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Speth, Jana; Speth, Clemens

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Motor imagery in REM sleep is increased by transcranial direct current stimulation of the left motor cortex (C3)

Jana Speth, Clemens Speth

Psychology, School of Sciences, University of Dundee, DD1 4HN UK

Keywords

Brain stimulation; Motor system activation; tDCS; Consciousness; Effects of tDCS on human consciousness; Phenomenology; Mentation reports; Quantitative linguistic analysis; Motor agency analysis

Abstract

This study investigates if anodal transcranial direct current stimulation (tDCS) of areas above the motor cortex (C3) influences the quantity and quality of spontaneous motor imagery experienced in REM sleep. A randomized triple-blinded design was used, combining neurophysiological techniques with a tool of quantitative mentation report analysis developed from cognitive linguistics and generative grammar. The results indicate that more motor imagery, and more athletic motor imagery, is induced by anodal tDCS in comparison to cathodal and sham tDCS. This insight may have implications beyond basic consciousness research. Motor imagery in REM sleep has been hypothesized to serve the rehearsal of motor movements, which benefits later motor performance. Electrophysiological manipulations of motor imagery in REM sleep could in the long run be used for rehabilitative tDCS protocols benefitting temporarily immobile clinical patients, especially those who cannot perform specific motor imagery tasks - such as dementia patients, infants with developmental and motor disorders, and coma patients.

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Introduction

This study investigates if the potentially beneficial motor imagery of rapid eye movement (REM) sleep can be further increased by manipulating motor cortical excitability through transcranial direct current stimulation (tDCS). This could in the long run help the clinical treatment of motor system disorders.

REM sleep exhibits high activation of several motor regions, including primary motor and premotor cortices, the cerebellum, and basal ganglia (Braun et al., 1997 and Hobson and Pace-Schott, 2002; Maquet et al., 2000). This motor area activation is remarkable given that sleepers normally lie motionless in bed, and are mostly prevented from acting out any motor movements by input-output gating mechanisms in the brain stem (Pompeiano, 1967; Hobson, 1994; Hobson et al., 1998). It is assumed that the high motor system activation of REM sleep is converted into imaginative rather than actual movement, and indeed strong motor imagery has been shown to be typical for REM sleep (Hobson, 2009, Porte and Hobson, 1996, Speth et al., 2013 and Speth and Speth, 2016). The strong motor imagery of REM sleep has been proposed to be more than a byproduct of physiology, serving motor development and the rehearsal of motor movements for corresponding real situations even if the rehearsed situations are not remembered as such (Hobson and Stickgold, 1994, Hobson, 2009, Hobson et al., 2000, Revonsuo, 2000a and Revonsuo, 2000b; Valli and Revonsuo, 2009). Direct empirical evidence of the role of REM sleep for the consolidation of motor skills has been proposed, and is still being debated (Genzel et al., 2009, Rasch et al., 2009 and Wamsley et al., 2010).

The spontaneous motor simulations of REM sleep may thus hold a special role even compared to waking motor imagery, which itself is increasingly recognized as a crucial component of the motor system, and a back door to enhancing motor performance after impairment, usually via task-designs (Abbruzzese et al., 1999, Bonnet et al., 1997, Decety, 1996, Fadiga et al., 1999, Jackson et al., 2001, Jeannerod, 1995, Jeannerod, 2001 and Kober et al., 2014; Lehericy et al., 2004, Porro et al., 1996 and Schnitzler et al., 1997; Sharma et al., 2006 and Yoo et al., 2008). Note for example that motor control has been shown to be temporarily restored during REM sleep in Parkinson's patients who also exhibit REM sleep behaviour disorder (RBD; De Cock et al., 2007). RBD is a condition where motor movements are not output-gated, and motor imagery is therefore realized during sleep (Desseilles et al., 2011 and Oudiette et al., 2009).

The stimulation technique used in the current study to enhance motor-cortical activity, tDCS, is non-invasive, has limited side effects, and is experienced as more comfortable and less irritating by participants than other brain-stimulation techniques. The excitability of the motor cortex has been shown to be increasable via anodal tDCS, and reducible via cathodal tDCS (Nitsche and Paulus, 2000). TDCS is considered a safe brain stimulation technique, with minor adverse events reported in only a few cases that range from itching under the electrodes and skin irritation to mild headaches (Nitsche et al., 2008). TDCS is not known to cause epileptic seizures or decrease the seizure threshold in healthy participants (Liebetanz et al., 2006 and Nitsche et al., 2008).

TDCS of the motor cortex in waking has previously been shown to increase motor task performance (Boggio et al., 2006 and Reis et al., 2008), and to facilitate implicit motor learning in healthy people (Nitsche et al., 2003). Jang et al. (2011) report that tDCS of the primary motor cortex during a motor task (involving hand movements) increases motor cortical excitability. TDCS applied in sleep has been shown to enhance memory consolidation (Marshall et al., 2011; Marshall et al., 2004). However, studies that stimulate motor areas in REM sleep are scarce.

Nitsche et al. (2010) report tDCS of pre-motor areas during REM sleep to facilitate motor memory consolidation. Electrical stimulation has been applied to frontal areas in REM sleep to study the effect on dream mentation (Stumbrys et al., 2013; Voss et al., 2014). The present study is among the very first to investigate the effect that tDCS of motor areas has on sleep mentation. This effect could be an increase in motor imagery, and specifically athletic motor imagery, as indicated by a recent study that uses anodal tDCS of areas above the motor cortex (C3) in the waking resting state (Speth et al., 2015).

In summary, the current study uses mentation reports to establish the effects of tDCS of areas above the motor cortex (C3) on human mentation, specifically on motor imagery. Reports from REM sleep with anodal tDCS, cathodal tDCS, and sham stimulation are quantitatively analysed by independent, blind raters by means of a linguistic tool that has been validated in previous studies (Speth et al., 2013, Speth et al., 2015, Speth et al., 2016a and Speth et al., 2016b).

