Woord vooraf

Voor u ligt mijn Master Thesis, welke de afsluiting vormt van de master Revalidatiewetenschappen en Kinesitherapie aan de Universiteit te Hasselt.

Graag wil ik de personen die hebben bijgedragen aan de totstandkoming van deze Master Thesis bedanken. Allereerst een woord van dank voor mijn promotor, Prof. Dr. Raf Meesen, en co-promotor, Dr. Koen Cuypers, voor de begeleiding tijdens het onderzoek en het schrijven van deze thesis.

Daarnaast wil ik de deelnemers van deze studie bedanken voor de moeite en voor de tijd die ze hebben vrijgemaakt om te kunnen deelnemen aan ons onderzoek. En niet te vergeten ook mijn collega-studenten voor de goede raad, kritische blik en stimulatie tijdens het schrijven van deze thesis.

Hapert, 3 mei 2014 A. v. R.

Research context

This master thesis is situated in the field of neurological rehabilitation, more specific the underlying fundamental mechanisms. The main focus of this pilot study is to investigate the effect of different stimulation intensities of transcranial direct current stimulation (tDCS) on the inhibition and facilitation and to explore the differences between young and older adults.

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 masterthesis is part of the ongoing studies about the underlying mechanism of neuroplasticity and motor control, in function of exploring new rehabilitation strategies for neurodegenerative diseases by modulation of neuroplasticity in the lab of Movement Control and Neuroplasticity.

The experiment was conducted in REVAL, building A, Agoralaan, 3590 Diepenbeek and was approved by the ethical medic commission of the university of Hasselt on 11/01/2013. The equipment needed to perform our measurements: TMS stimulator and neuronavigator, tDCS stimulator and an EMG recorder.

Our experiment is a double-blind cross-over repeated measures experiment, which research question and research design was already formed. Part one of the master thesis and the data-acquisition was done together with Joren Meulemans. For the data-acquisition we spent, together with the co-promotor, 6 weeks in the lab. After the data was gathered the thesis was separated in two different topics, short intracortical inhibition and intracortical facilitation, and the data processing and writing was done individually. Feedback was given by promotor and co-promotor.

Reference list

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The effect of tDCS intensity on short-interval intracortical inhibition in young and older healthy subjects.

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

Abstract

Background: tDCS can result in modifications in cortical excitability and may influence short interval intracortical inhibition (SICI) and intracortical facilitation (ICF) mechanisms; therefore it might have implications for the rehabilitation of people with neurodegenerative pathologies.

Objective: To explore the effect of different tDCS intensities on short-interval intracortical inhibition in young and older healthy subjects.

Method: 20 healthy adults (10 young adults, 13 older adults) were randomized to an order of interventions. The interventions were tDCS at 1 mA, 2.5 mA or as control intervention sham stimulation and were administered at 3 different days. SICI was measured at baseline, immediately after (post) and 30 (post 30) and 60 (post 60) minutes post intervention using TMS and compared between time points and between the age groups.

Results: SICI differed not significantly between the age groups, between the different interventions and over time. Post-hoc analysis over time revealed that only tDCS of 2.5 mA was able to induce a significant reduction in SICI 30 minutes after the stimulation in comparison with the pre measurement, and only in the older age group.

Conclusion: It is possible to reduce SICI 30 minutes after stimulation, in older adults, with anodal tDCS of 2.5 mA. There is no significant age-related change in SICI.

1. Introduction

Complex changes in the physiology of the human brain with aging are likely to underline the decline of perceptual, cognitive and motor abilities in older adults (Kossev, Schrader, Dauper, Dengler, & Rollnik, 2002). The most commonly seen effect of these changes is a decrease in motor skills (McGinley, Hoffman, Russ, Thomas, & Clark, 2010) and a decline in performance on accuracy and precision tasks in older adults when compared with younger adults (Marneweck, Loftus, & Hammond, 2011). Probably, peripheral changes play a role in this diminution, however recently interest has focused on functional changes in the cerebral cortex that also might contribute to this decline (Hortobagyi, del Olmo, & Rothwell, 2006). Specifically, more attention has been set to age-related changes of inhibitory processes in motor cortex that might diminish motor performance, since it is well established that fine motor control necessitate suppression of the activation of antagonistic or irrelevant muscles (Marneweck et al., 2011). In addition, in comparison to younger adults, older adults need more complex activation of the motor system to have similar performance. Such deficits regularly limit common tasks of daily living and can negatively influence the independence of elderly (Ward & Frackowiak, 2003). Since the increasing life expectancy and retirement age, there is a rising demand to identify strategies to preserve the neuromuscular function with normal aging (Goodwill, Reynolds, Daly, & Kidgell, 2013).

The application of transcranial direct current stimulation (tDCS) represents a noninvasive brain stimulation technique that delivers a small electric current, which can modulate brain excitability in healthy subjects (Nitsche & Paulus, 2000; Priori, Berardelli, Rona, Accornero, & Manfredi, 1998). 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). The current flows from the cathode (negative charged electrode) to the anode (positive charged electrode). 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). Only a few studies investigated the effects of different current intensities on inhibition and facilitation mechanisms, but these effects remain unclear. There seems to be no difference in inhibition and facilitation mechanisms with stimulation at low current intensities on the hand motor cortex (B. Cengiz, N. Murase, & J. Rothwell, 2013; Kidgell et al., 2013). However, these results are in contrast with stimulation of the leg motor cortex, whereby enhanced excitability can be achieved by 2 mA anodal tDCS but not with 1 mA tDCS, probably because not enough current penetrates deep enough to effect the leg area (Jeffery, Norton, Roy, & Gorassini, 2007). Transcranial direct current stimulation of the motor cortex may also have functional consequences and possible clinical utility in motor disorders. For instance, it appears that 1 mA anodal tDCS can enhance dexterity in the non dominant hand (Boggio et al., 2006) and results 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 (Hummel et al., 2010). Besides, tDCS of 1,5 mA applied to left regions could be useful in enhancing memory function in aging (Manenti, Brambilla, Petesi, Ferrari, & Cotelli, 2013). Finally, not much is known about the effects of different intensities tDCS, but it seems that lower current intensities have no different effect on inhibition and facilitation (Bülent Cengiz et al., 2013; Kidgell et al., 2013). Therefore, in this study, intensities of 1 mA and 2.5 mA are used to explore the effects of higher intensities on the inhibition mechanism in the human brain. We expect that different current intensities would differentially modulate cortical excitability. More specifically, we expect decreased inhibition with higher current intensity.

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 after-effects (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 after-effects 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).

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. A powerful and rapidly alternating current passes through the coil, creating a brief alternating magnetic field, which penetrates through the cranium. Subsequently, this alternating magnetic field induces a current in the brain and penetrates the membranes of the neurons, resulting in an action potential or excitatory postsynaptic potential (Terao & Ugawa, 2002). These action potentials result in motor-evoked potentials, which are measured by electromyography (EMG) recordings of the target muscle. A motor evoked potential (MEP) can be reduced, called inhibition (SICI), or enhanced, called facilitation (ICF), with a conditioning-test stimulus by using paired-pulse TMS. The time between the conditioning stimulus and test stimulus is called inter stimulus interval (ISI). For example, at ICF a subthreshold conditioning pulse precedes a test pulse by 7-20 ms and the MEP amplitude associated with the test pulse is increased as compared to a single pulse. On the contrary, at SICI, the ISI is 1-6 ms and the conditioning pulse reduces the MEP amplitude (Kujirai et al., 1993; Ziemann, Rothwell, & Ridding, 1996). Consequently SICI consists of two phases that are physiologically distinct. The first phase is thought to be due refractoriness of cortical axons activated by the conditioning stimulus. The conditioning stimulus will activate some of the same axons as recruited by the test stimulus. If the test stimulus is given 1 ms later these axons will be refractory and results in temporally dispersed synaptic

input arriving at the corticospinal neurons, leading to reduced motor evoked potential (Kujirai et al., 1993). The cause of the second phase of inhibition is thought to be due to gamma-amino-butyl-acid (GABA) inhibition (Fisher, Nakamura, Bestmann, Rothwell, & Bostock, 2002; Kujirai et al., 1993). GABA is an inhibitory neurotransmitter in the brain tissue with an abundant structural selection of receptors and a dense representation of interneurons in the cortex (Blatow, Caputi, & Monyer, 2005). Inhibition may be related to the activation of pre-synaptic GABAb receptors, who have a specific role in controlling GABA release from inhibitory interneurons (McDonnell, Orekhov, & Ziemann, 2006). It is likely that, because different mechanisms of inhibition operate at the two phases, it is differently affected in diseases (Fisher et al., 2002). In summary, the effect of the conditioning stimulus depends on the intensity of both the conditioning and test stimulus and the interstimulus interval between the two stimuli (Kujirai et al., 1993; Ziemann et al., 1996).

