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Close to threshold transcranial electrical stimulation preferentially activates inhibitory networks before switching to excitation with higher intensities

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ABSTRACT

Background: Recently we have shown that transcranial random noise (tRNS) and 140 Hz transcranial alternating current stimulations (tACS), applied over the primary motor cortex (M1) and using 10 min stimulation duration and 1 mA intensity, significantly increases cortical excitability as measured by motor evoked potentials at rest before and after stimulation.
Objective/hypothesis: Here, by decreasing the stimulation intensity in 0.2 mA steps from 1.0 mA, we investigate to what extent intensity depends on the induced after-effects.
Methods: All twenty-five subjects participated in two different experimental sessions each. They received tACS using 140 Hz frequency and full spectrum tRNS at five different intensities on separate days. Sham stimulation was used as a control.
Results: Instead of receiving a simple threshold, unexpectedly, in these two independent data sets at threshold intensities of 0.4 mA we found a switch of the already known excitation achieved with an intensity of 1 mA to inhibition. The intermediate intensity ranges of 0.6 and 0.8 mA had no effect at all. Interestingly, the inhibition produced by 140 Hz tACS was stronger than that induced by tRNS.
Conclusions: In summary, we have shown here the possibility of selectively controlling the enhancement or reduction of M1 excitability by applying different intensities of high frequency transcranial electrical stimulation.

Introduction

Apart from repetitive transcranial magnetic stimulation (rTMS) and its more efficient variant "theta burst stimulation" (TBS) [1], transcranial direct current stimulation (tDCS) is the most well-known method currently used to influence motor cortex (M1) excitability [2]. Recently it was shown that transcranial random noise stimulation (tRNS), in a range between either 0.1 Hz and 640 Hz or between 100 and 640 Hz, is a highly effective method of increasing cortical excitability and avoiding directional sensitivity of standard tDCS [3]. tRNS induces a consistent excitability increase lasting at least 60 min after 10 min of stimulation, as demonstrated by both physiological measures and behavioural tasks [3].

Sinusoidally varying transcranial stimulation (transcranial alternating current stimulation: tACS) may be particularly able to interact with ongoing rhythms in the cortex. Recently, tACS applied with a frequency of 140 Hz, the so-called "ripple frequency", was shown to increase excitability in a similar way to both anodal tDCS and tRNS [4]. In this paper we argued that plastic after-effects induced by tRNS can possibly be explained by a 140 Hz interference with cortical ripple frequency oscillations.

So far in all paradigms we controlled for the duration of the after-effects by varying stimulation duration [3,5–9]. Controlled reductions of stimulation intensity have been performed only for tDCS [7]. Therefore, the lowest intensity capable of inducing observable after-effects is so far unknown for tACS and tRNS. To answer this question, we applied different stimulation intensities (0.2, 0.4, 0.6 and 0.8 mA, respectively) in order to determine whether a low intensity of tRNS and 140 Hz can also induce a change in cortical excitability. We hypothesized that the intensity reduction of externally applied high frequency oscillations will end up with a threshold intensity which should not be undercut in therapeutic studies.

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Table 1
Stimulation paradigms, electrode sizes, subject's characteristics and baseline values for the performed experiments.

Experiment	Number of subjects	Baseline (single TMS) amplitudes (mV) ± SEM	Sex (f/m)
Experiment 1	Sham: 14	1.06 ± 0.04	11/3
Full spectrum tRNS	0.2 mA: 14	1.03 ± 0.03	
	0.4 mA: 14	1.03 ± 0.03	
	0.6 mA: 14	1.01 ± 0.03	
	0.8 mA: 14	1.03 ± 0.02	
	1.0 mA: 14	1.05 ± 0.02	
Experiment 2	Sham: 11	1.06 ± 0.04	8/3
140 Hz tACS	0.2 mA: 11	1.03 ± 0.02	
	0.4 mA: 11	1.07 ± 0.03	
	0.6 mA: 11	1.04 ± 0.03	
	0.8 mA: 11	1.02 ± 0.03	
	1.0 mA: 11	0.95 ± 0.02	

(*S: stimulation electrode; R: Reference electrode*).
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. f, female; m, male; R, reference electrode (frontopolar); S, motor cortex stimulation electrode; FDI, First Dorsal Interosseous muscle.

