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FACULTEIT GENEESKUNDE EN LEVENSWETENSCHAPPEN

master in de revalidatiewetenschappen en de kinesitherapie

Masterproef

The effect of transcranial direct current stimulation on the level of excitability of the human motor cortex in healthy subjects.

Promotor :
Prof. dr. Raf MEESEN

Copromotor :
dr. Koen CUYPERS

Cathy Vandermeeren

Proefschrift ingediend tot het behalen van de graad van master in de revalidatiewetenschappen en de kinesitherapie

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KNOWLEDGE IN ACTION

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Woord vooraf

Graag zou ik een woord van dank richten aan een aantal mensen, zonder wiens hulp deze thesis niet tot stand zou gekomen zijn.

Vooreerst mijn promotor Prof. Dr. Raf Meesen voor zijn coördinatie en hulp bij zowel het onderzoek als bij de analyse en het schrijven van de eindverhandeling. Ik apprecieer de grote mate waarin ik zelf mocht beslissen over het hele proces.

Onze copromotor Dr. Koen Cuypers wil ik bedanken voor zijn raadgeving en hulp tijdens het onderzoek en zijn grote hulp bij de verwerking van onze data en het schrijven van de eindverhandeling. U wist een pasklaar antwoord op mijn vragen en bracht oplossingen voor mijn problemen.

Ik zou ook zeker al onze proefpersonen van harte willen bedanken voor hun toewijding gedurende de onderzoekswerken. Zonder hen was er van een onderzoek geen sprake geweest.

Tot slot wil ik ook mijn collega-studenten voor de goede raad, kritische blik en stimulatie tijdens het schrijven van deze thesis.

Hasselt, 10 februari 2015 C.V.

Research context

The experiment can be situated within the domain of fundamental neurological research, more specific the underlying fundamental mechanisms. Interestingly about fundamental research is the fact that our findings might be useful to support the domain of neurological rehabilitation.

This thesis is based on fundamental knowledge, from earlier investigations in the field of non-invasive brain stimulation. In earlier studies outcomes were often ambiguous, but overall there is consensus towards the hypothesis that tDCS can be used as a valuable technique for neuro-rehabilitation (Nitsche and Paulus, 2001; Paulus 2003).

tDCS is a novel, noninvasive brain stimulation technique that delivers a small electric current, which can modulate brain excitability in patient populations and healthy adults (Jefferson, Mistry et al. 2009). Because it allows a painless, focal, noninvasive excitability modulation of the cortex, it can be a promising tool for neural rehabilitation (Nitsche and Paulus 2000).

This master thesis takes part in the existing project “Underlying mechanisms of neuroplasticity and motor control: Modulation of neuroplasticity in the development of novel rehabilitation strategies in neurodegenerative diseases (project number R-1993)”. The project was started in 2008 by Koen Cuypers, under supervision of Prof. Dr. Raf Meesen.

The experiment was completed in campus REVAL of the ‘University Hasselt’ situated in Diepenbeek and was approved by the ethical commission of the university of Hasselt on 11/01/2013. A PC, TMS stimulator and neuronavigator, tDCS stimulator and an EMG recorder was the equipment needed to perform our measurements.

The experiments were a double-blind cross-over repeated measures experiment of which the research question and protocol was already formed. The first part, the data-acquisition and part 1 of the master thesis, was done together with Ine De Smedt. Part two we did separately, Ine analyzed the data of the, young subjects, whereas I focused on the older subjects.

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The effect of transcranial direct current stimulation on the level of excitability of the human motor cortex in healthy subjects

In accordance with the guidelines of the *Behavioural Brain Research: http://www.journals.elsevier.com/behavioural-brain-research/*

Abstract

Background: tDCS (transcranial Direct Current Stimulation) can result in modifications in cortical excitability. Therefore it might have implications for the rehabilitation of people with neurodegenerative pathologies. Anodal tDCS (a-tDCS) has been shown to improve motor skills after stroke when combined with motor rehabilitation (Hummel et al., 2005), to decrease absolute reaction time in a serial reaction time test (Nitsche et al., 2003) and to induce sustainable increases in cortical excitability (Nitsche and Paulus, 2000, 2001). In most of the studies more intensive protocols, with an increased duration and intensity (2 mA), were used. It was assumed that more intensive stimulation would enhance the efficiency of the stimulation effects (Batsikatze et al., 2013). Although the more intensive protocols have shown their effectiveness in numerous studies, the physiological mechanisms and changes remain unclear (Fregni et al., 2006; Ferrucci et al., 2009; Brunoni et al., 2001; Bueno et al., 2011). Kidgell et al. (2013) claim that different a-tDCS current intensities do not differentially modulate cortical excitability at any time period.

Objective: To investigate whether the tDCS intensity has an effect on the level of the excitability of the human motor cortex in older healthy subjects. There are less experiments with older subjects comparison with younger subjects, most investigations were done with young subjects. tDCS might be useful for the older population to be more active and healthy.

