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ORIGINAL ARTICLE

Safety and effects on motor cortex excitability of five anodal transcranial direct current stimulation sessions in 24 hours

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Summary
Background and objective. — Application parameters of transcranial direct current stimulation (tDCS) for therapeutic purposes are relatively restricted. The aim of this study was to assess safety and effects on motor cortex excitability of an intensive anodal-tDCS protocol.
Methods. — In 26 healthy subjects, five 15-minute anodal-tDCS sessions were delivered, at increasing time intervals, over 24 hours. Safety was defined as absence of serious adverse events including brain tissue alterations on magnetic resonance imaging. Effect on motor cortex excitability was evaluated by motor evoked potential (MEP) amplitude, measured eight times.
Results. — No serious adverse events occurred. Mild adverse events, such as reversible scalp erythema or transient metallic taste, were observed in 27% of subjects. MEP amplitudes did not change in any of the recording periods. When inter-individual variability was taken into account and threshold values defined, 50% of subjects were classified as responders, 15% were inverse responders, and 35% non-responders. In the responders, normalized MEP was increased by 57% 1 hour after the first anodal-tDCS and increased by 50% three hours after two stimulations delivered 1 hour apart. Intra-individual, inter-sessional consistency of MEP response over four measurements was 61—77%.

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Discussion. — Five anodal-tDCS delivered in 24 hours are safe and well tolerated, expanding the safety standard of tDCS. However, only half of subjects respond to anodal-tDCS with a robust and durable MEP augmentation. On the other hand, the response to a single anodal-tDCS predicts fairly well the response to other sessions in the same subject.
Conclusions. — These findings should be considered in clinical trials utilizing repeated anodal-tDCS.

Introduction

Transcranial direct current stimulation (tDCS), a non-invasive brain modulation technique that can induce long lasting changes in cortical excitability, has been increasingly used for therapeutic purposes in neurological and psychiatric diseases [14]. Moreover, several reports have suggested that tDCS can improve memory, verbal and mathematical skills, and performances in other fields such as sports and even military training [4,7,9,18,25].

The common view is that anodal-tDCS enhances cortical excitability, as assessed by motor evoked potentials (MEPs) after transcranial magnetic stimulation (TMS), whereas cathodal-tDCS decreases cortical excitability [20,21]. However, the reality is much more complex. To obtain greater effects, duration and intensity of stimulations have been increased, but more is not always better. Indeed, doubling the duration of anodal-tDCS may decrease cortical excitability; in addition, doubling the intensity of cathodal stimulation may convert an inhibitory effect into an excitatory one [2,19]. Another way to increase the after effects is to employ repeated stimulations. In a previous paper, we assessed the effects on motor cortex excitability of five cathodal-tDCS sessions delivered over 25 hours [30]. Goal of this study was to explore the possibility of safely employing closely repeated cathodal-tDCS, which could be relevant for example in the treatment of epilepsy, or in the prospective of translating into clinical practice the neuroprotective effects found in the acute stroke phase in rodents [30]. Since in therapeutic applications anodal stimulation is much more frequently employed than cathodal [14], we planned a study that would also assess the safety and effects on motor cortex excitability of five anodal-tDCS sessions in 24 hours.

Methods

Subjects

The study was carried out in accordance with the Declaration of Helsinki and approved by the Ethics Committee of ''G. d'Annunzio'' University of Chieti-Pescara. All subjects signed written informed consent. Exclusion criteria included standard contraindications for tDCS and TMS [3,26]. Twenty-six healthy volunteers (12 males, aged 25.7 ± 6.1 years), were enrolled in the study. Handedness was ascertained using the Edinburgh Handedness Inventory [24].

