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RESEARCH ARTICLE

Efficacy of tRNS and 140 Hz tACS on motor cortex excitability seemingly dependent on sensitivity to sham stimulation

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Abstract

This study investigates the effect of corticospinal excitability during sham stimulation on the individual response to transcranial non-invasive brain stimulation (tNIBS). Thirty healthy young adults aged 24.2 ± 2.8 S.D. participated in the study. Sham, as well as 1 mA of tRNS and 140 Hz tACS stimulation were applied for 10 min each at different sessions. The effect of each stimulation type was quantified by recording TMS-induced, motor evoked potentials (MEPs) before (baseline) and at fixed time points after stimulation (T0, T30, T60 min.). According to the individual response to sham stimulation at T0 in comparison to baseline MEPs, subjects were regarded as responder or non-responder to sham. Following, MEPs at T0, T30 and T60 after verum or sham stimulation were assessed with a repeated measures ANOVA with the within-subject factor stimulation (sham, tRNS, 140 Hz tACS) and the between-subjects factor group (*responder* vs *non-responder*). We found that individuals who did not show immediately changes in excitability in sham stimulation sessions were the ones who responded to active stimulation conditions. On the other hand, individuals who responded to sham condition, by either increases or decreases in MEPS, did not respond to active verum stimulation. This result suggests that the presence or lack of responses to sham stimulation can provide a marker for how individuals will respond to tRNS/tACS and thus provide an explanation for the variability in interindividual response. The results of this study draw attention to the general reactivity of the brain, which can be taken into account when planning future studies using tNIBS.

Keywords Sham stimulation · tRNS · 140 tACS · Plasticity · MEP

Introduction

Transcranial electrical stimulation (tES) is a widely used method which influences cortical excitability. To date, the majority of studies in humans use transcranial direct current stimulation (tDCS) to modulate cortical activity [for a review see (Paulus 2011; Paulus et al. 2016)]. More recently, two additional types of tES have been introduced: transcranial random noise stimulation (tRNS) and transcranial alternating current stimulation (tACS) [for review see (Antal and Herrmann 2016)]. Both 1 mA tRNS and tACS at a frequency of 140 Hz may cause significant increases in cortical excitability (Terney et al. 2008; Moliadze et al. 2010a, 2012). The physiological mechanisms of tRNS are not completely clarified yet; as yet, it is unclear, if tRNS may interfere with ongoing network oscillations, as mentioned in the original publication (Terney et al. 2008), with homeostatic mechanisms (Fertonani et al. 2011) or induces plastic changes in the brain. With regard to changes during or after tACS, there is only little evidence for the effects of tACS on brain plasticity (Antal and Herrmann 2016).

Most of the studies investigating effects of tES compare group means, which do not offer much information about the within-subject response variability. Yet, there is a substantial variability of responses to tES across subjects on an individual level. This represents one of the most important limitations of tES (Horvath et al. 2014; Krause and Cohen Kadosh 2014; Li et al. 2015; Fertonani and Miniussi 2016). The individual effects of tES are dependent on intensity, electrode montage (Moliadze et al. 2010b; Teo et al. 2011; Moliadze et al. 2012; Batsikadze et al. 2013; Sehm et al. 2013; Scheldrup et al. 2014; Mehta et al. 2015; Brauer et al.

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2018), stimulation duration and frequency (Nitsche and Paulus 2000; Kanai et al. 2010; Stagg and Nitsche 2011; Brignani et al. 2013; Feurra et al. 2013; Wach et al. 2013; Cabral-Calderin et al. 2016; Brauer et al. 2018), and on the timing of stimulation relative to task engagement (Pirulli et al. 2013; Scheldrup et al. 2014; Cabral-Calderin et al. 2016; Brauer et al. 2018).

Apart from the methodological factors of stimulation, which generally affect both inter- and intra-subject variability. There are a number of other physiological determinants which have been extensively studied to find out about their influence on individual responses to tDCS, e.g. anatomical features of the head and brain, initial level of brain function, genetics, development and aging (Antal et al. 2007; Horvath et al. 2014; Krause and Cohen Kadosh 2014; Horvath et al. 2015; Moliadze et al. 2015; Horvath et al. 2016; Moliadze et al. 2018) [for review see (Li et al. 2015)]. As a consequence, several studies have reported that not all people respond to tES protocols as expected leading to a differentiation between subjects as responders vs non-responders to active stimulation (Ridding and Ziemann 2010; López-Alonso et al. 2014; Wiethoff et al. 2014; Guerra et al. 2017). Baseline neurophysiological states should also affect sham stimulation. However, this has not been studied as a predictor of response to active stimulation.

In addition, several studies show that individual reactions to stimulation protocols may indicate a general sensitivity to tES: The response to theta burst stimulation (TBS) was predicted by the latency of motor evoked potentials (MEPs) to single TMS pulses of different orientations (Hamada et al. 2013). In case of tDCS, individuals who showed greater responsiveness to tDCS also showed greater cortical responses following the repetitive TMS (Lang et al. 2004; Siebner et al. 2004; Bocci et al. 2014). Labruna et al. (2016) recently reported that subjects who are more sensitive to single-pulse TMS displayed greater tDCS effects (Labruna et al. 2016). However, there are studies that were not able to demonstrate the relationship between corticospinal excitability and reactivity at baseline and effects of tES (López-Alonso et al. 2014; Tremblay et al. 2016).