Methods: 16 healthy adults were randomized to an order of interventions. These interventions were tDCS at 1mA, 2.5mA or sham stimulation as control intervention. The interventions were administered at 3 different days. An recruitment curve was measured at baseline, after (post), and 30 (post30) and 60 (post 60) minutes post intervention using a TMS device. The effect of the intervention was analyzed by comparing the different conditions.

Results: Excitability differed not significantly between the different conditions, within each condition and over time. Post-hoc analysis over time revealed a significant difference at 1 mA 30 minutes after the stimulation. For the behavioral characteristics between the different conditions, a significant difference for Quality of Sleep (QOS) was revealed in the sham condition.

Conclusion: No difference was found in excitability between conditions and over time. Only 1 mA anodal tDCS leaded to more excitability after 30 minutes stimulation.

Keywords:

Motor cortex, Transcranial direct current stimulation, Cortical excitability, Current intensity, Input-Output curve, Transcranial magnetic stimulation

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1. Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive technique to induce changes in the cortical excitability and can be a promising tool for neural rehabilitation (Nitsche and Paulus, 2000). It is a non-invasive and painless technique with no or minimal side effects and it can be applied by an inexpensive battery-operated device, which is very simple to operate (Jeffery et al., 2007; Bolognini et al., 2009). tDCS involves an application of very-low-amplitude direct currents (2mA or less) via surface scalp electrodes (Webster et al., 2006). This produces a sub-sensory level of electrical stimulation which remains imperceptible by most people during its application. In a small percentage of subjects it may cause minimal discomfort with a mild tingling sensation, which usually disappears after a few seconds (Nitsche et al., 2003). The effects of weak electric currents on brain and neuronal function are determined by the electrode position and the polarity of the current flow (Nitsche & Paulus, 2000). Current flows from the anode to the cathode, some being diverted through the scalp and some moving through the brain (Pedro Cavaleiro et al., 2006). With the anode electrode positioned over the primary motor cortex and the cathode over the contralateral orbit, inducing an anterior-posterior current flow, the excitability is enhanced. On the contrary, the excitability is reduced with a posterior-anterior current flow (Nitsche et al., 2008). The amplitude of the excitability changes depends on a few factors, including the current-intensity (Nitsche et al., 2008; Nitsche & Paulus, 2000).

Transcranial direct current stimulation of the motor cortex may also have functional consequences and possible clinical utility in motor disorders. Recent research proved that anodal tDCS (a-tDCS) increases corticomotor excitability in both healthy individuals and subjects with stroke (Bastani et al. 2012). It is well known that excitability is increased by a-tDCS, and diminished by cathodal tDCS (Nitsche and Paulus, 2000). Other therapeutic studies in neuropsychiatric disorders have shown promising results in epilepsy (Fregni et al., 2006; Nitsche and Paulus, 2009), chronic pain (Williams et al., 2009) and major depression (Boggio et al., 2008 ; Ferrucci et al., 2009; Brunoni et al., 2011b), for example. One mA anodal tDCS enhanced dexterity in the non-dominant hand (Boggio et al., 2006) and resulted immediate in improvements in error awareness (Harty et al., 2014) and motor performance (Goodwill et al., 2013). Also 1 mA anodal tDCS might facilitate motor function in older subjects, which effect was more pronounced with advancing age. Compared to other therapies, tDCS is safer than invasive brain stimulation that is associative with surgical risks and has increased costs.

The after-effects of tDCS on excitability changes depend on the targeted cortical area, duration of the stimulation and the current intensity (Nitsche et al., 2008; Nitsche & Paulus, 2000). Stimulation duration of at least 3 min at 1 mA or an intensity of 0.6 mA for 5 min is necessary to obtain aftereffects (Nitsche & Paulus, 2000). Additionally, increasing stimulation duration leads to an evident enlargement of MEP amplitude and endurance of the effect (Nitsche & Paulus, 2000), like indicated by the study of Nitsche et al. (2001), where 5 and 7 minutes of 1 mA tDCS resulted in after-effects of no longer than 5 minutes and stimulation from 9 to 13 minutes elevations of MEP amplitudes revealed from, respectively, 30 to 90 minutes. However, longer tDCS shows stable MEPs before reverting to baseline (Nitsche & Paulus, 2001). With the intensities used in the current study, we expect aftereffects slightly longer than 30 minutes after stimulation for 10 minutes with 1 mA tDCS and longer after-effects after

2.5 mA, since it established that after-effects also depends on current intensity (Nitsche et al., 2008; Nitsche & Paulus, 2000).
A-tDCS can lead to increased corticomotor excitability in both healthy individuals and subjects with stroke. (Bastani et al., 2012) A-tDCS is cheap and easy-to-apply and can be used as a stand-alone technique or as an adjuvant technique to enhance corticomotor excitability and the efficacy of motor training approaches (Bastani et al., 2012). The combination of tDCS with motor training has shown to enhance motor function (Bastani et al., 2012).