Study plan

The experimental plan is shown in Fig. 1. Anodal-tDCS was delivered five times over a 24-hour period at increasing time intervals. Transcranial magnetic stimulation (TMS) was performed 8 times: immediately before each one of the five anodal-tDCS: (T0, T2, T3, T4, T6), immediately after the first and fourth stimulation (T1 and T5), and two hours after the fifth stimulation (T7). Magnetic resonance imaging (MRI) was performed three times: the day before the first stimulation, within 30 min after the third and within 3 hours after the fifth anodal-tDCS.

Safety and tolerability

Safety was defined as the absence of serious adverse events described as severe or medically significant events including the requirement of hospitalization and brain tissue alterations detectable by MRI and related to tDCS [1,3]. Safety and tolerability were assessed by a modified Italian version of a questionnaire proposed by Fertonani et al., 2015 [8,30]. To evaluate the local effect, the skin under the electrodes was examined before and after each stimulation.

Magnetic resonance imaging

MRI was performed using a 3 Tesla scanner equipped with a 8-channel SENSE receiver head coil (Philips Achieva, The Netherland). First a 3D acquisition of the whole brain was obtained using a 1 mm T1-weighted FFE sequence. Then 3 mm axial T2-weighted fluid-attenuated inversion recovery (T2w FLAIR), DWI and susceptibility-weighted (SWI) images were obtained. FLAIR, DWI and SWI images were examined in a real time manner by an experienced neuroradiologist (MC) to rule-out cytotoxic edema (using DWI) and microhemorrhages (using SWI), [6,27] to halt the protocol if necessary and minimize any risk to the participants.

Anodal transcranial direct current stimulation

Direct current of 2 mA intensity was delivered using a battery-driven constant-current stimulator (BrainSTIM E.M. S. Bologna, Italy) for 15 min. The electrode (7×10 cm) connected with the anode was positioned over the dominant hemisphere with the 7 cm long side parallel to the central sulcus and centered on the optimal cortical representation

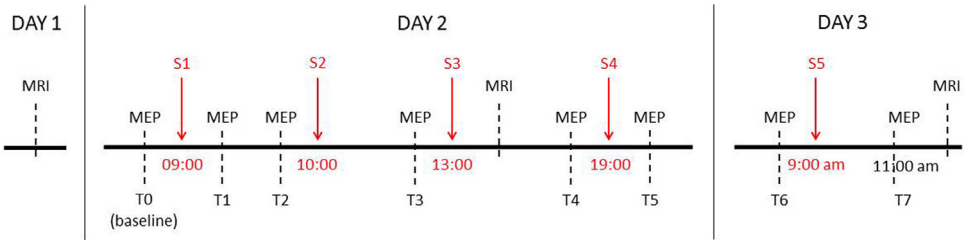

Figure 1 Experimental plan of the study. MEP: motor evoked potential, T: time of electrophysiological recordings; S: time of anodal transcranial direct current stimulation, MRI: magnetic resonance imaging.

of the first dorsal interosseus muscle (FDI), as determined by the highest MEP amplitude recorded from FDI after TMS. The electrode (7×5 cm) connected with the cathode was positioned on the ipsilateral shoulder. At the cephalic electrode the calculated current density was $0.286\,A/m^2$, the charge density for one stimulation was $257\,C/m^2$ and the cumulative charge density of 5 stimulations was $1285\,C/m^2$.