In conclusion, the aim of this study is to evaluate the impact of tDCS intensity on the motor cortex inhibitory processes in different age groups. For this purpose, we studied SICI in a group of young people and older people and compared the data. Another aim of this study is to measure the effect and the after-effect of different intensities tDCS on the inhibition mechanism in the human brain, therefore we used intensities of 1 mA and 2.5 mA and compared these data with sham stimulation and at different time points, in the young and older adults.

2. Material and methods

2.1 Participants

Twenty-three healthy adults, aged between 18-35 (N = 10) or older than 60 year (N = 13), with no history of neurological impairment participated in the study. Only male subjects were included, since there is evidence that female hormones play an important role in the human brain and causes more variability between the MEPs (M. J. Smith et al., 1999). The participants, recruited from the local community, were relatives or were recruited by flyers or e-mails. All participants were tested for right-handedness with the Edinburgh Handedness Inventory, taken into account a cut-off of 60% (Oldfield, 1971). The older participants were free of any cognitive impairment as assessed by the Mini-mental State Examination (MMSE), with a cut-off value of ≥ 26/30. (Folstein Mf, 1983). In addition, the older adults were also screened for depression with the Geriatric Depression Scale (GDS) (Yesavage & Sheikh, 1986). All participants filled in a safety-screening questionnaire to determine their suitability for TMS and tDCS application. Additionally, the participants completed a general questionnaire about their hobbies, work, sports, medication and more. And 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. All participants provided written informed consent prior to participation in the study, which was approved by the local ethics committee of the university of Hasselt and the central ethics committee of U.Z. Leuven.

2.2 Experimental design

This study consisted of a double-blind crossover repeated measurement design (see figure 1), containing four sessions, spread out over a couple of weeks. There was a washout period of at least three days between sessions. The first session was a familiarization session whereby the participants got familiar with the measurements. The intervention sessions were anodal tDCS delivered either at 1 mA or 2.5 mA or sham stimulation. The examiners and the subjects were both blinded to whether the interventions. The stimulators were coded using a five-letter code, preprogrammed by the principal investigator. Sealed envelope containing cards with codes for the order of the intervention was used for randomization to intervention. Another researcher entered the code in the stimulator, so that every researcher and participant was blinded to the intervention. At every intervention session inhibition was measured before stimulation, immediately after stimulation and 30 and 60 min after stimulation. Attention, tiredness and discomfort or pain was reported before every measurement.

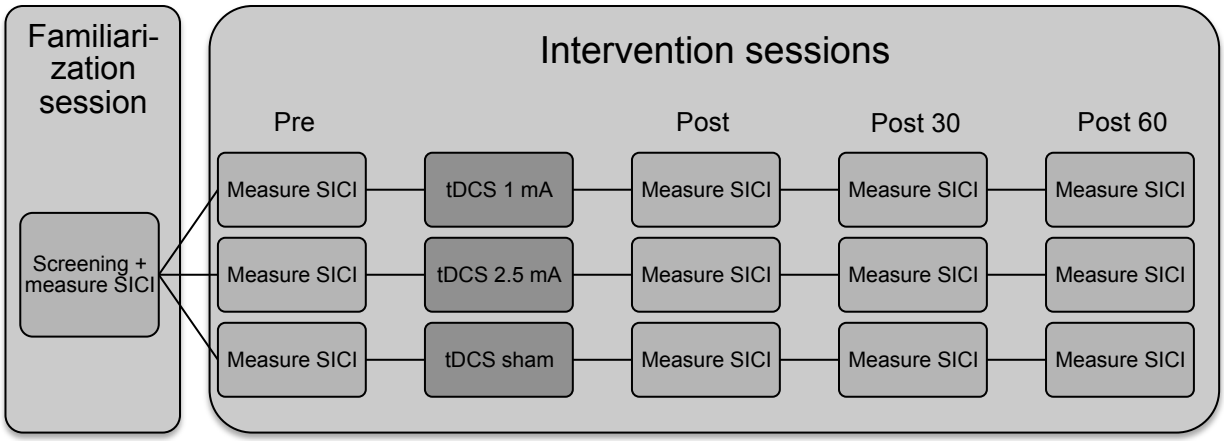

Figure 1. Research design

2.3 Transcranial direct current stimulation

tDCS was applied over the left motor cortex using a battery-driven constant current stimulator (Eldith, Neuroconn Germany). The stimulator delivered a current strength of 1mA, 2.5mA or sham through rubber electrodes. The anodal electrode was attached over the primary motor cortex on the hotspot for the FDI muscle, as identified by TMS over the left cortex, by use of a zero gel (Newronika, Italy). The cathodal electrode, the reference electrode, covered in a sponge and soaked with natrium chloride 0,9% (Versol), was placed on the contralateral orbit. The subjects received 1mA, 2.5mA or sham stimulation at random. Stimulation of 1mA and 2.5mA endured 10 minutes; the sham stimulation only took 20 seconds. The current was always ramped up or down over the first and the last 15 seconds of stimulation.

2.4 Recording EMG activity

Surface electromyographic (EMG) signals were recorded from the first dorsal interosseus (FDI) muscle with a 16-channel “Bagnoli” Delsys EMG system (bandwith 20-450 Hz, Boston, USA). The skin surface was shaved and cleaned with alcohol before the electrode, with 1 cm interelectrode distance, was placed on the muscle belly. To ensure optimal relaxation of the 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. The data was stored for offline analysis.

2.5 Transcranial magnetic stimulation

MEPs were elicited in the right FDI by single-pulse TMS over the left primary motor cortex, conducted by a Magstim 200 magnetic stimulator (Magstim Bistim 200; maximal stimulator output of 113%) with a figure of eight coil (diameter of 70 mm). The coil was held at an angle of 45 degrees from the midline, tangentially to the skull. The coil was connected to two Magstim stimulators through a bistim module for the paired-pulse TMS protocols.

The volunteers were seated in a comfortable chair with armrests. First, a cap with a coordinate system with one centimeter along the medio-lateral and antero-posterior axe was made. The optimal coil placement (hotspot) was defined as the site where TMS resulted in the largest MEPs of the contralateral FDI. The hotspot was recorded with TMS neuronavigation (ANT, VISOR Neuronavigational System) to ensure that the position was the same during the whole experimental session. Second, the rest motor threshold (rMT) of the single pulse was determined. RMT is defined as the lowest percentage output of the stimulator needed whereby at least 5 of the 10 consecutive trials elicited a MEP larger than 0.005 mV.

After defining of the rMT, an input-output curve was determined using TMS intensities of 90%, 110%, 130%, 150%, 170% and 190% rMT. In total 36 stimuli are given at random (six stimuli per intensity).

Then, the middle of the input-output curve was used to determine the test stimulus. The test stimulus is defined as the percentage stimulator output needed to get the MEP at the middle of the input-output curve. Next, the coil was connected to two Magstim stimulators for the paired-pulse protocol, and the rMT for the double pulse was determined. The condition stimulus is calculated by the rMT x 0.80 (Kujirai et al., 1993). The condition stimulus preceded the test stimulus and reduced the MEP with at least 50%. If this was not the case, the condition stimulus was adjusted so that there was a reduction of 50% MEP. Finally, the subjects received at random 24 pulses: 12 single pulses (SP) and 12 pulses whereby the condition stimulus precedes the test stimulus with an interstimulus interval of 3 ms (inhibition). The measurements of short interval intracortical inhibition were performed prior the stimulation (pre), immediately after the stimulation (post) and 30 (post 30) and 60 minutes after stimulation (post 60).

