The task has been adapted from Shamay-Tsoory and Aharon-Peretz (2007) version which additionally included 'First Order' stimuli. The first four types of stimuli illustrated here were included in the 'test item' analysis and the fifth and sixth stimuli (Phy2) were used in the 'control item' analysis.



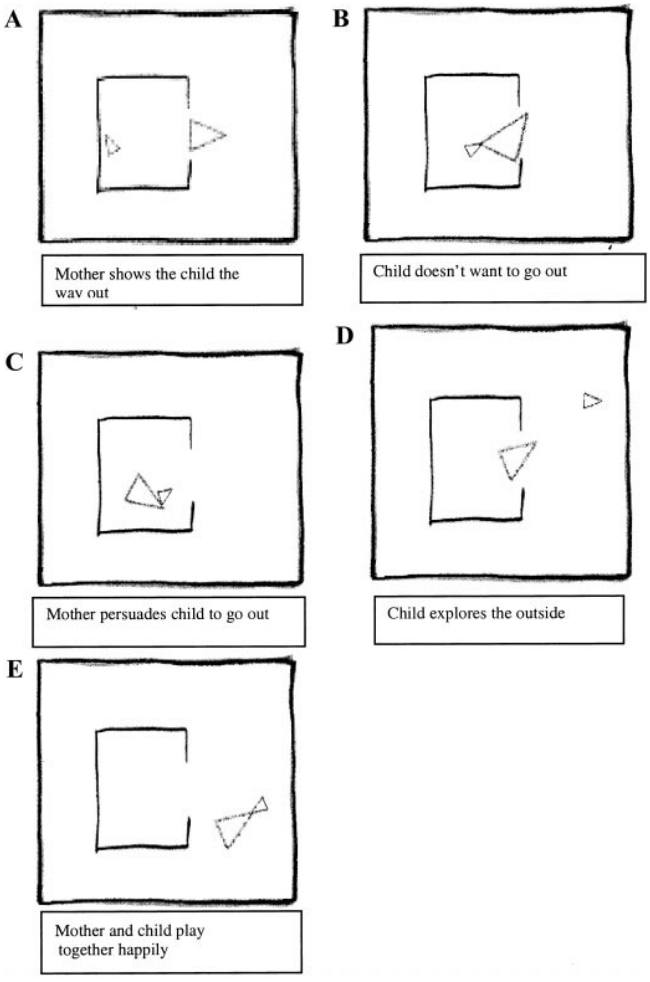
Reference: Shamay-Tsoory, Simone G, & Aharon-Peretz, Judith. (2007). Dissociable prefrontal networks for cognitive and affective theory of mind: a lesion study. *Neuropsychologia, 45*(13), 3054-3067.

Appendix B: Tricky Triangles

Tricky Triangles Animation Example

The stills below illustrate a "Tricky Triangles" animation.

"The animation was designed following a script in which Big Triangle is coaxing the reluctant Little Triangle to come out of an enclosure. Subjects were presented with the animations without any suggestion relative to a story or characters' roles. The captions have been added here for clarification" *Taken from Castelli et al. 2000, pg. 323.*



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Tricky Triangles Multiple Choice Questions

Taken from (White, Coniston, Rogers, & Frith, 2011, pg. 152-153)

After each animation the following question will be presented:

"Which of these 3 categories best fits the film clip you have just seen"

(a) No interaction
(b) Physical interaction
(c) Mental interaction

After each Mental Interaction animation the following questions will be asked (if the participant gets the above question correct).
The correct answers are displayed in bold:

Coaxing:
How do you think the Little Triangle feels at the end of the clip?
(a) Proud; (b) No feelings; **(c) Secure**;
(d) Annoyed; (e) Unsure
How do you think the Big Triangle feels at the end of the clip?
(a) Frustrated; **(b) Loving**; (c) Tense; (d) Frivolous;
(e) No feelings

Mocking:
How do you think the Little Triangle feels at the end of the clip?
(a) No feelings; (b) Fulfilled; (c) Anxious;
(d) Mischievous; (e) Devious
How do you think the Big Triangle feels at the end of the clip?
(a) Aggravated; (b) Puzzled; (c) Lonely;
(d) Satisfied; (e) No feelings

Seducing:
How do you think the Little Triangle feels at the end of the clip?
(a) Lost; **(b) Cunning**; (c) Scared; (d) Composed;
(e) No feelings
How do you think the Big Triangle feels at the end of the clip?
(a) Excited; (b) Eager; (c) No feelings; (d) Cheerful;
(e) Foolish

Surprising:
How do you think the Little Triangle feels at the end of the clip?
(a) Provoked; (b) No feelings; (c) Bored;
(d) Pleased; (e) Lucky
How do you think the Big Triangle feels at the end of the clip?
(a) No feelings; (b) Terrified; **(c) Delighted**; (d) Disappointed;
(e) Uneasy

Appendix C: Reading the Mind in the Voice – Revised

The stimuli in the table below was presented to participants via audio. Participants must choose the correct answer from the four options. The correct answers are *italicized.*

The table below was taken from Golan, Baron-Cohen, Hill, and Rutherford (2007).