Materials and methods

We conform to the Declaration of Helsinki, and the experimental protocol was approved by the Ethics Committee of the University of Göttingen.

Subjects

A total of 25 subjects (age 25.9 ± 2.35 years, range: 23–30 years) participated in this study (for details see Table 1). All subjects were right-handed, according to the Edinburgh handedness inventory [10], and they were naïve with regard to the aim of the study. Those who were ill, pregnant, suffering from drug abuse, had metallic implants/implanted electrical devices were excluded by an interview and a short physical examination that encompassed assessments of in particular symmetry of gait and vigilance. All gave written informed consent. Subjects, but not the investigator, were blinded for stimulation conditions in all of the studies.

Stimulation techniques

tACS and tRNS

tRN and 140 Hz tAC stimulations were delivered by a battery-driven electrical stimulator (Version DC-Stimulator-Plus, NeuroConn GmbH, Ilmenau, Germany) through conductive-rubber electrodes placed in two saline-soaked sponges. The waveform of the 140 Hz stimulation was sinusoidal. For 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. Since high frequency oscillations did not induce a flickering sensation, subjects were kept blinded with regard to the type of the experiment.

The size of the stimulation electrode over the left M1 was 4 × 4 cm and of the reference electrode 6 × 14 cm, which was placed over the contralateral orbit; both were fixed on 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 frontopolar cortex – has been shown to be the optimal combination to enhance excitability of the M1 [7,11].

Measuring corticospinal excitability

To examine changes in corticospinal excitability, motor evoked potentials (MEPs) of the right first dorsal interosseus muscle (FDI) were recorded following stimulation of its motor-cortical representation field by single-pulse TMS. These were induced using a Magstim 200 magnetic stimulator (Magstim Company, Whiteland, Wales, UK) with a figure-of-eight standard double magnetic coil (diameter of one winding, 70 mm; peak magnetic field, 2.2 T; average inductance, 16.35 μH). Surface electromyogram (EMG) was recorded from the right FDI through a pair of Ag–AgCl surface electrodes in a belly-tendon montage. Raw signals were amplified, band-pass filtered (2 Hz–2 kHz; sampling rate, 5 kHz), digitized with a micro 1401 AD converter (Cambridge Electronic Design, Cambridge, UK) controlled by Signal Software (Cambridge Electronic Design, version 2.13), and stored on a personal computer for offline analysis. Complete relaxation was controlled through visual feedback of EMG activity and whenever it was necessary, the subject was instructed to relax. The coil was held tangentially to the skull, with the handle pointing backwards and laterally at 45° from the midline, resulting in a posterior–anterior direction of current flow in the brain. This orientation of the induced electrical field is thought to be optimal for a predominantly transverse activation of pyramidal tract neurons in the anterior wall of M1, targeting preferentially the new M1 [12]. The optimum position was defined as the site where TMS resulted consistently in the largest MEP in the resting muscle. The site was marked with a skin marker to ensure that the coil was held in the correct position throughout the experiment.

Experimental design

Subjects participated in two different experimental studies. The order of the stimulation conditions with regard to all experiments occurred in a counterbalanced fashion.

Experiment 1: tRNS

Fourteen subjects participated in 6 experimental sessions, on separate days, at least 3 days apart to avoid carry-over effects. The subjects received sham stimulation and tRNS using different intensities in a randomized order. Stimulus intensities (in percentage of maximal stimulator output) of TMS were determined at the beginning of each experiment. Immediately following stimulation, 25 single test-pulse MEPs were recorded at 0.25 Hz, i.e. approximately 0 min, 5 min, 10 min post stimulation, and then every 10 min up to 60 min and then again at 90 min.

Experiment 2: 140 Hz stimulation

Eleven subjects participated in 6 experimental sessions. Apart from the stimulation technique, the experimental procedure was the same as for the 10-min tRNS. Twenty-five MEPs were recorded at the following intervals: before, 0 min, 5 min, 10 min post stimulation and then every 10 min up to 60 min and then again at 90 min.