In this study, we investigate, whether response-variability to tES is related to individual differences in sensitivity to sham stimulation reflecting the physiological state of the brain. Our research question is based on previous findings which have shown that for both the motor and cognitive domains, variability in the response rate to tDCS is relatively high and it is unclear how much of the observed effectiveness is solely due to a placebo/sham effect (López-Alonso et al. 2014; Wiethoff et al. 2014; Chew et al. 2015; Hordacre et al. 2016; Turi et al. 2017; Dissanayaka et al. 2018; Hordacre et al. 2018). Therefore, sham stimulation might provide an additional source of variability, since sham stimulation might have physiological effects (Fonteneau et al. 2019). For example, it was shown that expectations induced by verbal instructions as induced with sham/placebo conditions may influence the effects of tDCS (Turi et al. 2017). However, it remains unclear how the physiological state of the brain itself impacts a subject's responsiveness to tES.

The present study aimed to investigate the impact of individual sensitivity to sham stimulation on electrophysiological responsiveness to tRNS and 140 Hz tACS when stimulating the primary motor cortex. We hypothesized that (1) based on the individual physiological state of brain there are different reaction types with respect to sham, i.e., *responder* or *non-responder* and that (2) this reaction is associated with the plasticity-like response to tRNS and 140 Hz tACS. Due to homeostatic metaplasticity, we expect that the group of *responders* to sham will not show a response to actual stimulation with 1 mA tRNs and 140 Hz tACS. In addition, and based on the current research, we expected that the group of *non-responders* shows excitatory effects for both stimulation methods.

Materials and methods

The study was in accordance with the latest revision of the Declaration of Helsinki. Experimental procedures were approved by the local ethics committee of the Kiel University, Kiel, Germany. Prior to the experiment, subjects gave their written informed consent.

Subjects

In total, 30 healthy subjects (age 24.2 ± 2.8 S.D., years, range 18–30 years, 10 females, 20 males) participated in this study (for details see Table 1). To calculate our sample size, we used g*Power (Faul et al. 2007) with the following settings: effect size $f = 0.25$, α level $= 0.05$, power $= 0.95$, correlation among repeated measures $= 0.7$. The minimum sample size was found to be 27, which we increased to 30. All participants were right-handed according to the Edinburgh Handedness Inventory (Oldfield 1971) and completed the entire study protocol. Exclusion criteria were pregnancy, history of migraine, brain-related injury or unexplained loss of consciousness, IQ < 90, history or family history of epileptic seizures, history of other neurological, psychiatric or chronic internal disorders, intake of medication effecting the central nervous system, brain or cardiac pacemakers, or permanent metal implants (cochlear implants, clips, dental braces).

Experimental design and procedure

A double-blind, randomized, sham-controlled, within-subject design was implemented conducting all stimulation

Table 1 Subject characteristics and thresholds before stimulation for each group and separated in responders according to the direction of their response

Experimental session	Subjects			SI_{1mV} (%)[b] ± SD	RMT (%)[b] ± SD	AMT (%)[b] ± SD	Baseline MEP amplitude (mV) ± SD
	n	Sex[a]	Age ± SD				
Non-responder to sham stimulation							
Sham	13	4F/9 M	25.2 ± 3.5	54.7 ± 11.2	47.23 ± 9.5	38.6 ± 8.7	0.98 ± 0.08
tRNS				57.3 ± 12.3	48.8 ± 10.3	39.0 ± 9.5	0.97 ± 0.18
140 Hz tACS				55.7 ± 12.3	47 ± 10.5	37.3 ± 9.3	0.96 ± 0.12
Responder (positive) to sham stimulation							
Sham	9	4F/5 M	23.3 ± 2.1	59.1 ± 9.4	49.6 ± 6.1	42.9 ± 7.0	0.93 ± 0.08
tRNS				55.6 ± 9.4	47.7 ± 8.4	41.6 ± 7.6	0.96 ± 0.1
140 Hz tACS				56.4 ± 7.8	49.2 ± 6.6	41.3 ± 6.6	1.0 ± 0.06
Responder (negative) to sham stimulation							
Sham	8	2F/6 M	22.6 ± 2.4	57.3 ± 8.7	50.1 ± 7.9	43.3 ± 7.4	0.98 ± 0.1
tRNS				59.3 ± 10.3	51.5 ± 8.2	43.8 ± 8.7	0.99 ± 0.1
140 Hz tACS				56.3 ± 10.1	48.0 ± 8.2	40.5 ± 7.6	1.00 ± 0.09

Data are presented in mean ± SD; *F* female, *M* male. Baseline MEP amplitude means of about 1 mV were calculated for each experimental condition. The single test-pulse TMS intensity was adjusted to achieve a baseline MEP of SI 1 mV

[a]Chi-square responder vs non-responder n.s.

