Brain Stimulation 13 (2020) 1588–1599

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

Age-related differences of motor cortex plasticity in adults: A transcranial direct current stimulation study

Ensiyeh Ghasemian-Shirvan [a, b], Leila Farnad [a, c], Mohsen Mosayebi-Samani [a, d], Stefanie Verstraelen [e], Raf L.J. Meesen [e, f], Min-Fang Kuo [a], Michael A. Nitsche [a, g, *]

[a] Department of Psychology and Neurosciences, Leibniz Research Center for Working Environment and Human Factors, Dortmund, Germany
[b] International Graduate School of Neuroscience, Ruhr-University Bochum, Bochum, Germany
[c] Institute of Cognitive Neuroscience, Ruhr-University Bochum, Bochum, Germany
[d] Institute of Biomedical Engineering and Informatics, Ilmenau University of Technology, Ilmenau, Germany
[e] Neuroplasticity and Movement Control Research Group, Rehabilitation Research Institute (REVAL), Hasselt University, Diepenbeek, Belgium
[f] Movement Control and Neuroplasticity Research Group, Department of Movement Sciences, Group Biomedical Sciences, KU Leuven, Belgium
[g] Department of Neurology, University Medical Hospital Bergmannsheil, Ruhr-University Bochum, Bochum, Germany

ARTICLE INFO

Article history:
Received 23 May 2020
Received in revised form
21 August 2020
Accepted 9 September 2020
Available online 17 September 2020

Keywords:
Aging
Neuroplasticity
tDCS
MEP
TMS

ABSTRACT

Background: Cognitive, and motor performance are reduced in aging, especially with respect to acquisition of new knowledge, which is associated with a neural plasticity decline. Animal models show a reduction of long-term potentiation, but not long-term depression, in higher age. Findings in humans are more heterogeneous, with some studies showing respective deficits, but others not, or mixed results, for plasticity induced by non-invasive brain stimulation. One reason for these heterogeneous results might be the inclusion of different age ranges in these studies. In addition, a systematic detailed comparison of the age-dependency of neural plasticity in humans is lacking so far.
Objective: We aimed to explore age-dependent plasticity alterations in adults systematically by discerning between younger and older participants in our study.
Methods: We recruited three different age groups (Young: 18–30, Pre-Elderly: 50–65, and Elderly: 66–80 years). Anodal, cathodal, or sham transcranial direct current stimulation (tDCS) was applied over the primary motor cortex with 1 mA for 15 min to induce neuroplasticity. Cortical excitability was monitored by single-pulse transcranial magnetic stimulation as an index of plasticity.
Results: For anodal tDCS, the results show a significant excitability enhancement, as compared to sham stimulation, for both, Young and the Pre-Elderly groups, while no LTP-like plasticity was obtained in the Elderly group by the applied stimulation protocol. Cathodal tDCS induced significant excitability-diminishing plasticity in all age groups.
Conclusion: Our study provides further insight in age-related differences of plasticity in healthy humans, which are similar to those obtained in animal models. The decline of LTP-like plasticity in higher age could contribute to cognitive deficits observed in aging.

1. Introduction

As health care strategies have improved during the last century, life expectancy has grown accordingly. However, advanced age also leads to physical and cognitive decline, which gradually constrain daily activities and independent living in the elderly population [1]. One relevant underlying mechanism is presumed to be altered plasticity, which refers to a structural and functional alteration of the strength of synaptic connections in response to environmental or internal demands, due to age-related changes in synaptic function and neurotransmission [2].

A respective age-related decline of plasticity has been described in animals, with an increase of the synaptic threshold for the induction of long-term potentiation (LTP), and an increased probability for the induction of long-term depression (LTD) [3], which might partially be caused by the reduction of spine density, number

* Corresponding author. Department of Psychology and Neurosciences, Leibniz Research Center for Working Environment and Human Factors, Dortmund, Germany.
E-mail address: nitsche@ifado.de (M.A. Nitsche).

https://doi.org/10.1016/j.brs.2020.09.004

of synapses [4,5], reduction of receptor density, functionality [6] and reduced amount of available neurotransmitters [7,8]. In adult humans, similar plasticity alterations have been described, with a progressive decline of plasticity throughout the lifespan [9]. Furthermore, the respective plasticity alterations in humans might be caused by mechanisms similar to those revealed in animal models, including reduction of synaptic connections [5], number of neurons [10], volume of cortical grey matter [11,12], deterioration of white matter fibres [13,14], decrease of available neuromodulators [15] and neurotransmitters [16], which results in decline of motor and cognitive functions [13,17,18]. The targeted modulation of age-related plasticity decline might therefore be suited to improve respective motor and/or cognitive processes, moreover, it might improve rehabilitation results in old patients with neurological diseases.

Age-related cortical plasticity and/or excitability alterations in humans have already been investigated in several studies. However, the results are heterogeneous so far. For plasticity, repetitive transcranial magnetic stimulation (TMS)-induced plasticity has been shown to be affected by age. The effects of LTP-like plasticity-inducing PAS over the motor cortex have been shown to be decreased [19] by age. The latter effect was however only found in older women, but not men, in another study [20], and contrary results were reported for the model of the primary somatosensory cortex, where an enhancement of PAS-induced LTP-like plasticity effects was revealed in older adults, as compared to a younger age group [21]. For intermittent TBS (iTBS) over the primary motor cortex, an enhancement of MEP amplitudes was reported in older adults in one study [22], while no age-related impact on iTBS-induced MEP alterations was found in another study [23]. Likewise, the neuroplastic effects of LTD-like plasticity-inducing continuous theta burst stimulation (cTBS), 1 Hz, and 6 Hz repetitive TMS (rTMS), and paired associative stimulation (PAS) protocols have been shown to be diminished, or abolished for the model of the motor cortex, in elderly in a couple of studies [24–27], whereas, in another study unimpaired LTD-like plasticity was reported following cTBS in old participants [28]. This heterogeneity of the effects of TMS-related plasticity induction protocols might be due in part to the altered levels of intra-cortical inhibition and facilitation in older adults compared with young populations. However, these findings are also inconsistent. Intra-cortical facilitation (ICF), which is associated with glutamatergic NMDA receptor-dependent activity, has been found to be decreased [29] or unaltered [30,31] in older adults, as compared to young healthy controls. Short interval intra-cortical inhibition (SICI), which is related to GABAergic plasticity mechanisms, has been shown to be increased [29], decreased [30,32] or remained unchanged [33–35] in higher age, as compared with young populations.

Transcranial direct current stimulation (tDCS) is another non-invasive brain stimulation (NIBS) technique, which induces LTP- and LTD-like plasticity in a polarity-dependent way, via application of weak direct electrical currents through the scalp. For the primary motor cortex, but also other areas, anodal tDCS, which refers to surface inward current over the target area, results in enhancement of cortical excitability, whereas cathodal tDCS, which refers to outward current over the target area, reduces it at the macroscopic level, with standard stimulation protocols [36,37]. Respective after-effects can last for about 1 h or longer [37,38]. Pharmacological and neuroimaging studies revealed that tDCS induces calcium-dependent plasticity of glutamatergic synapses and that NMDA receptors are crucially involved in these effects [39–42]. Moreover, GABAergic activity is reduced by both, anodal and cathodal tDCS [41]. Beyond these regional effects, tDCS has also been shown to alter functional connectivity, as explored by electroencephalography, and functional neuroimaging techniques [43,44], which might be relevant for the impact of tDCS on cognitive processes.

Similar to the TMS-related neuroplasticity induction protocols, tDCS-induced plasticity has been shown to be affected by age. In one study, young and elderly adults received 1 mA anodal tDCS for 30min over the left M1. The results, for the elderly group, indicated a 30 min delay of LTP-like plasticity, in comparison with the young group [45]. Another study explored the neuromodulatory effects of anodal tDCS with 1 mA for 20min over the primary motor cortex with respect to GABA activity, which has been suggested to contribute to age-related motor and cognitive functional decline, in a sample of old and young participants via SICI. While inhibition decreased in young participants, a reversal of effects was found in the older ones [46].

While the above-mentioned preliminary studies conducted with different NIBS techniques indicate an impact of age on neuroplasticity in humans, nevertheless the specific results are partially conflicting, and systematic studies are rare. This might be partially due to stimulation protocol differences, but also related to the definition of young, and old age groups. In the present study we aimed to assess age-related differences of motor cortex plasticity induced by tDCS with standard protocols, which have been shown to induce LTP-and LTD-like plasticity in young adults lasting for about one hour [47,48], and compared one young control group with two groups of older adults. Sixty participants, divided into 3 groups of Young (18–30 years), Pre-Elderly (50–65 years) and Elderly (66–80 years), were included. These age groups were selected based on the assumed time course of alteration of plasticity mechanisms in advanced age [9]. Each group received 1 mA anodal, cathodal, and sham tDCS for 15min in different sessions. Due to the heterogeneous findings of studies in humans, which explored the impact of age on NIBS outcomes, definitive hypotheses about the age-dependent effects of tDCS on plasticity could not be derived. However, based on previous findings, we anticipated an age-dependent decline of tDCS-induced plasticity effects. In addition, as outlined in previous studies [49,50], tDCS is subject to a relevant inter-individual variability, similar to other NIBS modes [51,52]. We thus investigated the amount of inter-individual variability for each age group, to identify the effects of this factor on the neurophysiological outcomes of tDCS.

2. Materials and methods

2.1. Participants

To the best of our knowledge, no studies with regard to age-dependently altered tDCS-induced plasticity in the human motor cortex were available for estimation of effect size, which took into account the 3 different age groups included in our study. We conducted therefore a Power analysis (G*Power 3.1), based on a medium effect size (0.25), and critical alpha and β-errors of 0.05, which resulted in a sample size of 18. We added 2 participants per group to compensate for dropouts, and unforeseen variability. Therefore, sixty healthy, non-smoking participants were recruited and divided into three different age groups of young (20 participants between 18 and 30 years, 9 females, mean age 25.95 ± 3.37), Pre-Elderly (20 participants between 50 and 65 years, 9 females, mean age 58.6 ± 5.07), and Elderly (20 participants between 65 and 80 years, 9 females, mean age 74.45 ± 4.61). All participants were right-handed according to the Edinburgh handedness inventory [53]. Prior to participation, volunteers were clinically screened by a certified neurologist for neurological, and cognitive performance, a history of neurological and psychiatric diseases, and absence of exclusion criteria for non-invasive electrical and magnetic brain

stimulation [54,55]. Central nervous system-acting medication or respective recreational substances served also as exclusion criteria. This study was approved by the local ethics committee of IfADo, and is in accordance with the Declaration of Helsinki. All participants gave written informed consent before starting the experiment.