Analysis and statistics

For both studies first the TMS intensity resulting in MEP amplitudes of 1 mV was established. A repeated measure of analysis of variance (ANOVA) (a given current condition versus sham × time

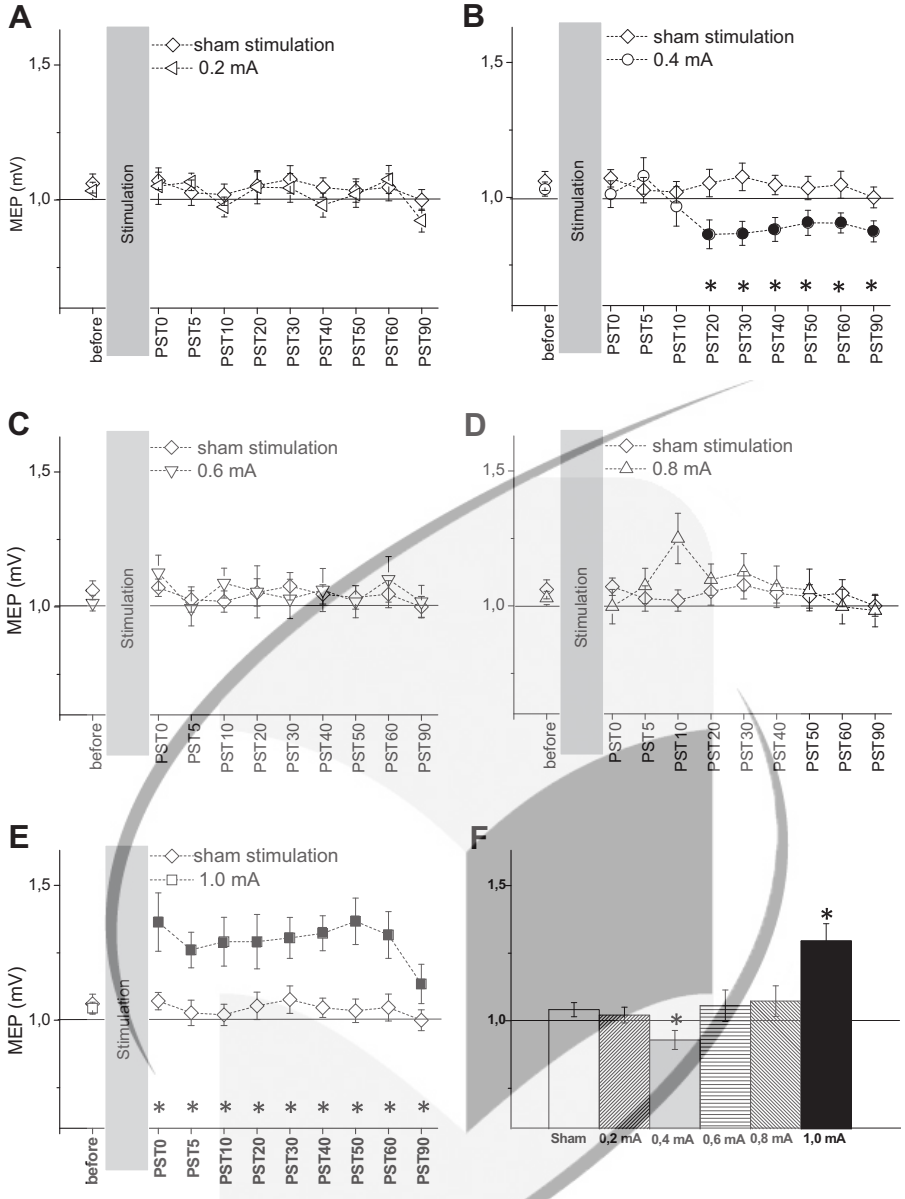

Fig. 1. Full spectrum tRNS. 1 mA tRNS (E) significantly increased MEPs at the PST0–PST90 time points compared to the sham stimulation. Significantly decreased MEPs were observed with 0.4 mA stimulation between 20 min and 90 min post stimulation (PST20–PST90) compared to sham stimulation (Fischer-LSD test, $^*P < 0.05$). 0.2, 0.6 and 0.8 mA stimulation (A, C and D) were without any effect. The figure shows mean amplitudes of MEPs and their SEMs before and after stimulation up to 90 min. (F) Recalculated data of (A), (B), (C), (D) and (E) in order to sum up the different intensities of tRNS induced effects on cortical excitability. Post hoc tests showed that the 1 mA tRNS applied induced a significant elevation in MEP compared to sham stimulation, whereas 0.4 mA decreased it (Fishers LSD $P < 0.05$). Error bars indicate standard errors. The bar graphs show the MEP amplitude values from PST0 to PST90. $^*P < 0.05$.

points of MEP recordings; dependent variable: mean amplitude of MEPs) was calculated. If a significant main effect of INTENSITY OF STIMULATION or the interaction of TIME and INTENSITY OF STIMULATION occurred, a Fischer-LSD test was performed.