[b]% of maximal TMS-output

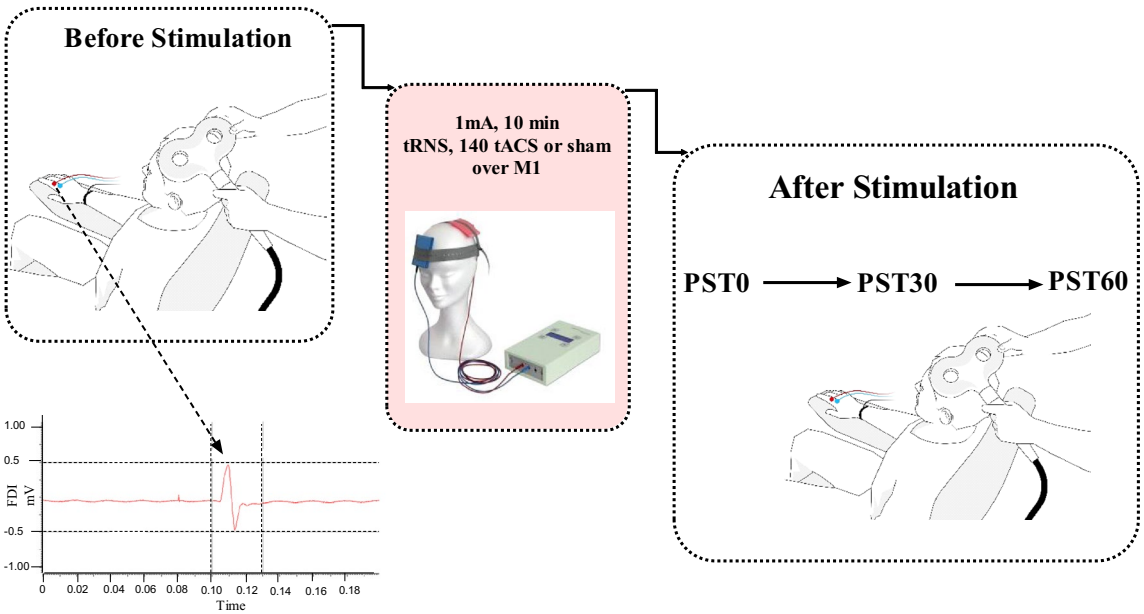

Fig. 1 Design of the study. 1 mA tRNS, 140 Hz tACS or sham stimulation was applied over the left primary motor cortex (M1, over C3 according to the international 10–20 system) for 10 min. The reference electrode was placed over the contralateral supraorbital area. In the beginning of each stimulation session, 20 single-pulse TMS were administered. The intensity required to evoke a MEP of ~ 1 mV peak-to-peak amplitude (SI1 mV) and a baseline of TMS-evoked MEPs (20 stimuli) were recorded at 0.25 Hz prior to stimulation. Afterwards, 1 mA tDCS was administered over 10 min, and then the above-mentioned parameters were recorded again directly as well as 30 and 60 min after stimulation (PST0–PST60)

conditions in each participant. The order of the stimulation conditions (tRNS, 140 Hz tACS, sham) was counterbalanced across subjects. Sessions were separated by at least 7 days to avoid carry over effects. In each subject, the experimental sessions were performed at the same time during the day.

Stimulus intensities (in percentage of maximal stimulator output) of TMS were determined at the beginning of each experiment. Immediately following stimulation, 20 single test-pulse MEPs were recorded at 0.25 Hz at intervals of directly after (T0), 30 min (T30), 60 min (T60) (see Fig. 1).

After finishing each experimental session, the participant was asked to complete a stimulation side effects questionnaire adapted from (Poreisz et al. 2007). The questionnaire contains items pertaining to the presence and severity of headaches, change or difficulties in concentration, mood, visual perception, presence of fatigue, and discomforting sensations such as pain, tingling, itching and burning.

Stimulation techniques

140 Hz tACS and tRNS

1 mA 140 Hz tACS and 1 mA of tRNS were administered for 10 min by a battery-driven electrical stimulator (Version DC-Stimulator-Plus, NeuroConn GmbH, Ilmenau, Germany) through conductive-rubber electrodes placed in two saline-soaked sponges. For the tACS, the waveform of the 140-Hz stimulation was sinusoidal. For the tRNS in the stimulation mode "noise", there was a random level of current generated for every sample (sampling rate 1280 sps). The random numbers were normally distributed; the probability density function followed a bell-shaped curve. In the frequency spectrum, all coefficients had a similar size ("white noise"). The noise signal contained all frequencies up to half of the sampling rate, i.e. a maximum of 640 Hz. Due to the statistical characteristics, the signal had no DC offset. The current was ramped up and down over the first and last 5 s of stimulation.

The size of the stimulation electrode over the left M1 and the reference electrode, which was placed over the contralateral supraorbital area, were 5 × 7 cm; both were fixed to the head by elastic bands. The position of the stimulation electrode was determined prior to stimulation by single pulses of transcranial magnetic stimulation (TMS). This electrode set-up—active electrode over the M1 and reference electrode over the contralateral supraorbital area—has been shown to be the optimal combination to enhance excitability of the M1 (Moliadze et al. 2010b). Subjects and the investigator, who made the MEP measurements, were blinded for stimulation conditions in all of the studies. The stimulations were done by another investigator.