Nitsche and Paulus (2000) investigated that a minimum of 0.6 mA current intensity stimulation is needed to induce stimulation after-effects. They argue that increasing the current intensity leads to prolonged and larger after-effects (Nitsche and Paulus, 2000). There seems to be no difference in inhibition and facilitation mechanisms with stimulation at low current intensities on the hand motor cortex (Nitsche and Paulus, 2000; Uy and Ridding, 2003; Hummer et al 2005, Kidgell et al., 2013). Findings of Jeffery et al. (2007) showed enhanced excitability of the leg motor cortex when a stimulation intensity of 2 mA a-tDCS was applied. A plausible examination for these results might be that the current (at 1 mA) penetrated deep enough to have an effect on the leg area (Jeffery et al., 2007). Until now it is unclear whether intensities higher than 2 mA lead to prolonged and/or larger after-effects, therefore we investigated the effect of high intensity tDCS on corticomotor excitability. The change of cortical excitability in the motor cortex can be measured by motor evoked potentials (MEPs), elicited by transcranial magnetic stimulation (TMS) (Priori et al., 1998). Over the past decade TMS has gained increasing popularity as a method to study the excitability of the human motor cortex. TMS is a non-invasive brain stimulation technique which provides a quantitative measure of corticomotor excitability (Paulus et al., 2003). It is a painless and non-invasive technique to allow the quantification of neuron responses. TMS uses electromagnetic induction to induce weak electric currents using a rapidly changing magnetic field. These tiny currents cause stimulation of corticospinal neurons in specific parts of M1 and produces muscle responses (MEPs) in contralateral target muscles (Chiappa, 1997). For the assessment of the excitability of the human cortex, a representation of the amplitude of the motor evoked potential (MEP) was used (Nitsche and Paulus, 2000).

The protocol of the experiment was determined by the promoter and co-promotor but the students adapted it for the publication in this thesis.

In summary, the aim of the experiment is to evaluate the impact of tDCS intensity on the motor cortex excitability processes in healthy, older subjects. For this purpose, we studied the recruitment curve (Area under the curve). Another aim of this experiment is to study the effect and the after-effect of different intensities tDCS on the excitability mechanism in the human brain, therefore we used intensities of 1 mA and 2.5 mA and compared with sham stimulation. And at different time points, before the stimulation, after the stimulation (post) and 30 and 60 minutes after stimulation.

2. Materials and methods

2.1 Participants

Sixteen healthy male subjects older than 60 year participated in this study (mean age ± 69.5 years; range 64-83). Patients were recruited from the local community. Participants who had metallic fixtures, cardiac problems, history of brain problems, a history of neurological impairment or reported symptoms of dementia or depression were excluded from the study. The participants, from the local community, were recruited by flyer or e-mail. All participants were right-handed. They were tested for handedness with the Edingburgh Handedness inventory (Oldfield, 1971). At the beginning of every session the subjects filled in a questionnaire about the hours they have slept last night, the quality of the sleep and alcohol and caffeine use.

Finally, subjects were screened for TMS contra-indications. Each participant provided their informed consent for participation in the experimental procedures which were approved by the medical ethics committee of the University Hasselt.

The reason for including only male subjects was because it is proven that the hormone cycle in female subjects plays an important role in the human brain and causes more variability of MEP's (Smith et al., 1999). If they met the exclusion criteria, they were excluded.

2.2 Experimental design

The experiment was accomplished in 4 different sessions, consisting of the familiarization session of the subjects to the experimental procedures and 3 experimental sessions in which the stimulation with different current intensities will be applied (see figure 1). The order of used tDCS intensity was quasi-randomized, and the study is a cross-over, double-blind, repeated measurements design. The subject, as well the researcher, were blinded from the tDCS characteristics. The principal investigator was not blinded and did not perform any measurements.