Results

As expected, 140 Hz tACS and full spectrum tRNS applied over primary motor cortex and using 10 min stimulation duration and 1 mA intensity showed the classical behaviour and induced excitability increase. An important finding of this study is that 0.4 mA tRNS, as well as 140 Hz stimulation, significantly suppressed MEP amplitudes compared to baseline and sham stimulation.

Experiment 1: tRNS

For 1 mA stimulation, repeated measures ANOVA revealed significant main effects of INTENSITY OF STIMULATION ($F_{1.13} = 10.36$, $P = 0.007$) and TIME ($F_{9.12} = 2.66$, $P = 0.008$). The interaction between INTENSITY OF STIMULATION and TIME was also significant ($F_{9.12} = 2.13$, $P = 0.03$).

According to the Fisher-LSD analysis, 1 mA stimulation induced a significant increase of MEPs compared to the sham stimulation at time points PST0-PST90 ($P < 0.05$). Compared to baseline, MEPs were increased at the time points PST0–PST90 (Fisher LSD, $P < 0.05$). See Fig. 1E.

For 0.4 mA stimulation, repeated measures ANOVA revealed significant main effects of INTENSITY OF STIMULATION ($F_{1.13} = 6.01$,

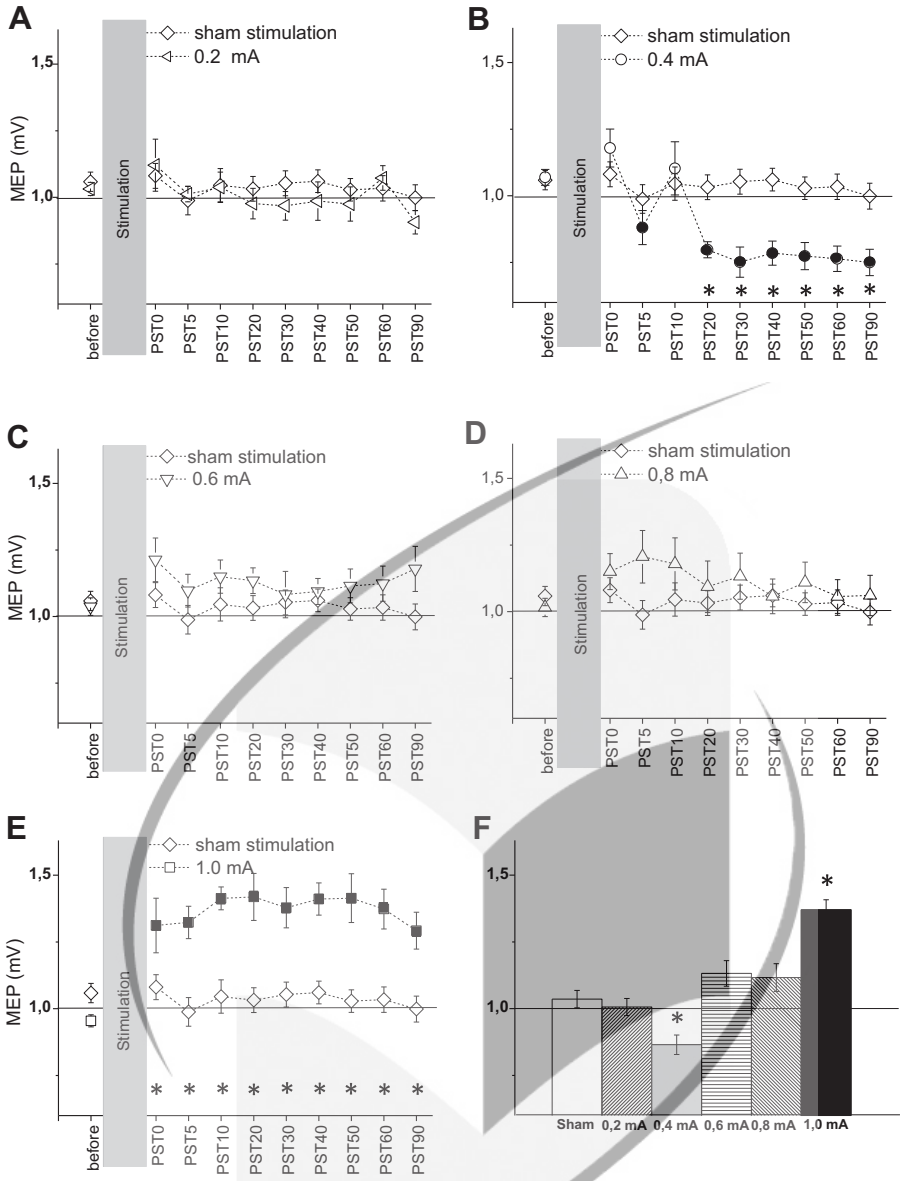

Fig. 2. 140 Hz stimulation. 1 mA 140 Hz (E) significantly increased MEPs at the PST0–PST90 time points compared to the sham stimulation. Significantly decreased MEPs were observed with 0.4 mA intensity stimulation between 20 min and 90 min post stimulation (PST20–PST90) compared to sham stimulation (Fischer-LSD test, $^*P < 0.05$). 0.2, 0.6 and 0.8 mA stimulation (A, C and D) were without any effect. The figure shows mean amplitudes of MEPs and their SEMs before and after stimulation up to 90 min. (F) Recalculated data of (A), (B), (C), (D) and (E) in order to sum up the different intensities of 140 Hz stimulation induced effects on cortical excitability. Post hoc tests showed that the 1 mA 140 Hz induced a significant elevation in MEP compared to sham stimulation, whereas 0.4 mA decreased it (Fishers LSD $P < 0.05$). Error bars indicate standard errors. The bar graphs show the MEP amplitude values from PST0 to PST90. $^*P < 0.05$.

$P = 0.03$) and TIME ($F_{9.12} = 2.31$, $P = 0.02$). The interaction between INTENSITY OF STIMULATION and TIME was also significant ($F_{9.12} = 2.56$, $P = 0.01$).