Transcranial magnetic stimulation

To detect changes in corticospinal excitability, MEPs of the right FDI (first dorsal interosseous muscle) were recorded following a single-pulse TMS of its representation area on M1. A Magstim 200 magnetic stimulator (Magstim Company, Whiteland, Wales, UK) with a figure-eight standard double magnetic coil (diameter of one winding: 70 mm; peak magnetic field: 2.2 T; average inductance: 16.35µH) was used. A surface electromyogram (EMG) was recorded from the right FDI through a pair of Ag–AgCl surface electrodes in a belly tendon montage. The amplified raw data were band-pass filtered (2 Hz–2 kHz; sampling rate, 5 kHz) and digitized with a micro 1401 AD converter (Cambridge Electronic Design, Cambridge, UK) controlled by Signal Software (Cambridge electronic Design, version 2.13). For offline analysis, data were stored on a computer. Complete relaxation was controlled through visual feedback of EMG activity, and, in case of tension, the subject was reminded to relax. The eight-curved coil was held tangentially to the skull at 45° from the sagittal line which resulted in a posterior–anterior direction of current flow in the brain. The optimum position was defined as the site where TMS consistently resulted in the largest and most stable MEP in the resting muscle. The spot was marked with a skin marker pencil to ensure that the coil was held in the correct position throughout the experiment. The intensity required to evoke a MEP of ~ 1 mV peak-to-peak amplitude (SI1 mV) and a baseline of TMS-evoked MEPs (20 stimuli) were recorded at 0.25 Hz prior to stimulation.

Data analysis and statistics

First, MEP analysis was completed manually by visual inspection of offline EMG data. Traces showing any muscle activity prior to the stimulus were removed from the analysis.

All statistical analyses were performed using SPSS Version 24.

Throughout all analyses, results were regarded as statistically significant with a two-tailed p value of less than 0.05.

Classification of response to sham stimulation based on MEP measurements

Response to sham was evaluated based on change in MEP directly after stimulation (T0), compared to baseline MEP with a Wilcoxon signed rank test for matched samples. Based on the result, subjects were categorized as either *"responder"* or *"non-responder"* to sham stimulation.

Age and parameters of TMS baseline measurement were compared between these groups using one-way ANOVA.

Effect of response to sham on verum stimulation

We performed a repeated measures (rm) ANOVA with two within-subject factors, TIME (4 levels: before (baseline), and "directly after (T0), 30 min after (T30), "60 min after (T60) stimulation) and STIMULATION (3 levels: "tRNS", "140 Hz tACS", "sham"), and the between-subject factor GROUP (*"responder"* vs. *"non-responder"* to sham). MEP amplitude served as the dependent variable.

In case of a significant interaction effect of GROUP × STIMULATION or GROUP × STIMULATION × TIME,

rm ANOVA with the aforementioned two within-subject factors TIME and STIMULATION was repeated for each group separately. In case of significant results, simple contrasts were computed, comparing both verum (tRNS, tACS) to sham stimulation as well as each time point after stimulation ("directly after", "30 min after", "60 min after" stimulation) to baseline.

In both rm ANOVAs, the assumption of sphericity was tested with Mauchly's test.

Adverse events questionnaire

The incidence of each side effect was coded in a binary system (yes = 1, no = 0). The severity of each side effect was rated on a numerical analogue scale (NAS) from one to five, one being very mild and five being an extremely high intensity of any given side-effect. Adverse events were compared between *responders* and *non-responders* to sham. As we wanted to explore possible differences between excitatory and inhibitory response to sham, we differentiated a "positive" and a "negative" response group.

Because the variable failed normal distribution (Kolmogoroff–Smirnoff test), the number of adverse events as well as the severity of each adverse events were compared between conditions using the Kruskal–Wallis test.

Results

Classification of response to sham stimulation based on MEP measurements

Non-parametric matched samples test classified 17 subjects as *responders* to sham (see Fig. 2).

Age and baseline parameters of TMS did not differ between responders and non-responders to sham (for subjects characteristics see Table 1).

Effect of response to sham on verum stimulation

In the three-way rm ANOVA, Mauchly's test indicated that the assumption of sphericity had not been violated for

