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Age-dependent non-linear neuroplastic effects of cathodal tDCS in the elderly population: a titration study

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ABSTRACT

Background: Neuromodulatory effects of transcranial direct current stimulation (tDCS) in older humans have shown heterogeneous results, possibly due to sub-optimal stimulation protocols associated with limited knowledge about optimized stimulation parameters in this age group. We systematically explored the association between the stimulation dosage of cathodal tDCS and induced after-effects on motor cortex excitability in the elderly.

Method: Thirty-nine healthy volunteers in two age groups, namely Pre-Elderly (50–65 years) and Elderly (66–80 years), participated in the study. Ten sessions of cathodal tDCS, with a combination of four intensities (1, 2, 3 mA and sham) and three durations (15, 20, 30 min) were conducted over the M1 in each participant. Cortical excitability changes were monitored with TMS-induced motor evoked potentials (MEPs) for up to 2 h after stimulation.

Results: Motor cortex excitability was reduced by cathodal stimulation intensities of 1 and 3 mA in both age groups, in accordance with results observed in the younger age groups of previous studies. For the 2 mA stimulation condition, an age-dependent conversion of plasticity into a stimulation duration-dependent excitability enhancement was observed in the Pre-Elderly group, whereas in the Elderly group, LTD-like plasticity was preserved, or abolished, depending on stimulation duration.

Conclusion: The LTD-like plasticity effects induced by cathodal tDCS originally described in young adults are also observable in older humans, but non-linearities of the resulting plasticity were partially preserved only in the Pre-Elderly, but not the Elderly group. These results aid in understanding age-dependent plasticity dynamics in humans, and to define more efficient tDCS protocols in the aging brain.

1. Introduction

Aging in otherwise healthy humans is associated with a decline in cognitive and motor performance, which negatively affects the quality of life [1–3]. These age-dependent alterations are caused by structural and neurophysiological alterations of the brain at the cellular, circuit, and systems level [4–6]. Former studies have stressed an impact of age on neuroplasticity, which is the structural and functional alteration of the strength of synaptic connections in response to environmental or internal demands, as a critical factor for age-related cognitive, and motor decline [7,8].

In this regard, animal studies have shown an age-related decline of motor functions, including a decline of motor coordination [9], or motor slowing and parkinsonian symptoms [10], as well as a decline of cognitive performance, including impairment of visual recognition memory [11], executive functions [12], or discrimination learning [13]. These deficits have been linked to age-dependent alterations of plasticity mechanisms, including an increase of the synaptic threshold for the induction of long-term potentiation (LTP), and increased probability for the induction of long-term depression (LTD) [14], potentially due to synaptic density

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reduction [4,15] as well as a decrease of available neurotransmitters and/or receptors [5,16].

A respective age-dependent decline of motor/cognitive functions observed in human adults might be explained by mechanisms similar to those revealed in animal models [17,18]. Such mechanisms may include the reduction of synaptic connections/morphology, number of neurons [4,19], and a decrease of neuromodulator and neurotransmitter availability [20,21], resulting in altered neuroplasticity. Therefore, a target for counteracting the decline of motor/cognitive performance in healthy aging might be modulation of neuroplasticity.

Neuroplasticity can be induced by non-invasive brain stimulation techniques (NIBS), which do not disrupt the integrity of the skull [22]. One of these techniques, transcranial direct current stimulation (tDCS), induces LTP- and LTD-like plasticity in a polarity-dependent way, by delivering weak direct electrical currents through the scalp via electrodes placed on the head. Studies in young healthy participants have shown that for the primary motor cortex, anodal tDCS, which refers to surface inward current over the target area, results in an enhancement of cortical excitability at the macroscale level. Contrarily, outward current over the target area, labeled cathodal tDCS, reduces excitability with standard stimulation protocols for about 1 h or longer after intervention [23–26]. Following these findings, there has been an increased interest for the use of tDCS in basic and clinical settings [27–29]. However, the overall efficacy of the technique is limited at present [30], which might be attributed to the inconsistency of intervention parameters, and other methodological differences between studies. One important limitation of current protocols is the application of uniform dosages, independent from individual preconditions, which might lead to suboptimal interventions in individuals characterized by differences of brain physiology, and anatomy, as compared to healthy young adults, such as in higher age, or patients with brain pathologies. Knowledge about how to adapt tDCS in these groups to optimize efficacy, is however limited [31].

Recent systematic neurophysiological tDCS titration studies in *healthy young adults* have revealed a gradual improvement of anodal stimulation efficacy by intensifying the stimulation dosage [32]. However, for cathodal tDCS, intensity-dependent nonlinear after-effects were observed, with low (1 mA) and high (3 mA) intensity stimulation protocols resulting in a significant motor cortical excitability reduction and medium dosages (2 mA) resulting in an induction of LTP-like plasticity [33,34]. Furthermore, in comparison with lower dosages, longer lasting excitability-diminishing after-effects were observed with higher stimulation intensity (3 mA). However, taking into account age-related alterations of brain anatomy, physiology, and neuroplasticity, as outlined above [35,36], whether and to what degree the findings obtained in young healthy humans can be extrapolated to the *elderly population* is not yet clear.

Only a few studies have assessed the direct neurophysiological effects of tDCS in elderly adults. We recently tested the effects of anodal and cathodal tDCS of 1 mA for 15min applied over the primary motor cortex in three age groups: young (18-30 years-old), Pre-Elderly (50-65 years-old) and Elderly (66-80 years-old) humans. The results showed no age-dependent differences for the excitability-diminishing effects of cathodal tDCS, while the excitatory effect of anodal tDCS declined depending on age [37] for this specific tDCS protocol. Apart from this, other studies for age-dependent cathodal tDCS effects are not available. However, the results of this study do not exclude that cathodal tDCS effects differ between young and elderly humans with respect to other tDCS dosages, especially given the dosage-dependent non-linearities of tDCS effects, which in young healthy adults are observed under specific dosages [34]. Here, gradual age-dependent alterations of cerebral connectivity, and transmitter availability could have relevant effects. Systematic dosing studies for cathodal tDCS in elderly adults are thus required to identify age-adapted optimized intervention protocols.