According to the Fischer-LSD test, significantly decreased MEPs were observed with 0.4 mA tRNS between 20 min and 90 min post stimulation (PST20–PST90) compared to sham stimulation ($P < 0.005$). We compare MEP amplitudes at the single time points during and post stimulation to the baseline MEP amplitudes. 0.4 mA tRNS induced a significant decrease in MEP amplitude compared to baseline at the time points PST20–PST90, (Fisher LSD, $P < 0.05$). See Fig. 1B.

In contrast to the effect of 0.4 mA and 1 mA stimulation, 0.2, 0.6 did not modify the MEP amplitudes significantly, when compared to sham stimulation. For 0.2 mA stimulation, there was no main effect of INTENSITY OF STIMULATION ($F_{1.13} = 0.64$, $P = 0.4$) and TIME ($F_{9.12} = 1.13$, $P = 0.3$). The interaction between INTENSITY OF STIMULATION and TIME was also not significant ($F_{9.12} = 0.7$, $P = 0.7$). For 0.6 mA stimulation, there was no main effect of INTENSITY OF STIMULATION ($F_{1.13} = 0.03$, $P = 0.9$) and TIME ($F_{9.12} = 0.96$, $P = 0.5$). The interaction between INTENSITY OF STIMULATION and TIME was also not significant ($F_{9.12} = 0.57$, $P = 0.8$). For 0.8 mA stimulation, there was no main effect of INTENSITY OF STIMULATION ($F_{1.13} = 0.17$, $P = 0.7$) and TIME ($F_{9.12} = 1.53$, $P = 0.1$). The interaction between INTENSITY OF STIMULATION and TIME was also not significant ($F_{9.12} = 1.89$, $P = 0.06$) See Fig. 1A, C and D.

Experiment 2: 140 Hz stimulation

After-effects of different intensities of 140 Hz stimulation are represented in Fig. 2. When 140 Hz tACS of 1 mA intensity was

applied to the M1 cortical excitability increased up to 40% above baseline. Repeated measures ANOVA revealed significant main effects of INTENSITY OF STIMULATION ($F_{1,10} = 47.46$, $P < 0.001$) and TIME ($F_{9,90} = 3.10$, $P = 0.003$). The interaction between INTENSITY OF STIMULATION and TIME was also significant ($F_{9,90} = 3.81$, $P < 0.001$). Stimulation with 1 mA intensity induced a significant increase of MEPs compared to the sham stimulation at time points PST0–PST90 ($P < 0.05$). Compared to baseline, MEPs were increased at the time points PST0–PST90 (Fisher LSD, $P < 0.005$). See Fig. 2E.