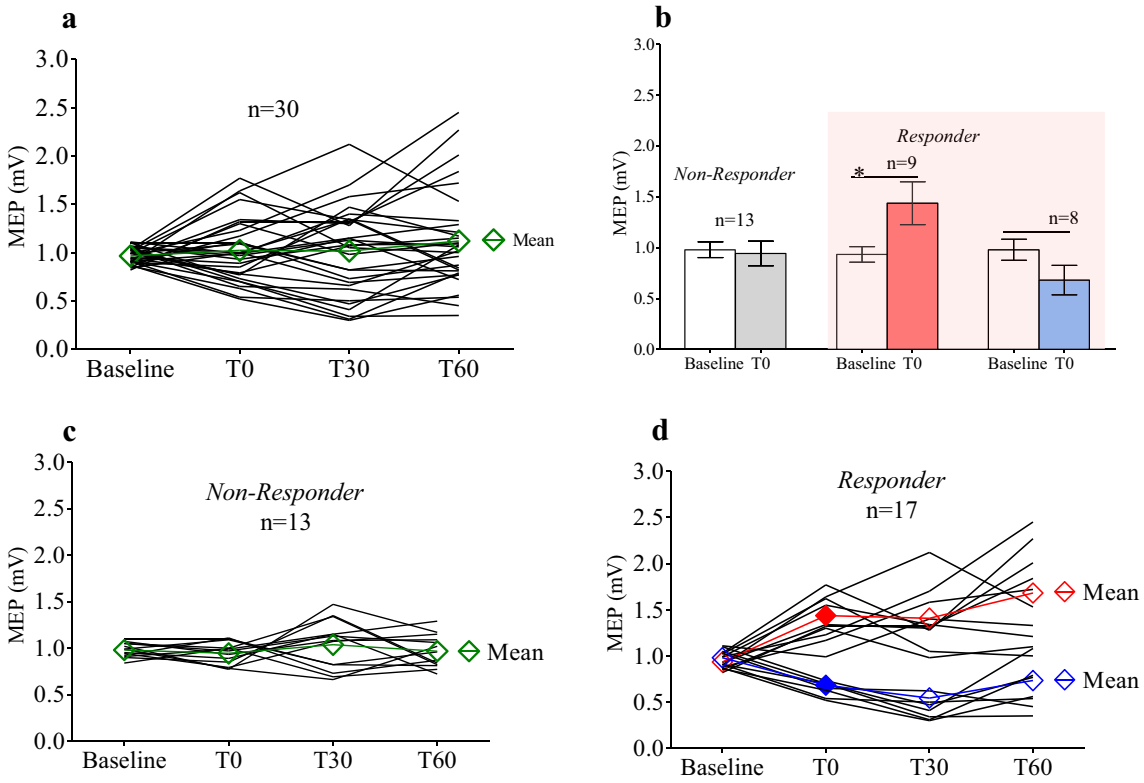

Fig. 2 Sham stimulation. **a** Changes of the individual MEP amplitude during sham condition for all subjects show considerable variability, while the mean implies no changes compared baseline. **b** Response to sham was evaluated based on change in MEP directly after stimulation, compared to baseline MEP with a Wilcoxon signed rank test for matched samples. Based on the occurrence of significant changes, the sample was divided into two groups, *"responder"* and *"non-responder"* to sham stimulation. **c** and **d** The individual MEP amplitude changes at sham condition for each group. Red lines are for a "positive" and blue lines are for a "negative" response group indicating a stability of the response throughout post-stimulation measurement

Table 2 Repeated measures ANOVAs with MEP amplitude as dependent variable

	df	*F*	*p*
Three-way rm ANOVA			
STIMULATION	2	2.30	0.110
STIMULATION × GROUP	2	6.60	**0.003**
TIME	3	8.34	**<0.001**
TIME x GROUP	3	6.32	**0.001**
STIMULATION × TIME[a]	4.20	0.98	0.424
STIMULATION × TIME × GROUP[a]	4.20	3.44	0.010
Two-way rm ANOVA non-responder			
STIMULATION	2	10.05	**0.001**
TIME	3	10.46	**<0.001**
STIMULATION × TIME	6	2.66	**0.022**
Two-way rm ANOVA responder			
STIMULATION	2	1.46	0.248
TIME	3	1.77	0.165
STIMULATION × TIME	6	1.47	0.198

Bold values indicate significant results ($p < 0.05$)

[a]The assumption of sphericity was violated and degrees of freedom were corrected using the Greenhouse–Geisser estimates

the effect of stimulation and time ($p > 0.119$), but for their interaction $\chi^2(20) = 38.00$, $p = 0.009$, therefore, the degrees of freedom were corrected only for the respective interaction using Greenhouse–Geisser estimates of sphericity ($\varepsilon = 0.699$).

There was a significant three-way interaction of STIMULATION, TIME and GROUP $F(4.2, 117.5) = 3.44$, $p = 0.010$. The main effect for TIME and the interaction effects for TIME with GROUP and for STIMULATION with GROUP were also significant (all $p \leq 0.003$).

We then performed a two-way rm ANOVA separately for each group; Mauchly's did not indicate a violation of the assumption of sphericity. The analyses showed that only in *non-responders* to sham there was a significant interaction of STIMULATION and TIME $F(6, 72) = 2.66$, $p = 0.022$. The main effects were significant as well (STIMULATION $F(2, 24) = 10.05$, $p = 0.001$. TIME $F(3, 36 = 10.46$, $p < 0.001$; see Table 2 and Fig. 3).

All contrasts in non-responders were significant (all $p \leq 0.025$) indicating that both for tRNS as well as tACS, MEP amplitudes increased for all timepoints compared to baseline (after stimulation), see Table 3 for all comparisons.