2.2. Motor cortical excitability monitoring

For monitoring of motor cortex excitability, single-pulse biphasic TMS at a frequency of 0.25 Hz with a 10% jitter was applied by a PowerMag magnetic stimulator (Mag&More, Munich, Germany) with a figure-of-eight-shaped coil (diameter of one winding 70 mm; peak magnetic field, 2T), to induce anterior-posterior to posterior-anterior current flow in the brain. The coil was held tangentially to the left side of the skull and the handle was held 45° from midline pointing backward. Surface motor evoked potentials (MEPs) were recorded from the right ADM muscle, in a belly-tendon montage, through a pair of gold cup electrodes. The signals were amplified and filtered (1000; 3 Hz- 3 KHz) by a D440-2 (Digitimer, Welwyn Garden City, UK), and were digitized (sampling rate, 5 kHz) with a micro 1401 AD converter (Cambridge Electronic Design, Cambridge, UK), controlled by Signal Software (Cambridge Electronic Design, v. 2.13). A waterproof pen was used to mark the position of the TMS coil on the head to guarantee its constant position throughout the experiment.

2.3. Transcranial direct current stimulation (tDCS)

TDCS was delivered by a battery-powered constant current stimulator (neuroCare, Ilmenau, Germany) using two saline-soaked surface sponge electrodes (5 × 7cm, 35 cm^2) placed on the scalp. One electrode was fixed over the motor cortex representational area of the right abductor digiti minimi muscle (ADM), as identified by TMS, and the other electrode contralaterally over the supraorbital area [36,37]. The participants received anodal and cathodal tDCS of 1 mA intensity for 15min with 10sec ramping up and down at the beginning and the end of stimulation, respectively. For sham stimulation, 1 mA was delivered for 30 s, with a10sec ramp up and down followed by 15 min stimulation with 0.0 mA.

2.4. Experimental procedure

The study was performed in a cross-over, single-blinded, randomized design. Each session approximately started at either 10am or 2pm, based on preferences of the participants, and this start time was then maintained constant for each participant throughout the entire experiment. In each session, participants were seated first in a comfortable and adjustable chair with head- and armrests. Then, a single pulse TMS with medium intensity was applied over the left motor cortex to identify the representational area of the right ADM, in which the largest MEPs were produced (hot-spot determination). TMS intensity was adjusted to elicit peak-to-peak MEP amplitudes of on average 1 mV (SI1mV), and baseline cortical excitability was then determined by recording 30 MEPs. Prior to tDCS, to reduce somatosensory perception of the stimulation, and improve the level of blinding, a topical anaesthetic cream (EMLA, 2.5% lidocaine+2.5% prilocaine) was applied over the stimulation site [56,57]. Afterwards, tDCS electrodes were placed over the left M1 and the supra-orbital area and anodal, cathodal or sham tDCS, in random order between sessions, was applied. Immediately after tDCS, electrodes were removed, and 30 MEPs were provoked every five minutes for up to 30 min, and at the time-points of 60, 90 and 120 min after the intervention (Fig. 1). At the end of the experiment, participants were asked about side effects during, and 24 h after tDCS with a respective post stimulation questionnaire [58,59]. To avoid carry-over effects, tDCS protocols were applied with a minimum one-week interval between each session [39].

2.5. Data analysis and statistics

MEP amplitudes were first visually inspected offline to exclude those with muscle activity prior to the TMS pulse (time window including 100 ms before the TMS pulse artifact; see supplementary material, Table 1, for mean numbers of included MEP). In each session, for each post-stimulation time-point, the individual means of MEP amplitudes were calculated and then normalized to the baseline MEP amplitudes (quotient of post-intervention versus pre-intervention MEP amplitudes).

2.5.1. Testing the equivalence of 'SI_{1mV}' and 'baseline MEP' between groups, and sessions

To exclude that baseline measures differed between sessions, two separate mixed model ANOVAs were calculated with 'session' (3 levels) as within-subject factor, 'SI_{1mV}' or 'baseline MEP' as dependent variables, and 'age groups' as between-subject factor.

2.5.2. Effect of age on tDCS-induced neuroplasticity

To determine if age affected the tDCS-induced neuroplastic after-effects, a mixed model ANOVA was conducted with normalized post-stimulation MEP amplitudes as dependent variable, 'condition' (3 levels) and 'time-points' (10 levels) as within-subject factors, and 'age group' (3 levels) as between subject factor. In addition, to exclude a difference of sham tDCS effects between age groups, a mixed model ANOVA was conducted with 'time-points' (10 levels) as within-subject factor, and 'age group' as between-subject factor. Furthermore, to test if the post-stimulation MEPs changed compared to baseline, one-sample t-tests were conducted between each post-stimulation time-point and baseline.

2.5.3. Effect of age on early and late tDCS after-effects

To better define the time course of plasticity induced by tDCS and compensate for variability between single time-points, the normalized post-stimulation MEP amplitudes of all time-points were grand-averaged and pooled into two epochs: first 30min after stimulation (early epoch), and 60–120min after stimulation (late epoch). A mixed model ANOVA was calculated with 'condition' (3 levels) and 'epoch' (2 levels) as within-subject factors, normalized post-stimulation MEPs as dependent variable and age as between subject factor. In addition, to exclude differences of sham tDCS effects between age groups, a mixed model ANOVA was conducted with 'epoch' (2 levels) as within-subject factor, normalized post-stimulation MEPs as dependent variable and 'age group' as between-subject factor. Moreover, post hoc one-sample t-tests were conducted, to evaluate tDCS-altered MEP changes compared to baseline.

2.5.4. Inter-individual variability

To investigate the amount of variability in each age group, based on the normalized grand average (GA) of the MEP obtained in the first 30 min post-stimulation, participants were assigned to four groups, of 1) Anode-Responder-Cathode-Responder (ARCR), 2) Anode-Responder-Cathode-Non-Responder (ARCN), 3) Anode-Non-Responder-Cathode-Responder (ANCR), and 4) Anode-Non-responder-Cathode-Non-responder (ANCN), which were defined according to their individual response to anodal and cathodal tDCS (>1, facilitation; <1, inhibition) [49]. In addition, Pearson correlation coefficients were calculated, to test if responders to one polarity were also likely to respond to the other tDCS polarity.

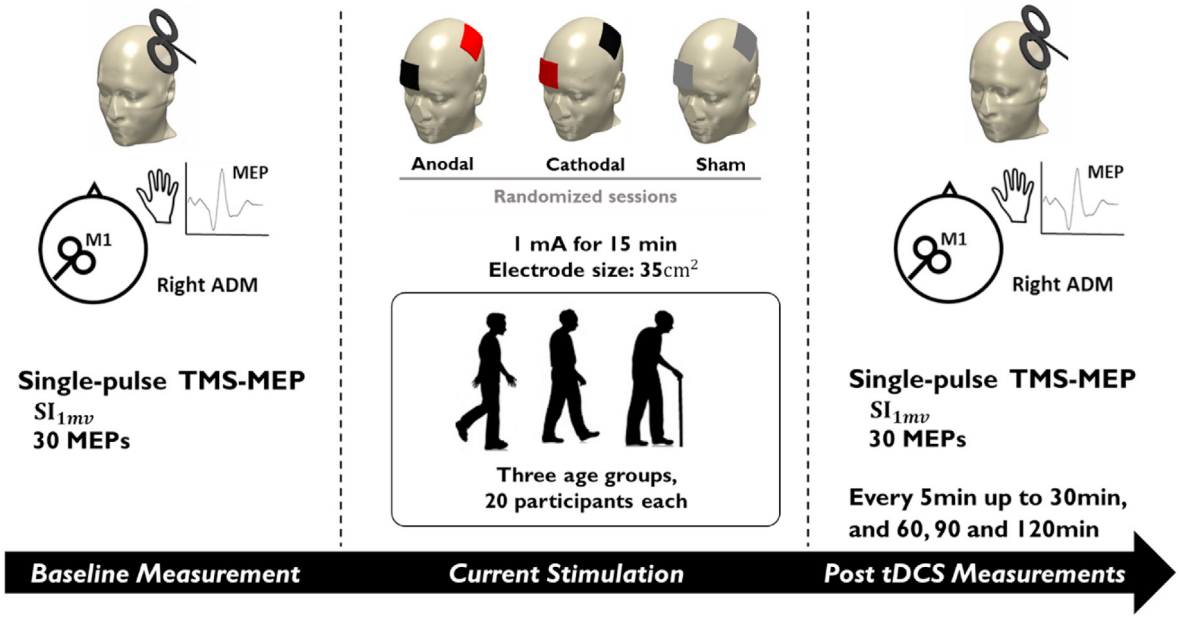

Fig. 1. Experimental procedure. Single-pulse TMS was conducted at a frequency of 0.25 Hz to the left motor cortex. First, the representational area of the right ADM, in which the largest MEPs were produced, was identified. The intensity of the TMS pulses was then adjusted to elicit MEPs with a peak-to-peak amplitude of on average 1 mV (SI1mV). Finally, baseline cortical excitability was determined by measuring 30 MEPs. Afterwards, 15min of anodal, cathodal or sham was applied in random order. The after-effects were then monitored with TMS-induced MEPs (each time-point 30 MEPs) every 5 min for up to 30 min and 60, 90 and 120 min after stimulation.

2.5.5. Assessment of tDCS side-effects, and blinding

In each session, the subjects filled in a questionnaire which contained: 1. Guessed intensity of applied direct current (0, 1 mAik), 2. Rating scales for the presence and intensity of visual phenomena, itching, tingling and pain during stimulation, and 3. Rating scales for the presence and intensity of skin redness, headache, fatigue, concentration difficulties, nervousness and sleep problems within 24 h after stimulation. The side-effects were rated on a numerical scale from zero to five, zero representing no and five extremely strong sensations. A Chi-square test was used to evaluate if the participants could correctly guess the respective stimulation conditions. The presence of each side-effect, during and after tDCS, was analyzed by a mixed model ANOVA with 'condition' (3 levels) as within-subject factor, 'age group' as between subject factor, and rating scores (0–5) as dependent variable.