Method

Dream reports conceived in the sleep lab upon forced awakenings from REM sleep under anodal, cathodal, and sham stimulation were analysed. Motor imagery was measured with a quantitative linguistic tool: Motor agency analysis. Agency analysis has been used in previous studies, and has been shown to be a reliable tool (Speth et al., 2013, Speth et al., 2015 and Speth et al., 2016a; Speth and Speth, 2016b; Speth et al., 2016b; Speth and Speth, 2015a and Speth and Speth, 2015b). The current study is triple-blind in so far as neither the participant delivering the report, nor the investigator recording it, or the raters analysing it, knew the stimulation condition. This study was approved by the University Research Ethics Committee (UREC) of the University of Dundee, and conducted in the Dundee Sleep and Consciousness Laboratory. The present study uses the same method as a previous experiment (conducted on the effect of tDCS of areas above the motor cortex (C3) in relaxed wakefulness) in terms of the strength of the stimulation, the position of stimulation electrodes, EEG measurements, and the linguistic tool of mentation report analysis (Speth et al., 2015; Speth & Speth, 2016). The current study was however conducted with sleeping participants, in a sleep laboratory setting.

Participants

Participants were male and female undergraduate volunteers. All 11 participants (4 male, 7 female) were undergraduate students and native speakers of English (age range: 19–32; mean=22.82 (SD=3.74)). Participants were issued with an information sheet on the experimental procedure, and were told that they would be awoken during the night and asked to answer a series of questions. They were assured that they would be able to refuse answers, without further explanation, at any time. They were informed about the possibility that they would feel a slight tingling sensation during the tDC stimulation. Participants with diagnoses of epilepsy or severe migraines would be excluded from the study, along with participants who reported a history of allergic skin reactions. Written informed consent was obtained from all participants prior to the experiment.

Questionnaires

A short open-answer questionnaire issued prior to the experiment assessed participants' age, gender, native language, nationality, education, medication, as well as the time of last caffeine consumption, the general level of caffeine consumption, physical exercise on the day of testing, average amount of physical exercise per week, and meditation experience. Handedness was tested with a short version of the Edinburgh handedness inventory (EHI-short; Veale, 2014). Further, participants' personality traits were assessed using the NEO Five-Factor-Inventory (NEO-FFI). The NEO-FFI tests for five personality dimensions (neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness) in 60 items (Costa and McCrae, 1992).

Experimental design

The sleep lab provided a low-stimulus environment for the experiment. Participants were invited to the sleep lab for three consecutive nights, whereby the first night served as an acclimation night to avoid the first night effect (Touissant et al., 1995). During the acclimation night, EEG and tDCS equipment was attached, but the data were discarded. About an hour before participants would normally go to bed on a typical night, the investigator would begin to attach the EEG and tDCS electrodes. Participants would then go to sleep in single bedrooms. The investigator would monitor the sleep EEG and initiate the computer-randomized tDCS from the observation room. REM sleep was detected according to the AASM manual (Iber et al., 2007): The beginning of a REM phase was thus scored upon detection of conjugate, irregular, sharply-peaked eye movements in combination with a low chin EMG tone and sawtooth-like mixed frequency EEG waves. The REM phase continues over all subsequent periods of 30 s where a low EMG amplitude is exhibited in combination with mixed EEG frequency, in the absence of indicators of other sleep phases (such as sleep spindles, K-complexes), or indicators of awakening or arousal. Stimulation was applied after the sleeper's transition into REM sleep was confirmed, usually after two 30 s periods of REM sleep. Anodal tDCS, cathodal tDCS, or sham stimulation was applied for a duration of 5 min. REM sleep was monitored during stimulation, and data were not included if the participant had transitioned from REM sleep to another sleep phase or waking. The investigator would then enter the participant's room, wake the participant, and ask for a dream report while offering a voice recorder. The investigator kept verbal, mimic, and gestural interaction with participants to a minimum while the report was being delivered. When participants stopped reporting, without showing the wish to continue, the investigator would stop the recording. This procedure was repeated for up to four REM phases during each of the two test nights. Participants could sleep in the morning, and were offered the opportunity for a debriefing. The reports were later transcribed and handed to three blind, independent raters for the Motor agency analysis.

TDCS

The tDC stimulation was carried out with a NeuroConn DC-Stimulator Plus (neuroConn GmbH, Illmenau, GER). The positioning of the tDCS rubber electrodes was the same for all three testing conditions. A smaller stimulation electrode (25 cm²) was applied at C3, while a larger (35 cm²) reference electrode was positioned contralaterally between Fp2 and F8.

The current study uses a stimulation strength of 1.5 mA, as Cuypers et al. (2013) report that tDCS only significantly affects motor learning tasks at a current of 1.5 mA. In order to minimize the perception of current by the participant, the current was ramped up linearly over the course of 15 s at the beginning, and ramped down over the course of 15 s at the end of the session. In order

to avoid adverse effects from a sudden increase in skin resistance, the tDC stimulation would automatically stop above an impedance of 20 k Ω . In the sham condition, current was ramped up and immediately ramped down again in order to promote a perception of current onset similar to the stimulation conditions.

EEG

Sleep phases were monitored with a 31-channel EEG device (QuickAmp, Brain Products GmbH, Gilching, GER). An electrode cap fixated the electrodes according to the international 10–20 electrode positions. The electrode position C3 was not recorded, as it was taken up by the tDC stimulation electrode. Ag/AgCl ring electrodes were used. Participants' scalps were prepared with a 70% isopropyl solution and an abrasive electrolyte gel (Abralylt LIGHT) in order to achieve impedance levels below 10 k Ω . In line with the AASM guidelines (Iber et al., 2007), EOG electrodes were placed 2 cm left and beneath the left eye, and 2 cm right and above the right eye, and two EMG electrodes were applied to the chin. The recording was carried out via BrainVision Recorder Professional (v.1.2.0.0601). The sampling rate was 512 Hz. A band pass low-cutoff filter for frequencies <0.5 Hz, a high-cutoff for frequencies >70 Hz, and a 50 Hz notch filter were applied. All filters apply a Butterworth slope of 12 dB per octave.