2.6 Data and statistical analysis

The statistical analysis were performed using SPSS (IBM SPSS Statistics, Version 22). Mann-Whitney test procedures were used to determine group differences (young vs. old). The Friedmann test was used to calculate significant differences between the three different conditions (sham vs. 1 mA vs. 2.5 mA) and the Wilcoxon Signed Rank Test was used to determine the effect of stimulation on inhibition between different time points (pre vs. post vs. post 30 vs. post 60). For all analysis, significance was set at p-value < 0.05.

Inhibition is expressed in percentage as the ratio of the mean MEP amplitude evoked by the paired-pulse condition to that evoked by the single pulse alone. Data are presented as means ± standard deviation (SD).

Boxplots were made to determine possible outliers and three subjects were excluded from the data-analysis. Two participants were outliers with values between 1.5 and 3 box lengths away from the upper border. One participant was considered as an extreme outlier with values more than three times the length of the box away from the upper border of the boxplot.

3. Results

3.1 Participants

Subjects' characteristics are shown in table 1. At baseline, significant differences between groups were noticed for age ($p < 0.05$; Mann-Whitney *U* test) indicating that there is a significant difference in age between the groups.

Table 1. Demographic data at baseline

	Young Mean (SD)	**Old** Mean (SD)	*p*-value
N	9	11	
Age	25.60 (5.10)	69.64 (6.05)	0.000*
Laterality quotient	95.56(13.33)	91.09 (11.77)	0.174
MMSE		29.09 (0.83)	
GDS		3.36 (4.11)	

Demographic data at baseline (Mann- Whitney test). * $p < 0.05$ (significance difference between young and old subjects). MMSE = minimal mental state examination; GDS = geriatric depression scale

3.2 Effect of stimulation intensity on inhibition

Data describing the mean inhibition of both groups at baseline, post, post 30 and post 60 are shown in figure 2.

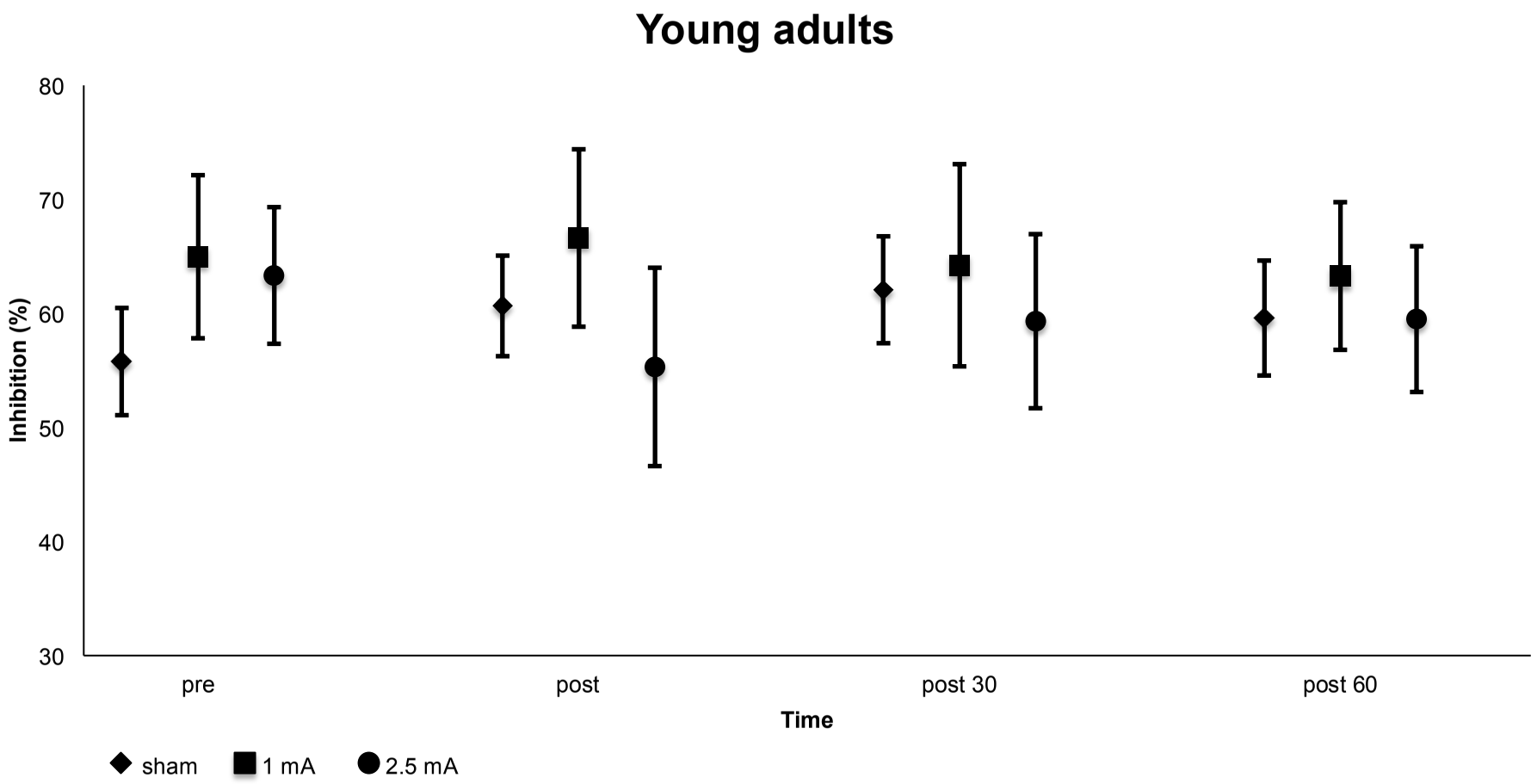

Figure 2a. Modulations of inhibition at baseline (pre), after (post) and 30 (post 30) and 60 (post 60) minutes after stimulation. Inhibition expressed in percentages. Data plotted as group mean ± standard deviation (SD).
* Significant ($p < 0.05$) differences in inhibition between time points.