For the ANOVAs, Mauchly's test of sphericity was conducted, and the Greenhouse-Geisser correction was applied when necessary. The critical significance level was set at $P \leq 0.05$. In case of significant results of the ANOVAs, post-hoc Students t-tests were conducted, which were Bonferroni-corrected for multiple comparisons. In addition, one-sample t-tests were used to compare post-stimulation with baseline MEP amplitudes, which were also Bonferroni-corrected for multiple comparisons. Statistical analyses were performed with SPSS (IBM Corp. Version 26.0).

3. Results

All participants completed the entire study.

3.1. Equivalence of SI_{1mV} and baseline MEP

Baseline MEP and SI_{1mv} are displayed in Table 1. The ANOVA results showed no significant differences of SI_{1mv} and baseline MEPs and their interactions with 'age group' across sessions. For SI_{1mV}, neither the main effects 'condition' ($F_{(1.785,\ 101.768)} = 1.661$, $p = 0.198$, $\eta_p^2 = 0.028$), and 'age group' ($F_{(2,\ 57)} = 0.452$, $p = 0.638$, $\eta_p^2 = 0.016$), nor their interaction ($F_{(3.571,\ 101.768)} = 1.631$, $p = 0.178$, $\eta_p^2 = 0.054$) were significant. Similarly, for baseline MEPs, the main effects of 'condition' ($F_{(2,114)} = 0.054$, $p = 0.947$, $\eta_p^2 = 0.001$), 'age group' ($F_{(2,57)} = 1.700$, $p = 0.192$, $\eta_p^2 = 0.056$), and their interaction ($F_{(4,\ 114)} = 2.432$, $p = 0.051$, $\eta_p^2 = 0.079$) showed no significances.

Table 1
Baseline MEP values and TMS stimulation intensities: Data are presented as mean ± SD; SI_{1mV} refers to the percentage of maximal stimulator output (%MSO) which was required for generating ~1 mV MEP. The results of the ANOVAs show no significant differences of baseline MEP and SI_{1mV} across sessions, and between age groups.

Experimental group	Experimental session	SI_{1mV} (%)	Baseline MEP (mV)
Young	**Anodal**	57.45 ± 13.53	0.98 ± 0.08
	Cathode	56.10 ± 14.21	1.01 ± 0.09
	Sham	56.72 ± 13.39	1.04 ± 0.09
Pre-Elderly	**Anode**	60.65 ± 12.79	1.04 ± 0.10
	Cathode	60.62 ± 12.52	1.04 ± 0.10
	Sham	60.87 ± 12.54	1.05 ± 0.10
Elderly	**Anode**	58.50 ± 13.20	1.09 ± 0.09
	Cathode	58.50 ± 13.43	1.05 ± 0.11
	Sham	58.47 ± 13.46	1.1 ± 0.12

3.2. Effect of age on tDCS-induced neuroplasticity, detailed time course

The results of the ANOVA revealed significant main effects of 'condition' ($F_{(2,114)} = 88.865$, $p < 0.001$, $\eta_p^2 = 0.609$), 'time-points' ($F_{(5.852,333.587)} = 3.260$, $p < 0.004$, $\eta_p^2 = 0.054$), and 'age group' ($F_{(2,57)} = 5.788$, $p < 0.005$, $\eta_p^2 = 0.169$), and significant interactions between 'condition' × 'time-points' ($F_{(22.286,643.302)} = 6.103$, $p < 0.001$, $\eta_p^2 = 0.0.097$), 'condition' × 'age group' ($F_{(4,114)} = 15.961$, $p < 0.001$, $\eta_p^2 = 0.359$), and 'condition' × 'time-points' × 'age group' ($F_{(36,1026)} = 2.250$, $p < 0.001$, $\eta_p^2 = 0.73$), but no significant interaction of 'time-points' × 'age group' ($F_{(11.705,333.587)} = 1.427$, $p = 0.153$, $\eta_p^2 = 0.048$), Fig. 2, and Table 2A. The additional repeated measures ANOVA conducted for sham stimulation resulted in no significances for the main effects of 'time-points' ($F_{(6.774,386.124)} = 1.442$, $p = 0.189$, $\eta_p^2 = 0.025$), and 'age group' ($F_{(2,57)} = 1.285$, $p = 0.284$, $\eta_p^2 = 0.043$), and the interaction 'time-points' × 'age group' ($F_{(13.548,386.124)} = 0.965$, $p = 0.487$, $\eta_p^2 = 0.033$), Fig. 2, and Table 2B.

Post-hoc tests comparing sham tDCS with the anodal stimulation protocols revealed a significant increase of cortico-spinal excitability lasting for about 1.5 h after stimulation in the young and about 2 h (but not for all time-points) in Pre-Elderly groups, but no significant excitability alteration in the Elderly group. Furthermore, post-hoc tests comparing MEP alterations between different age groups for anodal tDCS revealed a significant reduction of after-effects for the Pre-Elderly group (for about 15min after stimulation) and the Elderly group (for about 2 h after stimulation), in

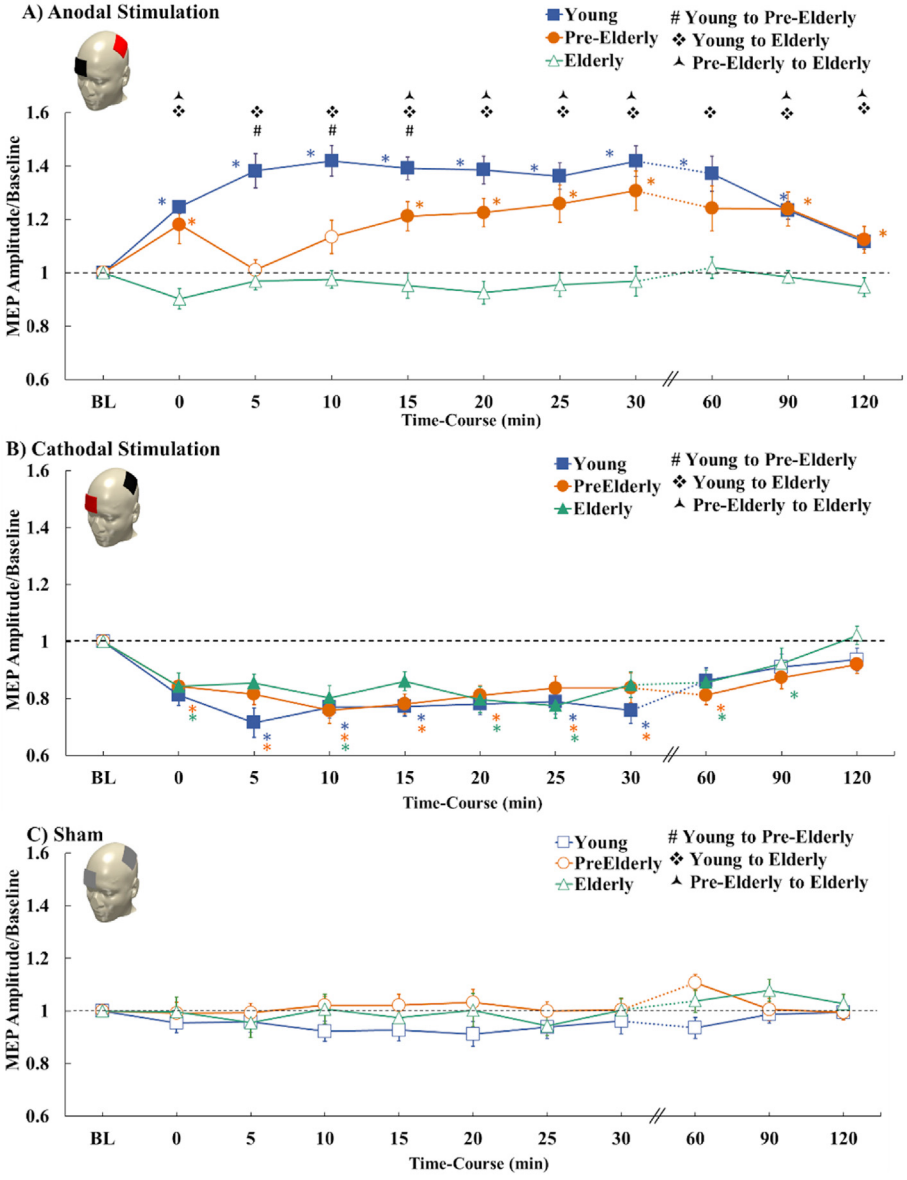

Fig. 2. Impact of age on post-tDCS motor cortical excitability alterations, detailed time course: A). Anodal stimulation, B) Cathodal stimulation and C) Sham condition. Error bars represent standard error of mean (SEM). Filled symbols indicate a significant difference of cortical excitability after tDCS, as compared to the respective baseline values. Asterisks indicate a significant difference between the active and respective sham stimulation conditions. The symbols at the top line represent the results of one-to-one time-point comparisons between age groups for single time-points. The critical significance level was set at P < =0.05. Post hoc t-tests were Bonferroni-corrected for multiple comparisons. BL = baseline.

Table 2
Results of the ANOVAs for the tDCS-induced neuroplastic after-effects. A) The mixed model ANOVA performed to test for the impact of age on the tDCS-generated motor cortical excitability alterations revealed significant main effects of 'condition', 'time-points', and 'age group', as well as significant interactions between these factors with the exception of 'time-points' × 'age group'. B) The mixed model ANOVA conducted to exclude differences of sham tDCS results between the respective age groups indicated no significances. C) The mixed model ANOVA conducted for the grand-averaged pooled MEPs revealed significant main effects of 'condition', and 'age group', but not 'epoch', and respective interactions between these factors with the exception of 'epoch' × 'age group'. D) The mixed model ANOVA conducted for the grand-averaged pooled MEPs, to exclude differences of sham tDCS results between the respective age groups indicated no significances. Asterisks indicate significant results. d.f. = degrees of freedom, η_p^2 = partial eta squared.