Quantitative linguistic analysis of mentation reports

The raters were asked to quantify specific linguistic references to motor imagery in the participants' mentation reports. The linguistic tool used for the rating is based on linguistic theta theory (Gruber, 2001, Reinhart, 2002 and Reinhart and Siloni, 2005). The tool has been used to successfully link degrees of linguistic references to simulated motor activity in reports of subjective experience with characteristic motor cortical activation of states of consciousness (Speth et al., 2013 and Speth et al., 2015; Speth et al. 2016a).

The current study measures motor imagery by means of linguistic Motor agency analysis. In linguistic theta system theory, the initiator of an event takes on a specific thematic (theta) role within a sentence or phrase. He or she is the agent who performs an action. In the phrase "Mimi throws a ball", Mimi is the agent. The agent is defined through his or her relationship to the predicate of a phrase: He or she is performing the action described by the predicate. Mimi is the one who is doing something. The agent is described by a noun phrase, but the agent is not necessarily congruent with the grammatical subject. Consider the following phrases, where Mimi is the agent in both (i) and (ii), but the syntactic subject only in the active version (i).

(i) Mimi opens the box.

(ii) The box is opened by Mimi.

As participants were reporting on their mental experience, it was expected that raters would encounter instances of moderated-simulated agency. Moderated-simulated agency describes how the agent engages in explicitly mental processes concerned with simulated motor action. Consider for example the phrase "I imagined myself running". In this instance, "I" is the agent of the verb phrase "imagined...", which moderates the explicitly mental, simulated activity of "running". Raters were advised to count instances of moderated-simulated agency as normal instances of simulated motor activity.

The following phrases, (iii), (iv), and (v), are samples from mentation reports. They contain instances of normal simulated motor agency as well as moderated-simulated motor agency:

(iii) And he was coming home and like holding a bunch of flowers I guess.

(iv) I imagined myself running.

(v) I think I was... I think I was walking along a corridor.

Doubly or vaguely moderated action verbs however cannot be recognized as simulated agency. The following phrase (vi) for example (double moderation emphasized in bold print) would not be counted as agency, as its interpretation does not necessarily entail that the person simulated actively carrying out the tasks.

(vi) "thinking about what I needed to do"

Note that the following example (vii) does contain two motion verbs, but no agency (as there is no agent even remotely connected to the motion verbs):

(vii) Um, just like running through like a film, and stuff that I'd watched recently and stuff like that... thought of things to do on the bicycle.

Raters were instructed to count repetitions separately. The following sample (viii) contains two instances of simulated agency:

(viii) I was going along... I think I was going home with my friends.

Raters were instructed to count instances of agency that are described by seemingly static verb phrases such as "to be" if they can be linked to simulated agency rather than static events, as in example (ix):

(ix) ... how I'd be in the swimming pool, you know...

Raters were asked to specify the perspective of each instance of simulated motor agency as that of the experimental subject in their imagination (first person singular, first person plural), of mentations on the temporarily absent researchers which occur in experimental settings (second person singular and plural), or as simulated motor agency of non-present real persons or imaginative characters (third person singular and plural). Ratings would thus discriminate between simulated agency experienced from a distinct first, second, or third person point of view as in example (x), and participants' experiences of witnessing motor imagery as though in a movie or slideshow as in example (xi):

(x) I imagined skiing... I was skiing down a mountain.

(xi) I had this vision of rugby I think...

Raters were asked to count as normal simulated agency such instances of apparently inhibited motor agency as in example (xii), which entail the planning, attempt, or beginning of simulated action:

(xii) I tried to run.

As the English language comprises a vast number of fixed movement and motion idioms and metaphors, we ensured that those simulated motor agencies that describe distinct and intense simulations of motor movement would be captured. For example, the phrase "Mimi walked to Rome" more clearly describes physical action than "Mimi went to Rome". Raters were

thus asked to determine the intensity of the motor simulations by classifying motor agencies as athletic disciplines where applicable (according to the list of Olympic disciplines and World Games disciplines; see <http://www.olympic.org/sports> and <https://www.theworldgames.org/the-sports/sports>).

Report rating instructions

All raters were asked to rate all reports. Along with a hard copy of the reports, raters were handed an instruction manual in which they were asked to identify instances of simulated motor agency. The instruction manual contained the definitions of simulated motor agency that are given above. Raters were asked to use their best judgement and decide how to deal with particular phrases, as not all possible verbal references to simulated motor agency possible in natural speech can be pre-defined.

Statistical analyses

Nonparametric Kruskal-Wallis tests were conducted to ensure that the randomly-assigned groups did not differ with respect to gender, native language, nationality, education, medication intake, time of last caffeine consumption, physical exercise prior to testing, and the average amount of physical exercise per week. A one-way analysis of variance (ANOVA) was conducted to test for differences in age, laterality scores of the EHI-short, and scores for neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness of the NEO-FFI between the experimental groups. Inter-rater agreement for the three raters on the number of motor agencies per report was assessed by means of Cronbach's α . The mean of the ratings of the three raters was calculated for each report. This number was used for further analysis: An ANOVA with Welch-adjusted degrees of freedom (df) was conducted to test for significant differences in the number of motor agencies between the stimulation conditions (anodal, cathodal, and sham). Pairwise Games-Howell tests were used post-hoc.

In a second step of analysis, only motor agencies referring to athletic motor imagery were analysed. Here, a Welch-adjusted ANOVA testing could not be conducted as the variance in one condition (sham) was zero. Pairwise Welch t-tests were used instead.