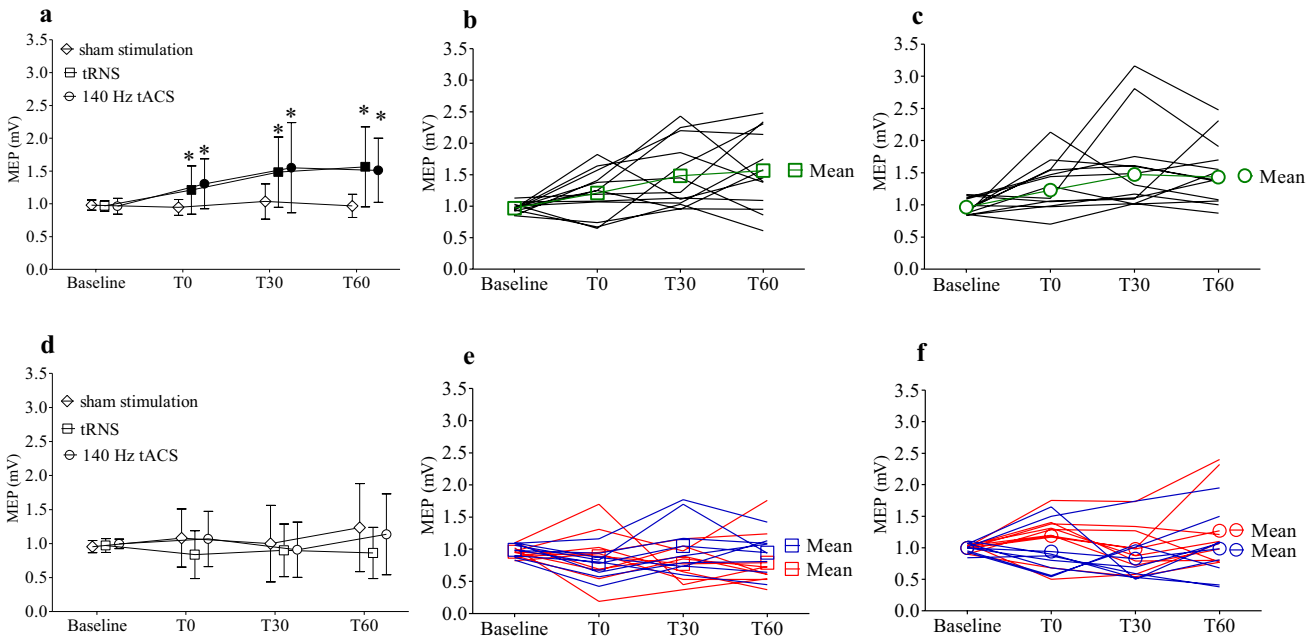

Fig. 3 After-effects of 1 mA 140 Hz tACS, 1 mA tRNS, and sham stimulation. Filled symbols indicate significant deviations of the measurements after MEP amplitudes from baseline values ($p < 0.05$). The figure shows mean amplitudes of MEPs and their SEMs before and after stimulation up to 60 min. **a** *Non-responder* group ($n = 13$). The analyses showed that only in non-responders to sham there was a significant interaction of STIMULATION and TIME ($p = 0.022$). The main effects were significant as well (STIMULATION $F(2, 24) = 10.05$, $p = 0.001$. TIME ($p < 0.001$). All contrasts in non-responders were significant (all $p \leq 0.025$) indicating that both for tRNS as well as 140 Hz tACS, MEP amplitudes increased for all timepoints compared to baseline (after stimulation). The individual MEP amplitude changes for tRNS **b** and 140 Hz tACS **c** for each subject. **d** For *responders* to sham ($n = 17$), the interaction as well as the main effects remained non-significant ($p > 0.165$). The individual MEP amplitude changes for tRNS **e** and 140 Hz tACS **f** for each subject

Table 3 Contrasts for the interaction effect of the rm ANOVA with factors STIMULATION × TIME (Table 2)

	STIMULATION	TIME	*df*	*F*	*p*
Non-responder to sham	„tRNS“ vs. „sham“	T0 vs. baseline	1	6.53	**0.025**
		T30 vs. baseline	1	7.96	**0.015**
		T60 vs. baseline	1	10.72	**0.007**
	„140 Hz tACS“ vs. „sham“	T0 vs. baseline	1	12.22	**0.004**
		T30 vs. baseline	1	7.06	**0.021**
		T60 vs. baseline	1	17.37	**0.001**
*Responde*r to sham	tRNS“ vs. „sham“	T0 vs. baseline	1	4.09	0.060
		T30 vs. baseline	1	0.38	0.545
		T60 vs. baseline	1	3.83	0.068
	„140 Hz tACS“ vs. „sham“	T0 vs. baseline	1	0.26	0.615
		T30 vs. baseline	1	0.98	0.338
		T60 vs. baseline	1	0.68	0.423

Bold values indicate significant results ($p < 0.05$)