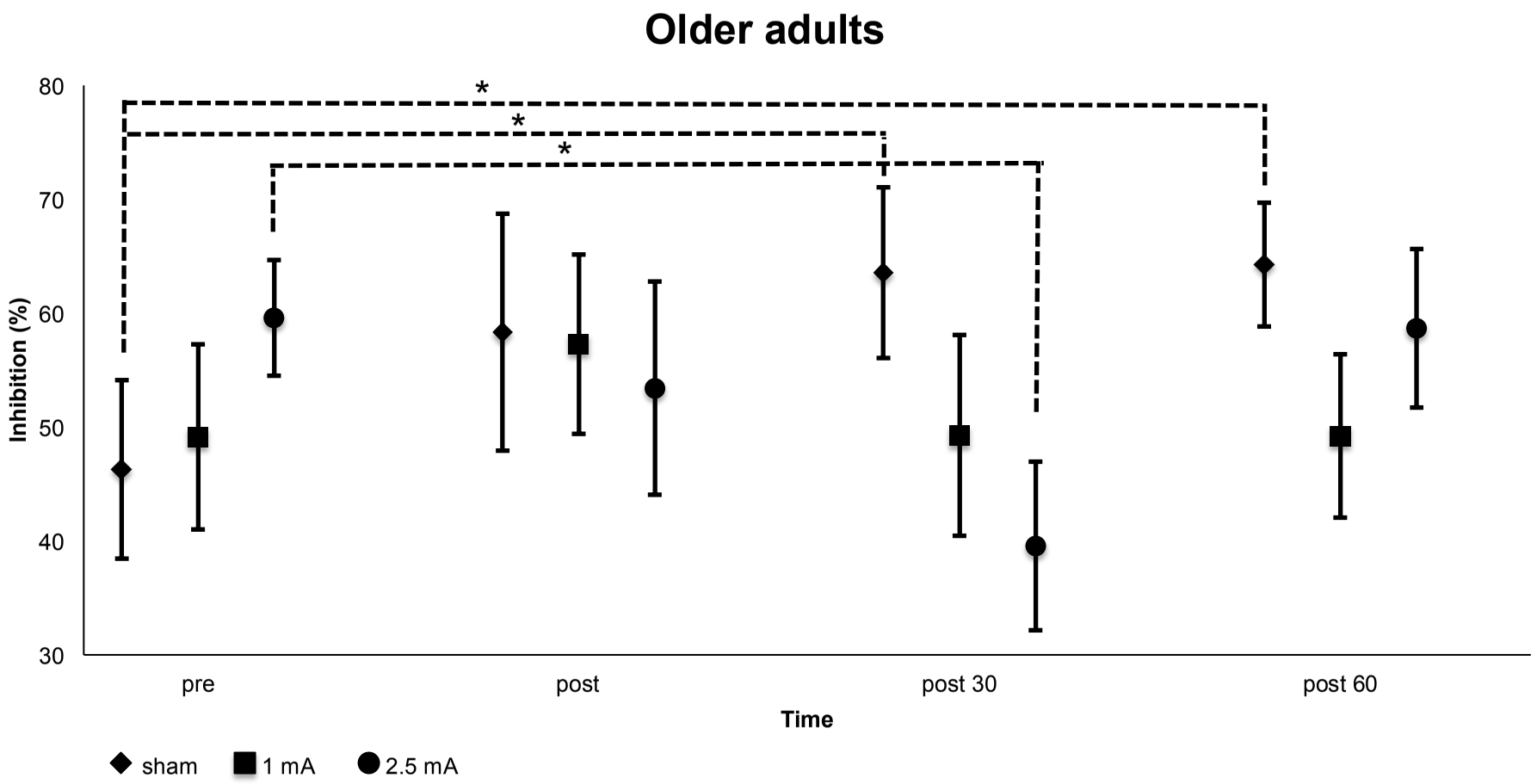

Figure 2b. Modulations of inhibition at baseline (pre), after (post) and 30 (post 30) and 60 (post 60) minutes after stimulation. Inhibition expressed in percentages. Data plotted as group mean ± standard deviation (SD).
* Significant ($p < 0.05$) differences in inhibition between time points.

3.2.1 Comparisons between conditions

Table 2. Comparison of inhibition between the conditions

Young				
	Sham	**1 mA**	**2.5 mA**	
	Mean (SD)	Mean (SD)	Mean (SD)	*p*-value
pre	55.78 (4.70)	64.97 (7.17)	63.32 (5.99)	0.097
post	60.67 (4.41)	66.63 (7.79)	55.31 (8.70)	0.459
post 30	62.06 (4.68)	64.21 (8.87)	59.31 (7.62)	0.459
post 60	59.59 (5.04)	63.27 (6.48)	59.49 (6.38)	0.717

Old				
	Sham	**1 mA**	**2.5 mA**	
	Mean (SD)	Mean (SD)	Mean (SD)	*p*-value
pre	46.27 (7.84)	49.12 (8.12)	59.58 (5.08)	0.078
post	58.33 (10.41)	57.29 (7.88)	53.41 (9.37)	0.441
post 30	63.56 (7.50)	49.27 (8.81)	39.54 (7.40)	0.060
post 60	64.27 (5.44)	49.21 (7.16)	58.67 (6.95)	0.441

Comparison (Friedman test) of inhibition between the conditions for both young and old adults. * $p < 0.05$ (significance difference between conditions). Inhibition expresses in percentages.

Age group means for inhibition are given in table 2. No significant differences were observed ($p < 0.05$; Friedmann test) for the different stimulation intensities in both young and older subjects, suggesting that stimulation intensity does not influence inhibition.

3.2.2 Comparisons between groups

Table 3. Comparison between age groups

		Young	**Old**	
		Mean (SD)	Mean (SD)	*p*-value
Sham	pre	55.78 (4.70)	46.27 (7.84)	0.342
	post	60.67 (4.41)	58.33 (10.41)	0.970
	post 30	62.06 (4.68)	63.56 (7.50)	0.909
	post 60	59.59 (5.04)	64.27 (5.44)	0.970
1 mA	pre	64.97 (7.17)	49.12 (8.12)	0.184
	post	66.63 (7.79)	57.29 (7.88)	0.382
	post 30	64.21 (8.87)	49.27 (8.81)	0.425
	post 60	63.27 (6.48)	49.21 (7.16)	0.138
2.5 mA	pre	63.32 (5.99)	59.58 (5.08)	0.97
	post	55.31 (8.70)	53.41 (9.37)	0.849
	post 30	59.31 (7.62)	39.54 (7.40)	0.138
	post 60	59.49 (6.38)	58.67 (6.95)	0.621

Comparison (Mann-Whitney U test) between age groups. * $p < 0.05$ (significance difference between young and old adults). Inhibition expressed in percentages.

Table 3 summarizes the mean inhibition for the different stimulation intensities in young and old subjects. No significant differences ($p < 0.05$; Mann-Whitney U test) in inhibition were found between young and old subjects, meaning that inhibition was similar in both groups.

3.2.3 Comparisons over time

Table 4. Comparison of inhibition over time

Young					
	pre	post	post 30	post 60	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean(SD)	*p*-value
Sham	55.78 (4.70)	60.67 (4.41)	62.06 (4.68)	59.59 (5.04)	0.954
1 mA	64.97 (7.17)	66.63 (7.79)	64.21 (8.87)	63.27 (6.48)	0.954
2.5 mA	63.32 (5.99)	55.31 (8.70)	59.31 (7.62)	59.49 (6.38)	0.435

Old					
	pre	post	post 30	post 60	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*p*-value
Sham	46.27 (7.84)	58.33 (10.41)	63.56 (7.50)	64.27 (5.44)	0.053
1 mA	49.12 (8.12)	57.29 (7.88)	49.27 (8.81)	49.21 (7.16)	0.921
2.5 mA	59.58 (5.08)	53.41 (9.37)	39.54 (7.40)	58.67 (6.95)	0.220

Comparisons (Friedmann test) of inhibition over time. * $p < 0.05$ (significance difference over time). Inhibition expressed in percentages.

Between time comparisons ($p < 0.05$; Friedmann test) revealed no significant differences of inhibition over time in both young and older adults, indicating that inhibition does not change over time. An overview is given in table 4.

3.2.4 Post-hoc analysis over time

Post-hoc analysis over time, table 5, revealed significant differences ($p < 0.05$; Wilcoxon Signed Ranks Test) in older adults for the comparisons between pre – post 30 and pre – post 60 at the sham condition and for the pre – post 30 at the 2.5 mA condition. These results suggest that, in the older adults, there is more inhibition at post 30 and post 60 in comparison with pre at the sham condition, and there is less inhibition post 30 in comparison with pre at the 2.5 mA condition.

3.3 Behavioral characteristics

Table 6 gives an overview of the behavioral characteristic differences between the different conditions for both age subgroups. These results reveal a difference in QOS in the sham condition for the older age group. Differences between the age subgroups are shown in table 7. Significance differences were observed in behavioral characteristics for QOS, alcohol, attention post 30 in the sham condition and for coffee in the 1mA condition ($p < 0.05$; Mann-Whitney *U* test). These differences may have an influence on the results.

Comparisons for the behavioral characteristics attention, fatigue and pain over time (table 8) revealed significant differences ($p < 0.05$; Friedmann test) between attention over time in the 2.5 mA condition, only for young adults.

Post-hoc analysis of the behavioral characteristics over time are shown in table 9, indicating significant differences ($p < 0.05$; Wilcoxon Signed Ranks Test). For the young adults, attention was significantly different between pre and post 60 at the sham condition, 1 mA condition and 2.5 mA condition. In addition, there was also a significant difference in attention between pre and post 30 at the 2.5 mA condition. For the older adults, there was only a significant difference between post 30 and post 60 at the sham condition.