To allow for better comparability between the present results and earlier studies on sleep mentation, the relative number of reports that contained at least one instance of motor agency was compared between anodal, cathodal, and sham stimulation in a third set of analyses. For a report to be counted as showing motor agency, two of the three raters had to have identified at least one instance of motor agency. A non-parametric Kruskal-Wallis H test was conducted to test if the stimulation conditions differed with respect to the reports containing at least one instance of motor agency. Non-parametric Mann-Whitney U tests were used post-hoc. Further, the relative number of reports that contained at least one instance of athletic motor imagery were compared between the stimulation conditions. Again, Kruskal-Wallis H and Mann-Whitney U tests were used.

In addition, changes in the perspective from which simulated motor agency was carried out, or perceived, were analysed. A one-way ANOVA was used to compare differences in the absolute number of first, second, and third person perspective agencies, separately for singular and plural, between the conditions. Further, analyses of covariance (ANCOVAs), with the number of motor agencies as a covariate, were used to account for differences in the absolute number of

motor agencies between conditions, and to be able to detect changes in relative patterns of motor agency perspective irrespective of those differences in the absolute number of motor agencies.

To account for the potential artefact that tDC stimulation of motor areas resulted in more (motor) speech output, a one-way ANOVA was conducted to test for differences in the report length. Further, an ANCOVA with the report length as covariate was conducted to test if differences in agency persisted after correcting for report length. Fisher's least significant difference (LSD) tests were used post-hoc.

For differences analysed by means of ANOVAs, η^2 was used to estimate effect sizes. Effect sizes for differences analysed by means of ANCOVAs were estimated via partial η^2 .

To assess how motor agency and athletic motor agency performed in detecting reports from anodal stimulation, receiver operating characteristic (ROC) curves were plotted for both tools for the classification between anodal stimulation versus cathodal and sham stimulation. The area under the curve (AUC) values were used as an indicator of classification performance of Motor agency analysis. Discrimination performance was interpreted according to Hosmer et al. (2013), an interpretation that has been used in numerous studies in the past (e. g. Speth et al., 2015, Kim-Dorner et al., 2010, Nichols et al., 2005 and Shatnawi et al., 2010): an AUC of <0.7 was interpreted as "poor"; an $0.7 \leq \text{AUC} < 0.8$ as "acceptable"; an $0.8 \leq \text{AUC} < 0.9$ as "excellent", and an $\text{AUC} \geq 0.9$ as "outstanding" discrimination performance.

Results

In two REM phases a participant spontaneously awoke during anodal stimulation, before the five minutes of stimulation were over. In one case this happened during sham stimulation. In two cases a participant awoke during cathodal stimulation, and in one of these instances the participant reported a "stinging" sensation below the reference electrode. Mild itching, tingling sensations, and similar sensations under the electrodes are not unusual in tDCS studies (Nitsche et al., 2008), which in the current experiment seems to have been caused by the fact that (due to prior movements, or hair being in the way) the contact area under the electrode was smaller than usual. These data are not included in the analysis.

A total of 44 reports from REM sleep were collected: 18 reports from anodal, 14 from cathodal, and 15 from sham stimulation. The groups did not differ in age, gender, native language, nationality, education, medication intake, time of last caffeine consumption, physical exercise prior to testing, and the amount of physical exercise per week. Further, the groups neither differed with respect to their laterality, nor their neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness scores. The agreement of the raters on the number of motor agencies was Cronbach's $\alpha = .907$. Linguistic samples from reports after anodal tDCS, cathodal tDCS, and sham can be seen in Table 1.

Table 1: Dream report samples from anodal tDCS, cathodal tDCS, and sham. The type of agency (athletic, non-athletic) and the grammatical perspective (first, second, third) from which the agency is reported are given. Transcription notation in line with system used by Du Bois (1991).

Linguistic sample	Agency type and grammatical perspective
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Anodal tDCS	<p>We were like flowing around in space @ .. couple of friends .. feel like, I can't remember who they were though .. ermh, (H) they were just sort of like flowing around, flowing around, pushing each other doing some like crazy stunt (H) erh, that was generally, and I felt like very warm feel really warm weirdly warm... still like .. oddly like, I don't know movie like I feel like running, heart's a bit racing.</p> <p>Aah, I heard a little people were just like having fun, running around having joy for once @ ermh, some reason they are just running don't know what for just like running it's pretty fun though. (H) don't really say much .. erm feel like I'm feeling exhilaration just like I don't know, cocky ah, I feel we were like in kind of in a sweat, uh. That's about it really.</p> <p>Erm, yea so running around I just been doing my shopping and was trying carrying a load of eggs at the same time and they were duck eggs as well, so really didn't wanna to drop them because their shells, the shells are really thin for some reason and .. and yea just resolving and falling over not dropping the eggs, that's it @@@</p> <p>running around some mountains, running around some mountains, try to renovation something I think</p> <p>surfing, did some... it has gone really quickly</p> <p>I was walking my dog and in a field and it caught up... yea year yea and an animal or something</p> <p>Aah it was like couldn't move .. like I was kind of paralysed and there's like a suffocate feeling of dreads like something like you could tell it was a nightmare you know what it feels when</p>	<p>1 x non-athletic first person plural</p> <p>2 x non-athletic third person plural</p> <p>1 x athletic third person plural</p> <p>1 x athletic first person singular</p> <p>3 x athletic first person plural</p> <p>1 x athletic first person singular</p> <p>4 x non-athletic first person</p> <p>2 x athletic first/second/third person singular or plural</p> <p>1 x non-athletic first/second/third person singular or plural</p> <p>1 x athletic first person singular</p> <p>1 x athletic first person singular</p> <p>1 x non-athletic third person singular</p> <p>1 x non-athletic first person</p>
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you've a nightmare, you can't move and you just kind of panic

Cathodal tDCS	I was just like walking to this village it was like empty secluded .. eh it was pretty lonely felt pretty cold .. (H) (Ex) erh, obviously no one there, sort of just tried to sit in a bar .. and just look at empty glasses and that was about it so I had my thoughts to myself. (H)	1 x athletic first person singular 1 x non-athletic first person singular
	I was playing a football match... Yeah and I was winning.	1 x athletic first person singular
	Erm not much like literally just nothing	N/A