		Factor	d.f., Error	F value	η_p^2	p value
A	**Effect of age on tDCS-induced neuroplasticity**	Condition	2, 114	88.865	0.609	<0.001*
		Time-points	5.852, 333.587	3.260	0.054	0.004*
		Age group	2, 57	5.788	0.169	0.005*
		Condition × age group	4, 114	15.961	0.359	<0.001*
		Condition × time-points	11.286, 643.302	6.103	0.097	<0.001*
		Time-points × age group	11.705, 333.587	1.427	0.048	0.153
		Condition × time-points × age group	36, 1026	2.250	0.73	<0.001*
B	**Sham session**	Time-points	6.774, 386.124	1.442	0.025	0.189
	Overall	Age group	2, 57	1.285	0.043	0.284
		Time-points × age group	13.548, 386.124	0.965	0.033	0.487
C	**Effects of age on Early and Late after effects of tDCS (Pooled MEPs)**	Condition	2, 114	57.487	0.502	<0.001*
		Epoch	1, 57	0.011	0.001	0.915
		Age group	2, 57	7.055	0.198	0.002*
		Condition × epoch	2, 114	17.681	0.237	<0.001*
		Condition × age group	4, 114	17.946	0.386	<0.001*
		Epoch × age group	2, 57	1.657	0.055	0.200
		Condition × epoch × age group	4, 114	3.382	0.106	0.012*
D	**Sham session (Pooled MEPs)**	Epoch	1, 57	2.469	0.042	0.122
		Age group	2, 57	2.017	0.066	0.142
		Epoch × age group	2, 57	0.010	0.001	0.990

comparison with the young group. Similarly, a significant difference was found between the Pre-Elderly, and Elderly groups, with lower MEP amplitudes of the Elderly group, Fig. 2A. The one-sample t-tests comparing anodal stimulation after-effects to respective baseline cortical excitability revealed a significant enhancement of cortical excitability lasting for about 2 h after stimulation in the young and Pre-Elderly (with about 10 min delay) groups, but no significant excitability alteration for the Elderly group, Fig. 2A.

In addition, post-hoc tests comparing sham tDCS with the cathodal stimulation protocols revealed a significant decrease of cortico-spinal excitability for about 30 min after stimulation in the young, for about 1 h in the Pre-Elderly, and for 1.5 h (but not for all time-points) in the Elderly group. Furthermore, the results of the post-hoc tests for cathodal tDCS revealed no significant differences of MEP alterations between different age groups, Fig. 2B. The one-sample t-tests comparing cathodal stimulation after-effects to respective baseline cortical excitability revealed a significant decrease of cortical excitability in the young (for 1 h after stimulation), Pre-Elderly (for 2 h after stimulation), and Elderly (for 1 h after stimulation) groups, Fig. 2B.

3.3. Effect of age on early and late tDCS effects, epoched data

To compensate for the intrinsic variability of MEP amplitudes, we pooled the respective single time bin results into two epochs of early (time-points 0–30 min) and late (60–120 min) effects. The ANOVA results revealed significant main effects of 'condition' ($F_{(2,114)} = 57.487$, $p < 0.001$, $\eta_p^2 = 0.502$), and 'age group' ($F_{(2,57)} = 7.055$, $p = 0.002$, $\eta_p^2 = 0.198$), and significant interactions of 'condition' × 'epoch' ($F_{(2,114)} = 17.681$, $p < 0.001$, $\eta_p^2 = 0.237$), 'condition' × 'age group' ($F_{(4,114)} = 17.946$, $p < 0.001$, $\eta_p^2 = 0.386$), and 'condition' × 'epoch' × 'age group' ($F_{(4,114)} = 3.382$, $p = 0.012$, $\eta_p^2 = 0.106$), but no significant effects of 'epoch' ($F_{(1,57)} = 0.011$, $p = 0.915$, $\eta_p^2 = 0.001$), and 'epoch' × 'age group'($F_{(2,57)} = 1.657$, $p = 0.200$, $\eta_p^2 = 0.055$), Fig. 3, Table 2C. The mixed model ANOVA conducted to exclude differences between sham tDCS over the different age groups revealed no significant main effects of 'epoch' ($F_{(1,57)} = 2.469$, $p = 0.122$, $\eta_p^2 = 0.042$), and 'age group' ($F_{(2,57)} = 2.017$, $p = 0.142$, $\eta_p^2 = 0.066$), and no significant 'epoch' × 'age group' interaction ($F_{(2,57)} = 0.010$, $p = 0.990$, $\eta_p^2 = 0.001$), Fig. 3C, Table 2D.

Post-hoc tests comparing sham tDCS with the anodal stimulation protocols revealed a significant increase of cortico-spinal excitability for about 2 h (both early and late epochs) after stimulation in young and Pre-Elderly populations, but no significant differences in the Elderly group. Furthermore, post-hoc tests comparing MEP alterations between the different age groups for anodal tDCS revealed a significant reduction of after-effects for the Pre-Elderly group (for the early epoch) and the Elderly group (for the early and late epochs) in comparison with the young group. Similarly, a significant difference, in both the early and late epochs, was observed between the Pre-Elderly and Elderly groups. In addition, the one-sample t-tests comparing anodal stimulation after-effects with respective baseline cortical excitability measures revealed a significant enhancement of cortical excitability for the early and late epochs in the young and Pre-Elderly groups, but no significant effects for the Elderly group, Fig. 3.

Post-hoc tests comparing sham tDCS with the cathodal stimulation protocols revealed a significant decrease of cortico-spinal excitability for about 30min (early epoch) after stimulation in the young, and for about 2 h (for both, early and late epochs) in the Pre-Elderly and Elderly groups. Furthermore, post-hoc tests conducted for cathodal tDCS effects revealed no significant differences of MEP alterations between the different age groups. The one-sample t-tests comparing cathodal stimulation after-effects with the respective baseline cortical excitability revealed a significant

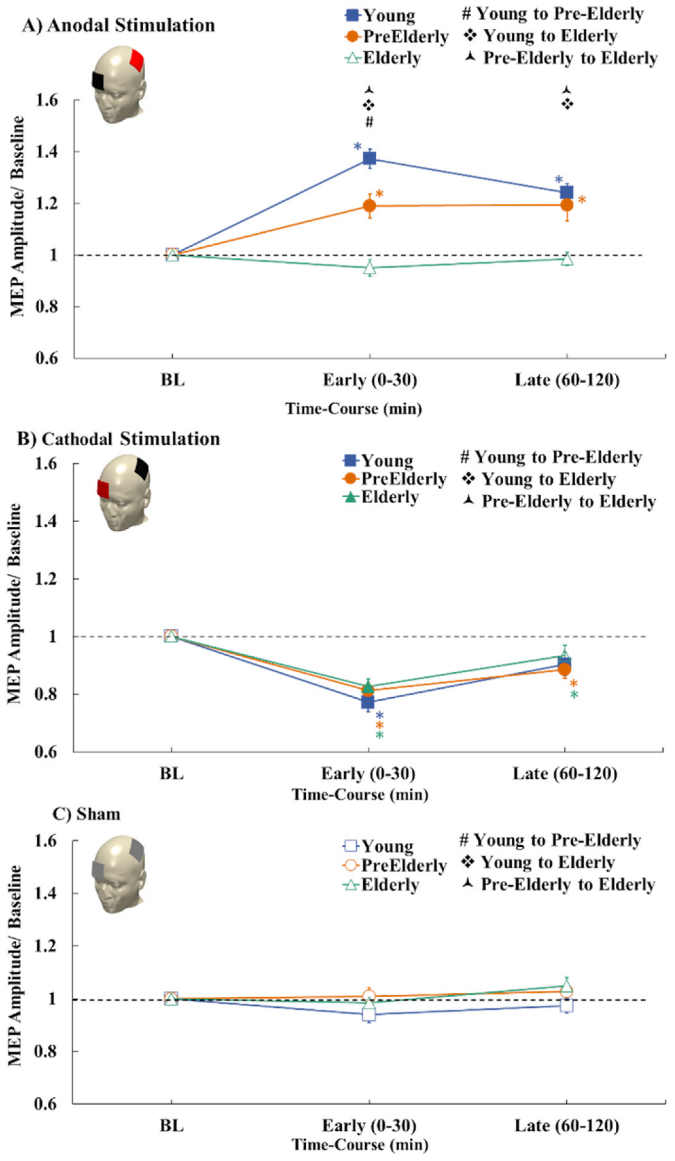

Fig. 3. Pooled MEP amplitudes, early and late tDCS post-stimulation effects. MEPs were pooled into two epochs of early (0–30 min), and late (60–120 min) effects. A) Anodal stimulation, B) Cathodal stimulation and C) Sham condition. Error bars represent standard error of means. Filled symbols indicate a significant difference of cortical excitability after tDCS, as compared to the respective baseline values. Asterisks indicate a significant difference between the respective active and sham stimulation conditions. The symbols of the top row show the results of one-to-one time-point comparisons between groups. The critical significance level was set at $P < =0.05$. Post hoc t-tests were Bonferroni-corrected for multiple comparisons. BL = baseline.

decrease of cortical excitability in the young (for early and late epochs), Pre-Elderly (for early and late epochs), and Elderly (for the early epoch) groups, Fig. 3.

3.4. Inter-individual variability

The individual results of the normalized post-stimulation MEP amplitudes, and responder and non-responder rates are available in the suppl. material, Fig. 1. In addition, the results of Pearson correlations indicated low correlations between anodal and cathodal tDCS effects in the young ($r = 0.166$), Pre-Elderly ($r = -0.011$), and Elderly groups ($r = 0.335$).

3.5. Assessment of tDCS side-effects, and blinding efficacy

For blinding, we explored by a chi-square test if the participant groups could correctly guess the respective stimulation conditions, and found no significant heterogeneity ($x^2 = 0.787$, $p = 0.675$), which showed that blinding was not compromised. Table 3 shows the results of guessed intensities versus actual intensities.

The ANOVAs conducted for the side-effects showed no significant effects for visual phenomena, itching, tingling and pain during the stimulation, and redness of skin, headache, fatigue, difficulty in concentration, nervousness and sleep problems 24 h after stimulation. The respective results are presented in Tables 4 and 5.