Transcranial magnetic stimulation

TMS was delivered by a Bistim 200[2] stimulator through an 8 cm figure-of-eight coil (Magstim Co, Whiteland, Dyfed, UK) and MEPs were recorded from the FDI muscle contralateral to the stimulated hemisphere as previously reported [30]. The mean peak-to-peak amplitude of 20 MEP trials was calculated at each recording time. For recording times from T1 to T7 MEP amplitudes were normalized to baseline (T0) MEP value. To take into account the inter-individual variability, the standard errors of the mean (SEM) amplitudes were calculated in each subject from 20 MEPs at baseline (T0) for both stimulated and non-stimulated hemisphere and divided for the corresponding individual mean MEP amplitude at T0. Then, the average across subjects of normalized SEM values was calculated (nSEM). The normalized MEP amplitude at T2 was considered to be significantly changed if its value exceeded the 95% confidence interval of inter-individual variability around 1 (the reference value at T0), i.e. the normalized MEP was significantly increased if it was higher than 1 + 1.96*nSEM and decreased if its value was lower than 1−1.96*nSEM [28,30]. In the former case, as following anodal-tDCS a MEP amplitude increase is expected, the subjects were classified as ''responders'' whereas in the latter case they were classified as ''inverse responders''. When the normalized MEP amplitude was between 1−1.96*nSEM and 1 + 1.96*nSEM, subjects were classified as ''non-responders''. For statistical analyses, inverse and non-responders were merged into a single group named ''non/inverse responders''. Although the influence of tDCS can extend beyond the stimulated brain region to connected areas and networks, [12,23] the effects on cortico-motor system have been reported to be limited to the stimulated hemisphere [13]. Therefore, we used as an internal control condition, the MEP values recorded after stimulation of the hemisphere that did not receive anodal-tDCS.

Statistical analysis

The Gaussian distribution of data was verified by the Kolmogorov-Smirnov test. To assess differences in MEP amplitude due to multiple stimulations over time, a repeated-measures analysis of variance (ANOVA) design was performed on MEP amplitude values with Time (T0 to T7) as within-subject factor and Group (responders, non/inverse responders) as between-subject factor. In the case that a significant interaction with the factor Group was found, reduced models were separately applied to responders and combined non/inverse responders, with Hemisphere and Time as within-subject factors. The Greenhouse-Geisser correction was applied when non-sphericity of data was verified. Post-hoc comparisons were computed by paired *t*-test using the Bonferroni correction to take into account multiple comparisons. Normalized MEP amplitudes were judged to be significantly changed from baseline values, by comparing the mean values at each time (T1 to T7) with respect to the value at T0 through the two-tailed *t*-test. Bonferroni correction was applied. Differences of mean values of different groups were assessed by two-tailed independent *t*-tests, separately for different times.

Results

Safety and tolerability

All 26 enrolled subjects completed the study. No serious adverse effects occurred. MRI was performed in the first eight enrolled subjects (all right handed) and the visual inspection of FLAIR, SWI and DWI sequences did not reveal signal abnormalities within 30 min after S3 and 3 hours after S5. All subjects reported mild to moderate tingling or burning sensation under the cephalic electrode that in 3 (11%) subjects lasted during the entire stimulations. Reversible mild scalp erythema, not requiring medical intervention, was observed in 6 (23%) subjects. One subject (4%) complained of transient metallic taste.

MEP amplitude analysis

Anodal-tDCS was delivered on the dominant hemisphere (left in 25 subjects, right in one). At baseline the MEP amplitudes of the stimulated hemisphere (mean ± standard error: $1309 \pm 132\,\mu V$) and of the non-stimulated hemisphere ($1344 \pm 173\,\mu V$) were not different (paired *t*-test $t(25) = -0.212$; $P = 0.834$) (Table 1). The inter-individual variability of MEP amplitudes was high and no significant changes of MEP amplitudes were found at any time in either hemisphere (Table 1, Fig. 2A). Even when MEPs were normalized, anodal-tDCS failed to induce significant

Table 1 Mean (in parentheses the standard error) of MEP amplitudes (μV) for the stimulated and non-stimulated hemisphere in all 26 subjects (whole sample), in the 13 responders and 13 non/inverse responders at the different recording times.