For 0.4 mA stimulation, Repeated measures ANOVA revealed significant main effects from INTENSITY OF STIMULATION ($F_{1,10} = 9.14$, $P = 0.01$) and TIME ($F_{9,90} = 7.76$, $P < 0.001$). The interaction between INTENSITY OF STIMULATION and TIME was also significant ($F_{9,90} = 7.5$, $P < 0.001$).

According to the Fischer-LSD test, significantly decreased MEPs were observed using 0.4 mA tACS between 20 min and 90 min post stimulation (PST20–PST90) compared to sham stimulation ($P < 0.005$). See Fig. 2B

140 Hz tACS with 0.2, 0.6 and 0.8 mA intensities did not modify the MEP amplitudes significantly, when compared with sham stimulation. For 0.2 mA stimulation, there was no main effect of INTENSITY OF STIMULATION ($F_{1,10} = 0.43$, $P = 0.5$) and TIME ($F_{9,90} = 1.65$, $P = 0.1$). The interaction between INTENSITY OF STIMULATION and TIME was also not significant ($F_{9,90} = 0.55$, $P = 0.8$). For 0.6 mA stimulation, there was no main effect of INTENSITY OF STIMULATION ($F_{1,10} = 2.77$, $P = 0.1$) and TIME ($F_{9,90} = 0.66$, $P = 0.7$). The interaction between TYPE OF STIMULATION and TIME was also not significant ($F_{9,90} = 1.09$, $P = 0.4$). For 0.8 mA stimulation, there was no main effect of INTENSITY OF STIMULATION ($F_{1,10} = 1.30$, $P = 0.3$) and TIME ($F_{9,90} = 0.63$, $P = 0.8$). The interaction between INTENSITY OF STIMULATION and TIME was also not significant ($F_{9,90} = 0.98$, $P = 0.5$). See Figure 2A, 2C and 2D.

Discussion

This study was designed to determine the minimal stimulation intensity required for induction of excitatory after-effects of transcranial high frequency electrical stimulation, both for 140 Hz tACS and for 0.1–640 Hz tRNS. Instead of receiving a simple threshold unexpectedly in these two independent data sets at threshold intensities of 0.4 mA we found a conversion of the already known excitation achieved with an intensity of 1 mA to inhibition. The intermediate intensity ranges of 0.6 and 0.8 mA had no effect at all.

This finding appears to be new when referred against the literature of tDCS, where so far anodal stimulation seemed to be excitatory when stimulated at a minimal threshold intensity of 0.6 mA and inhibitory with cathodal tDCS. When looking at the original data, a similar non-significant trend towards inhibition with 0.4 mA might have occurred in this early anodal tDCS study [7], whereas in contrast to the present findings, 0.6 and 0.8 mA anodal tDCS induced excitatory after-effects. It has, however, to be kept in mind that in the early studies we used electrodes of 35 cm^2 in size, whereas according to results by Nitsche et al., 2007, the size of the stimulation electrode can be reduced and the return electrode increased, the latter in order achieve subthreshold stimulation intensity at the return electrodes. The tRNS [3] and 140 Hz tACS [4] data were recorded with the same electrode set-up as used here with 16 cm^2 at M1. Another early study might be reconsidered in the present context as well. Priori et al. [13] found a decrease of MEP size after low intensity 7 s tDCS (<0.5 mA) with an electrode size of 25 cm^2, however, they used a different electrode mounting combined with a preceding cathodal stimulation. Another aspect that should be taken into account is that these data in Priori et al. [13] as well in the Nitsche and Paulus [7] studies were measured with clearly shorter stimulation duration. The longer stimulation duration of 10 min may prevent a direct comparability between tDCS and tACS, since the former allows a build up of a voltage gradient over time, which cannot be the case with tACS without DC offset. In other words, in contrast to tACS tDCS may act as a current integrator over time. Nevertheless studies on this are warranted.