4. Discussion

In this study, we investigated the impact of age on neuroplasticity of the human motor cortex with tDCS. In a sham-controlled repeated measures design, anodal and cathodal tDCS protocols of 1 mA were applied for 15min in 60 participants divided into three age groups. In general, the results of the present study show that all active tDCS protocols significantly altered cortical excitability, with anodal tDCS enhancing and cathodal tDCS reducing motor cortex excitability, except for anodal tDCS in the Elderly population, which resulted in no significant cortical excitability alteration. In addition, the results indicate a significant contribution of age on tDCS-induced neuroplastic after-effects. The excitatory effect of anodal tDCS, which was observed in the young group, was significantly diminished in the pre-Elderly, and abolished in the Elderly group. No significant age-dependent differences were however observed for the excitability-diminishing effects of cathodal tDCS. Furthermore, blinding was successful, and all participants tolerated tDCS well.

The results obtained in the young participant group are in accordance with those described in previous studies, in which excitability-enhancing and-diminishing after-effects following 1 mA with 15 min anodal and cathodal tDCS were observed [47,48]. However, neurophysiological studies exploring tDCS and/or other NIBS protocols in the older population are rare. In another study, a 30 min delay of cortico-spinal excitability enhancement has been reported for the old healthy participant group, (mean age ± SD: 68.3 ± 7.9) following 1 mA anodal tDCS for 30 min, as compared to the young healthy group [45]. This result fits relatively well with our outcomes for the Pre-Elderly group (mean age±SD: 58.6 ± 5.07), in which a 15 min delay of MEP increase after anodal tDCS was observed. In further general accordance with the results of the present study, paired associative stimulation, another NIBS tool suited to induce LTP-like plasticity, enhanced cortical excitability in young and middle-aged groups, but not in the elderly [19] (note that the age range of the pre-elderly group in our study was 50–65 years, while in the study conducted by Fathi and colleagues [19] it was 40–59 years). In addition, studies in aged animals have shown a decreased susceptibility to develop LTP [3,60,61]. Thus the age-dependent decline of LTP-like plasticity we found in the present study is in accordance with the results obtained by other plasticity induction tools in humans, and also with results from animal models. The missing effects of age on LTD-like plasticity induced by cathodal tDCS is in further accordance with the results obtained in animal models, which describe unaltered LTD, or a larger range of intervention parameters suited for the induction of LTD [3,6,62]. It contrasts however with results of some experiments in humans, where reduced LTD-like plasticity induction was described in elderly individuals [25,27,63], which might be due in part to the challenges of translating results from animal to human studies, as well as differences between stimulation protocols, or specifics of the participant groups [64,65]. Here, direct comparisons

Table 3
The participant's guessed/actual stimulation conditions. In each session, participants were asked to guess the intensity of the actually applied direct current (0, 1). The table contrasts actually applied intensity (rows) with perceived intensity (columns). The respective statistical test showed that blinding was not compromised.

			Intensity Guessed by Participants	
			0 mA	1 mA
Actual tDCS Intensity	**Sham**	**young**	**7**	**13**
		Pre-Elderly	**5**	**15**
		Elderly	**2**	**18**
	1 mA	**young**	**6**	**34**
		Pre-Elderly	**7**	**33**
		Elderly	**4**	**36**

of the effects of plasticity induction protocols in repeated measure studies might be valuable in future studies. According to the results of the present study, however standard protocols might be sufficient to induce LTD-like plasticity with tDCS in the elderly, whereas it has to be explored if stronger stimulation intensities are suited for the induction of LTP-like plasticity in this age group.

4.1. Proposed mechanisms

With respect to the mechanistic foundation of these effects, pharmacological, TMS, and neuroimaging studies revealed that neuroplasticity induced by tDCS depends on the glutamatergic system, and is calcium-dependent [40,66,67]. Interestingly, age-dependent reduction of LTP-, and also LTD-like plasticity has been shown to be associated with a reduction of NMDA receptor activity, and glutamate concentration [29,68] in earlier studies. It is therefore probable that the reduced LTP-like in elderly we saw in the present study is caused by a respective glutamatergic decline. The missing reduction of LTD-like plasticity in higher age in the present study might then be caused by a broader range of interventions resulting in LTD, as shown in animal models [3,61]. Furthermore, it has been shown in previous studies that dopamine, as well as serotonin, and noradrenaline modulate tDCS-induced plasticity. Specifically, reducing the amount of dopaminergic activity results in plasticity decline [69], whereas serotonin, and noradrenaline enhancement increase LTP-like, but reduce LTD-like plasticity [70,71]. Since these, beyond other neuromodulators, display reduced activity in elderly [72], it might be the case that they contribute to the age-dependent plasticity reduction observed in the present study. Respective mechanisms are however largely speculative at present, and should be explored in future studies.

Beyond age-dependent alterations at the local level, another factor that might affect plasticity in the aging brain is interregional functional connectivity (FC). Neuroimaging and neurophysiological studies have reported altered FC, at the local and global level, of the aged population in comparison with young adults, resulting in less

Table 4
Side effect ratings of the participants in the different stimulation conditions, including visual phenomena, itching, tingling and pain during stimulation and skin redness, headache, fatigue, difficulty in concentration, nervousness and sleep problems within 24 h after stimulation. The presence and intensity of side effects were rated on a numerical scale from zero to five, zero representing no, and five extremely strong sensations. Data are presented as mean ± SD.

	Side-effects	Group	Sham	Anode	Cathode
During Stimulation	**Visual**	Young	0.15 ± 0.48	0.10 ± 0.30	0.20 ± 0.52
		Pre-Elderly	0.25 ± 0.91	0.05 ± 0.22	0.00 ± 0.00
		Elderly	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Itching	Young	0.55 ± 0.88	0.75 ± 1.20	0.45 ± 0.60
		Pre-Elderly	0.05 ± 0.22	0.05 ± 0.22	0.00 ± 0.00
		Elderly	0.10 ± 0.44	0.30 ± 0.65	0.20 ± 0.61
	Tingling	Young	0.55 ± 0.94	1.10 ± 1.44	0.85 ± 1.18
		Pre-Elderly	0.45 ± 1.23	0.20 ± 0.41	0.10 ± 0.44
		Elderly	0.25 ± 0.63	0.35 ± 0.58	0.30 ± 0.80
	Burning	Young	0.55 ± 0.99	1.10 ± 1.41	0.50 ± 0.68
		Pre-Elderly	0.30 ± 0.73	0.10 ± 0.30	0.20 ± 0.52
		Elderly	0.10 ± 0.44	0.20 ± 0.52	0.15 ± 0.48
	Pain	Young	0.20 ± 0.52	0.30 ± 0.65	0.10 ± 0.44
		Pre-Elderly	0.00 ± 0.00	0.05 ± 0.22	0.05 ± 0.22
		Elderly	0.00 ± 0.00	0.05 ± 0.23	0.00 ± 0.00
24 h after Stimulation	**Redness**	Young	0.05 ± 0.22	0.05 ± 0.22	0.05 ± 0.22
		Pre-Elderly	0.05 ± 0.22	0.10 ± 0.30	0.05 ± 0.22
		Elderly	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Headache	Young	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.22
		Pre-Elderly	0.05 ± 0.22	0.05 ± 0.22	0.05 ± 0.22
		Elderly	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Fatigue	Young	0.15 ± 0.36	0.20 ± 0.89	0.25 ± 0.63
		Pre-Elderly	0.05 ± 0.22	0.15 ± 0.48	0.10 ± 0.44
		Elderly	0.05 ± 0.22	0.00 ± 0.00	0.05 ± 0.22
	Concentration	Young	1.10 ± 0.30	0.05 ± 0.22	0.05 ± 0.22
		Pre-Elderly	0.30 ± 0.97	0.05 ± 0.22	0.00 ± 0.00
		Elderly	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Nervousness	Young	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.22
		Pre-Elderly	0.00 ± 0.00	0.05 ± 0.22	0.00 ± 0.00
		Elderly	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Sleep Problems	Young	0.25 ± 0.71	0.15 ± 0.48	0.20 ± 0.61
		Pre-Elderly	0.00 ± 0.00	0.05 ± 0.22	0.00 ± 0.00
		Elderly	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 5
Side-effect ratings of the participants, ANOVA results. The results of the respective ANOVAs indicate no significant effect of stimulation conditions, and age group on visual phenomena, itching, tingling and pain during stimulation and redness of skin, headache, fatigue, difficulty in concentration, nervousness and sleep problems 24 h after stimulation.

	Side-effects	Factors	d.f., Error	F Value	*p* Value	η_p^2
During Stimulation	**Visual**	**Session**	1.473, 83.975	0.838	0.404	0.014
		Session × Age	2.946, 83.975	1.018	0.388	0.034
	Itching	**Session**	2, 114	1.615	0.203	0.028
		Session × Age	4, 114	0.553	0.697	0.019
	Tingling	**Session**	1.529, 87.159	0.688	0.476	0.012
		Session × Age	3.058, 87.159	1.801	0.133	0.059
	Burning	**Session**	1.641, 93.530	1.066	0.337	0.018
		Session × Age	3.282, 93.530	1.997	0.114	0.065
	Pain	**Session**	2, 114	1.304	0.275	0.022
		Session × Age	4, 114	0.652	0.627	0.022
24 h after Stimulation	**Redness**	**Session**	1, 57.000	1.000	0.322	0.017
		Session × Age	2, 57.000	1.000	0.374	0.034
	Headache	**Session**	2, 114	0.241	0.787	0.004
		Session × Age	4, 114	0.241	0.915	0.008
	Fatigue	**Session**	1.682, 95.884	0.357	0.664	0.006
		Session × Age	3.364, 95.884	0.357	0.807	0.012
	Concentration	**Session**	1.070, 60.979	2.002	0.161	0.034
		Session × Age	2.140, 60.979	1.234	0.300	0.042
	Nervousness	**Session**	1.600, 91.200	0.500	0.567	0.009
		Session × Age	3.200, 91.200	1.250	0.296	0.042
	Sleep Problem	**Session**	1.269, 72.358	0.081	0.835	0.001
		Session × Age	2.539, 72.358	0.443	0.691	0.015

efficient overall functional communication in the aging brain [13,73,74]. Furthermore, studies suggested a fine-tuned balance between local cortical and global network plasticity, due to compensatory mechanisms associated with aging [75,76]. It thus makes sense to speculate about an effect of age-dependent functional connectivity alterations on tDCS-induced neuroplasticity. Indeed, recent studies reported a reduction of sensorimotor network FC by anodal tDCS in old subjects [77], while an increase was observed in young adults [78]. This finding might help to explain the lack of anodal facilitatory effects in the old participants in the present study. However also here additional studies exploring respective mechanisms more directly are warranted.