Table 5. Post hoc analysis of inhibition over time

	Young			Old		
		Sham			**Sham**	
	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value
pre - post	55.78 (4.70)	60.67 (4.41)	0.314	46.27 (7.84)	58.33 (10.41)	0.062
pre - post 30	55.78 (4.70)	62.06 (4.68)	0.515	46.27 (7.84)	63.56 (7.50)	0.041*
pre - post 60	55.78 (4.70)	59.59 (5.04)	0.314	46.27 (7.84)	64.27 (5.44)	0.033*
post - post 30	60.67 (4.41)	62.06 (4.68)	0.953	58.33 (10.41)	63.56 (7.50)	0.424
post - post 60	60.67 (4.41)	59.59 (5.04)	0.594	58.33 (10.41)	64.27 (5.44)	0.213
post 30 - post 60	62.06 (4.68)	59.59 (5.04)	0.767	63.56 (7.50)	64.27 (5.44)	0.722
		1 mA			**1 mA**	
	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value
pre - post	64.97 (7.17)	66.63 (7.79)	0.678	49.12 (8.12)	57.29 (7.88)	0.062
pre - post 30	64.97 (7.17)	64.21 (8.87)	0.515	49.12 (8.12)	49.27 (8.81)	0.657
pre - post 60	64.97 (7.17)	63.27 (6.48)	0.859	49.12 (8.12)	49.21 (7.16)	0.374
post - post 30	66.63 (7.79)	64.21 (8.87)	0.953	57.29 (7.88)	49.27 (8.81)	0.657
post - post 60	66.63 (7.79)	63.27 (6.48)	0.767	57.29 (7.88)	49.21 (7.16)	0.534
post 30 - post 60	64.21 (8.87)	63.27 (6.48)	0.953	49.27 (8.81)	49.21 (7.16)	0.859
		2.5 mA			**2.5 mA**	
	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value
pre - post	63.32 (5.99)	55.31 (8.70)	0.314	59.58 (5.08)	53.41 (9.37)	0.374
pre - post 30	63.32 (5.99)	59.31 (7.62)	0.515	59.58 (5.08)	39.54 (7.40)	0.041*
pre - post 60	63.32 (5.99)	59.49 (6.38)	0.173	59.58 (5.08)	58.67 (6.95)	0.657
post - post 30	55.31 (8.70)	59.31 (7.62)	0.515	53.41 (9.37)	39.54 (7.40)	0.722
post - post 60	55.31 (8.70)	59.49 (6.38)	0.515	53.41 (9.37)	58.67 (6.95)	0.477
post 30 - post 60	59.31 (7.62)	59.49 (6.38)	0.767	39.54 (7.40)	58.67 (6.95)	0.050

Post hoc analysis of inhibition (Wilcoxon Signed Ranks Test) over time. * $p < 0.05$ (significance difference between time points)
Inhibition expressed in percentages.

Table 6. Behavioral characteristics: within group variability

		Young				Old			
		Sham	**1 mA**	**2.5 mA**		**Sham**	**1 mA**	**2.5 mA**	
		Mean (SD)	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	Mean (SD)	*p*-value
rMT SP (% SO)		36.89 (3.59)	37.22 (4.92)	36.00 (5.10)	0.748	38.00 (3.61)	38.90 (3.35)	38.82 (4.33)	0.196
rMT DP (% SO)		40.44 (4.33)	41.78 (5.72)	39.89 (6.60)	0.428	43.18 (5.42)	43.70 (3.92)	43.09 (5.34)	0.590
Conditioning stimulus (% SO)		33.56 (5.88)	35.11 (5.16)	32.44 (6.21)	0.045*	35.91 (5.61)	37.30 (4.19)	37.01 (4.00)	0.819
Test stimulus (% SO)		50.67 (6.30)	51.78 (7.76)	49.89 (8.12)	0.682	53.64 (5.83)	55.30 (4.45)	53.91 (6.22)	0.607
Hours sleep		6.72 (1.72)	7.33 (1.79)	7.22 (1.00)	0.618	7.59 (1.14)	7.35 (1.06)	7.27 (1.21)	0.895
QOS		7.44 (2.01)	7.89 (1.45)	7.22 (1.71)	0.733	9.18 (1.08)	7.72 (2.94)	8.45 (1.51)	0.030*
Alcohol		0.11 (0.33)	0.56 (1.13)	1.22 (2.59)	0.368	1.81 (1.47)	0.90 (1.19)	1.82 (1.60)	0.079
Coffee		0.78 (0.97)	1.33 (2.60)	1.44 (1.42)	0.705	1.63 (1.21)	2.40 (0.97)	2.27 (2.76)	0.519
Attention	pre	7.33 (2.18)	7.78 (1.20)	7.89 (1.17)	0.629	8.18 (1.08)	8.40 (1.17)	8.36 (1.29)	0.878
	post	6.89 (1.17)	7.33 (1.50)	7.33 (1.32)	0.091	8.09 (1.30)	8.40 (1.35)	8.09 (1.51)	0.396
	post 30	7.00 (1.32)	7.00 (1.41)	7.11 (1.27)	0.891	8.36 (1.03)	8.00 (1.63)	8.18 (1.33)	0.350
	post 60	6.44 (2.35)	6.78 (1.73)	7.00 (1.22)	0.810	8.00 (1.41)	7.90 (0.60)	7.82 (1.66)	0.957
Fatigue	pre	2.44 (1.67)	2.33 (2.00)	2.22 (1.86)	0.969	2.00 (2.45)	1.09 (1.92)	2.00 (1.73)	0.356
	post	3.11 (1.27)	2.44 (1.50)	2.67 (1.94)	0.435	2.91 (2.55)	1.60 (0.50)	2.09 (1.87)	0.504
	post 30	2.78 (1.39)	2.89 (2.09)	2.56 (1.74)	0.630	2.45 (2.50)	1.90 (1.97)	2.09 (1.92)	0.957
	post 60	3.11 (1.36)	2.78 (1.86)	2.56 (1.74)	0.483	2.72 (2.53)	2.40 (2.55)	2.72 (2.57)	0.857
Pain	pre	0.78 (1.56)	0.78 (1.56)	1.00 (1.80)	0.607	1.27 (2.10)	0.55 (1.51)	1.18 (1.78)	0.368
	post	0.89 (1.83)	1.00 (2.51)	0.89 (1.54)	0.926	1.72 (2.15)	0.70 (1.64)	1.72 (2.24)	0.210
	post 30	1.00 (1.80)	1.00 (1.73)	1.00 (1.50)	0.939	1.63 (2.06)	1.10 (1.73)	1.45 (2.25)	0.926
	post 60	1.00 (1.80)	1.11 (1.76)	1.33 (1.73)	0.504	1.72 (2.05)	1.20 (1.75)	1.36 (2.06)	0.810

Behavioral characteristics (Friedmann test). Within group variability. Differences between the conditions for young and old adults. * $p < 0.05$ (significant difference between conditions).
rMT = rest motor threshold; SP = single pulse; DP = double pulse; SO = stimulator output; QOS = quality of sleep