Interestingly in an epilepsy rat model, anodal DC stimulation at 100 and 200 μA induced inhibition but no excitation [14].

Data from a recent cTBS-300 study suggest that the intensity of stimulation is critical at threshold. The short duration cTBS is excitatory when applied with 70% rest motor threshold (RMT) intensity, however inhibitory at 65% MT and probably subthreshold at 60% RMT in [15]. Also, the classical short-interval intracortical inhibition paradigm makes use of a weak conditioning inhibitory stimulus. The best suppression was seen with small conditioning stimuli of 0.7–0.9 motor threshold in relaxed muscle. Increasing the intensity to motor threshold or above resulted in less suppression or even facilitation [16]. The reason for the observed reversal in the direction of MEP effects induced by high frequency oscillation at different intensities is not clear. It is possible that 140 Hz and tRNS at the lower intensity only facilitated intracortical inhibitory networks of corticospinal motoneurons, thus resulting in net inhibition of MEP amplitudes. However, we also cannot exclude the possibility that stimulation applied at this lower intensity may have inhibited intracortical facilitatory influences on corticospinal motoneurons. Moreover, the heterogeneity of inhibitory interneurons in the cerebral cortex suggests that each type of cell has different biophysical properties [17,18].

Indeed, the sensitivity of excitatory and inhibitory synapses to different frequencies and intensities of stimulation appears to be critical. Data from animal experiments indicate that iTBS and cTBS modulate the activity of different inhibitory cortical systems: iTBS primarily targets inhibition of pyramidal cell output activity by PV-expressing interneurons, while cTBS mostly affects the inhibitory activity of the CB-expressing interneurons [19].

Nonlinear excitation–inhibition integration caused by shunting of excitatory synaptic currents through activated GABAA channels has been shown experimentally [20–22] and theoretically [21,23,24]. Data from in vitro experiments conducted on slice preparations of the rat visual cortex suggest that the level of depolarization of the postsynaptic neuron can influence the response to a high frequency plasticity-inducing burst [25]. Specifically, LTD can be induced if the level of postsynaptic depolarization exceeds a certain threshold, but remains under a second, higher threshold critical for LTP induction [25].

Further evidence for a predominance of inhibition during electrical stimulation was published with regard to the rat cortex. When recording in a pyramidal neuron located in layer 5 of rat cortex the composite response to an electrical stimulation of various layers (2–3, 4 or 6), in terms of excitation–inhibition balance, resulted in conductance changes consisting of 20% excitation and 80% inhibition, independent from the stimulated layer [26,27].

Moreover, it was shown that excitatory circuits are strongly controlled by inhibitory circuits [28] of distinct types [29] by feedback and feed-forward connections [30].

Apart from intensity, another important parameter of stimulation is its duration. For example, anodal tDCS with a 13-min stimulation duration was necessary to produce a sustained excitability increase of 90 min post stimulation [8]. In contrast, only 9-min cathodal tDCS was required to induce a similar long excitability diminution, pointing towards a higher efficacy of cathodal as opposed anodal stimulation.

Interestingly, with regard to the build up of the after-effects of "low dose inhibitory" 140 Hz and full spectrum tRNS, both presented a delay, with maximum of MEP inhibition clearly observed