	T0	T1	T2	T3	T4	T5	T6	T7
Stimulated hemisphere								
Whole sample	1309 (132)	1389 (152)	1456 (134)	1572 (184)	1390 (179)	1456 (183)	1313 (167)	1375 (201)
Responders	1082 (121)	**1549*** **(247)**	**1661**** **(183)**	**1737*** **(286)**	1507 (282)	**1700*** **(302)**	1524 (265)	**1674*** **(349)**
Non/inverse responders	1537 (224)	1230 (177)	1252 (188)	1407 (236)	1274 (230)	1213 (196)	1102 (198)	1075 (180)
Non-stimulated hemisphere								
Whole sample	1344 (173)	1118 (136)	1250 (152)	1110 (120)	1203 (156)	1259 (167)	1215 (135)	1348 (183)
Responders	1142 (192)	1084 (175)	1137 (196)	1100 (182)	1180 (237)	1448 (288)	1191 (208)	1099 (173)
Non/inverse responders	1546 (286)	1154 (216)	1362 (237)	1120 (164)	1228 (213)	1070 (169)	1239 (181)	1597 (317)

Values significantly different from T0, as indicated by paired *t*-test, are in bold.
* $P < 0.05$
** $P < 0.005$, Bonferroni corrected.

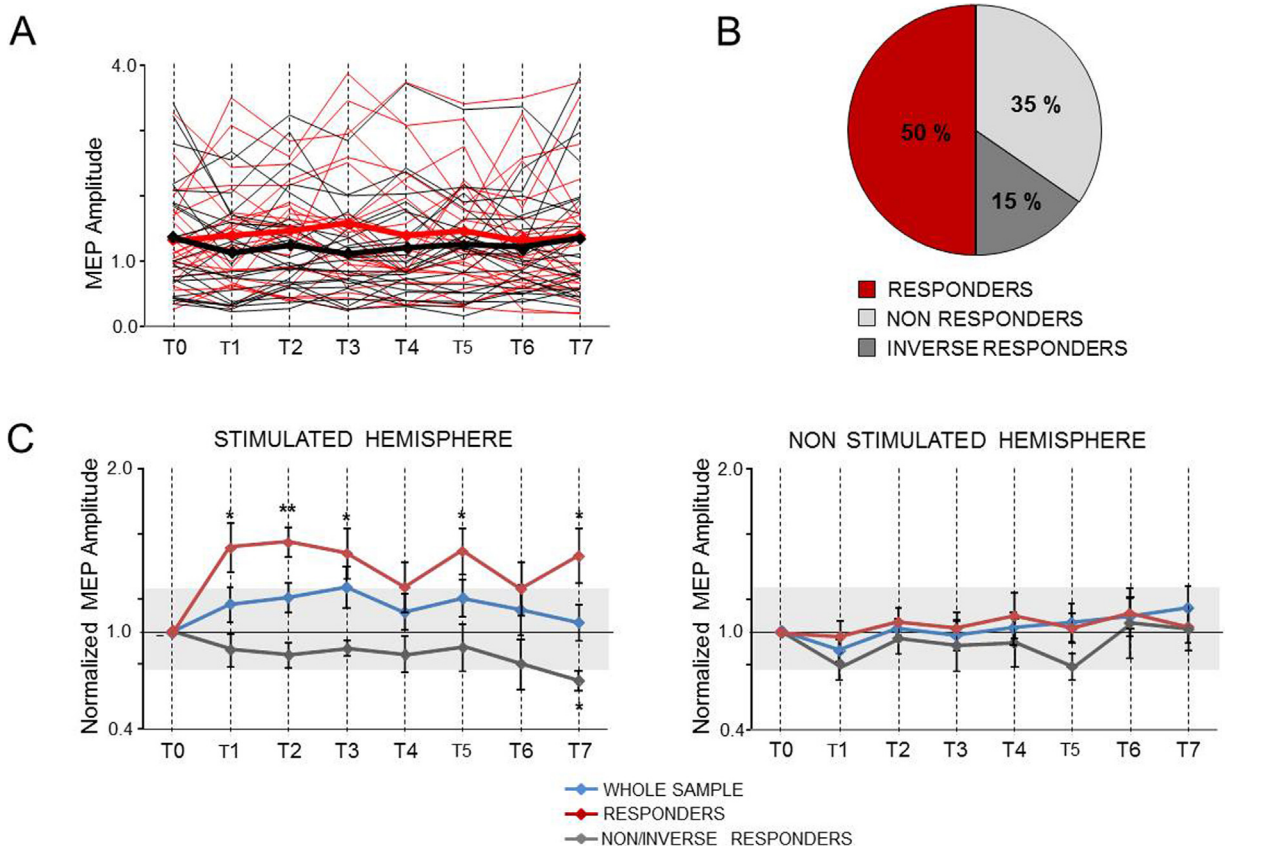

Figure 2 A. Variability of MEP amplitude in the stimulated (red lines) and non-stimulated (black lines) hemispheres at the eight recoding times (T0 to T7). The mean values are shown by thicker lines. B. Pie chart showing the response rate in 26 subjects for the three classification groups. C. Mean values (bars are standard errors) of normalized MEP amplitudes in the whole samples, in responders and non/inverse