Sham	Actually I got a feeling a little bit nauseous actually but in terms of anything that I noticed it's kind of sound really sad but it was actually a clip from a video game I watched the other day, erm, very, very bright, the characters looking in the mirror and it's yeah and they can hear the music #led and looned, and piano that's ###really. @@@	N/A
	Err I was in a cave flying remote control drone and there was sort of test some kind I don't know	1 x non-athletic first person singular
	Mm .. we were all really really really really really good at old school Tony Hawks games .. #daughter, let's talk about that but it's all very unlikely that it was kind of a weird dream	N/A
	Running around in bus. And they are random people just talking #trying to trick me and that'll mostly it	1 x athletic first/second/third person singular/plural
	What was the last, ###, lasted..... erm, did nothing really, I didn't really	N/A

Motor agency

The number of simulated motor agencies differed significantly between the three stimulation conditions (Welch's $F(2, 27.958)=4.58$, $p=.019$, $\eta^2=.243$). There were significantly more motor agencies under anodal stimulation ($M=1.83$, $SD=2.37$) than under cathodal stimulation ($M=0.19$, $SD=0.34$; $p=.024$) or sham stimulation ($M=0.11$, $SD=0.35$; $p=.018$). There was no significant difference between cathodal and sham stimulation.

Athletic motor agency

The numbers of athletic motor agencies were then analysed separately. There were significantly more athletic motor imageries under anodal stimulation ($M=0.59$, $SD=0.95$) than under sham stimulation ($M=0$, $SD=0$; Welch's $t(17)=2.66$, $p=.017$, $d=0.88$). There were further significantly more athletic motor imageries under anodal than under cathodal stimulation ($M=0.01$, $SD=0.28$; Welch's $t(20.58)=2.12$, $p=.047$, $d=0.72$). See Figure 1.

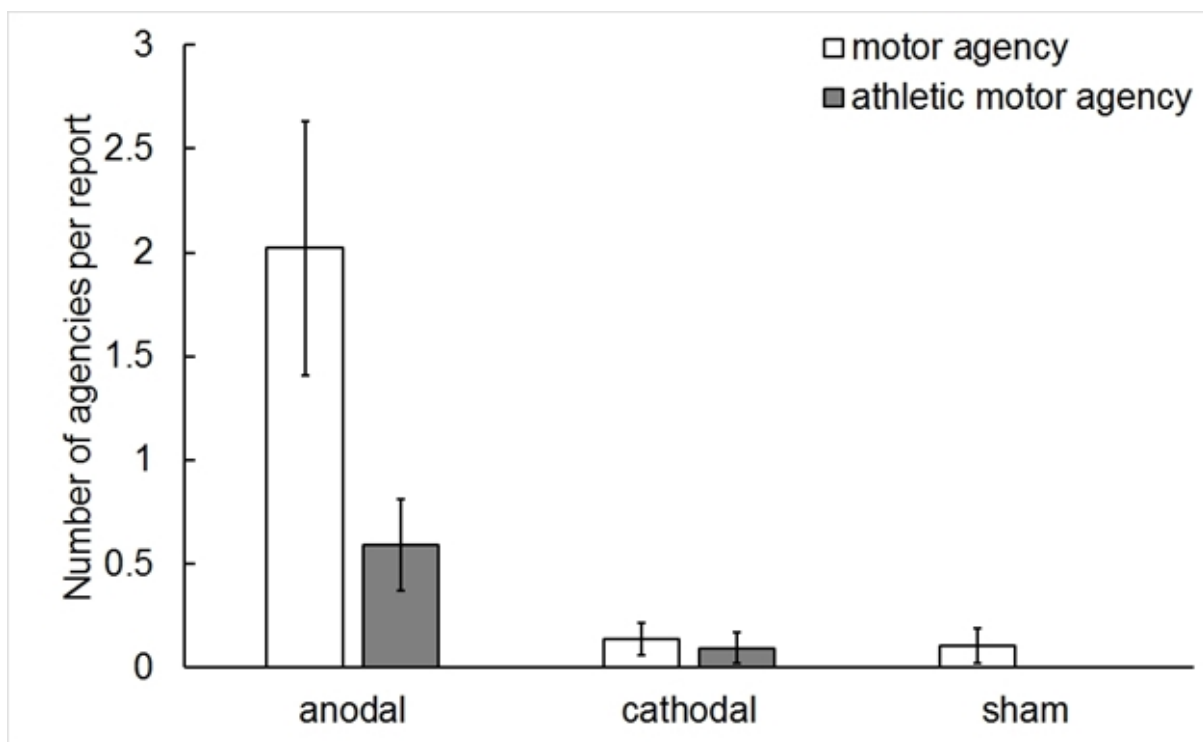


Figure 1: Number of motor and athletic motor agencies per report after anodal, cathodal, and sham stimulation of C3. Error bars indicate the standard error of the mean.

Reports containing versus not containing instances of motor agency

There was further a significant difference between stimulation conditions in the number of reports containing one instance or more of motor agency ($\chi^2(2)=11.91$, $p=.003$). There was a higher ratio of reports containing motor agency in the anodal stimulation condition than in the cathodal stimulation condition ($U=76$, $p=.027$). There were further more reports with motor agency from the anodal stimulation condition than from the sham stimulation condition ($U=61$, $p=.001$). There was no significant difference between cathodal and sham stimulation.

Further, the relative number of reports that contained at least one instance of athletic motor agency was compared between stimulation conditions. Again, there was a significant difference in the ratio of reports containing athletic motor agencies ($\chi^2(2)=7.94, p=.019$). There was a higher ratio of reports containing athletic motor agency from the anodal stimulation than from the sham stimulation condition ($U=90, p=.015$). Although the effect hinted towards the expected direction, there was no significant difference between anodal and cathodal stimulation ($U=93, p=.08$). Again, there was no significant difference between cathodal and sham stimulation. See Figure 2.