In the present study, we systematically explored the effect of cathodal tDCS on motor cortex plasticity with combinations of three stimulation intensities (1, 2, and 3 mA) and durations (15, 20, and 30 min), in a sham-controlled cross-over design in two age groups: Pre-Elderly and Elderly. According to previous findings, we anticipated a dosage-dependent non-linear effect of tDCS which is modulated by age, and an enhancement of respective neurophysiological outcomes by intensified tDCS dosages. The results of this study will provide further insights about the dependency of tDCS-induced LTD-like neuroplasticity from these stimulation parameters in the elderly population, and thereby deliver crucial information for future applications of cathodal tDCS in this group.

2. Methods

2.1. Participants

Thirty-nine healthy, non-smoking participants of two age groups were recruited: 20 Pre-Elderly participants (11 females; mean age (years ± SD) 58.65 ± 3.86) and 19 Elderly participants (10 females; mean age (years ± SD) 72.68 ± 5.12). These age ranges were selected based on previous findings looking at the impact of age on tDCS-generated plasticity [37,38], and is in line with the assumed course of plasticity alteration in advanced age (see these also for further details: [39,40]. All participants were right-handed according to the Edinburgh Handedness Inventory [41]. Prior to participation, volunteers were screened for history of neurological and psychiatric diseases, and the absence of exclusion criteria for non-invasive electrical and magnetic brain stimulation [42,43]. Central nervous system-acting medication or respective recreational substances served also as exclusion criteria. In addition, to ensure that cognitive functioning was within age-related norms, all participants underwent a cognitive screening, namely the Montreal Cognitive Assessment (MOCA) test [44]. Moreover, the amount of physical activity of the participants was quantified using an adapted version of the Lüdenscheid Activity Questionnaire [45]. Additionally, all participants were instructed not to consume drinks containing caffeine at least 2 h prior to each session, and to avoid alcohol one day prior to each session. The study conformed to the Declaration of Helsinki and was approved by the local ethics committee. All participants gave written informed consent prior to study participation, and were financially compensated.

2.2. Motor cortical excitability monitoring

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique that can induce action potentials in surface-near cortical neurons [46]. The motor evoked potential (MEP) amplitude elicited by single pulse TMS is a global measure of excitability of the cortico-spinal system, not restricted to single neuronal subgroups, and neurotransmitter or -modulator systems. In principle, single pulse TMS-evoked MEP could also be affected via transmission alterations at cortico-spinal synapses, and at the neuromuscular junction. However, the direct effects of tDCS are missing at this level, which make this measure appropriate for screening the cortical effects of tDCS [23]. In this study, single pulse TMS at 0.25 Hz ± 10% jitter was delivered by a PowerMag magnetic stimulator (Mag&More, Munich, Germany) with a figure-of-eight magnetic coil (diameter of one winding, 70 mm; peak magnetic field, 2T). The coil was held tangentially to the skull, with the handle pointing backwards and laterally at 45° from the midline

and was applied to the left primary motor cortex. Surface MEPs were recorded from the right Abductor Digiti Minimi muscle (ADM) with gold cup electrodes in a belly-tendon montage. The signals were amplified, and filtered (1000; 3Hz - 3 KHz) via the D440-2 (Digitimer, Welwyn Garden City, UK), and 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).

2.3. Transcranial direct current stimulation (tDCS)

tDCS was applied with a constant current battery-powered stimulator (neuroConn, Ilmenau, Germany), through a pair of saline-soaked surface sponge electrodes (5 × 7 cm) placed on the scalp. Based on previous studies, one electrode was fixed over the motor cortex representational area of the right ADM as identified by TMS (the long axis medio-lateral, and with an angle of 45° in relation to midline to align with the motor strip orientation [47], and the other was placed contralaterally over the right orbit [23,26]. Prior to stimulation, a topical anaesthetic cream (EMLA®, 2.5% lidocaine, 2.5% prilocaine) was applied to the stimulation site to ensure sufficient blinding of the participants (Guleyupoglu, Febles, Minhas, Hahn, & Bikson, 2014). All participants received cathodal tDCS at an intensity of 1.0, 2.0 or 3.0 mA for 15, 20 or 30 min, with a 30 s ramp-up and down at the start and end of stimulation. For sham stimulation, a current strength of 1.0 mA was delivered for 30 s, with a 30 s ramp up and down, followed by 15 min with 0 mA stimulation. All intensity-duration combinations, including sham stimulation, resulted in 10 experimental sessions per participant.

2.4. Experimental procedure

All volunteers went through a 2-h introductory session to examine their medical and cognitive state as well as to familiarize them with the experimental procedure, including tDCS. This session was separated at least one week from the principal experimental sessions.

At the beginning of each experimental session, participants were seated in a comfortable chair with head and armrests. TMS was applied to the left motor cortex to identify the representational area of the right ADM in which the largest MEPs were produced (motor hot spot), and the respective coil position was marked with a waterproof pen. The intensity of the TMS pulses was adjusted to elicit MEPs with a peak-to-peak amplitude of 1 mV (SI_{1mV}) on average, which was determined at the beginning of each session, and kept constant throughout the experiment in each participant. Baseline cortical excitability was determined by measuring 30 MEPs. Afterwards, tDCS electrodes were mounted and tDCS was applied. tDCS with different intensities and durations (as outlined above) was applied in a randomized order with a minimum of seven days between each session to avoid carry-over effects [48,49]. After intervention, tDCS electrodes were removed and corticospinal excitability was assessed by TMS (30 stimuli per time-point) every 5 min for up to 30 min, then at 60 min, 90 min, and 120 min post tDCS (Fig. 1).

2.5. Calculations and statistics

MEP amplitudes were first visually inspected to exclude trials in which background electromyographic activity was present. Then, the individual means of MEP amplitudes recorded at each time point were calculated for all subjects and all conditions separately. The post-intervention mean MEP amplitudes were then normalized to the respective individual mean baseline MEP amplitude.