responders in the stimulated and non-stimulated hemisphere. The grey area represents the inter-individual variability calculated on the basis of the standard error of the mean of MEP amplitudes at T0. The significance of two-tailed paired *t*-test (Bonferroni corrected) with respect to the value of 1 (i.e. a significant variation of the MEP amplitude with respect to the value at T0) is indicated (*$P < 0.05$; **$P < 0.005$).

Table 2

	Factor	GL	F	*P*
Whole	Time	4.3; 103.7	0.440	>0.200
sample	Hemisphere	1; 24	1.760	0.197
	Time*Hemisphere	7; 168	1.784	0.093
	Group	1; 24	0.129	>0.200
	Time*Group	**7; 168**	**3.489**	**0.002**
	Hemisphere*Group	1; 24	2.372	0.137
	Time*Hemisphere*Group	**7; 168**	**2.302**	**0.029**
Responders	Time	7; 84	2.100	0.052
	Hemisphere	1; 12	3.506	0.086
	Time*Hemisphere	**7; 84**	**2.108**	**0.049**
Non/inverse	Time	7; 84	1.482	0.090
responders	Hemisphere	1; 12	0.028	>0.200
	Time*Hemisphere	7; 84	1.992	0.066

Results of the ANOVA design applied on MEP amplitude of the whole sample, with Time (T0 to T7), Hemisphere (stimulated, non-stimulated) as within-subject factor and Group (responder, non/inverse responder) as between-subject factor. Reduced models separately applied to each group with Time and Hemisphere as within-subject factors are also shown. Significant main effects or interactions are evidenced in bold.