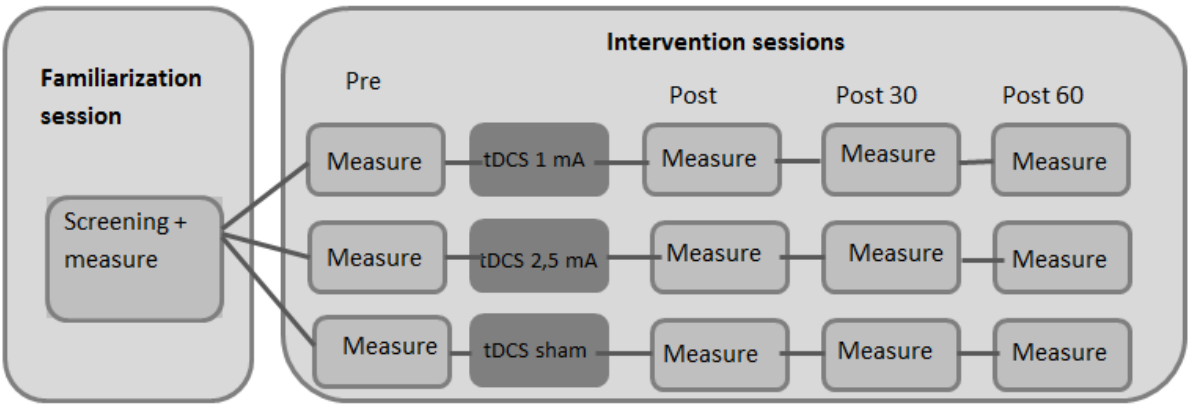

Figure 1. Research design

General setup. Subjects were comfortably seated in a chair behind a table with a computer screen on it. The right forearm was supported comfortably on a cushion on the table. To make sure the muscle was relaxed, EMG was recorded with a 16-channel "Bagnoli" Delsys EMG system (bandwith 20-450Hz, USA). Surface electromyography (EMG) was recorded from the right first dorsal interosseus muscle (FDI). The EMG was displayed on a computer screen in front of the subject and the

investigator. To ensure optimal relaxation of the FDI muscle, background EMG was controlled (<0.005 mV) during the entire experiment. All cables were fastened with tape to prevent movement artifacts. The signals were measured with a frequency of 5000 Hz and filtered with a Humbug noise eliminator (Quest Scientific, North Vancouver, BC, Canada), which removes noise of 50/60 Hz.

Transcranial magnetic stimulation. A 90-mm figure-8 coil connected to a TMS stimulator (Magstim 200 Company, Whiteland, Dyfed, UK) was used to elicit MEPs in a single pulse paradigm. The coil was positioned over the contralateral hemisphere to the target muscle, while holding the handle pointing backwards and 45 degrees away from the mid-sagittal line (Brasil-Neto et al., 1992). Subjects put on a swimming cap. Tape was stacked on and a 1 by 1 cm grid was drawn on it.

The first thing that needed to be done was finding the “hotspot”. The hotspot is defined as the optimal coil position at the skull at which the mean peak-to-peak amplitude of the MEPs evoked by five successive pulses was largest and most constant (Marneweck et al., 2011). To obtain the hotspot several points on the grid were stimulated. Secondly, the “rest motor threshold (rMT) was determined. rMT is defined as the minimum intensity of the stimulator needed for evoking MEPs larger than 0.005 mV in at least five out of ten consecutive trials (Rossini et al., 1994).

The Advanced Neuro Technology (ANT) (Visor Neuronavigational System) was used for our experiment. It provides industry-leading precision, reliability, and consistency for TMS sessions. With its meticulous digitization procedures and advanced 3D visualization it allows users to easily fine-tune, analyze and replicate any previous procedure with absolute confidence. It is also easier for the application of magnetic stimulation and positioning of the TMS coils in order to reproduce effects. The ANT was used to ensure that the position was the same during the whole experimental session.

Next, an “Input-Output curve” (I-O curve) was obtained at 6 intensity levels: 90% rMT, 110% rMT, 130% rMT, 150% rMT, 170% rMT and 190% rMT. There were six stimuli of each intensity and the order of these were pseudo-randomized. Between every pulse there was a pause of approximately seven seconds, with a randomization of 20%. In total 36 pulses were given. The I-O curve describes the relation between the intensity of the TMS-output and the MEP-amplitudes. The curve reflects an index of the excitability of larger neuronal populations

Note that it is very important to be aware of the fact that the target muscle is completely relaxed. This is measured by the EMG signal that has to be beneath 0.005mV. Otherwise the MEP threshold will be reached with a lower pulse intensity. Measurements where the EMG signal was too high, were removed from analysis.

2.3 Transcranial direct current stimulation.

tDCS was applied by a battery-driven constant current stimulator (Eldith, DC-stimulator, Neuronn Germany). Two surface electrodes were placed on the skull, the anodal electrode (25 cm^2) on the previously located hotspot and the cathodal sponge electrode (50 cm^2) on the supra-orbital area ipsilateral to the target muscle.

The anodal electrode was attached by the use of ‘Zero Gel’ (Newronika, Italy), while the cathodal sponge was soaked with 0.9% NaCl (Versol) and attached with an elastic cap and tape.

The stimulator delivers a current strength of 1 mA, 2.5 mA or sham through the electrodes attached on the head. The anodal electrode is attached over the primary motor cortex on the hotspot for the FDI muscle, by use of a zero gel. The subjects received this non-invasive stimulation, which can cause an excitatory sensation on the head. The stimulation of 1mA and 2.5 mA lasts 10 minutes, the sham stimulation only takes 20 seconds.

2.4 Data and statistical analysis

The statistical analysis was performed using SPSS (IBM SPSS Statistics, Version 22). Mann-Whitney test procedures were used to calculate at one time point (e.g. pre) for differences between the stimulation groups (2.5 mA, 1 mA and sham). The same was done for post, post 30 and post 60.. The Friedman test was used to calculate significant differences within each condition (2.5 mA, 1 mA and sham) over time. And the Wilcoxon Signed Rank Test was used to determine the effect of stimulation on excitability between different time points (pre vs. post vs. post 30 vs. post 60). For every analysis, the significance was set at p-value < 0.05.