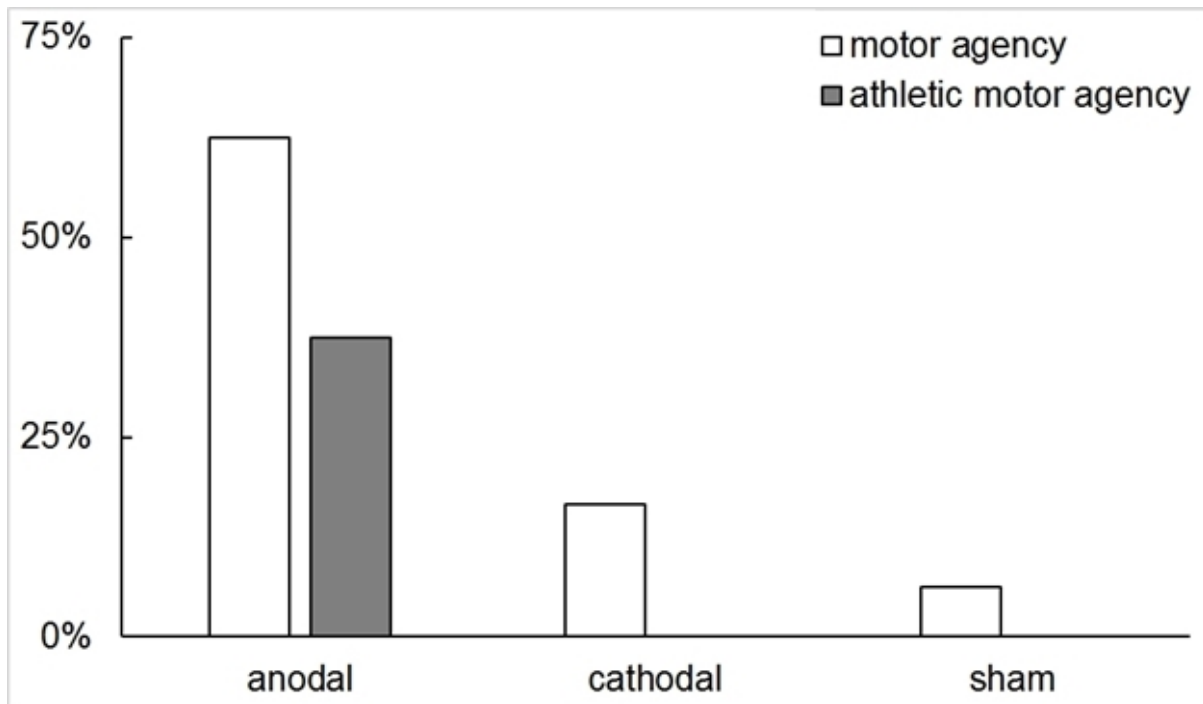


Figure 2: Percentage of reports containing one instance or more of motor agency or athletic motor agency per report after anodal, cathodal, or sham stimulation.

Agency perspective

There were no differences in the perspective from which the agency was perceived between the conditions. The three conditions did not differ significantly in the absolute number of first person and third person motor agencies, both for singular and plural (no second person singular and plural agencies were identified in any report). Further, after correcting for differences in the number of motor agencies between conditions, the patterns of motor agency perspectives did not change significantly.

Report length

The three stimulation conditions did not differ in report length. There was no significant difference in the word counts of the reports. Correcting for word count still yielded significant differences in motor agency between conditions ($F(2, 42)=4.21, p=.022, \text{partial } \eta^2=.167$). There were still significantly more references to motor agency in reports from anodal than from

cathodal stimulation ($p=.029$), and significantly more references to motor agency in reports from anodal versus from sham stimulation ($p=.011$).

Classification performance

The ROC curves for both motor agency and athletic motor agency for the classification between anodal stimulation versus cathodal and sham stimulation can be seen in Figure 3. The AUC value as an indicator of classification performance for motor agency analysis was $AUC = .782$, and was significantly different from .5 ($p=.007$). For athletic motor agency, the AUC value ($AUC = .067$) was not significantly different from .5 ($p=.637$).