Table 4 Side-effects and sensations

Sensations/side effects Incidence (intensity:mean ± SD)	*Non-responder* to sham stimulation $n = 13$			*Responder* to sham stimulation $n = 17$					
				Positive $n = 9$			Negative $n = 8$		
Lightening bold	2	–	–	–	–	1	1	–	–
Tingling	3	–	–	1	–	1	2	1	2
	1.3 ± 0.58			1		1	1	2	2 ± 1.41
Itching	1	–	1	–	–	–	–	–	2
	2		1						2 ± 1.41
Burning	1	1	1	1	–	–	1	–	2
	2	1	1	1			2		2 ± 1.41
Pain	–	1	–	–	–	–	3	3	1
		1					1.7 ± 0.58	1	3
Headache	–	–	–	–	1	–	1	–	1
					1		1		2
Fatigue	2	4	3	2	4	2	4	3	2
	1.5 ± 0.71	1	1.7 ± 1.15	3	2 ± 0.82	2 ± 1.41	3 ± 1.41	2 ± 1	1 ± 0
Difficulties in concentrating	–	1	–	–	1	–	1	1	–
		2			1		1	1	
Nervousness	–	1	–	–	–	–	1	1	1
		2					1	1	1
Flickering	3	1	1	2	1	–	2	1	–
	2 ± 1	1	2	1	3		2 ± 1.41	1	

There were no significant differences between groups regarding all types of adverse effects, indicating no noxious effect of both tRNS and tACS and a successful sham arrangement

For responders to sham, the interaction as well as the main effects remained non-significant ($p > 0.165$).

Adverse effects: side-effects and sensations

Adverse effects reported by the participants are displayed in Table 4. There were no significant differences between groups regarding all types of adverse effects, indicating no noxious effect of both tRNS and tACS and a successful sham arrangement (see Table 4).

When asked (“Did you feel any difference between the first, second and third stimulation sessions”?) none of the subjects could distinguish between stimulation conditions. The order of sessions had no effect on guess rate concerning

the experimental condition. Sham stimulation as well as 140 Hz tACS and tRNS were indistinguishable regarding side effects (Kruskal–Wallis test).Thus, the blinding procedure was judged as being successful.

Discussion

The goal of this study was to determine whether individual differences in the efficacy of tRNS and 140 Hz tACS stimulation are related to individual differences in sensitivity to sham stimulation. Previous research on response-variability to tES has focused mainly on tDCS studies, while our study focuses on tRNS and tACS.

As we hypothesized, we found that subjects who responded to sham stimulation turned out to be non-responders to verum stimulation when applying tRNS and 140 Hz tACS. This effect did not depend on the direction of the response to sham; both groups of participants who had either shown an increase or decrease in MEPs as a result of sham stimulation, did not show any significant response to verum stimulation. This was not due to a lack of effectiveness of the verum stimulation as in contrast, individuals that reacted as expected and did not show a response to sham stimulation, yielded the typical excitatory effects subsequent to active stimulation.

The results in the *"non-responders"* to sham are consistent with the previous literature. In the case of tRNS, there are other studies that have shown a consistent increase in excitability lasting at least 60 min after 10 min of stimulation. This was demonstrated by both physiological measures and behavioural tasks (Terney et al. 2008; Moliadze et al. 2010a, 2012; Fertonani et al. 2011). The same applies to the 140 Hz tACS. tACS over 10 min at 140 Hz with 1 mA at the motor cortex caused an hour-long MEP increase. This increase in corticospinal excitability was paralleled by a relative decrease in short latency intracortical inhibition (SICI), an electrophysiological marker of $GABA_A$ receptor mediated inhibition (Moliadze et al. 2010a). A recent study has reported sustained beneficial after-effects when 140 Hz tACS applied over the left frontal cortex during explicit word-pair encoding (Ambrus et al. 2015). However, a later study demonstrated a nonlinear dependency between the intensity of 140 tACS and tRNS protocol and the observed effects on motor excitability. Specifically, low stimulation intensities given at 140 Hz over the primary motor cortex resulted in cortical inhibition, as assessed with increased motor thresholds during simultaneous recordings of motor evoked potentials with single-pulse TMS (Moliadze et al. 2012).

As we hypothesized for the *"responders"* to sham, they did not show significant corticospinal responses to both tRNS and tACS. Our hypothesis was based on the fact that the polarity and strength of activity-dependent synaptic plasticity can be modified as a function of the prior general cortical reactivity (Karabanov et al. 2015; Muller-Dahlhaus and Ziemann 2015). In subjects with distinct corticospinal reactivity to sham stimulation, both tRNS and tACS may cause a deviant response to tES which may differ from that in subjects with no significant reactivity to sham. These findings may be explained by the theory of metaplasticity. The basic idea of metaplasticity is that the threshold for activity-dependent synaptic plasticity is not static but dynamic and it is also a function of the integrated prior activity of the post-synaptic neuron. It refers to synaptic or cellular activity that primes the ability to induce subsequent synaptic plasticity, such as long-term potentiation (LTP) or depression (LTD) (for review see (Karabanov et al. 2015; Muller-Dahlhaus and Ziemann 2015). Our results add further support the Bienenstock–Cooper–Munro (BCM) theory (Bienenstock et al. 1982), which claims that high levels of prior activity favor the induction of LTD, while low levels of prior activity favor LTP (Lang et al. 2004; Siebner et al. 2004); for review see (Ziemann et al. 2008; Karabanov et al. 2015).