To correct for inter-individual differences in absolute MEP amplitudes, MEP’s were normalized to the maximum MEP value (i.e. mean of 5 pulses) measures at baseline for each intervention session. For the assumption of normality of the data, the Kolmogorov-Smirnov test was used. An statistical analyze was made of the area under the recruitment curve (AURC). AURC is a robust marker of overall corticospinal output and projection strength and was calculated with the following algorithm: yi(xi+ 1 − xi) + (1/2)(yi+ 1 − yi)(xi+ 1 − xi), where y is the stimulus intensity and x is the MEP amplitude at a given intensity. To calculate the entire curve, each intensity level was summed (90% rMT + 110% rMT + 130% rMT + 150% rMT + 170% rMT + 190% rMT).

Excitability is expressed in AURC. Data are presented as means ± standard deviation (SD).

3. Results

All participants successfully finished the entire protocol. At the end of the experiment all participant received the three different stimulations (2.5 mA, 1 mA, Sham). At different time moments, pre-post-post30-post60.

3.1 Effect of stimulation intensity on excitability

Data describing the mean excitability at baseline, post, post 30, post 60 are shown in figure 2a and 2b.

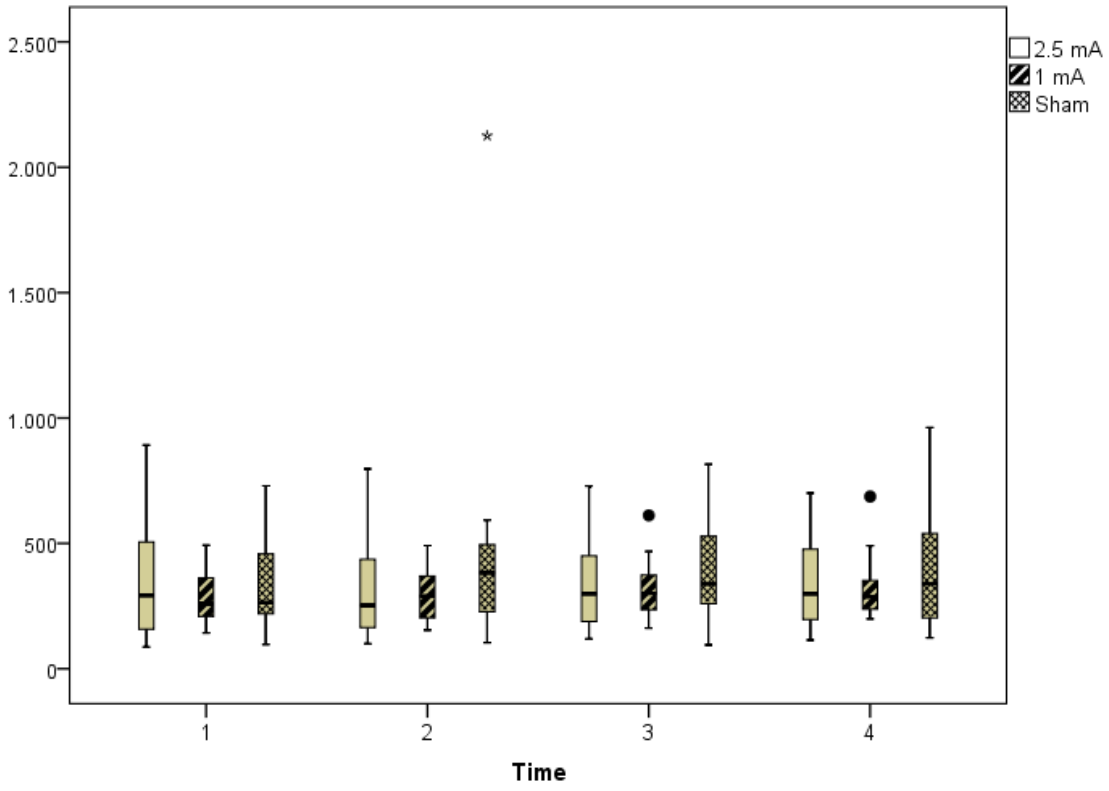

Figure 2a. Modulations of excitability at baseline (pre), after (post) and 30 (post 30) and 60 (post 60) minutes after stimulation with extreme outlier. Excitability expressed in area under the curve (AURC). Data plotted as group mean ± standard deviation (SD). * = outlier sham, ° = outlier 1mA

A boxplot (figure 2a) was made to determine possible outliers. One participant was considered as an extreme outlier with more than three times the length of the box away from the upper border of the boxplot. Two participants were outliers with values less than 1 box lengths away from the upper border. Different measurements were done, with the outliers and without the outliers in the data. There was a significant difference between the measurements. The extreme outlier was excluded from the data-analysis (figure 2b). The data shown on figure 2b were used for the data-analyses.