In addition to the proposed compensatory mechanisms, age-dependent dedifferentiation might also affect the functional organization/activity of the brain [79]. Indeed, a generalized spread of brain activity has been described in older adults, resulting in increased correlated activation of larger brain regions independent from the specific cognitive process [80–83], which was shown to be either irrelevant for task performance or associated with reduced motor/cognitive performance [79]. At the cellular level, this is associated with deficits in neurotransmission, and results in a reduction of the signal-to-noise (SNR) ratio, but also loss of neural specialization [84]. In this line, previous aging studies have suggested that age-related over-activation of cerebral regions during motor performance is in better accordance with the compensation than the dedifferentiation hypothesis [79]. To what degree these two mechanisms are associated with the neuroplastic effects of tDCS requires however further detailed exploration.

Age-related plasticity decline, which might be one important reason for respective motor and/or cognitive decline, is one major focus of NIBS studies aiming to improve motor and/or cognitive performance in higher age. In accordance, anodal tDCS applied over the primary motor cortex enhanced motor consolidation [85] in older adults, when performed prior to task performance, but also enhanced acquisition of a complex motor skill [86], performance of skilled hand motor function [87], and reduced the tracking error in a visuo-motor tracking task [88], when performed during task performance. Thus up-regulation of likely age-dependently declined LTP-like plasticity by anodal tDCS seems to be valuable in higher age to improve performance, and at least partially independent from the timing of stimulation. Mechanistically, this effect could be due to counterbalancing the age-dependent reduction of glutamatergic activity [29] by anodal tDCS applied before, or during task performance, which would support the formation of task-dependent LTP-like plasticity. Contrasting results were however also reported, including a lack of online anodal tDCS effects on the rate or accuracy of motor learning in old populations [89], or functional performance of the dominant hand [90]. These contrasting functional effects of tDCS might be due to the application of suboptimal stimulation protocols. Specifically, the reduced propensity for LTP-like plasticity in the elderly might require larger stimulation intensities, as compared to stimulation in young adults. Although systematic tDCS titration studies in higher age have not been conducted so far, preliminary information is available for the impact of anodal tDCS on working memory performance, where stimulation with 1 mA had an improving effect in young adults, but 2 mA were required in patients suffering from Parkinson's disease, who show dopaminergic decline, which is to some degree also present in healthy aging [91,92]. Making use of homeostatic effects of cathodal LTD-like plasticity-inducing stimulation before task performance to enhance task-related LTP-like plasticity might be another option, which however likely faces limitations, as shown by heterogeneous effects on motor learning in previous studies [93], and reduced glutamatergic activity in elderly humans, which might limit task-related counter-regulation.

Beyond performance-improving effects of anodal tDCS over the motor cortex in elderly, stimulation over the prefrontal cortex increased awareness of performance errors [94], and strengthened verbal episodic memories [95]. Stimulation over the left inferior frontal significantly improved overt semantic word generation performance [96], and right temporo-parietal cortex stimulation enhanced learning in an associative learning paradigm [97]. Despite promising findings in these pilot studies, results of other studies are at least partially heterogeneous. Anodal tDCS applied over the primary somatosensory cortex, which enhanced performance of proprioceptive accuracy in a young subject group, reduced performance in elderly participants [98], and prefrontal anodal tDCS did not change working memory performance in older

adults in another study [99]. The results of the present study would suggest that these negative results might be caused by the reduced efficacy of tDCS in higher age, and thus it might be required to adapt stimulation protocols including intensity, duration, and repetition rate [47,48,100–105]. Therefore, a respective titration of stimulation parameters also in healthy old adults might be beneficial to identify efficient intervention protocols in this age group.

4.2. Limitations and future directions

This study should be interpreted within the context of a few limitations. In the present study, we probed the neurophysiological effects of tDCS at the group level. Individual characteristics however also affect the outcomes of tDCS and other NIBS protocols [49,51]. Accordingly, the data obtained in the present experiment show some inter-individual variability, which was slightly higher in the older groups (supplementary materials Fig. 1). Potentially contributing factors are anatomical and biophysical differences of individual brains, including genetics, time of day of the intervention, and brain state [106–108]. The higher heterogeneity of effects in the older participants might be due to age-related brain atrophy, and decline of neurotransmitter activity. Thus, to improve stimulation efficacy at the level of the individual, an important next step would now be to understand/control for individual factors affecting the physiological and behavioral outcome of tDCS [108]. In addition, the study was performed in a sham-controlled single blinded design. A double-blinded design would have been preferable to prevent an observer bias more definitely. Furthermore, we explored age-dependent tDCS effects over the motor cortex in healthy humans; a one-to-one transferability of our results to other cortical areas, as well as patient groups, should not be taken for granted, because of differences of cerebral architecture, neurotransmitter, and -modulator activities, among other factors. Moreover, pathological age-related decline of cognitive performance such as in mild cognitive impairment, or dementia, might affect cortical excitability [2,109], and subsequently affect the neuromodulatory effects of tDCS. A further limitation of the current study is therefore the lack of formal screening for cognitive performance, which might potentially affect our results. To exclude this confounding factor in future studies, a more formal cognitive performance evaluation via standardized screening tests (e.g. Mini Mental State Examination (MMSE) or Montreal-Cognitive Assessment (MoCA) should be considered. Also, the proposed mechanisms for the revealed age-dependent effects are largely speculative at present, and should be explored more directly by pharmacological and/or neuroimaging, and brain stimulation techniques [9,110], in future studies. Future studies should furthermore explore how adaptation of tDCS protocols can help to enhance the efficacy of this tool also in higher age, which might include intensification of interventions [9,111].

5. Conclusion

The results of this study show a significant effect of age on tDCS-induced neuroplasticity. Anodal tDCS-induced LTP-like plasticity, which was observed in the young participant group, was significantly diminished, or abolished in Pre-Elderly and Elderly groups, respectively, while largely identical LTD-like effects were observed in all age groups under study. These age-related changes of neuroplasticity, which have been also shown in respective animal models, might be caused by respective age-dependent alterations of glutamatergic, and neuromodulatory activity. Our study thus provides further insights in the age-dependency of neuroplasticity in healthy humans, and delivers important information for future applications of tDCS.

CRediT authorship contribution statement

Ensiyeh Ghasemian-Shirvan: Conceptualization, Data curation, Formal analysis, Investigation, Writing - original draft, Visualization. **Leila Farnad:** Data curation, Formal analysis, Writing - review & editing. **Mohsen Mosayebi-Samani:** Formal analysis, Methodology, Validation, Writing - review & editing. **Stefanie Verstraelen:** Formal analysis, Writing - review & editing, Writing - review. **Raf L.J. Meesen:** Supervision, Writing - review & editing, Writing - review. **Min-Fang Kuo:** Conceptualization, Supervision, Writing - review & editing. **Michael A. Nitsche:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

Declaration of competing interest

MA Nitsche is a member of Advisory Boards of Neuroelectrics and NeuroDevice. None of the remaining authors have potential conflicts of interest to be disclosed.

Acknowledgements

This work was supported by a research grant from the German Federal Ministry of Education and Research (BMBF) (GCBS grant 01EE1501, TRAINSTIM grant 01GQ1424E). We thank Nicole Rück and Eva Strzelec for their help in recruiting elderly participants.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2020.09.004.

References

[1] Grady C. The cognitive neuroscience of ageing. Nat Rev Neurosci 2012;13(7):491–505.
[2] Burke SN, Barnes CA. Neural plasticity in the ageing brain. Nat Rev Neurosci 2006;7(1):30–40.
[3] Foster TC. Dissecting the age-related decline on spatial learning and memory tasks in rodent models: N-methyl-D-aspartate receptors and voltage-dependent Ca2+ channels in senescent synaptic plasticity. Prog Neurobiol 2012;96(3):283–303.
[4] van der Zee EA. Synapses, spines and kinases in mammalian learning and memory, and the impact of aging. Neurosci Biobehav Rev 2015;50:77–85.
[5] Adams I. Comparison of synaptic changes in the precentral and postcentral cerebral cortex of aging humans: a quantitative ultrastructural study. Neurobiol Aging 1987;8(3):203–12.
[6] Kumar A. Long-term potentiation at CA3–CA1 hippocampal synapses with special emphasis on aging, disease, and stress. Front Aging Neurosci 2011;3(7).
[7] Segovia G, Porras A, Del Arco A, Mora F. Glutamatergic neurotransmission in aging: a critical perspective. Mech. Ageing Dev. 2001;122(1):1–29.
[8] Schmidt S, Redecker C, Bruehl C, Witte OW. Age-related decline of functional inhibition in rat cortex. Neurobiol Aging 2010;31(3):504–11.
[9] Antonenko D, Nierhaus T, Meinzer M, Prehn K, Thielscher A, Ittermann B, et al. Age-dependent effects of brain stimulation on network centrality. Neuroimage 2018;176:71–82.
[10] Henderson G, Tomlinson BE, Gibson PH. Cell counts in human cerebral cortex in normal adults throughout life using an image analysing computer. J Neurol Sci 1980;46(1):113–36.
[11] Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage 2001;14(1 Pt 1):21–36.
[12] Fjell AM, Westlye LT, Amlien I, Espeseth T, Reinvang I, Raz N, et al. High consistency of regional cortical thinning in aging across multiple samples. Cerebr Cortex 2009;19(9):2001–12. New York, NY : 1991.
[13] Goh JOS. Functional dedifferentiation and altered connectivity in older adults: neural accounts of cognitive aging. Aging Dis 2011;2(1):30–48.
[14] Sullivan EV, Pfefferbaum A. Diffusion tensor imaging and aging. Neurosci Biobehav Rev 2006;30(6):749–61.
[15] Kaasinen V, Vilkman H, Hietala J, Nagren K, Helenius H, Olsson H, et al. Age-related dopamine D2/D3 receptor loss in extrastriatal regions of the human brain. Neurobiol Aging 2000;21(5):683–8.
[16] Heise KF, Zimerman M, Hoppe J, Gerloff C, Wegscheider K, Hummel FC. The aging motor system as a model for plastic changes of GABA-mediated