changes in the whole sample (Fig. 2C). We then classified the individual motor responses on the basis of SEM of MEPs at T0 [28,30]. The grand average value of nSEM of 26 subjects was 0.23. Thus, the 95% confidence interval of inter-individual variability of normalized MEP was 0.77—1.23. On the basis of these threshold values, the normalized MEP value at T2 was used for the classification of the individual response to anodal-tDCS (Fig. 2B). Thirteen subjects (50%) had normalized MEP >1.23 and were classified as ''responders'', 4 subjects (15%) had normalized MEP <0.77 and were classified as ''inverse responders'', and 9 (35%) subjects had normalized MEP values between 0.77 and 1.23 and were classified as ''non-responders''. On the basis of this categorization we found no differences between the values of MEP amplitude at T0 of responders and non/inverse responders, both in stimulated and non-stimulated hemispheres (independent two-tailed *t*-test $t(24) = -1.674$, $P = 0.107$, $t(24) = -1.189$, $P = 0.246$ respectively). The ANOVA with Hemisphere (stimulated and non-stimulated) and Time (T0 to T7) as within-subject factors and Group (responders, non/inverse responders) as between-subject factor showed a significant triple interaction Time X Group X Hemisphere, as well as a significant Time X Group interaction (Table 2). When ANOVA was separately repeated for the responders and the non/inverse responder groups, a significant Time X Hemisphere interaction was found only in the responders indicating that MEP amplitudes significantly changed over time differently in the two hemispheres and thus dependently on stimulation (Table 2). Indeed, a post-hoc comparison showed that in the stimulated hemisphere MEP amplitudes increased, with respect to T0, at T1, T2, T3, T5, and T7 (Table 1). No difference was found in the non-stimulated hemisphere of the subjects classified as responders (Table 1). Anodal-tDCS induced in the stimulated hemisphere of responders 54% increment of normalized MEP immediately after the first stimulation (T1) and 57% increment 1 hour after (T2) (Fig. 2C). Three hours after the second stimulation (T3) the increment was still 50%. No significant MEP change was found 6 hours after the third stimulation (T4), but MEP facilitation (52%) reappeared soon after the fourth stimulation (T5). At T6, 14 hours after the fourth stimulation, the normalized MEP was not significantly changed but MEP facilitation (49%) was evident 2 hours after the fifth stimulation (T7). The MEP increment at T5 and T7 with respect to the baseline were comparable. In the non/inverse responders group, the normalized MEP at T7 was significantly decreased (Fig. 2C). In the stimulated hemisphere, the normalized MEP values of the responders were increased compared with the values of non/inverse responders at T1 ($P = 0.049$), at T2 ($P < 0.001$), at T3 ($P = 0.011$), at T5 ($P = 0.035$), and at T7 ($P = 0.002$) (*P*-values corrected). Considering the subjects classified as responders, the normalized MEP values of the stimulated hemisphere were greater than the values of non-stimulated hemisphere at T1 ($P = 0.048$), T2 ($P = 0.002$), and T3 ($P = 0.007$) (*P*-values corrected). At T5 and T7, significance did not survive to multiple comparison correction (*P*-vales corrected: $P = 0.090$; $P = 0.082$). The normalized MEP values of non/inverse responders in stimulated and non-stimulated hemisphere were not different ($P > 0.2$) at all times. We also analysed the intra-individual consistency of the subject classification at T2 with three additional recording times (Table 3). Sixty-one percent of the subjects that were classified as responsive and 77% of subjects that were classified as non/inverse responders at T2 maintained their response modality at T1, T5 and T7.

Discussion

In the tDCS protocol employed here, the current and charge density values were respectively 500 and 204 times inferior to the values necessary to induce a cerebral lesion in the rat [15]. The cumulative charge density of five 15 min stimulations in 24 hours was about 40 times inferior to the value of the single stimulation necessary to induce a lesion [15].

Table 3

	T1	T5	T7	Number of subjects (%)
Responders	R	R	R	8 (61%)
(13)	R	R	N-IR	1 (8%)
	R	N-IR	N-IR	1 (8%)
	R	N-IR	R	1 (8%)
	N-IR	R	N-IR	2 (15%)
Non/inv.	N-IR	N-IR	N-IR	10 (77%)
responders (13)	N-IR	R	N-IR	3 (23%)

Intra-individual consistency of MEP response classification at different times. Number (in parentheses the percentage) of subjects classified as responders (R) and non/inverse responders (N-IR) at times T1, T5 and T7 are shown for the group of responders and non/inverse responders classified on the basis of normalized MEP at T2.

As there are issues and concerns on basing human safety standard only on animal histology thresholds [3], it seemed important to verify the safety of closely repeated sessions of anodal-tDCS.

Safety data for tissue injury are usually grouped independently from polarity [3] and we merged the results of the current study with those from a companion study employing five cathodal-tDCS in 25 hours [30] for a total of 58 subjects and 274 sessions. All enrolled subjects completed the study. No serious adverse events occurred and MRI, performed in 33% of subjects, showed no abnormalities. Reversible mild scalp erythema was observed in 19% of subjects and transient metallic taste in 5%. These effects can be classified as mild adverse events [1]. Overall, five closely repeated tDCS can be considered safe and well tolerated and our observations considerably extend the safety standards of tDCS previously obtained with only one stimulation [22].