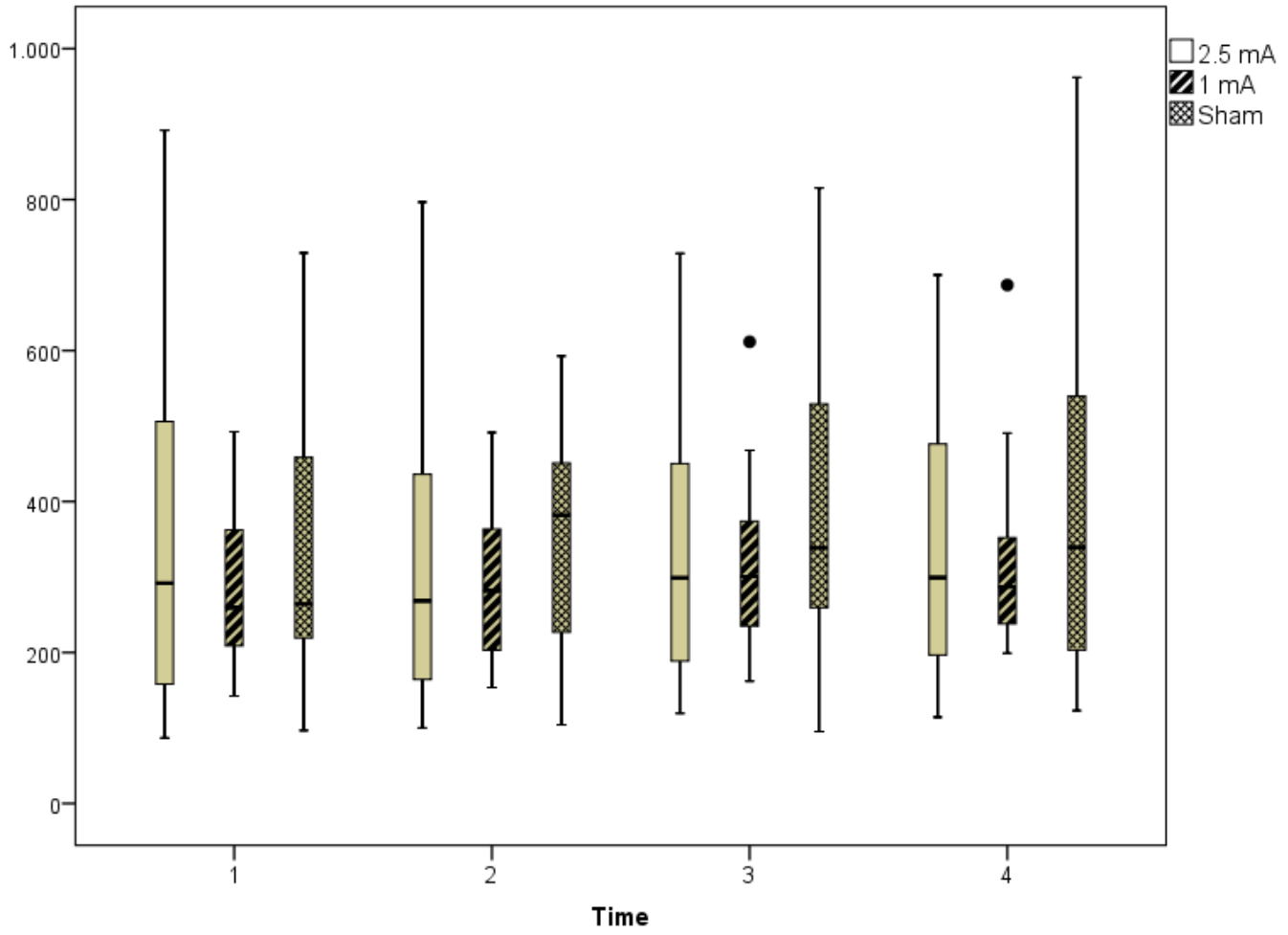

Figure 2b. Modulations of excitability at baseline (pre), after (post) and 30 (post 30) and 60 (post 60) minutes after stimulation without extreme outlier. Excitability expressed in area under the curve (AURC). Data plotted as group mean ± standard deviation (SD). * = outlier sham, ° = outlier 1mA

3.1.1 Comparison between the conditions

Table 1 comparison of excitability between the conditions

	Sham	**1 mA**	**2,5 mA**	
	Mean (SD)	Mean (SD)	Mean (SD)	*p*-value
Pre	337.7 (188.7)	283.5 (95.5)	352.5 (245.5)	0.485
Post	460.9 (466,1)	287.7 (96.5)	317.2 (200.7)	0.534
Post 30	390.5 (217,4)	313.5 (117.8)	340.3 (186.2)	0.485
Post 60	379.2 (230,8)	318.0 (124.5)	341.7 (182.9)	0.983

Table 1. Comparison (Friedman test) of excitability between the conditions. Area under de curve (AURC). * $p < 0.05$ (significance difference between conditions).

No significant differences were observed (p <0.05; Friedman test) for the different stimulation intensities, suggesting that stimulation intensity does not influence excitability.