An additional explanation of our observed effect of a response reaction to sham on verum stimulation may be related to the concept of state dependency (Silvanto et al. 2008). It was argued that repetitive transcranial magnetic stimulation (rTMS)-induced activity or "neural noise" (Ruzzoli et al. 2010) is not totally random and may not be independent of the task-induced neural activity or brain state (Silvanto et al. 2008). Such an "optimal level of noise" is usually used to explain "unexpected" facilitation/inhibition effects of TMS protocols. Similar effects of state dependency have also been suggested for transcranial electrical stimulation techniques [e.g. (Nguyen et al. 2018; Moliadze et al. 2019) with the assumption that stimulation-induced modulation of ongoing brain activity can be defined as noise induction (Miniussi et al. 2013)]. Therefore, it seems likely that the corticospinal excitability and reactivity (in the sense of reaction or excitation readiness) may determine individual response to tES (Horvath et al. 2014; Ammann et al. 2017).

It could be argued, that the physiological response to sham stimulation would be similarly activated in the active conditions, which would then influence the ability to induce plasticity-like responses. However, based on MEP measurements this assumption would be too speculative, since measuring MEPs (at rest) is itself already a quite variable outcome measure (Horvath et al. 2016). Therefore, an alternative explanation is that some people are inherently variable to TMS and that passage of time, i.e. boredom/arousal state, may be responsible for changes detected with TMS. In our study, we did not measure task-evoked state dependency but rather physiological state dependency. Some current papers and reviews refer to this as "baseline activity",

interchangeably it is also called "individual physiological brain state" [for a review see (Krause and Cohen Kadosh 2014)].

Another relevant methodological issue to consider is the setup of the comparison group for stimulation. Similar to other studies, our sham stimulation consisted in delivering a short period of active stimulation at the beginning of the stimulation session followed by no stimulation for a total duration equal to the duration of the active stimulation. However, several studies have investigated tDCS effects with parameters similar to those of sham parameters (i.e., short stimulation duration), with mixed findings (Furubayashi et al. 2008; Javadi et al. 2012; Fonteneau et al. 2019). Therefore, we do not strictly have a physiological state dependency, as a slight stimulation took place. Nevertheless, taking the neuromodulatory effects elicited by our sham as a possible predictor for the response to verum stimulation remains valid within our sham setup. Furthermore, even though a few studies have reported that sham stimulation might also have elicited some neurophysiological effects, it has been proven as a reliable widely accepted protocol.

Our findings are in accordance with previous studies, showing that the mean group results do not offer much information about the single person response variability. Inter-individual differences such as genetic and gender differences may modulate the effects of tES. The brain-derived neurotrophic factor (BDNF) has been shown to play a role in the mechanisms of neuroplasticity induction, giving rise to cellular events resulting in LTP and LTD (Schinder and Poo 2000; Mei et al. 2011). Antal and colleagues identified a different efficacy of stimulation by comparing individuals with different alleles of the Val66Met single nucleotide polymorphism of the BDNF gene (Antal et al. 2010): The heterozygotes (Val66Met) reacted more strongly to tDCS and independently of stimulation polarity. It would be interesting to test whether the BDNF Val66Met polymorphism correlated with sham-sensitivity. In other words, if subjects from the responder groups are different from the "No-responder" group in with regard to a BDNF-polymorphism.

Limitations

This study is characterized by some limitations. Most importantly, the small number of subjects in the groups may limit the generalizability of the results. Using an overall statistical method, we tried to minimize alpha accumulation and increase power.

Another possible limitation is that we did not measure MEPs online during sham stimulation. It would be interesting to see whether online effects of sham also influence response to active stimulation. However, previous research shows that effects directly after 140 Hz tACS are similar to online effects (Moliadze et al. 2010a). Since we classified responder vs non-responder directly after stimulation (T0), we can argue that online effects would be similar. This is also reflected in the fact, that variability at T0 seems lower than later effects, 30 and 60 min after stimulation.

Furthermore, the use of neuro-navigation would have been preferable to objectively monitor the coil position and reduce any possible bias introduced by the examiner.

Generally, it is commonly acknowledged that MEP amplitude is naturally a variable measure. As such, it can be assumed that there will always be some shift away from baseline levels, regardless of intervention. Accordingly, the utilization of a control condition to differentiate between natural fluctuation and tES-engendered effects is imperative and has been implemented in this study.

Finally, the results of this study may be specific to the primary motor cortex and tRNS/140 Hz tACS and not apply to every brain region or to all the varieties of transcranial brain stimulation. However, other studies have provided evidence in favor of similar BCM-like mechanisms (see above) in the visual cortex of human subjects (Fierro et al. 2005; Bocci et al. 2014).

Conclusion

The main finding of this study is that responsiveness to sham can predict the occurrence of a response to tRNS and 140 Hz tACS: the effect of sham stimulation in healthy young adults could be a predictor for classifying subjects as responders or non-responders to tES, namely that responders to sham are non-responders to tES. It is, therefore, likely that stimulation effects depend on the individual state of the brain. Thus, findings in healthy controls may not be directly transferable to patient populations. Further studies should characterize effects of tES in different healthy subjects, as well as in patients with regard to their potentially altered physiological state activity.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper. All the authors have read the manuscript and have approved this submission.

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