Table 2 The 25 items included in the revised version of 'Reading the Mind in the Voice' (*RMV-R*), their correct answers and foils

	Spoken phrase	Answers			
1	"No, honestly I do."	Defiant	Alarmed	*Earnest*	Convinced
2	"Collie said you were up here."	Surprised	Grateful	*Friendly*	Interested
3	"Your brother? I do not remember you ever speaking of a brother."	Desperate	*Confused*	Threatening	Angry
4	"Where did you get them?"	Frustrated	*Suspicious*	Intrigued	Doubtful
5	"Please! We must go."	*Worried*	Shy	Upset	Insulted
8	"I am afraid he is gone out, sir."	Concerned	*Apologetic*	Dazed	Hurried
9	"I swear I have."	Despairing	*Pleading*	Assertive	Horrified
10	"What on earth do you mean?"	*Perplexed*	Awe	Accusatory	Lured
11	"There's...uh,...there is something I want to ask you."	Assured	*Nervous*	Puzzled	Serious
12	"Keep the damn thing!"	Bossy	*Irritated*	Cruel	Surprised
14	"What a pair!"	*Joyous*	Scared	Playful	Desperate
15	"I have not doctor, but I will."	Decided	*Embarrassed*	Unsure	Furious
16	"Oh, my god!"	*Terrified*	Broken	Frustrated	Angry
17	"Why should I? Why should any of us?"	Arrogant	*Enraged*	Appalled	Scared
18	"But I had hoped..."	*Disappointed*	Unsure	Apologetic	Tearful
20	"I really am most grateful."	Impatient	Admiring	Bitter	*Sincere*
21	"I have no idea what she thought of me."	*Melancholy*	Ambivalent	Aggrieved	Resolved
24	"So, where are you off to now?"	Relaxed	Displeased	Competitive	*Concerned*
25	"I won't harm him, I promise you."	Deceitful	Menacing	Determined	*Sincere*
27	"I think she was trying to make some sort of...gesture."	Angry	*Derogatory*	Resentful	Nostalgic
28	"But I rather think that we have a few things to discuss."	*Stern*	Oppressive	Curious	Complacent
30	"Yeah, well, I know nothing about that."	Unconcerned	Joking	*Defensive*	Indignant
32	"What sort of people do you think we are?"	Impatient	*Insulted*	Disappointed	Curious
33	"Life must go on, Mr. Wilson."	Pitying	Brooding	Irritated	*Resigned*
37	"Katherine, perhaps you'd come to help."	Bossy	Fond	*Hopeful*	Irritated

Item numbers match those of the original task (Rutherford et al., 2002). Target answers are in italics.

Reference:

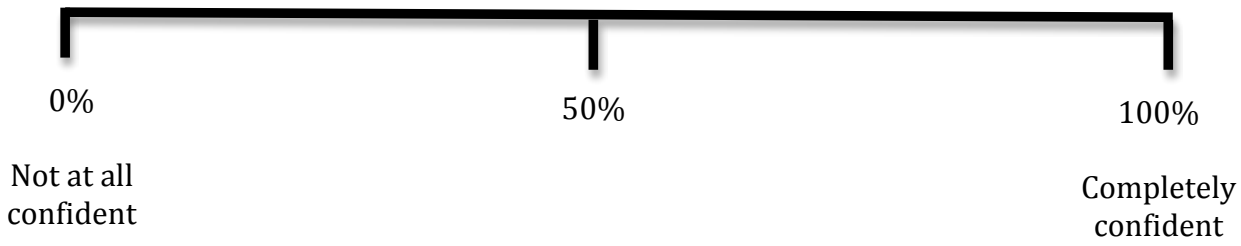
Golan, O., Baron-Cohen, S., Hill, J. J., & Rutherford, M.D. (2007). The 'Reading the Mind in the Voice'test-revised: a study of complex emotion recognition in adults with and without autism spectrum conditions. *J Autism Dev Disord, 37*(6), 1096-1106.

Appendix D: Metacognitive Measure

This measure will be incorporated into the 'Reading the Mind in the Voice' (RMVT) task. For each of the 25 questions in RMVT, the following two questions will be presented after the participant has selected their answer.

Meta-Cognitive Monitoring

"How confident are you that the answer you gave for the previous question was correct?"



Meta-Cognitive Control

"Imagine that you are on a game show. If you submit a correct answer for the previous animation you win $100, if you submit an incorrect answer you lose $100. If you do not submit an answer you do not win or lose any money."

"What would you do?"

☐ Submit answer

☐ Withhold answer

Word Count: 10,488