2.5.1. Equivalence of SI_{1mV} and baseline MEP between groups and measures

To test if baseline measures differed between sessions, and groups, two separate mixed model ANOVAs were calculated with "Condition" (10 levels) as the within-subject factor, "Age-Group" as the between-subject factor, and 'SI_{1mV}' and 'baseline MEP' as dependent variables.

2.5.2. 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

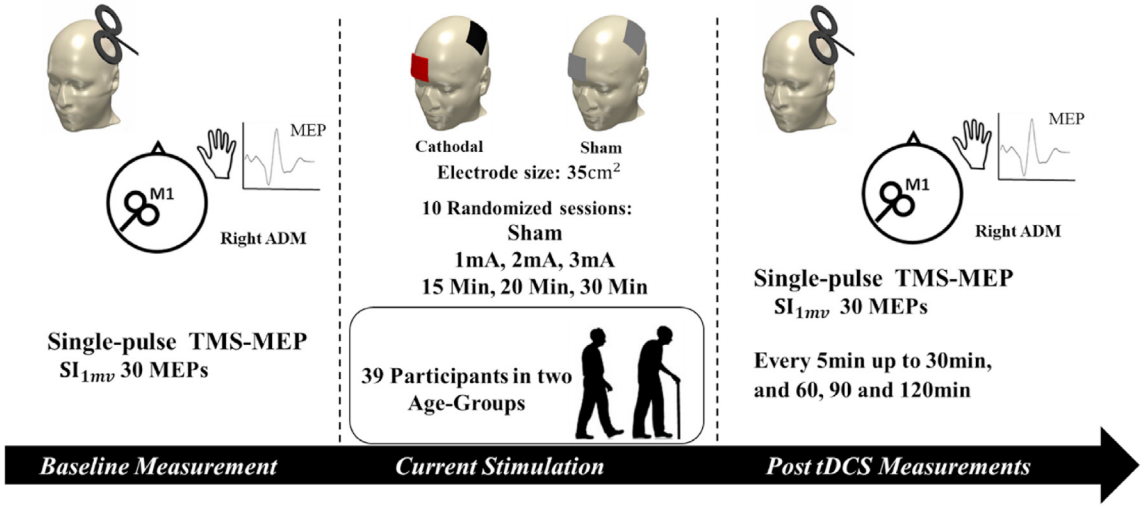

Fig. 1. Study course. Single-pulse TMS was conducted at a frequency of 0.25 Hz over the left motor cortex. The representational area of the right ADM, in which the largest MEPs were produced, was identified first. The intensity of the TMS pulses was adjusted to elicit MEPs with a peak-to-peak amplitude of 1 mV (SI_{1mV}) on average before intervention, and kept constant until the end of the session. Baseline cortical excitability was determined by measuring 30 MEPs. Afterwards, sham, or cathodal real tDCS was applied in one of three different intensities (1, 2 and 3 mA) and durations (15, 20 and 30 min), in random order in 20 Pre-Elderly and 19 Elderly participants. The after-effects were monitored with TMS-induced MEPs (each time point with 30 MEPs) every 5 min up to 30 min, then 60 min, 90 min, 120 min after tDCS.

normalized post-stimulation MEP amplitudes of all time-points were grand-averaged and pooled into two epochs: 30min after stimulation (early epoch), and 60–120min after stimulation (late epoch). Then, to test if the respective active stimulation condition effects differed from those of sham stimulation, and between age groups, a mixed model ANOVA was calculated with 'Condition' (10 levels) and 'Epoch' (3 levels) as the within-subject factors, 'Age-Group' as the between-subject factor, and normalized post-stimulation MEPs as the dependent variable. In addition, to disentangle the effects of tDCS intensity and duration, a mixed-model ANOVA was calculated with normalized MEPs as the dependent variable, 'Intensity' (3 levels), 'Duration' (3 levels), and 'Epoch' (3 levels) as within-subject factors, and 'Age-Group' as between-subject factor. Furthermore, we examined the effect of 'Session-Order' as a covariate in ANCOVA analyses conducted separately for all dosage combinations with 'Session-Order' as the covariate, 'Age-Group' as between-, and 'Epoch' as within-subject factors. Additionally, we conducted an ANCOVA to test a possible effect of session/condition interval duration on the results defining 'Epoch' as within-subject factor (3 levels), 'Age-Group' as the between-subject factor, and 'Session-Interval' as a covariate. Eventually, to explore a possible effect of SI_{1mV}, and thus baseline motor cortex excitability on tDCS-driven motor cortical excitability alterations, first we averaged the 10 SI_{1mV} of one participant over sessions, then the mixed model ANCOVA with 'Condition' (10 levels) and 'Epoch' (3 levels) as the within-subject factors, 'Age-Group' as the between-subject factor, and 'SI_{1mV}' as covariate was calculated.

2.5.3. Assessment of tDCS side-effects, and blinding

After each session, participants were asked to fill in a questionnaire which contained: (1) their guess as to which intensity of tDCS was applied (0, 1, 2, and 3 mA), (2) rating scales for the presence, and amount, of visual phenomena, itching, tingling and pain during stimulation, and (3) rating scales for the presence, and amount, 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 of sensations from zero ('none') to five ('extremely strong'). To identify whether participants correctly guessed tDCS intensities, a Chi-square test was conducted. The presence of side-effects during and after tDCS was analysed separately for each side effect by mixed-model ANOVAs with 'Condition' (10 levels) as the within-subject factor, 'Age-Group' as the between-group factor and rating scores (0–5) as a dependent variable. In case of significant effects, follow-up exploratory post-hoc paired t-tests were conducted to examine if an active tDCS session resulted in a significant difference in sensation relative to sham tDCS.

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 ANOVA results, exploratory *post-hoc* Fisher's Least Significant Difference (LSD) tests were conducted. Statistical analyses were performed with SPSS (IBM Corp. Version 27.0).