intracortical inhibition and their behavioral relevance. J Neurosci : Off J Soc Neurosci 2013;33(21):9039–49.
[17] Young-Bernier M, Kamil Y, Tremblay F, Davidson PS. Associations between a neurophysiological marker of central cholinergic activity and cognitive functions in young and older adults. Behav Brain Funct : BBF 2012;8:17.
[18] Gomes-Osman J, Rice J, Cabral DLF, Fried PJ, Nissim NR, Aksu S, et al. Non-invasive brain stimulation: probing intracortical circuits and improving cognition in the aging brain. Front Aging Neurosci 2018;10:177.
[19] Fathi D, Ueki Y, Mima T, Koganemaru S, Nagamine T, Tawfik A, et al. Effects of aging on the human motor cortical plasticity studied by paired associative stimulation. Clin Neurophysiol : Off J Int Fed clin Neurophysiol 2010;121(1):90–3.
[20] Tecchio F, Zappasodi F, Pasqualetti P, Gennaro L, Pellicciari MC, Ercolani M, et al. Age dependence of primary motor cortex plasticity induced by paired associative stimulation. Clin Neurophysiol : Off J Int Fed clin Neurophysiol 2008;119(3):675–82.
[21] Pellicciari MC, Miniussi C, Rossini PM, De Gennaro L. Increased cortical plasticity in the elderly: changes in the somatosensory cortex after paired associative stimulation. Neuroscience 2009;163(1):266–76.
[22] Gedankien T, Fried PJ, Pascual-Leone A, Shafi MM. Intermittent theta-burst stimulation induces correlated changes in cortical and corticospinal excitability in healthy older subjects. Clin Neurophysiol : Off J Int Fed clin Neurophysiol 2017;128(12):2419–27.
[23] Dickins DSE, Sale MV, Kamke MR. Plasticity induced by intermittent theta burst stimulation in bilateral motor cortices is not altered in older adults. Neural Plast 2015. 2015:323409.
[24] Freitas C, Perez J, Knobel M, Tormos JM, Oberman L, Eldaief M, et al. Changes in cortical plasticity across the lifespan. Front Aging Neurosci 2011;3(5).
[25] Muller-Dahlhaus JF, Orekhov Y, Liu Y, Ziemann U. Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. Exp Brain Res 2008;187(3):467–75.
[26] Bashir S, Perez JM, Horvath JC, Pena-Gomez C, Vernet M, Capia A, et al. Differential effects of motor cortical excitability and plasticity in young and old individuals: a Transcranial Magnetic Stimulation (TMS) study. Front Aging Neurosci 2014;6(111).
[27] Todd G, Kimber TE, Ridding MC, Semmler JG. Reduced motor cortex plasticity following inhibitory rTMS in older adults. Clin Neurophysiol : Off J Int Fed clin Neurophysiol 2010;121(3):441–7.
[28] Lee NJ, Ahn HJ, Jung K-I, Ohn SH, Hong J, Kim YJ, et al. Reduction of continuous theta burst stimulation-induced motor plasticity in healthy elderly with COMT Val158Met polymorphism. Ann Rehabil Med 2014;38(5):658–64.
[29] McGinley M, Hoffman RL, Russ DW, Thomas JS, Clark BC. Older adults exhibit more intracortical inhibition and less intracortical facilitation than young adults. Exp Gerontol 2010;45(9):671–8.
[30] Peinemann A, Lehner C, Conrad B, Siebner HR. Age-related decrease in paired-pulse intracortical inhibition in the human primary motor cortex. Neurosci Lett 2001;313(1–2):33–6.
[31] Shibuya K, Park SB, Geevasinga N, Huynh W, Simon NG, Menon P, et al. Threshold tracking transcranial magnetic stimulation: effects of age and gender on motor cortical function. Clin Neurophysiol : Off J Int Fed clin Neurophysiol 2016;127(6):2355–61.
[32] Marneweck M, Loftus A, Hammond G. Short-interval intracortical inhibition and manual dexterity in healthy aging. Neurosci Res 2011;70(4):408–14.
[33] Oliviero A, Profice P, Tonali PA, Pilato F, Saturno E, Dileone M, et al. Effects of aging on motor cortex excitability. Neurosci Res 2006;55(1):74–7.
[34] Opie GM, Semmler JG. Age-related differences in short- and long-interval intracortical inhibition in a human hand muscle. Brain Stimul 2014;7(5):665–72.
[35] Cirillo J, Todd G, Semmler JG. Corticomotor excitability and plasticity following complex visuomotor training in young and old adults. Eur J Neurosci 2011;34(11):1847–56.
[36] Nitsche MA, Nitsche MS, Klein CC, Tergau F, Rothwell JC, Paulus W. Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. Clin Neurophysiol 2003;114(4):600–4.
[37] Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 2000;527(3):633–9.
[38] Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 2001;57(10):1899–901.
[39] Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: state of the art 2008. Brain stimulation 2008;1(3):206–23.
[40] Stagg CJ, Nitsche MA. Physiological basis of transcranial direct current stimulation. Neuroscientist : Rev J Neurobio Neurol Psych 2011;17(1):37–53.
[41] Stagg CJ, Best JG, Stephenson MC, O'Shea J, Wylezinska M, Kincses ZT, et al. Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. J Neurosci : Off J Soc Neurosci 2009;29(16):5202–6.
[42] Mosayebi-Samani M, Melo L, Agboada D, Nitsche MA, Kuo M-F. Ca2+ channel dynamics explain the nonlinear neuroplasticity induction by cathodal transcranial direct current stimulation over the primary motor cortex. Eur Neuropsychopharmacol 2020. in press.
[43] Polania R, Nitsche MA, Paulus W. Modulating functional connectivity patterns and topological functional organization of the human brain with transcranial direct current stimulation. Hum Brain Mapp 2011;32(8):1236–49.
[44] Zheng X, Alsop DC, Schlaug G. Effects of transcranial direct current stimulation (tDCS) on human regional cerebral blood flow. Neuroimage 2011;58(1):26–33.
[45] Fujiyama H, Hyde J, Hinder MR, Kim S-J, McCormack GH, Vickers JC, et al. Delayed plastic responses to anodal tDCS in older adults. Front Aging Neurosci 2014;6:115.
[46] Heise K-F, Niehoff M, Feldheim J-F, Liuzzi G, Gerloff C, Hummel FC. Differential behavioral and physiological effects of anodal transcranial direct current stimulation in healthy adults of younger and older age. Front Aging Neurosci 2014;6(146).
[47] Mosayebi Samani M, Agboada D, Jamil A, Kuo M-F, Nitsche MA. Titrating the neuroplastic effects of cathodal transcranial direct current stimulation (tDCS) over the primary motor cortex. Cortex 2019;119:350–61.
[48] Agboada D, Mosayebi Samani M, Jamil A, Kuo M-F, Nitsche MA. Expanding the parameter space of anodal transcranial direct current stimulation of the primary motor cortex. Sci Rep 2019;9(1). 18185.
[49] Wiethoff S, Hamada M, Rothwell JC. Variability in response to transcranial direct current stimulation of the motor cortex. Brain Stimul 2014;7(3):468–75.
[50] Strube W, Bunse T, Nitsche MA, Nikolaeva A, Palm U, Padberg F, et al. Bidirectional variability in motor cortex excitability modulation following 1 mA transcranial direct current stimulation in healthy participants. Physiological reports 2016;4(15).
[51] Ridding MC, Ziemann U. Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. J Physiol 2010;588(Pt 13):2291–304.
[52] Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC. The role of interneuron networks in driving human motor cortical plasticity. Cerebr Cortex 2013;23(7):1593–605. New York, NY : 1991.
[53] Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9(1):97–113.
[54] Bikson M, Grossman P, Thomas C, Zannou AL, Jiang J, Adnan T, et al. Safety of transcranial direct current stimulation: evidence based update 2016. Brain Stimul 2016;9(5):641–61.
[55] Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol : Off J Int Fed clin Neurophysiol 2009;120(12):2008–39.
[56] Guleyupoglu B, Febles N, Minhas P, Hahn C, Bikson M. Reduced discomfort during high-definition transcutaneous stimulation using 6% benzocaine. Front Neuroeng 2014;7:28.
[57] McFadden JL, Borckardt JJ, George MS, Beam W. Reducing procedural pain and discomfort associated with transcranial direct current stimulation. Brain Stimul 2011;4(1):38–42.
[58] Poreisz C, Boros K, Antal A, Paulus W. Safety aspects of transcranial direct current stimulation concerning healthy subjects and patients. Brain Res Bull 2007;72(4–6):208–14.
[59] Brunoni AR, Amadera J, Berbel B, Volz MS, Rizzerio BG, Fregni F. A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. Int J Neuropsychopharmacol 2011;14(8):1133–45.
[60] Kelly KM, Nadon NL, Morrison J, Thibault O, Barnes CA, Blalock E. The neurobiology of aging. Epilepsy Res 2006;68(Suppl 1):S5–20.
[61] Barnes CA. Long-term potentiation and the ageing brain. Philos Trans R Soc Lond B Biol Sci 2003;358(1432):765–72.
[62] Murphy GG, Fedorov NB, Giese KP, Ohno M, Friedman E, Chen R, et al. Increased neuronal excitability, synaptic plasticity, and learning in aged Kvβ1.1 knockout mice. Curr Biol 2004;14(21):1907–15.
[63] Bashir S, Perez JM, Horvath JC, Pena-Gomez C, Vernet M, Capia A, et al. Differential effects of motor cortical excitability and plasticity in young and old individuals: a Transcranial Magnetic Stimulation (TMS) study. Front Aging Neurosci 2014;6.
[64] Nitsche MA, Muller-Dahlhaus F, Paulus W, Ziemann U. The pharmacology of neuroplasticity induced by non-invasive brain stimulation: building models for the clinical use of CNS active drugs. J Physiol 2012;590(19):4641–62.
[65] Brunoni AR, Fregni F, Pagano RL. Translational research in transcranial direct current stimulation (tDCS): a systematic review of studies in animals. Rev Neurosci 2011;22(4):471–81.
[66] Liebetanz D, Nitsche MA, Tergau F, Paulus W. Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain : J Neurol 2002;125(Pt 10):2238–47.
[67] Clark VP, Coffman BA, Trumbo MC, Gasparovic C. Transcranial direct current stimulation (tDCS) produces localized and specific alterations in neurochemistry: a (1)H magnetic resonance spectroscopy study. Neurosci Lett 2011;500(1):67–71.
[68] Sailasuta N, Ernst T, Chang L. Regional variations and the effects of age and gender on glutamate concentrations in the human brain. Magn Reson Imaging 2008;26(5):667–75.
[69] Nitsche M, Lampe C, Antal A, Liebetanz D, Lang N, Tergau F, et al. Dopaminergic modulation of long-lasting direct current-induced cortical excitability changes in the human motor cortex. Aktuelle Neurol 2006;33.