Regarding the effect on motor cortical excitability, when the whole sample was considered, there was no evidence of MEP amplitude reduction at any time. After taking into account inter-individual variability, it was evident that only half of subjects responded in the expected way, with a significant increase of both absolute and normalized MEP amplitude limited to the stimulated hemisphere. Recent studies utilizing anodal-tDCS, with different stimulation paradigms and employing various methods to classify MEP response according to inter-individual variability, showed that the rate of increased MEP varies from 20 to 60% of subjects [16,17,28,29]. Similarly, in a study employing repeated cathodal-tDCS, we found that only 56% of subjects showed the expected MEP reduction [30]. Particularly worrisome is that in the current study and in the previous one with cathodal-tDCS 15—19% of subjects were inverse responders [30]. This reversal of polarity-dependent effect has been documented in up to 40% of subjects [2,28]. We deem that the high inter-individual variability in the response is a crucial issue in the t-DCS field and likely accounts for absence of group effect and inconsistencies of results that raised doubts on the effective applicability and utility of tDCS [10,11]. Inter-individual variability of the response to tDCS has been mainly assessed by MEP measurement and it is uncertain whether the effect on motor cortex excitability may be extrapolated to behavioral or cognitive tasks. In any case, absence or reversal of polarity-dependent effects should be taken into account at the individual level in therapeutic applications. In responders, MEP augmentation was robust (around 50%) and the effect durable up to at least three hours after two stimulations delivered one hour apart. MEP increment was no longer evident with intervals from the last stimulation of 6 and 14 hours but reappeared when anodal-tDCS was repeated. Although the time intervals of tDCS and MEP recording were slightly different, this time course was very similar to what we observed with repeated cathodal-tDCS [30]. Another critical issue for the therapeutic applications of tDCS is whether the response is replicable over time. We found that, in the short term, the intra-individual consistency of response to repeated anodal-tDCS at four assessments is fairly good and comparable with the results obtained in two separate sessions 6—12 months apart [17]. However, the response reliability was lower than in a similar study with cathodal-tDCS where 88—92% of subjects classified as responders maintained the expected MEP suppression at three assessments [30]. Intra-individual variability may be influenced by the conditions of the nervous system during tDCS and the interaction with task-induced activity task may be even more important than stimulation polarity [5]. It seems that intra-individual variability, at least in terms of MEP response, is lower than inter-individual variability.

Conclusion

This study expands the safety standards of tDCS. MEP amplitudes did not change in the whole sample and only half of subjects responded to anodal-tDCS with the expected increased MEP with a non-negligible percentage even showing the opposite direction, confirming that tDCS effects on cortical excitability are highly variable across different subjects. Intra-individual inter-sessional consistency is fairly good, and in therapeutic trials on the motor system, the result of one tDCS could be used to assess whether a subject is a responder or not, thus avoiding the possibility of an undesirable opposite effect. These results should be considered in trials utilizing repeated anodal-tDCS.

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Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Antal A, Alekseichuk I, Bikson M, Brockmöller J, Brunoni AR, Chen R, et al. Low intensity transcranial electric stimulation: safety, ethical, legal regulatory and application guidelines. Clin Neurophysiol 2017;128:1774—809.

[2] Batsikadze G, Moliadze V, Paulus W, Kuo M-F, Nitsche MA. Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. J Physiol 2013;591:1987—2000.

[3] 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;9:641—61.

[4] Borducchi DM, Gomes JS, Akiba H, Cordeiro Q, Borducchi JH, Valentin LS, et al. Transcranial direct current stimulation effects on athletes' cognitive performance: an exploratory proof of concept trial. Front Psychiatry 2016;7:183.

[5] Bortoletto M, Pellicciari MC, Rodella C, Miniussi C. The interaction with task-induced activity is more important than polarization: a tDCS study. Brain Stimul 2015;8:269—76.