3.1.2 Comparison within each condition (contrast)

Table 2. Comparison within each condition

Contrast	***p*-value**
Δ pre 2.5-1mA	0.987
Δ pre 2.5-sham	0.867
Δ pre 1-sham	0.564
Δ post 2.5-1mA	0.780
Δ post 2.5-sham	0.202
Δ post 1-sham	0.119
Δ post30 2.5-1mA	0.985
Δ post 30 1-sham	0.564
Δ post60 2.5-1mA	0.431
Δ post60 2.5-sham	1.000
Δ post60 1-sham	0.809

Table 2. Comparison (Mann-Whitney U test) within each condition (contrast). * $p < 0.05$ (significance difference between each condition).

Two groups were compared with each other. For pre stimulation we compared 2.5 mA with 1 mA, 2.5 mA with sham and 1 mA with sham. The same was done for post, post 30 and post 60. No significant differences (*p< 0.05; Mann-Whitney U test)* in excitability were found. This means that there are no differences between the stimulations at each time point. Table 2 summarizes the *p*-values of each condition (contrast).

3.1.3 Comparison over time

Table 3. Comparison of excitability over time

	Pre	**Post**	**Post 30**	**Post 60**	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*p*-value
Sham	353.5(184.1)	349.9 (147.1)	408.4 (212.6)	398.3 (228.2)	0.208
1 mA	283.5 (95.5)	287.7 (96.5)	313.5 (117.8)	318.0 (124.5)	0.337
2,5 mA	352.5 (245.5)	317.2 (200.7)	340.3 (186.2)	341.7 (182.9)	0.599

Table 3. Comparisons (Friedman test) of excitability over time. Area under the curve (AURC). *p< 0.05 (significance difference over time).

Between time comparisons (p< 0.05; Friedman test) revealed no significant differences of excitability over time. Indicating that excitability does not change over time. An overview is given in table 3.

3.1.4 Post-hoc analysis over time

Table 4. Post-hoc analysis of excitability over time

	p-value					
	Pre – post	**Pre – post 30**	**Pre – post 60**	**Post – post 30**	**Post – post 60**	**Post 30 – post 60**
Sham	1.000	0.093	0.211	**0.035***	0.421	0.597
1 mA	0.821	0.193	0.252	**0.044***	0.252	0.464
2.5 mA	0.669	0.669	0.900	0.348	0.193	0.900

Table 4. Post-hoc analysis of inhibition (Wilcoxon Signed Ranks Test) over time. *p< 0.05 (significance difference between time points

Post-hoc analysis over time, table 4, revealed a significant difference (p< 0.05; Wilcoxon Signed Ranks Test) in older adults for the comparison between post – post 30 at 1 mA and sham condition. This result suggests that, in older adults, there is more excitability at post 30 in comparison with post at the 1 mA condition and at the sham condition

3.2 Behavioral characteristics

Table 5 gives an overview of the behavioral characteristics between the different conditions. These results reveal a difference in QOS in the sham condition.

Table 5. Behavioral characteristics

	Sham	**1 mA**	**2,5 mA**	
	Mean (SD)	Mean (SD)	Mean (SD)	*p*-value
Hours sleep	7.66 (1.05)	7.57 (0.98)	7.40 (1.06)	0.724
QOS	9.20 (1.01)	8.80 (1.42)	8.60 (1.35)	**0.045***
Alcohol	1.33 (1.59)	0.73 (1.11)	1.20 (1.52)	0.183
Coffee	2.07 (1.33)	2.67 (0.98)	2.47 (2.07)	0.432

Table 5. Behavioral characteristics (Friedman test). Differences between the conditions. *$p < 0.05$ (significant difference between conditions). QOS = Quality of Sleep

4. Discussion

The objective of the current study was to evaluate the impact of tDCS intensity on the motor cortex excitability processes in healthy elderly. We hypothesized that different current intensities would differentially affect cortical excitability. An important finding in this study was that the size of change of excitability was not significantly different between the conditions and over time, illustrating that current intensities of 1 mA, 2.5 mA or sham stimulation do not influence cortical plasticity.

We used the same TMS intensity for the test stimulus before, after, 30 minutes after and 60 minutes after tDCS, while other studies changed the intensity of the test stimulus at each time point to ensure similar MEP amplitude of the test stimulus prior to and following tDCS (Nitsche & Paulus, 2008; Nitsche et al., 2008; Nitsche et al., 2005). It is possible that in our study, like in the research of Ogata et al. (2007), changes in amplitudes of the conditioned MEPs are in proportion with the change of the test MEPs. This means that the ratio of the MEP amplitude evoked by the paired-pulse condition to that evoked by the single pulse alone did not change significantly (Ogata et al., 2007).

As mentioned above, there is no effect of tDCS on excitability measured over time. Remarkably, post-hoc analysis over time revealed significant differences, indicating more excitability post 30 in comparison with post at the 1 mA condition and the sham condition. However, we found a significant result in QOS, more in the sham condition comparison with 1 mA and 2.5 mA. This can have an influence on the outcome, therefore other factors like state-dependent differences (McGinley et al., 2010) or a probable interaction-effect of behavioral characteristics might play a role.