3. Results

All participants attended all experimental sessions, except for one participant in the Elderly group that dropped out due to the COVID-19 pandemic. In addition, because of the COVID-19 lockdown, some experimental sessions had to be postponed for nine participants. Therefore, single inter-session intervals between experimental sessions had to be extended for up to 120 days (actual session intervals reported in the supplementary material, table S1).

The results of the MOCA test were in the normal range for all participants without significant differences between the two groups. In addition, a Mann-Whitney *U* test indicated no significant between-group differences for 'Physical Activity Level' (Table 1). (For the results of the distribution of participants to Chronotype, please refer to supplementary materials. table S2).

3.1. No difference of SI_{1mV} and baseline MEPs between conditions, and participant groups

Baseline MEP and SI_{1mV} are listed in Table 3. The respective ANOVA results showed no significant differences of SI_{1mV} for the factors 'Condition' ($F_{(4.870,\ 180.183)} = 1.463$, $p = 0.206$, $\eta_p^2 = 0.038$), 'Age-Group' ($F_{(1,\ 37)} = 1.020$, $p = 0.319$, $\eta_p^2 = 0.027$) and their interaction, 'Condition' × 'Age-Group' $F_{(4.870,\ 180.183)} = 1.099$, $p = 0.362$, $\eta_p^2 = 0.029$). Furthermore, the ANOVA conducted for baseline MEPs did not show significant differences for 'Condition' ($F_{(9,\ 333)} = 0.553$, $p = 0.835$, $\eta_p^2 = 0.015$), 'Age-Group' ($F_{(1,\ 37)} = 3.546$,

Table 2
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 showed no significant differences of baseline MEP and SI_{1mV} across sessions, and between Age-Groups.

Age Group	Experimental Session	Baseline MEP	SI_{1mV} (%)
Pre-Elderly	Sham	1.00 ± 0.12	60.75 ± 12.45
	1 mA-15min	1.06 ± 0.14	59.60 ± 13.35
	1 mA-20min	1.02 ± 0.09	59.65 ± 13.36
	1 mA-30min	1.04 ± 0.10	59.60 ± 12.43
	2 mA-15min	1.01 ± 0.10	59.60 ± 12.97
	2 mA-20min	1.01 ± 0.11	59.70 ± 13.46
	2 mA-30min	1.05 ± 0.12	59.70 ± 13.23
	3 mA-15min	1.06 ± 0.11	58.67 ± 13.51
	3 mA-20min	1.01 ± 0.09	59.82 ± 13.50
	3 mA-30min	1.02 ± 0.08	59.20 ± 13.03
Elderly	Sham	1.06 ± 0.13	55.78 ± 11.70
	1 mA-15min	1.04 ± 0.11	55.78 ± 12.56
	1 mA-20min	1.05 ± 0.07	55.52 ± 12.19
	1 mA-30min	1.02 ± 0.07	55.42 ± 12.38
	2 mA-15min	1.05 ± 0.10	55.34 ± 12.57
	2 mA-20min	1.11 ± 0.09	55.21 ± 12.38
	2 mA-30min	1.06 ± 0.11	55.63 ± 12.58
	3 mA-15min	1.06 ± 0.12	55.63 ± 12.37
	3 mA-20min	1.08 ± 0.15	55.47 ± 12.13
	3 mA-30min	1.04 ± 0.12	55.47 ± 12.40

Table 1
Demographic characteristics of participants. Mean age (±SD), mean MOCA score (±SD): comparisons between groups were performed using *Student's* unpaired t-tests for 'Age' and 'MOCA Score'. For physical activity level (ranging from 1.0 [low] to 4.0 [high]), a Mann-Whitney *U* test was conducted.

Factor	Pre-Elderly	Elderly	Test	*P* Value
Age (years)	11 females/58.65 ± 3.86	10 females/72.68 ± 5.12	*Student's* unpaired *t*-test	*P<0.001**
MOCA score	27.78 ± 1.90	27.11 ± 1. 67	*Student's* unpaired *t*-test	*P= 0.522*
Physical Activity Level	1.85	1.89	*Mann-WhitneyU* test	*P= 0.825*

$p = 0.068$, $\eta_p^2 = 0.087$), and the respective interaction, 'Condition' × 'Age-Group' ($F_{(9, 333)} = 1.275$, $p = 0.249$, $\eta_p^2 = 0.033$).

3.2. tDCS effects on motor cortex excitability

The respective mixed-model ANOVA was conducted to test if the respective active stimulation conditions results differ from those of sham stimulation, and if results differ between age groups. The results showed significant main effects of 'Condition' ($F_{(9, 333)=}9.641$, $p < 0.001$, $\eta_p^2 = 0.207$), and 'Epoch' ($F_{(2, 74)} = 41.310$, $p < 0.001$, $\eta_p^2 = 0.528$)', but not 'Age-Group' ($F_{(1, 37)} = 3.883$, $p = 0.056$, $\eta_p^2 = 0.095$). In addition, the results revealed significant interactions of 'Condition' × 'Age-Group' ($F_{(9, 333)} = 4.097$, $p < 0.001$, $\eta_p^2 = 0.100$), 'Condition' × 'Epoch' ($F_{(18, 666)} = 6.467$, $p < 0.001$, $\eta_p^2 = 0.149$), and 'Condition' × 'Epoch' × 'Age-Group' ($F_{(18, 666)} = 2.524$, $p < 0.001$, $\eta_p^2 = 0.064$) (Table 3A). The post-hoc tests comparing active tDCS conditions with the effects of sham stimulation *for the Pre-Elderly group* revealed a significant reduction of MEP amplitudes after 1 mA-15min, 1 mA-20min, 1 mA-30min, 3 mA-15min, 3 mA-20min, and 3 mA-30min (all conditions for early and late epochs), while MEP amplitudes were enhanced after 2 mA-20min (only in the early epoch), but unaltered after 2 mA-15min and 2 mA-30min. However, *for the Elderly group*, cortical excitability reductions were observed after 1 mA-15min, 1 mA-20min, 1 mA-30min, 3 mA-15min, 3 mA-20min and 3 mA-30min (all in the early epoch), but no significant MEP alterations emerged for the 2 mA-15min, 2 mA-20min and 2 mA-30min stimulation dosages. In addition, the post-hoc tests comparing tDCS conditions between age groups showed significant larger MEP after 2 mA-20min and 2 mA-30min cathodal tDCS in the Pre-Elderly in comparison to the Elderly group in both early and late epochs (Fig. 2).