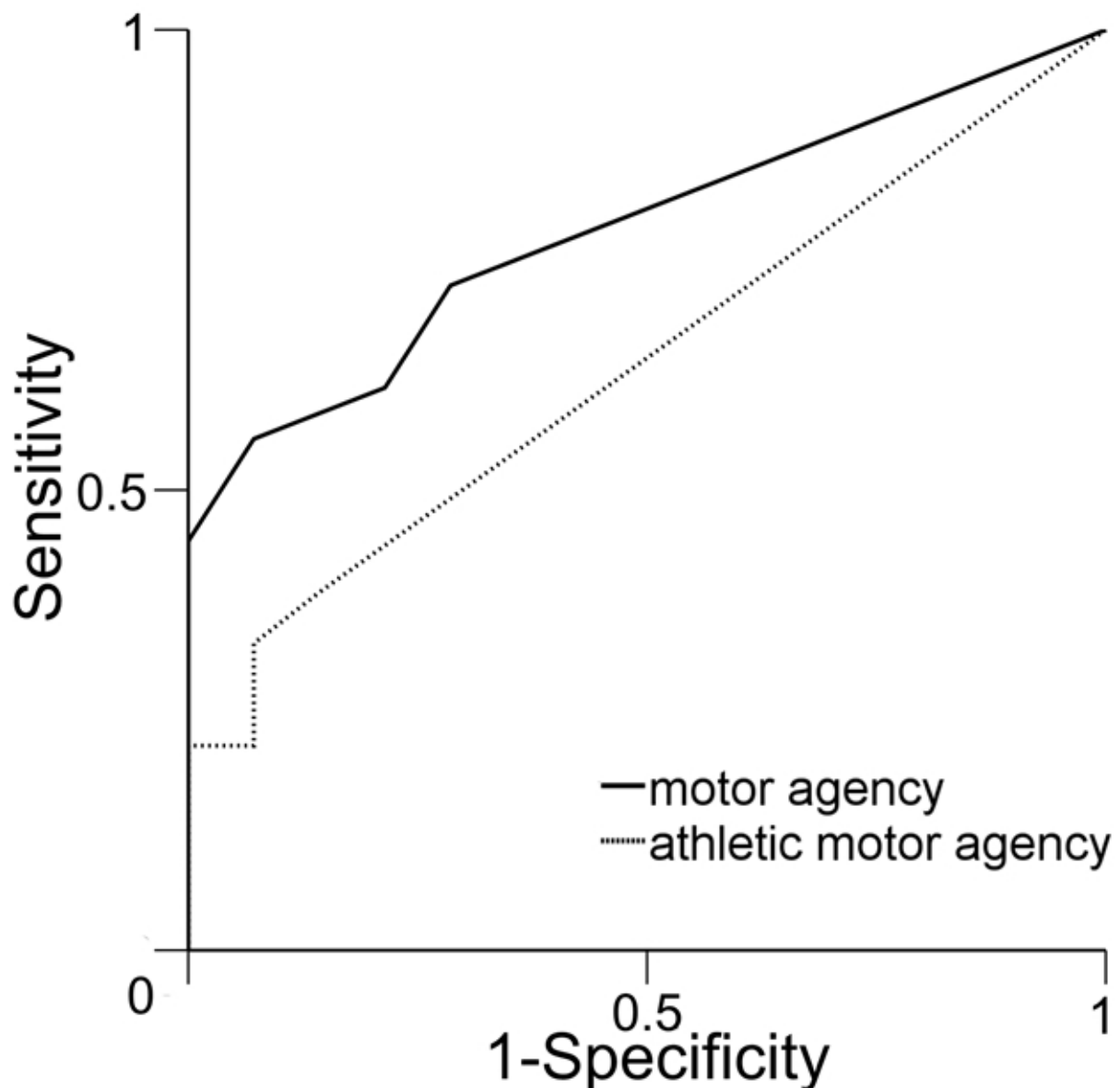


Figure 3: Receiver operating characteristic (ROC) curve for detecting reports from anodal tDCS, using motor agency and athletic motor agency as classifiers.

Discussion

This study investigated the effects of tDCS on human consciousness in REM sleep, aiming to compare the levels of motor imagery under three different stimulation conditions (anodal, cathodal, and sham tDCS). An implicit method (third person analysis of first person mentation reports) was used to measure motor imagery. Anodal, but not cathodal, tDCS of areas above the motor cortex (C3) in REM sleep increased motor imagery in general, as well as imagery of particularly athletic motor movements (classified as athletic disciplines). Anodal tDCS is known to increase motor cortical excitability (Nitsche and Paulus, 2000), and the present findings now indicate that it also enhances motor imagery. This ties in with a previous study where motor cortical tDCS in waking resting state increased motor imagery (Speth et al., 2015).

Neuroimaging studies, as well as studies that observe differences in dream mentation as functions of waking activities, indicate that dream movement and waking movement share the same neural substrate (Dresler et al., 2011 and Schredl and Erlacher, 2006). Having manipulated the neural substrate in question (the motor cortex) and observed accompanying changes in the features of dream reports (linguistic references to motor imagery), the present study can be interpreted as strengthening these findings.

As established previously, the fact that the English language comprises a remarkably large number of fixed motion and movement metaphors and idioms, had to be accounted for (Speth et al., 2013, Speth et al., 2015 and Speth et al., 2016a; Speth and Speth, 2016a). Raters were therefore again asked to differentiate between general versus athletic motor imagery, the rationale being that this allows for a rough distinction between weaker and stronger motor agency, while keeping the room for interpretation as well as the cognitive load for the raters to a minimum. The tool of Motor agency analysis would, for example, treat the idiom “going somewhere” as a possible linguistic artefact (as it does not necessarily denote motor imagery), but pick up “I was walking somewhere” (as a clearer description of athletic motor imagery, in this case experienced from a first person point of view). As explained in Speth et al. (2015), it is entirely possible that this objectivity was bought at the cost of a few cases where the tool did not pick up strong motor imagery (such as chopping vegetables or washing a car). Both general motor imagery and athletic motor imagery could differentiate between the stimulation conditions in the current study as well as in the previous study.

The present data reinforce the notion that the increase in linguistic references to motor agency in the reports is not an artefact of a general increase in motor speech output (again, see Speth et al., 2015). First, the length of the reports did not differ between stimulation conditions. Second, the present effect is robust to the control for word count, a procedure which despite the criticism of Hobson et al. (2000) concerning the control for report length in the study of dream reports, may have been appropriate in the current study, as verbal reporting involves motor action and could have been influenced by the stimulation of motor areas. Anodal stimulation however still resulted in significantly more reported motor imagery than cathodal and sham stimulation.

While the present study computer-randomized the participants to experimental groups and tested for differences in the groups that could potentially influence motor imagery (age, gender, handedness, personality, caffeine consumption, meditation experience, physical exercise, etc.), a replication of the experiment, preferably with a within-subject design and with a larger number of participants, will strengthen the claim that tDC stimulation in REM sleep affects motor imagery.

There was no difference in the perspective from which motor imagery was experienced. Note that in waking, tDCS of areas above the motor cortex led to motor imagery being experienced more often from a first person (and less often from a third person point of view) in the two tDC

stimulation conditions, as compared to the sham condition (Speth et al., 2015) – which ties in with the observation of Sirigu and Duhamel (2001) that first person motor simulations are more likely to involve motor activation than third person motor simulations. However, it seems that in REM sleep, perspectives may be less defined than they are in waking mentation.

The present results indicate that anodal tDCS of areas above the motor cortex enables us to further enhance motor imagery in REM sleep, a state which has long been assumed to be crucial for motor development and the rehearsal of motor movements for later performance.

There are certain limitations to the present study: Although it did control that the experimental groups did not differ in caffeine consumption, handedness, personality traits, and more, this study did not control for alcohol consumption. Although it seems unlikely that participants varied in their acute alcohol intake, it could still be possible that the groups differed systematically in their general habits of alcohol consumption.