For the 2.5 mA conditions in the present study, the missing effect of significance might be due to the fact that the parameters were solely obtained immediately after tDCS, when the stimulation might have had only minor effects on cortical excitability, as can be derived from the missing effect on single-pulse MEP amplitudes. Furthermore, in contrast to the above-mentioned TMS protocols, which are influenced by glutamatergic mechanisms, I-wave facilitation and cortical silent period are primarily controlled by the GABAergic system (Paulus et al. 2008), on which tDCS might have no major impact (Nitsche et al. 2004b).

Most studies applied tDCS in younger subject below the age of 50. This was in contrast with this study, where we used healthy subjects above 60 years. Some studies included women, but our reason for including only male subjects is because it is proven that the hormone cycle in female subjects plays an important role in the human brain and causes more variability of MEP's (Smith et al., 1999).

Anodal 1 mA stimulation is the most common used stimulation in literature and has proven that a-tDCS can be effective in increasing corticomotor excitability in healthy individuals (Nitsche and Paulus, 2000; Uy and Ridding, 2003; Nitsche et al., 2005; Antal et al., 2007; Jeffery et al., 2007; Boros et al., 2008; Furubayashi et al., 2008). In this study we didn't found any effect in increasing corticomotor using 1 mA stimulation. A few aspects might play a role, like the age of the participants, small number of participants, different muscle being tested or longer stimulation. Furubayashi et al. found increased MEP's at 1 minute and 15 minutes for long (10minutes) 1 mA anodal stimulation. Measurements at more different time periods might also play a role (e.g. 15 minutes or 90 minutes post stimulation). Anodal tDCS at 2 mA resulted in a significant increase of MEP amplitudes (Batsikatze et al., 2013). However 2 mA anodal stimulation resulted in no change of the I-O curve slope, which was obtained

only once immediately after the end of tDCS (Batsikatze et al., 2013). These results are not in accordance with those of a previous study with 1 mA protocols (Nitsche et al. 2005).

The results of the before mentioned studies do not necessarily contradict our present findings. The discrepancy in the results can be due to some differences in the methodology. First, there is a large difference between the studies in age and gender of the participants. Average age of the included participants in the current study was 69.5 year, whereas the participants in the other studies were respectively around 50 and 80 years old (Furubayashi et al., 2007; Hummel et al., 2010) or were younger participants, around 24 and 50 years old (Jeffery et al., 2007; Furubayashi et al., 2007; Batsikatze et al., 2013; Nitsche and Paulus, 2001). Second, there is an inequality between the studies in how the conditioning stimulus intensity was set, relative to resting motor threshold (McGinley et al., 2010), like in this present study, or relative to active motor threshold (Wassermann, 2002). Third, the coil used to deliver the magnetic stimulation on the cortex altered between the studies, varying between a figure-of-eight coil used in this study and a circular coil. The choice for a type of coil can possibly be a source for an experimental error. The hand-held figure-of-eight coils made contact with the scalp at a single point around which they are free to inclinate and pivot. This is in contrast with a round coil, where there is no effect of pivoting (Wassermann, 2002). A neuronavigation system to ensure similar coil position during the whole experimental session was used, because a figure of coil is more vulnerable for experimental error. Finally, there is dissimilarity between the muscles studied, exploring more distal hand muscles such the first dorsal interosseus (FDI) (Antal et al., 2007; Furubayashi et al., 2008; Uy and Ridding, 2003; Hummel et al., 2005) vs. proximal hand muscles, abductor digiti minimi (ADM) (Boros et al., 2008; Nitsche et al., 2005) or flexor carpi radialis (FCR) (Edwards et al., 2009) vs. lower extremities, the tibialis anterior (TA) and vastus lateralis (VAL) (Jeffery et al., 2007). Any of these factors may have accounted for differences in the outcomes of these studies, which make direct comparisons with our results difficult.

However TMS is commonly used, it is not without limitations. Intra- and inter-individual variability is high and it is likely that factors such as caffeine and alcohol intake, circadian rhythm, circulating hormone levels and other factors that influence neural function will contribute to variability in measurements (Wassermann 2002). Even we made an effort to control for factors that influence intracortical excitability, there were differences in the QOS between the conditions (table 6)

Another limitation of this study is the small sample size, which may have resulted in insufficient power to detect changes in the recruitment curve. However, past studies found significant effects with small samples of about 11 – 20 participants (Batsikatze et al. 2013; Boros et al., 2008; Kidgell et al., 2013; Nitsche et al., 2005).

Through the many methodological dissimilarities between the before mentioned studies and our study it is difficult to make a direct comparisons of the result. Therefore it is necessarily to develop one research protocol to study the influence of tDCS on the recruitment curve (AURC).

5. Conclusion

In this study we examined the effects of different current intensities on excitability in healthy elderly. No differences in excitability between conditions were reported over time. Sham and 1 mA anodal tDCS led to more excitability but only 30 minutes after the end of the stimulation.

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