The mixed-model ANOVA conducted to investigate the effects of different stimulation intensities and durations revealed significant main effects of 'Intensity' ($F_{(2, 74)=}28.873$, $p < 0.001$, $\eta_p^2 = 0.438$), 'Epoch' ($F_{(2, 74)} = 48.021$, $p < 0.001$, $\eta_p^2 = 0.565$), but not 'Age-Group' and 'Duration'. In addition, the results revealed significant 'Intensity' × 'Age-Group' ($F_{(2, 74)} = 13.251$, $p < 0.001$, $\eta_p^2 = 0.264$), 'Intensity' × 'Epoch' ($F_{(4, 148)} = 18.398$, $p < 0.001$, $\eta_p^2 = 0.332$), and 'Intensity' × 'Epoch' × 'Age-Group' ($F_{(4, 148)} = 7.704$, $p < 0.001$, $\eta_p^2 = 0.172$) interactions (Table 3B). Post hoc tests (Fisher LSD, $p < 0.05$) comparing tDCS intensities within each age group revealed a significant difference between 1 mA and 2 mA, 2 mA and 3 mA stimulation intensities in the Pre-Elderly group, but not in Elderly group. 1 mA and 3 mA resulted in excitability reduction, while 2 mA intensities resulted in excitability enhancement.

For the post-hoc tests comparing active tDCS protocols with respective baseline values, *the Pre-Elderly group* showed a significant reduction of motor cortical excitability after 1 mA-15min, 1 mA-20min, 1 mA-30min, 3 mA-15min, 3 mA-20min, and 3 mA-30min (for both, early and late epochs), but a motor cortical excitability enhancement was observed after 2 mA-20min in both early and late epochs, and no significant changes of cortical excitability were observed for the 2 mA-15min and 2 mA-30min conditions. F*or the Elderly group*, a reduction of cortical excitability was observed for the 1 mA-15min (early and late epoch), 1 mA-20min and 1 mA-30min stimulation conditions (only early epoch), and the 2 mA-20min, 2 mA-30min, 3 mA-15min, 3 mA-20min, and 3 mA-30min conditions (for both, early and late epochs). 2 mA-15min resulted in no significant alteration of cortical excitability. For an overview of the non-epoched data, please refer to Fig. 3 (furthermore, the individual data distribution is shown in the supplemental material, figure S1.).

The results of the ANCOVAs examining the effect of session order for the respective outcomes revealed no significant effects of 'Session-Order' or the 'Epoch' × 'Session-Order' interaction (all $p > 0.1$). The results of the ANCOVAs examining the effect of session/condition intervals for the respective outcomes revealed no significant effects of 'Session interval' or the respective interactions (all $p > 0.08$). Finally, to examine the effect of SI_{1mV} on motor cortical excitability alterations, we conducted a mixed model

Table 3
Effect of age group, and stimulation dosage on early and late tDCS effects, epoched data. The results of the respective mixed-model ANOVAs are shown. Asterisks indicate significant results ($p < 0.05$). 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 overall tDCS effects versus sham, epoched data (Early and late effects)	Condition	9, 333	9.641	0.207	<0.001*
	Condition × Age-Group	9, 333	4.097	0.100	<0.001*
	Epoch	2, 74	41.310	0.528	<0.001*
	Epoch × Age-Group	2, 74	2.461	0.062	0.092
	Condition × Epoch	18, 666	6.467	0.149	<0.001*
	Condition × Epoch × Age-Group	18, 666	2.524	0.064	<0.001*
	Age-Group	1, 37	3.883	0.095	0.056
B) Effect of age on the impact of different tDCS intensities and durations, epoched data (Early and late effects)	Intensity	2, 74	28.873	0.438	<0.001*
	Intensity × Age-Group	2, 74	13.251	0.264	<0.001*
	Duration	1.725, 63.834	0.447	0.012	0.613
	Duration × Age-Group	1.725, 63.834	1.362	0.035	0.262
	Epoch	2, 74	48.021	0.565	<0.001*
	Epoch × Age-Group	2, 74	2.767	0.070	0.069
	Intensity × Duration	4, 148	1.051	0.028	0.383
	Intensity × Duration × Age-Group	4, 148	0.987	0.026	0.417
	Intensity × Epoch	4, 148	18.398	0.332	<0.001*
	Intensity × Epoch × Age-Group	4, 148	7.704	0.172	<0.001*
	Duration × Epoch	4, 148	0.464	0.012	0.762
	Duration × Epoch × Age-Group	4, 148	0.753	0.020	0.557
	Intensity × Duration × Epoch	8, 296	1.575	0.041	0.132
	Intensity × Duration × Epoch × Age-Group	8, 296	0.777	0.21	0.623
	Age-Group	1, 37	3.523	0.087	0.068