Table 7. Behavioral characteristics: between group variability

		Sham			1 mA			2.5 mA		
		Young	Old		Young	Old		Young	Old	
		Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value
rMT SP (% SO)		36.89 (3.59)	38.00 (3.61)	0.536	37.22 (4.92)	38.90 (3.35)	0.460	36.00 (5.10)	38.82 (4.33)	0.168
rMT DP (% SO)		40.44 (4.33)	43.18 (5.42)	0.158	41.78 (5.72)	43.70 (3.92)	0.485	39.89 (6.60)	43.09 (5.34)	0.222
Conditioning stimulus (% SO)		33.56 (5.88)	35.91 (5.61)	0.491	35.11 (5.16)	37.30 (4.19)	0.389	32.44 (6.21)	37.01 (4.00)	0.073
Test stimulus (% SO)		50.67 (6.30)	53.64 (5.83)	0.237	51.78 (7.76)	55.30 (4.45)	0.411	49.89 (8.12)	53.91 (6.22)	0.170
Hours sleep		6.72 (1.72)	7.59 (1.14)	0.133	7.33 (1.79)	7.35 (1.06)	0.740	7.22 (1.00)	7.27 (1.21)	0.847
QOS		7.44 (2.01)	9.18 (1.08)	0.036*	7.89 (1.45)	7.72 (2.94)	0.670	7.22 (1.71)	8.45 (1.51)	0.095
Alcohol		0.11 (0.33)	1.81 (1.47)	0.002*	0.56 (1.13)	0.90 (1.19)	0.232	1.22 (2.59)	1.82 (1.60)	0.187
Coffee		0.78 (0.97)	1.63 (1.21)	0.105	1.33 (2.60)	2.40 (0.97)	0.011*	1.44 (1.42)	2.27 (2.76)	0.660
Attention	pre	7.33 (2.18)	8.18 (1.08)	0.393	7.78 (1.20)	8.40 (1.17)	0.273	7.89 (1.17)	8.36 (1.29)	0.378
	post	6.89 (1.17)	8.09 (1.30)	0.051	7.33 (1.50)	8.40 (1.35)	0.145	7.33 (1.32)	8.09 (1.51)	0.295
	post 30	7.00 (1.32)	8.36 (1.03)	0.021*	7.00 (1.41)	8.00 (1.63)	0.168	7.11 (1.27)	8.18 (1.33)	0.136
	post 60	6.44 (2.35)	8.00 (1.41)	0.095	6.78 (1.73)	7.90 (0.60)	0.125	7.00 (1.22)	7.82 (1.66)	0.330
Fatigue	pre	2.44 (1.67)	2.00 (2.45)	0.354	2.33 (2.00)	1.09 (1.92)	0.102	2.22 (1.86)	2.00 (1.73)	0.846
	post	3.11 (1.27)	2.91 (2.55)	0.787	2.44 (1.50)	1.60 (0.50)	0.424	2.67 (1.94)	2.09 (1.87)	0.489
	post 30	2.78 (1.39)	2.45 (2.50)	0.512	2.89 (2.09)	1.90 (1.97)	0.211	2.56 (1.74)	2.09 (1.92)	0.511
	post 60	3.11 (1.36)	2.72 (2.53)	0.590	2.78 (1.86)	2.40 (2.55)	0.619	2.56 (1.74)	2.72 (2.57)	0.908
Pain	pre	0.78 (1.56)	1.27 (2.10)	0.512	0.78 (1.56)	0.55 (1.51)	0.828	1.00 (1.80)	1.18 (1.78)	0.858
	post	0.89 (1.83)	1.72 (2.15)	0.324	1.00 (2.51)	0.70 (1.64)	0.598	0.89 (1.54)	1.72 (2.24)	0.465
	post 30	1.00 (1.80)	1.63 (2.06)	0.516	1.00 (1.73)	1.10 (1.73)	0.813	1.00 (1.50)	1.45 (2.25)	0.966
	post 60	1.00 (1.80)	1.72 (2.05)	0.359	1.11 (1.76)	1.20 (1.75)	0.850	1.33 (1.73)	1.36 (2.06)	0.707

Behavioral characteristics (Mann-Whitney test). Between group variability. Differences between young and old for the different conditions. * $p < 0.05$ (significant difference between young and old).
rMT = rest motor threshold; SP = single pulse; DP = double pulse; SO = stimulator output; QOS = quality of sleep

Table 8. Comparison of behavioral characteristics over time

Young		pre	post	post 30	post 60	
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*p*-value
Sham	Attention	7.33 (2.18)	6.89 (1.17)	7.00 (1.32)	6.44 (2.35)	0.135
	Fatigue	2.44 (1.67)	3.11 (1.27)	2.78 (1.39)	3.11 (1.36)	0.131
	Pain	0.78 (1.56)	0.89 (1.83)	1.00 (1.80)	1.00 (1.80)	0.194
1 mA	Attention	7.78 (1.20)	7.33 (1.50)	7.00 (1.41)	6.78 (1.73)	0.071
	Fatigue	2.33 (2.00)	2.44 (1.50)	2.89 (2.09)	2.78 (1.86)	0.202
	Pain	0.78 (1.56)	1.00 (2.51)	1.00 (1.73)	1.11 (1.76)	0.308
2.5 mA	Attention	7.89 (1.17)	7.33 (1.32)	7.11 (1.27)	7.00 (1.22)	0.023*
	Fatigue	2.22 (1.86)	2.67 (1.94)	2.56 (1.74)	2.56 (1.74)	0.322
	Pain	1.00 (1.80)	0.89 (1.54)	1.00 (1.50)	1.33 (1.73)	0.159

Old		pre	post	post 30	post 60	
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*p*-value
Sham	Attention	8.18 (1.08)	8.09 (1.30)	8.36 (1.03)	8.00 (1.41)	0.487
	Fatigue	2.00 (2.45)	2.91 (2.55)	2.45 (2.50)	2.72 (2.53)	0.081
	Pain	1.27 (2.10)	1.72 (2.15)	1.63 (2.06)	1.72 (2.05)	0.682
1 mA	Attention	8.40 (1.17)	8.40 (1.35)	8.00 (1.63)	7.90 (0.60)	0.435
	Fatigue	1.09 (1.92)	8.40 (1.35)	1.90 (1.97)	2.40 (2.55)	0.052
	Pain	0.55 (1.51)	0.70 (1.64)	1.10 (1.73)	1.20 (1.75)	0.158
2.5 mA	Attention	8.36 (1.29)	8.09 (1.51)	8.18 (1.33)	7.82 (1.66)	0.325
	Fatigue	2.00 (1.73)	2.09 (1.87)	2.09 (1.92)	2.72 (2.57)	0.147
	Pain	1.18 (1.78)	1.72 (2.24)	1.45 (2.25)	1.36 (2.06)	0.392

Comparisons (Friedmann test) of attention, fatigue and pain over time. * $p < 0.05$ (significance difference over time).

Table 9a. Post hoc analysis of behavioral characteristics over time, for the young adults