[70] Nitsche MA, Kuo M-F, Karrasch R, Wächter B, Liebetanz D, Paulus W. Serotonin affects transcranial direct current–induced neuroplasticity in humans. Biol Psychiatr 2009;66(5):503–8.
[71] Kuo H-I, Paulus W, Batsikadze G, Jamil A, Kuo M-F, Nitsche MA. Acute and chronic effects of noradrenergic enhancement on transcranial direct current stimulation-induced neuroplasticity in humans. J Physiol 2017;595(4):1305–14.
[72] Karp JF, Shega JW, Morone NE, Weiner DK. Advances in understanding the mechanisms and management of persistent pain in older adults†. Br J Addiction: Br J Anaesth 2008;101(1):111–20.
[73] Gazzaley A, Clapp W, Kelley J, McEvoy K, Knight RT, D'Esposito M. Age-related top-down suppression deficit in the early stages of cortical visual memory processing. Proc Natl Acad Sci USA 2008;105(35):13122–6.
[74] Achard S, Bullmore E. Efficiency and cost of economical brain functional networks. PLoS Comput Biol 2007;3(2):e17.
[75] Freitas C, Farzan F, Pascual-Leone A. Assessing brain plasticity across the lifespan with transcranial magnetic stimulation: why, how, and what is the ultimate goal? Front Neurosci 2013;7:42.
[76] Pascual-Leone A, Taylor MJ. A developmental framework of brain and cognition from infancy to old age. Brain Topogr 2011;24(3):183.
[77] Antonenko D, Schubert F, Bohm F, Ittermann B, Aydin S, Hayek D, et al. tDCS-induced modulation of GABA levels and resting-state functional connectivity in older adults. J Neurosci : Off J Soc Neurosci 2017;37(15):4065–73.
[78] Bachtiar V, Near J, Johansen-Berg H, Stagg CJ. Modulation of GABA and resting state functional connectivity by transcranial direct current stimulation. eLife 2015;4:e08789.
[79] Heuninckx S, Wenderoth N, Swinnen SP. Systems neuroplasticity in the aging brain: recruiting additional neural resources for successful motor performance in elderly persons. J Neurosci : Off J Soc Neurosci 2008;28(1):91–9.
[80] Cabeza R, Anderson ND, Locantore JK, McIntosh AR. Aging gracefully: compensatory brain activity in high-performing older adults. Neuroimage 2002;17(3):1394–402.
[81] Mitrushina M, Satz P. Analysis of longitudinal covariance structures in assessment of stability of cognitive functions in elderly. Brain Dysfunct 1991;4(4):163–73.
[82] Babcock RL, Laguna KD, Roesch SC. A comparison of the factor structure of processing speed for younger and older adults: testing the assumption of measurement equivalence across age groups. Psychol Aging 1997;12(2):268–76.
[83] Baltes P, Lindenberger U. Emergence of a powerful connection between sensory and cognitive functions across the adult life span: a new window to the study of cognitive aging? Psychol Aging 1997;12:12–21.
[84] Nilsson LG, Markowitsch HJ, editors. Cognitive neuroscience of memory. Hogrefe & Huber Publishers; 1999. p. 103–46.
[85] Rumpf J-J, Wegscheider M, Hinselmann K, Fricke C, King BR, Weise D, et al. Enhancement of motor consolidation by post-training transcranial direct current stimulation in older people. Neurobiol Aging 2017;49:1–8.
[86] Zimerman M, Nitsch M, Giraux P, Gerloff C, Cohen LG, Hummel FC. Neuroenhancement of the aging brain: restoring skill acquisition in old subjects. Ann Neurol 2013;73(1):10–5.
[87] Hummel FC, Heise K, Celnik P, Floel A, Gerloff C, Cohen LG. Facilitating skilled right hand motor function in older subjects by anodal polarization over the left primary motor cortex. Neurobiol Aging 2010;31(12):2160–8.
[88] Goodwill AM, Reynolds J, Daly RM, Kidgell DJ. Formation of cortical plasticity in older adults following tDCS and motor training. Front Aging Neurosci 2013;5:87.
[89] Raw RK, Allen RJ, Mon-Williams M, Wilkie RM. Motor sequence learning in healthy older adults is not necessarily facilitated by transcranial direct current stimulation (tDCS). Geriatrics 2016;1(4):32.
[90] Marquez J, Conley A, Karayanidis F, Lagopoulos J, Parsons M. Anodal direct current stimulation in the healthy aged: effects determined by the hemisphere stimulated. Restor Neurol Neurosci 2015;33(4):509–19.
[91] Boggio PS, Ferrucci R, Rigonatti SP, Covre P, Nitsche M, Pascual-Leone A, et al. Effects of transcranial direct current stimulation on working memory in patients with Parkinson's disease. J Neurol Sci 2006;249(1):31–8.
[92] Fregni F, Boggio PS, Nitsche M, Bermpohl F, Antal A, Feredoes E, et al. Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. Exp Brain Res 2005;166(1):23–30.
[93] Kuo M-F, Unger M, Liebetanz D, Lang N, Tergau F, Paulus W, et al. Limited impact of homeostatic plasticity on motor learning in humans. Neuropsychologia 2008;46(8):2122–8.
[94] Harty S, Robertson IH, Miniussi C, Sheehy OC, Devine CA, McCreery S, et al. Transcranial direct current stimulation over right dorsolateral prefrontal cortex enhances error awareness in older age. J Neurosci : Off J Soc Neurosci 2014;34(10):3646–52.
[95] Sandrini M, Brambilla M, Manenti R, Rosini S, Cohen LG, Cotelli M. Noninvasive stimulation of prefrontal cortex strengthens existing episodic memories and reduces forgetting in the elderly. Front Aging Neurosci 2014;6:289.
[96] Meinzer M, Lindenberg R, Antonenko D, Flaisch T, Flöel A. Anodal transcranial direct current stimulation temporarily reverses age-associated cognitive decline and functional brain activity changes. J Neurosci : Off J Soc Neurosci 2013;33(30):12470–8.
[97] Flöel A, Suttorp W, Kohl O, Kürten J, Lohmann H, Breitenstein C, et al. Noninvasive brain stimulation improves object-location learning in the elderly. Neurobiol Aging 2012;33(8):1682–9.
[98] Muffel T, Kirsch F, Shih P-C, Kalloch B, Schaumberg S, Villringer A, et al. Anodal transcranial direct current stimulation over S1 differentially modulates proprioceptive accuracy in young and old adults. Front Aging Neurosci 2019;11:264.
[99] Nilsson J, Lebedev AV, Lövdén M. No significant effect of prefrontal tDCS on working memory performance in older adults. Front Aging Neurosci 2015;7(230).
[100] Agboada D, Mosayebi-Samani M, Kuo M-F, Nitsche A M. Induction of long-term potentiation-like plasticity in the primary motor cortex with repeated anodal transcranial direct current stimulation – better effects with intensified protocols? Brain Stimul 2020;13(4):987–97.
[101] Mosayebi Samani M, Agboada D, Kuo MF, Nitsche MA. Probing the relevance of repeated cathodal transcranial direct current stimulation over the primary motor cortex for prolongation of after-effects. J Physiol 2020;598(4):805–16.
[102] Andrade C. Transcranial direct current stimulation for refractory auditory hallucinations in schizophrenia. J Clin Psychiatr 2013;74(11):e1054–8.
[103] Hoy KE, Arnold SL, Emonson MR, Daskalakis ZJ, Fitzgerald PB. An investigation into the effects of tDCS dose on cognitive performance over time in patients with schizophrenia. Schizophr Res 2014;155(1–3):96–100.
[104] Boggio PS, Ferrucci R, Rigonatti SP, Covre P, Nitsche M, Pascual-Leone A, et al. Effects of transcranial direct current stimulation on working memory in patients with Parkinson's disease. J Neurol Sci 2006;249(1):31–8.
[105] Monte-Silva K, Kuo MF, Hessenthaler S, Fresnoza S, Liebetanz D, Paulus W, et al. Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. Brain Stimul 2013;6(3):424–32.
[106] Kuo MF, Paulus W, Nitsche MA. Sex differences in cortical neuroplasticity in humans. Neuroreport 2006;17(16):1703–7.
[107] Sale MV, Ridding MC, Nordstrom MA. Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Exp Brain Res 2007;181(4):615–26.
[108] Huang YZ, Lu MK, Antal A, Classen J, Nitsche M, Ziemann U, et al. Plasticity induced by non-invasive transcranial brain stimulation: a position paper. Clin Neurophysiol : Off J Int Fed clin Neurophysiol 2017;128(11):2318–29.
[109] Prehn K, Flöel A. Potentials and limits to enhance cognitive functions in healthy and pathological aging by tDCS. Front Cell Neurosci 2015;9:355.
[110] Nitsche MA, Fricke K, Henschke U, Schlitterlau A, Liebetanz D, Lang N, et al. Pharmacological modulation of cortical excitability shifts induced by transcranial direct current stimulation in humans. J Physiol 2003;553(Pt 1):293–301.
[111] Fregni F, Boggio PS, Santos MC, Lima M, Vieira AL, Rigonatti SP, et al. Noninvasive cortical stimulation with transcranial direct current stimulation in Parkinson's disease. Mov Disord 2006;21(10):1693–702.