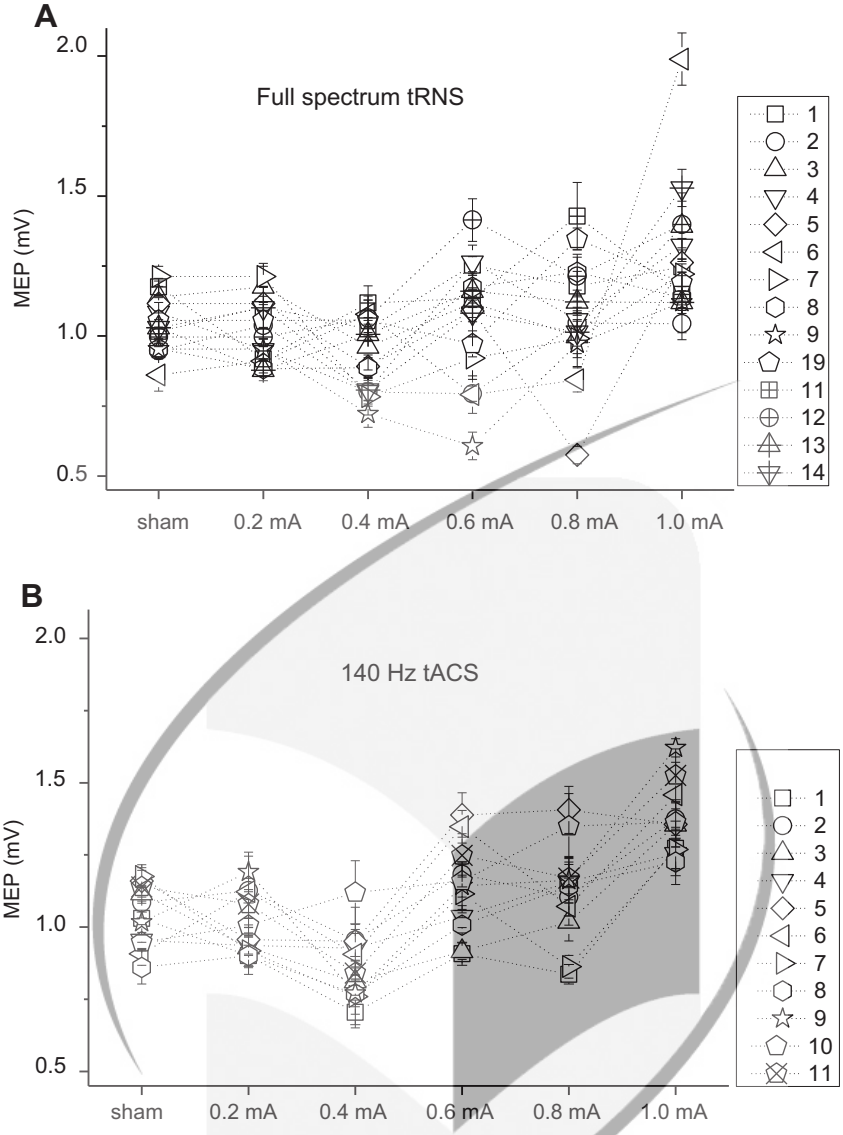

Fig. 3. The individual MEP amplitude changes at each intensity level for each subject.

20 min after stimulation. In contrast "high dose excitatory" 1 mA results occurred immediately after stimulation in both experiments. This difference has already been seen for TBS in the original study [1] and confirmed in a recent study [5]. Obviously inhibitory mechanisms may have a delayed onset when compared to excitatory protocols.

Skull thickness may be a source of error. Given inter-individual differences in skull and brain anatomy one might expect the "inhibitory" intensity to vary from subject to subject. We are not aware of a study with tDCS or tACS comparing skull thickness with response size. However when comparing EEG alpha power at frontal, temporal, and parietal sites and the thickness of the underlying skull there was only a mediocre association with correlations ranging between $r = -0.36$ and $r = 0.10$ [31]. Law [32] looked at skull thickness and resistivity variations over the upper surface in an adult human skull. Resistivity measurements ranged from 1360 to 21,400 Ohm-cm with an overall mean of 7560 ± 4130 Ohm-cm. The presence of sutures was found to decrease resistivity substantially. In another study skull thickness at parietal bone B coming closed to the motor cortex varied by about 35%: Left 4.78 mm ± 1.19 mm, Right: 4.64 ± 0.92 mm [33]. Interestingly the individual data variability was greater at 0.6 and 0.8 mA than at all other intensities. Thus skull thickness may contribution to individual variability at the "transition" intensities, but less at 0.4 and 1.0 mA. See Fig. 3.

A limiting factor with regard to the interpretation of our data is that the examiners were not blinded to the stimulation condition. Furthermore, the use of neuronavigation would have been preferable to objectively monitor coil position and reduce any possible bias introduced by the examiner. However since the results, in particular the inhibition at 0.4 mA and the "transition zone" at 0.6 and 0.8 mA were completely unexpected a bias seems to be very unlikely.

These data seem to be of importance for clinical stimulation protocols. In the context of epilepsy treatment, one essential necessity is to avoid unwanted excitation and its risk of even increasing seizure activity. Low-dose electric stimulation seems to pave the way towards this goal, as the high frequency methods investigated here avoid the possible risk of tDCS, with its polarity sensitive current flow [34]. The time courses of after-effects remain to be determined when considering longer treatment options in patients with epilepsy.

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