A further possible limitation to the current results is that some dream reports may have been refused or censored by participants in a way that reported dream content differed in motor imagery from content that did not get reported, or that got censored, thus resulting in an artefact. Of course we have to rely on (potentially censored) first person data in mentation research (which seems justified especially as long as mentation reports are obtained under ideal experimental conditions, see Windt, 2013). There is little reason to assume the above-mentioned systematic error in the current results: First of all, there does not seem to be a robust (alternative) hypothesis as to why anodal tDC stimulation of the motor cortex, as opposed to cathodal and sham stimulation, should result in a significant increase or decrease in such dream content that is likely to be censored by participants. To current knowledge, there is no neurophysiological explanation as to why tDCS should result in more censor-worthy dream content. It further seems unlikely that such censor-worthy content should differ from uncensored (reported) content in its levels of motor imagery. It is additionally noted that a report was delivered after every valid awakening, irrespective of the stimulation condition. Further, the experimental conditions did not differ with respect to report length. This would mean that if systematic censorship took place, the censored content would have been replaced by content of roughly equal length, which again seems unlikely.

The present study applied anodal stimulation to areas above the left motor cortex (C3), while a larger reference electrode is placed at a contralateral frontal position. This stimulation protocol was chosen to allow for maximum comparability with an earlier experiment on stimulating motor imagery in waking (Speth et al., 2015). Future studies on motor imagery enhancement could however test whether a dual hemisphere design yields additional effects on motor imagery, either by applying an anode on one and a cathode on the contralateral motor cortex, or by applying anodes to both cortices.

As the right and left primary motor cortices show an inhibitory interaction with each other (Perez and Cohen, 2009), it has been proposed that tDCS of one primary motor area can have a disinhibitory effect on the contralateral primary motor area (Schlaug et al., 2008). Empirical results however are to date inconclusive: Vines et al. (2008) for example report anodal stimulation of one motor cortex to increase motor skill performance of the contralateral hand, while cathodal stimulation of the contralateral motor cortex increases this effect further. Similar results suggest an additional benefit of bihemispheric tDCS for motor recovery in stroke patients (Bolognini et al., 2011; Lindenberg et al., 2010). Other studies however find no additional effect of bihemispheric tDCS (Goodwill et al., 2013; O'Shea et al., 2014).

In a recent study, a similar tDCS protocol was used in that an anode was placed at C3, a larger cathodal reference was placed in a contralateral supraorbital position, and a current of 1

mA was ramped up over ten seconds, followed by thirty minutes of stimulation (Lindenberg et al., in press). This protocol is similar to that of the current study, and Lindenberg et al. (in press) compare the effects on functional brain activity (measured via fMRI) to the effects of a dual hemisphere protocol in which the anode is placed at C3, and a cathode is placed at the ipsilateral C4 position. They note a great variance of motor and premotor cortex activation between participants in reaction to single and dual stimulation when participants were performing motor tasks. This observation could partly explain the inconclusive earlier results outlined above. These intra-individual differences in response to single or dual stimulation could be functions of differences in transcallosal connectivity, however dual tDCS leads to greater disinhibition of the motor cortex (Lindenberg et al., in press). In a no-task condition, dual stimulation led to higher resting state connectivity in both inferior frontal cortices, while single anodal stimulation of the left motor cortex led to higher functional connectivity in the left motor cortex, supplementary motor area, and precuneus – which indicated stronger local effects of single hemisphere tDCS on the motor system (Lindenberg et al., in press). While the results of Lindenberg et al. (in press) are interesting for the current study as they use a similar stimulation protocol and observe its effects via functional imaging, their study was conducted in waking, and partly involved motor and motor inhibition tasks. The present study on the other hand was conducted in sleep, and follows a no-stimulus, no-no task, no-response design, so that it still has to be investigated in how far the results of the two studies are comparable.

Although tDCS in conjunction with motor imagery has been studied before (e.g. Sasaki et al., 2016 and Quartarone et al., 2004), these studies use motor imagery as an independent variable, such as a task to imagine motor movement. The present study is among the very first to measure the effects of tDCS on motor imagery (as a dependent variable) in sleep.

The supplementary motor area (SMA) could play a critical role in primary motor and somatosensory cortex stimulation, as it has been reported that stimulation protocols that target primary motor areas in combination with the SMA result in changes in the excitability of primary sensorimotor areas, while protocols that only target the motor cortex do not result in such changes (Kirimoto et al., 2011). The current protocol may have resulted in a stimulation of the SMA in addition to the primary motor cortex: It uses a similar stimulation protocol and similar positioning as that of Kirimoto et al. (2011), but even larger stimulation electrodes. It is therefore possible that the current effects on motor imagery are not only a result of primary motor cortex stimulation, but also involve SMA stimulation to achieve the desired effect.

Raters show an almost absolute agreement in identifying the numbers of motor agency in the reports. The inter-rater reliability achieved by the raters in the present study, without intense training or a psycholinguistic background, affirms the reliability of the tool of linguistic Motor agency analysis to quantify simulated motor actions as they are expressed in the language of mentation reports.

The Receiver Operating Characteristic (ROC) indicates that motor imagery as quantified by linguistic Motor agency analysis has an acceptable accuracy in differentiating between anodal tDCS versus cathodal and sham stimulation (AUC =.782). Motor agency is a better classifier than athletic motor agency for stimulation conditions.

The tool of Motor agency analysis could be used to further explore targeted clinical treatments and disorders of the motor system by establishing which protocol for cortical tDC stimulation induces which kinds of motor simulations, and in which intensity. The ROC suggests that Motor agency analysis could also be advanced to serve individual clinical diagnoses. Individual diagnoses helped by mentation reports could benefit from the fact that reports from

REM sleep, as opposed to reports from quiet wake, sleep onset, and non-REM sleep, are usually the most elaborate (Stickgold et al., 2001).

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