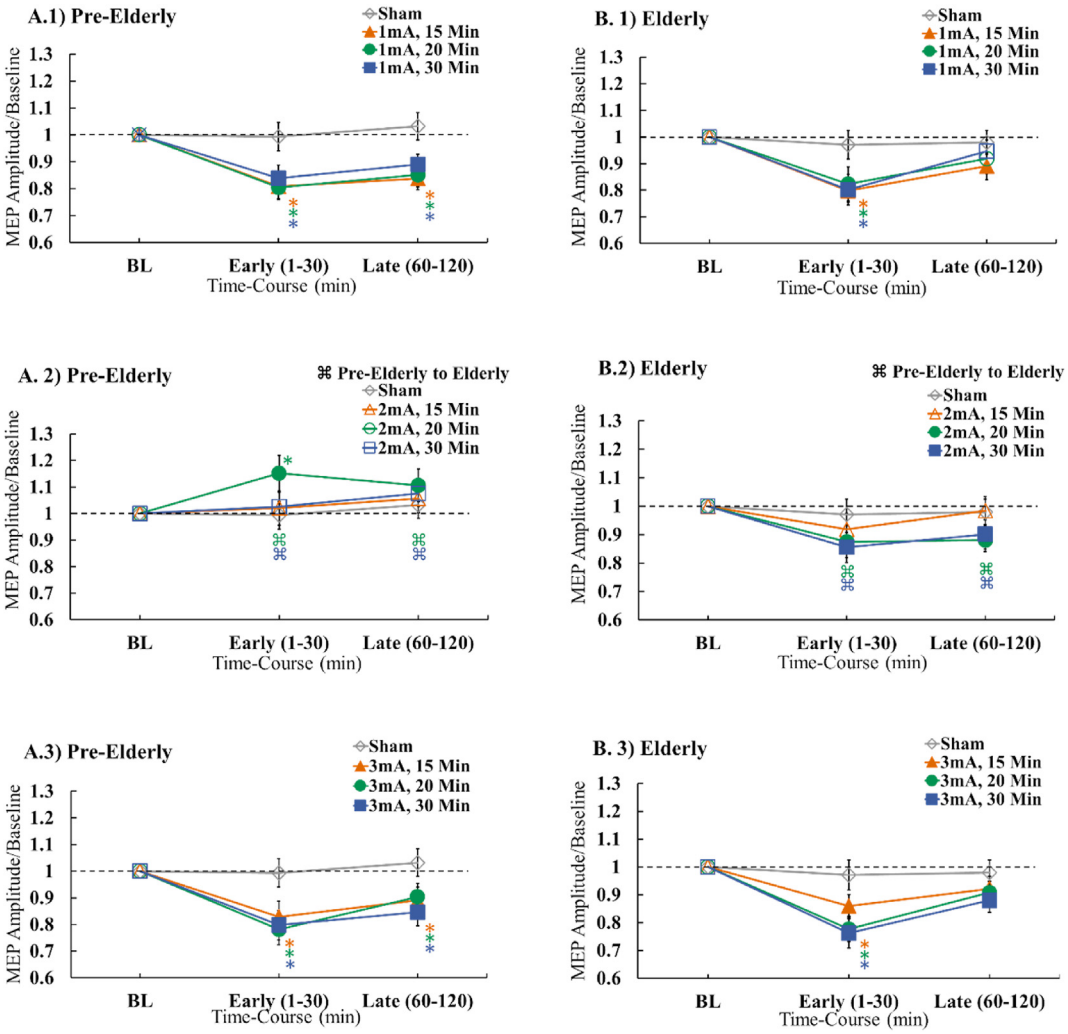

Fig. 2. Pooled MEP amplitudes for early and late post-tDCS effects. Grand-averaged MEPs were pooled into two time points of early (0–30 min) and late (60–120 min) excitability changes. Error bars represent standard error of means. A.1- A.3 for Pre-Elderly and B.1- B.3 for Elderly divided by Intensities. Filled symbols (■) indicate a significant difference of cortical excitability versus the respective baseline values. Asterisks (*) refer to each sub-figure, and indicate a significant difference between the respective active and sham stimulation condition. Significant differences between age groups are indicated by (⌘). BL= Baseline.

ANCOVA. The respective results showed no significant impact of SI_{1mV} on the outcomes (more details are provided in the supplementary material, table S3.).

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

The chi-square test revealed no significant heterogeneity with respect to blinding, suggesting successful blinding (Table 4). The results of the ratings of self-reported versus actual received stimulation intensities are reported in table S4 of the supplementary material.

Participant ratings for the presence and intensity of side effects during and within 24 h after stimulation are shown in Table S5 of the supplementary material. The mixed model ANOVAs showed no significant difference in the side effect ratings during or 24h after the end of stimulation, except for the tingling sensation, where higher scores of sensation were rated in Pre-Elderly compared to Elderly participants (Table S6 in the supplementary material). Further, Pearson correlations conducted for the tingling sensation with respective early epoch MEP amplitudes showed no significant correlation MEPs with tingling sensation for each dosage (please refer to supplementary material, Table S7).

4. Discussion

The main results of this study show that cathodal tDCS over the motor cortex in healthy humans with advanced age reduce cortical excitability with 1 and 3 mA stimulation intensity, with longer lasting effects in the Pre-Elderly group. Moreover, a non-linear pattern of plasticity induction was observed in both age groups. In contrast to stimulation with 1, and 3 mA, stimulation with 2 mA did not reduce cortical excitability in both age groups, and enhanced cortical excitability in the 20 min stimulation duration condition selectively in the Pre-Elderly group. In addition, all participants tolerated the intervention well, and blinding was successful.

For the *Pre-Elderly group*, the low (1 mA) and high (3 mA) intensity protocols resulted in an LTD-like plasticity lasting for about 120min after the end of stimulation, while for 2 mA protocols LTP-like plasticity induction (for 20min intervention duration) or no significant effects on MEP amplitudes (for 15min or 30min stimulation duration) were obtained. For the *Elderly group*, the low (1 mA) and high (3 mA) intensity protocols resulted in LTD-like plasticity for about 30min after tDCS, while for the 2 mA protocol a slight LTD-like plasticity (with 20min and 30min stimulation