	Young								
					Sham				
		Attention			**Fatigue**			**Pain**	
	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value
pre - post	7.33 (2.18)	6.89 (1.17)	0.340	2.44 (1.67)	3.11 (1.27)	0.222	0.78 (1.56)	0.89 (1.83)	0.317
pre - post 30	7.33 (2.18)	7.00 (1.32)	0.453	2.44 (1.67)	2.78 (1.39)	0.453	0.78 (1.56)	1.00 (1.80)	0.157
pre - post 60	7.33 (2.18)	6.44 (2.35)	0.038*	2.44 (1.67)	3.11 (1.36)	0.058	0.78 (1.56)	1.00 (1.80)	0.157
post - post 30	6.89 (1.17)	7.00 (1.32)	0.317	3.11 (1.27)	2.78 (1.39)	0.083	0.89 (1.83)	1.00 (1.80)	0.317
post - post 60	6.89 (1.17)	6.44 (2.35)	0.414	3.11 (1.27)	3.11 (1.36)	1.000	0.89 (1.83)	1.00 (1.80)	0.317
post 30 - post 60	7.00 (1.32)	6.44 (2.35)	0.180	2.78 (1.39)	3.11 (1.36)	0.414	1.00 (1.80)	1.00 (1.80)	1.000
					1mA				
		Attention			**Fatigue**			**Pain**	
	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value
pre - post	7.78 (1.20)	7.33 (1.50)	0.157	2.33 (2.00)	2.44 (1.50)	0.705	0.78 (1.56)	1.00 (2.51)	0.157
pre - post 30	7.78 (1.20)	7.00 (1.41)	0.066	2.33 (2.00)	2.89 (2.09)	0.059	0.78 (1.56)	1.11 (1.76)	0.157
pre - post 60	7.78 (1.20)	6.78 (1.73)	0.038*	2.33 (2.00)	2.78 (1.86)	0.157	0.78 (1.56)	1.00 (1.73)	0.180
post - post 30	7.33 (1.50)	7.00 (1.41)	0.461	2.44 (1.50)	2.89 (2.09)	0.157	1.00 (2.51)	1.00 (1.73)	1.000
post - post 60	7.33 (1.50)	6.78 (1.73)	0.336	2.44 (1.50)	2.78 (1.86)	0.518	1.00 (2.51)	1.11 (1.76)	0.655
post 30 - post 60	7.00 (1.41)	6.78 (1.73)	0.157	2.89 (2.09)	2.78 (1.86)	0.564	1.00 (1.73)	1.11 (1.76)	0.655
					2.5 mA				
		Attention			**Fatigue**			**Pain**	
	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value
pre - post	7.89 (1.17)	7.33 (1.32)	0.059	2.22 (1.86)	2.67 (1.94)	0.102	1.00 (1.80)	0.89 (1.54)	0.317
pre - post 30	7.89 (1.17)	7.11 (1.27)	0.034*	2.22 (1.86)	2.56 (1.74)	0.108	1.00 (1.80)	1.00 (1.50)	1.000
pre - post 60	7.89 (1.17)	7.00 (1.22)	0.020*	2.22 (1.86)	2.56 (1.74)	0.257	1.00 (1.80)	1.33 (1.73)	0.108
post - post 30	7.33 (1.32)	7.11 (1.27)	0.480	2.67 (1.94)	2.56 (1.74)	0.564	0.89 (1.54)	1.00 (1.50)	0.317
post - post 60	7.33 (1.32)	7.00 (1.22)	0.317	2.67 (1.94)	2.56 (1.74)	0.655	0.89 (1.54)	1.33 (1.73)	0.102
post 30 - post 60	7.11 (1.27)	7.00 (1.22)	0.317	2.56 (1.74)	2.56 (1.74)	1.00	1.00 (1.50)	1.33 (1.73)	0.180

Post hoc analysis of behavioral characteristics (Wilcoxon Signed Ranks Test) over time, for the young adults. * $p < 0.05$ (significance difference between time points)

Table 9b. Post hoc analysis of behavioral characteristics over time, for the older adults

	Old								
					Sham				
		Attention			**Fatigue**			**Pain**	
	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value
pre - post	8.18 (1.08)	8.09 (1.30)	0.792	2.00 (2.45)	2.91 (2.55)	0.063	1.27 (2.10)	1.72 (2.15)	0.276
pre - post 30	8.18 (1.08)	8.36 (1.03)	0.317	2.00 (2.45)	2.45 (2.50)	0.276	1.27 (2.10)	1.63 (2.06)	0.285
pre - post 60	8.18 (1.08)	8.00 (1.41)	0.480	2.00 (2.45)	2.72 (2.53)	0.131	1.27 (2.10)	1.72 (2.05)	0.197
post - post 30	8.09 (1.30)	8.36 (1.03)	0.317	2.91 (2.55)	2.45 (2.50)	0.414	1.72 (2.15)	1.63 (2.06)	0.317
post - post 60	8.09 (1.30)	8.00 (1.41)	0.792	2.91 (2.55)	2.72 (2.53)	1.000	1.72 (2.15)	1.72 (2.05)	1.000
post 30 - post 60	8.36 (1.03)	8.00 (1.41)	0.046*	2.45 (2.50)	2.72 (2.53)	0.083	1.63 (2.06)	1.72 (2.05)	0.564
					1 mA				
		Attention			**Fatigue**			**Pain**	
	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value
pre - post	8.40 (1.17)	8.40 (1.35)	1.000	1.09 (1.92)	1.60 (0.50)	0.357	0.55 (1.51)	0.70 (1.64)	0.317
pre - post 30	8.40 (1.17)	8.00 (1.63)	0.157	1.09 (1.92)	1.90 (1.97)	0.141	0.55 (1.51)	1.10 (1.73)	0.180
pre - post 60	8.40 (1.17)	7.90 (0.60)	0.197	1.09 (1.92)	2.40 (2.55)	0.102	0.55 (1.51)	1.20 (1.75)	0.083
post - post 30	8.40 (1.35)	8.00 (1.63)	0.194	1.60 (0.50)	1.90 (1.97)	0.083	0.70 (1.64)	1.10 (1.73)	0.285
post - post 60	8.40 (1.35)	7.90 (0.60)	0.257	1.60 (0.50)	2.40 (2.55)	0.066	0.70 (1.64)	1.20 (1.75)	0.102
post 30 - post 60	8.00 (1.63)	7.90 (0.60)	0.655	1.90 (1.97)	2.40 (2.55)	0.059	1.10 (1.73)	1.20 (1.75)	0.655
					2.5 mA				
		Attention			**Fatigue**			**Pain**	
	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value	Mean (SD)	Mean (SD)	*p*-value
pre - post	8.36 (1.29)	8.09 (1.51)	0.257	2.00 (1.73)	2.09 (1.87)	0.915	1.18 (1.78)	1.72 (2.24)	0.157
pre - post 30	8.36 (1.29)	8.18 (1.33)	0.317	2.00 (1.73)	2.09 (1.92)	0.783	1.18 (1.78)	1.45 (2.25)	0.564
pre - post 60	8.36 (1.29)	7.82 (1.66)	0.098	2.00 (1.73)	2.72 (2.57)	0.123	1.18 (1.78)	1.36 (2.06)	0.785
post - post 30	8.09 (1.51)	8.18 (1.33)	0.655	2.09 (1.87)	2.09 (1.92)	1.000	1.72 (2.24)	1.45 (2.25)	0.317
post - post 60	8.09 (1.51)	7.82 (1.66)	0.257	2.09 (1.87)	2.72 (2.57)	0.161	1.72 (2.24)	1.36 (2.06)	0.180
post 30 - post 60	8.18 (1.33)	7.82 (1.66)	0.157	2.09 (1.92)	2.72 (2.57)	0.084	1.45 (2.25)	1.36 (2.06)	0.317

Post hoc analysis of behavioral characteristics (Wilcoxon Signed Ranks Test) over time, for the older adults. * $p < 0.05$ (significance difference between time points)

4. Discussion

The objective of the current study was to evaluate the impact of tDCS intensity on the motor cortex inhibitory processes in healthy elderly. We hypothesized that different current intensities would differentially affect cortical excitability, with higher current intensities leading to less inhibition and thus greater effects on MEP responses. An important finding of this study was that the size of change of intracortical inhibition at ISI of 3 ms was not significant different between the conditions and over time, regardless of age groups, illustrating that current intensities of 1 mA, 2.5 mA or sham stimulation do not differently influence cortical plasticity. Because it is known that different mechanisms of SICI operate at distinct ISIs (Kujirai et al., 1993; Roshan, Paradiso, & Chen, 2003; Vucic, Cheah, Krishnan, Burke, & Kiernan, 2009), which mechanisms may be differently influenced by anodal tDCS, it is difficult to compare our results with studies using other ISIs. Further, we used the same intensity for the test stimulus between 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 (Boros, Poreisz, Munchau, Paulus, & Nitsche, 2008; Bülent Cengiz et al., 2013; Nitsche et al., 2008; Nitsche et al., 2005). It is possible that in our study, like in the research of Ogata et al. (2007), the change 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, Yamasaki, & Tobimatsu, 2007). This methodological difference in intensity of test stimulus makes it complex to compare our results with other studies, as proved, larger test stimuli results in less suppression (Lackmy-Vallee, Giboin, & Marchand-Pauvert, 2012). Nitsche et al. (2005) reported, contrary with our results, enhanced MEP amplitudes after 1 mA anodal tDCS applied for seven minutes at all ISIs tested (2, 3, 5, 10, and 15 ms ISIs). Stimulation of 1 mA anodal tDCS for 13 minutes leads to reduced inhibition, but seems to be more specific as it affected SICI not at all ISIs but merely at ISI of 3 ms (Nitsche et al., 2005). Stimulation of the premotor cortex with 1 mA anodal tDCS for 13 minutes demonstrated comparable results, reduced cortical inhibition, though only at ISIs of 2 ms and 3 ms (Boros et al., 2008). Less is known about the effects of different current intensities, Kidgell et al. (2013) reported a similar decreasing effect of anodal tDCS on intracortical inhibition, at ISI of 3 ms, with intensities of 0.8 mA, 1.0 mA and 1.2 mA (Kidgell et al., 2013). Besides, another recent published study, evaluating the effect of 1 mA and 2 mA anodal tDCS on the hand motor cortex revealed no differences between both intensities, leading to increased SICI at short intervals (1-2 ms) and decreased SICI at longer intervals (4-5 ms) (B. Cengiz, N. Murase, & J. C. Rothwell, 2013).