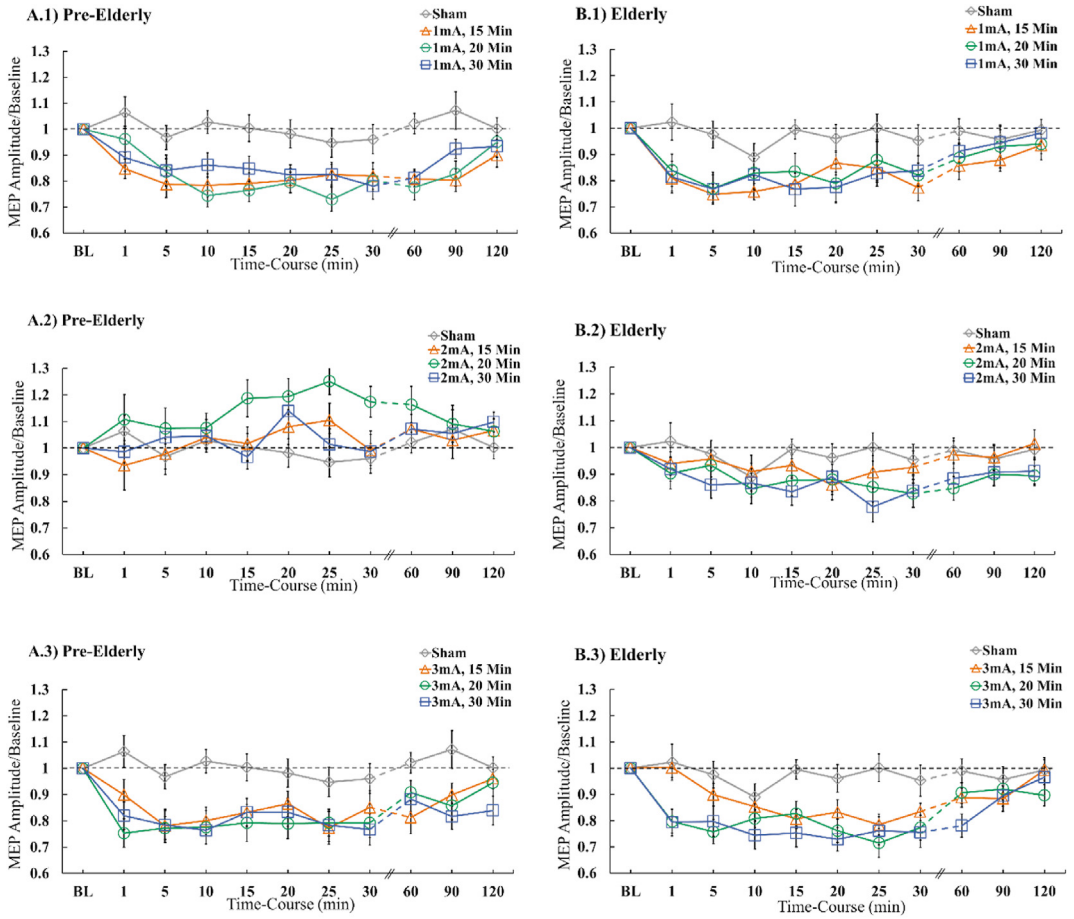

Fig. 3. Motor cortical excitability alterations induced by cathodal tDCS in Pre-Elderly (A1-3) and Elderly (B1-3) groups, non-grouped data. The time-series graphs show baseline-normalized MEPs, measured for up to 120 min after tDCS. Figures are grouped based on stimulation intensities: 1 mA (A1, B1), 2 mA (A2, B2) and 3 mA (A3, B3) with stimulation durations of 15, 20, and 30 min for each intensity, and sham tDCS. Error bars represent standard error of means. BL= Baseline.

duration compared to baseline excitability, but not the sham stimulation condition), or no significant effects on cortical excitability (for tDCS with 15min duration) were observed. The effects of 2 mA stimulation for 20min differed significantly between the two groups. While LTP-like plasticity was observed in the Pre-Elderly group, the same protocol resulted in minor LTD-like effects (significant vs baseline, but not the sham stimulation condition) in the Elderly population.

The results obtained in this study for the Pre-Elderly group are in accordance with previous findings in young healthy participants, in which intensity-dependent non-linear after-effects of cathodal motor tDCS were reported. Protocols with low intensity (1 mA-15min and 1 mA-20min) and high intensity (3 mA-20min) cathodal tDCS resulted in LTD-like plasticity, while an excitability enhancement was observed in conditions with 2 mA intensity for 20min [33,34]. The results are furthermore generally in line with results of a former study of our group with respect to the observed LTD-like plasticity after 1 mA-15min [37]. However, no neurophysiological data were available so far for 1 mA stimulation with longer duration or the higher cathodal motor tDCS intensities used (2 mA and 3 mA).

Table 4
Chi-square test results of responses about stimulation intensities (guessed vs actual).

		χ2 Value	d.f.	P value
Pearson Chi-Square				
	Pre-Elderly	11.056	6	0.087
	Elderly	6.609	9	0.678
	All	8.304	9	0.504

It should be noted that while it has been reported in some studies that advancing age increases motor threshold [50], no significant SI_{1mV} intensity differences between age groups were identified in the present study. This is similar to parallel studies of our group [37,38] and other groups [37,51–54]; furthermore SI_{1mV} had no significant impact on our results. Reasons which might explain this inconsistency are inter-individual variability of demographic parameters (e.g. genetic profile, sex, and age), and differences of inclusion criteria for recruiting participants between studies (e.g. smoking, taking medication, physical activity level), which can affect baseline cortical excitability [55].

4.1. Proposed mechanisms of action

Neuroimaging and pharmacological studies, in young healthy humans, have shown that the plasticity induction by tDCS, at the cellular level, is driven by the glutamatergic system, and involves NMDA receptors, which have calcium channel properties [56–60]. In addition, animal studies have demonstrated a dependency of the direction of plasticity from the amount of neuronal calcium influx [61]. Low-level Ca^{2+} influx has been shown to induce LTD, whereas a moderate calcium enhancement results in no synaptic modulation, a larger increase induces LTP, and maximum calcium influx might again abolish or convert plasticity due to counter-regulatory mechanisms [62,63]. Calcium dynamics might be thus a good

candidate mechanism to explain the dosage-dependent effects of tDCS.

These dynamics can not only explain dosage-dependent effects of cathodal tDCS, but also gradual age-dependent differences observed in the present study, since calcium dynamics are altered in higher age due to a decline of glutamatergic transmitter availability [64,65], and amount of calcium channels [66,67]. For the Pre-Elderly group, the results of the study are comparable to those obtained in young adults [34], and thus a relevant age-dependent decline of calcium influx most likely does not apply in this group. Here we suggest that 1 mA tDCS resulted in an LTD-inducing low calcium concentration, 2 mA in a calcium concentration sufficient for the induction of LTP-like plasticity, and 3 mA in LTD-like plasticity due to calcium overflow-induced counterregulatory mechanisms. Respective calcium-dependency of the effects of cathodal tDCS effects for the 3 mA stimulation condition have been recently described in young adults [59]. However, in contrast to the Pre-Elderly group, increasing tDCS dosage resulted in an almost uniform cortical excitability reduction for the Elderly population. One possible explanation for this effect is the decline of glutamate and calcium channel availability in the elderly, which will reduce calcium influx due to tDCS, and prevent calcium influx to an amount sufficient for the induction of LTP-like plasticity for the higher stimulation intensities. At present, these explanations are however speculative, and do not account fully for all details of the results. If decreased calcium influx in the elderly group would have been the only driving force for the limited conversion of tDCS effects on cortical excitability, a conversion effect would have been expected for stimulation with 3 mA in this age group, which did not take place. A more general reduction of the propensity to develop LTP-like plasticity in higher age was observed in animal models [68], and documented in a recent tDCS study in humans [37] which might contribute to this limited conversion. Furthermore, the re-establishment of LTD-like plasticity by stimulation with 3 mA might also be caused by effects of tDCS with this intensity on deeper cortical layers, which might not be affected by low intensity stimulation.

Apart from the calcium hypothesis mentioned above, other age-dependent neurophysiological and -anatomical changes might contribute to differences in the brain's responsiveness to stimulation [69]. This includes alterations of neurotransmitters, and -modulators, such as reduced availability of glutamate [64,70], GABA [21], dopamine [20], acetylcholine, serotonin, noradrenaline [71], which all have been shown to alter tDCS-generated plasticity responses [69,72]. Moreover, at the macroscale anatomical level, age-dependent cortical atrophy increases electrode to brain distance, which gradually reduces electrical field intensity at the target level [36], and at the microscale level, age-dependently altered electric parameters of dendrites [73] might reduce the responsiveness of neurons to an externally applied electrical current [74] as well as alter the distribution of current density [75,76]. These reasons for the gradually altered plasticity response to tDCS in higher age are speculative at present, and should be explored systematically in future studies.

4.2. Limitations and future directions

Some limitations of this study should be taken into account. First, this was an exploratory study. Data were acquired from a relatively small sample of 20 participants in each group over a couple of sessions involving different interventions. Thus, results are preliminary, and should be replicated in follow-up studies with larger samples. In addition, this study was performed in a sham-controlled single-blinded design. A double-blind design would have been preferable to prevent any observer bias more definitively. However, this would not be trivial due to the study's design, which included excitability measures immediately before and after stimulation with different durations. Furthermore, as mentioned above, we did not explore neurobiological mechanisms underlying age-dependent alterations of tDCS-induced neuroplasticity or investigate other aspects affecting the physiological impact of stimulation. Moreover, in neurological and psychiatric disorders, alterations of cerebral structures and functions, as well as altered neurotransmitter, and -modulator activities could affect the parameter range for optimal stimulation.

Furthermore, a possible limitation would be an excitability alteration induced by the TMS protocol applied to monitor excitability itself. Previous studies have however shown stable TMS single pulse MEPs over 24 h [77], that rTMS at the frequency of 0.25Hz induces no plasticity [78,79], and related studies of our own group also identified no effect of this TMS protocol on cortical excitability [80,81]. In addition, the sham tDCS session results in the present study show unaltered motor cortex excitability, which makes an impact of TMS itself on cortical excitability unlikely.

The proposed mechanisms of age-dependent plasticity alterations in the present study are speculative at present. They should be substantiated by more detailed mechanistic explorations in future, including monitoring the effects of pharmacologically defined systems by respective TMS protocols, magnetic resonance spectroscopy, and modelling studies exploring age-dependent differences of electrical field intensity, which might be caused by cerebral atrophy in higher age. The transfer of the physiological effects obtained in the present study to cognitive, and behavioural processes should not be taken for granted, and should be explored in future studies.

To maximize the comparability of results between age groups for exploring age-related plasticity alterations, we specified strict inclusion criteria (no relevant neurological, psychiatric, or internal organ disorders, no smoking, and no CNS-active medication). As a result, the generalizability of the results to the general elderly population might be limited. Consequently, the results obtained in the present study might also not be one-to-one transferable to clinical populations. Finally, the present study was conducted in the primary motor cortex, and taking into account anatomy differences, receptor- and neurotransmitter availability, and target-to-cortex distances for other areas, a one-to-one transferability of the results to other stimulation targets obtained in the present study cannot be taken for granted, and should be explored in future studies.

5. Conclusion

In the present study we expanded the parameter space of cathodal tDCS regarding current intensity and stimulation duration (up to 3 mA and 30min) while investigating age-related differences of tDCS-induced neuroplasticity in the motor cortex of healthy older adults from two different age groups. tDCS induced LTD-like plasticity in both age groups. However, the non-linear conversion effect of cathodal tDCS with an induction of LTP-like plasticity for a stimulation intensity of 2 mA was only observed in the Pre-Elderly group. These results might be explained by reduced glutamatergic activity in higher age, which reduces neuronal calcium influx, which is critical for plasticity induction in higher age. The results of the present study supplies relevant information for optimization of tDCS protocols to reduce cortical excitability. They might also help to improve the efficacy of tDCS as a therapeutic tool for the treatment of neurological and psychiatric disorders in elderly patients. However, a one-to-one transferability of these effects to other cortical areas and patient populations should not be taken for granted due to the state-dependency of tES effects, anatomical

differences, and differences of neuromodulator activities and cortical excitability between healthy humans and respective patients.

Declaration of competing interestCOI

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.

CRediT authorship contribution statement

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2022.01.011.

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