As mentioned above, there is no effect of tDCS on SICI measured over time. Remarkably, post-hoc analysis over time revealed significant differences for the older adults, indicating more inhibition post 30 and post 60 at the sham condition and less inhibition post 30 at the 2.5 mA condition in comparison with the pre measurement. Attention, fatigue or pain did not significantly differ at these time points, therefore other factors like state-dependent differences (McGinley et al., 2010) or a probable interaction-effect of these behavioral characteristics might play a role.

Another aim of this study was to compare the effects of stimulation on inhibition in young and older

adults. Previous investigations on age-related changes in cortical inhibition have reported inconsistent results (Kossev et al., 2002; Marneweck et al., 2011; McGinley et al., 2010; Oliviero et al., 2006; Peinemann, Lehner, Conrad, & Siebner, 2001; A. E. Smith, Ridding, Higgins, Wittert, & Pitcher, 2009; Stevens-Lapsley, Thomas, Hedgecock, & Kluger, 2013; Wassermann, 2002). Our results (table 3) were in agreement with the study of Oliviero et al. (2006) and Wasserman et al. (2002), where no significant effects of aging on SICI were found (Oliviero et al., 2006; Wassermann, 2002). As well, Smith et al. (2009) reported that age group comparison indicated no age-related change in SICI and in peak inhibition (A. E. Smith et al., 2009). In addition, even corticospinal and intracortical excitability of the lower extremity, more specifically the quadriceps muscle, were similar between older and younger adults (Stevens-Lapsley et al., 2013). However, the main finding of the study of Peinemann et al. (2001) was a substantial age-related net loss in intracortical paired-pulse inhibition. Included participants had no apparent neurologic deficits, so the age-related diminution in intracortical inhibition seems to be related to normal aging (Peinemann et al., 2001). The electrophysiological results of the study of Marneweck et al. (2011) were in line with the results of Peinemann et al. (2001), showing two changes in SICI associated with aging. First, the MEPs were more variable amongst the older adults. Second, the MEPs indicated a decline of SICI with advancing age (Marneweck et al., 2011). Decreased inhibition is probably the result of a compensatory phenomena in order to maintain an appropriate corticospinal motor output (Peinemann et al., 2001) and to keep it in a range that permits normal function in elderly (Oliviero et al., 2006). Completely opposite to the results of the aforementioned studies, Kossev et al. (2002) reported a significant increase of intracortical inhibition in middle-aged subjects. This is confirmed by the study of McGinley et al. (2010), indicating that advancing age leads to an increased SICI under resting conditions, which suggests increased GABAergic mediated inhibition with age (McGinley et al., 2010). 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, Peinemann et al. (2006) used a biphasic waveform (Peinemann et al., 2001), whilst this present study and the before mentioned studies used a monophasic stimulus (Kossev et al., 2002; Marneweck et al., 2011; McGinley et al., 2010; Oliviero et al., 2006; A. E. Smith et al., 2009; Stevens-Lapsley et al., 2013; Wassermann, 2002). Second, the ISIs studied were different. While this experiment is conducted with an ISIs of 3 ms like the studies of Kossev et al. (2002), McGinley et al. (2010) and Stevens-Lapsley et al. (2013) (Kossev et al., 2002; McGinley et al., 2010; Stevens-Lapsley et al., 2013), used the other studies an ISIs ranging from 1 ms up to 5 ms to explore SICI (Marneweck et al., 2011; Oliviero et al., 2006; Peinemann et al., 2001; A. E. Smith et al., 2009; Wassermann, 2002). Third, there is a large difference between the studies in age and gender of the older participants. Average age of the included participants in the current study was 69.64 year, whereas the participants in the studies of Peinemann et al. (2001) and Kossev et al. (2002) were respectively around 51 and 56 years old (Kossev et al., 2002; Peinemann et al., 2001). Besides, like in the study of Smith et al. (2009), only male participants were included (A. E. Smith et al., 2009), because it is proved that female hormones play an important role in the human brain and cause more variability between the MEPs (M. J. Smith et al., 1999). Though, also female participants were used in the other studies, which may have influenced the sizes of the MEPs and leads to

different results in inhibition (Kossev et al., 2002; Marneweck et al., 2011; McGinley et al., 2010; Oliviero et al., 2006; Peinemann et al., 2001; Stevens-Lapsley et al., 2013). Fourth, there is an inequality between the studies in how the conditioning stimulus intensity was set, relative to resting motor threshold (Marneweck et al., 2011; McGinley et al., 2010; Stevens-Lapsley et al., 2013), like in this present study, or relative to active motor threshold (Oliviero et al., 2006; Peinemann et al., 2001; A. E. Smith et al., 2009; Wassermann, 2002). Fifth, the coil used to deliver the magnetic stimulation on the cortex altered between the studies, varying between a circular coil (Kossev et al., 2002; Wassermann, 2002) and a figure-of-eight coil (Marneweck et al., 2011; McGinley et al., 2010; Oliviero et al., 2006; Peinemann et al., 2001; A. E. Smith et al., 2009) used in this study. The choice for a type of coil can possibly be a source for an experimental error. The hand-held figure-of-eight coils contact 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 figure of eight coil is more vulnerable for experimental error, therefore we used a neuronavigation system to ensure similar coil position during the whole experimental session. Finally, there is dissimilarity between the muscles studied, exploring more distal hand muscles such the first dorsal interosseus (FDI) (Marneweck et al., 2011; Oliviero et al., 2006; Peinemann et al., 2001; A. E. Smith et al., 2009) vs. proximal hand muscles, extensor carpi radialis (ECR) (Kossev et al., 2002) or flexor carpi radialis (FCR) (Kossev et al., 2002; McGinley et al., 2010), vs. lower extremity muscles like the quadriceps (Stevens-Lapsley et al., 2013). 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 (Horvath, Carter, & Forte, 2014; Wassermann, 2002) 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). In fact it is shown that stages of the menstrual cycles will differentially impact response to TMS protocols (M. J. Smith et al., 1999). In addition, within the circadian cycle there is a specific change of GABA-ergic inhibition in the motor cortex (Lang et al., 2011). Even we made an effort to control for factors that influence intracortical excitability, there were differences in the conditioning stimulus in the young adults and the QOS in the older adults between the conditions (table 6). As well, there were significant differences between the age groups in the sham condition for QOS, alcohol use and attention 30 minutes after stimulation, and coffee use in the 1 mA condition (table 7). Also between time points there are significant differences in attention for both young and older adults (table 8 and 9). Any of these factor differences may have confounded the results. Another limitation of this study is the small sample size, which may have resulted in insufficient power to detect changes in SICI. However, past studies found significant effects with small samples of about 11 – 20 participants (Boros et al., 2008; B. Cengiz et al., 2013; 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 results. Therefore it is necessarily to develop one research protocol to study the influence of tDCS on SICI.

5. Conclusion

In this study we examined the effects of different current intensities on SICI in young and older adults. There is no difference in SICI between the conditions and over time. Only 2.5 mA anodal tDCS is able to reduce inhibition 30 minutes after stimulation in the older adults. Another point of interest was the differences in inhibition between the age groups. Analysis of these results showed no difference between young and older adults, indicating no age-related change in SICI. Number of protocols used to study SICI is overwhelming, this raises the necessity for a standardized protocol to study SICI.

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