[6] Cheng AL, Batool S, McCreary CR, Lauzon ML, Frayne R, Goyal M, et al. Susceptibility-weighted imaging is more reliable than T2*-weighted gradient-recalled echo MRI for detecting microbleeds. Stroke 2013;44:2782—6.

[7] Choe J, Coffman BA, Bergstedt DT, Ziegler MD, Phillips ME. Transcranial direct current stimulation modulates neuronal activity and learning in pilot training. Front Hum Neurosci 2016;10:34.

[8] Fertonani A, Ferrari C, Miniussi C. What do you feel if I apply transcranial electric stimulation? Safety, sensations and secondary induced effects. Clin Neurophysiol 2015;126:2181—8.

[9] Hauser TU, Rutsche B, Wurmitzer K, Brem S, Ruff CC, Grabner RH. Neurocognitive effects of transcranial direct current stimulation in arithmetic learning and performance: a simultaneous tDCS-fMRI study. Brain Stimul 2016;9:850—8.

[10] Horvath JC, Forte JD, Carter O. Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy human subjects: a systematic review. Neuropsychologia 2015;66:213—36.

[11] Horvath JC, Forte JD, Carter O. Quantitative review finds no evidence of cognitive effects in healthy populations from single session transcranial direct current stimulation (tDCS). Brain Stimul 2015;8:535—50.

[12] Keeser D, Meindl T, Bor J, Palm U, Pogarell O, Mulert C, et al. Prefrontal transcranial direct current stimulation changes connectivity of resting-state networks during fMRI. J Neurosci 2011;31:15284—93.

[13] Lang N, Nitsche MA, Paulus W, Rothwell JC, Lemon RN. Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. Exp Brain Res 2004;156:439—43.

[14] Lefaucheur J-P, Antal A, Ayache SS, Benninger DH, Brunelin J, Cogiamanian F, et al. Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). Clin Neurophysiol 2017;128:56—92.

[15] Liebetanz D, Koch R, Mayenfels S, Konig F, Paulus W, Nitsche MA. Safety limits of cathodal transcranial direct current stimulationin rats. Clin Neurophysiol 2009;120:116—7.

[16] López-Alonso V, Cheeran B, Rio-Rodrıguez D, Fernandez-del-Olmo M. Inter-individual variability in response to non-invasive brain stimulation paradigms. Brain Stimul 2014;7:372—80.

[17] López-Alonso V, Fernández-del-Olmo M, Costantini A, Gonzalez-Henriquez JJ, Cheeran B. Intra-individual variability in the response to anodal transcranial direct current stimulation. Clin Neurophysiol 2015;126:2342—7.

[18] McKinley RA. Acceleration of image analyst training with transcranial direct current stimulation. Behav Neurosci 2013;127:936—46.

[19] 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 Stim 2013;6:424—32.

[20] Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 2001;57:1899—901.

[21] 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;114:600—4.

[22] Nitsche MA, Niehaus L, Hoffmann KT, Hengst S, Liebetanz D, Paulus W, et al. MRI study of human brain exposed to weak direct current stimulation of the frontal cortex. Clin Neurophysiol 2004;115:2419—23.

[23] Notturno F, Marzetti L, Pizzella V, Uncini A, Zappasodi F. Local and remote effects of direct current stimulation on the electrical activity on the motor cortical network. Hum Brain Mapp 2014;35:2220—32.

[24] Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9:97—113.

[25] Pisoni A, Cerciello M, Cattaneo Z, Papagno C. Phonological facilitation in picture naming: when and where? A tDCS study. Neuroscience 2017;352:106—21.

[26] 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 2009;120:2008—39.

[27] Simonsen CZ, Madsen MH, Schmitz ML, Mikkelsen IK, Fisher M, Andersen G. Sensitivity of diffusion- and perfusion-weighted imaging for diagnosing acute ischemic stroke is 97.5%. Stroke 2015;46:98—101.

[28] Tremblay S, Larochelle-Brunet F, Lafleur LP, El Mouderrib S, Lepage JF, Théoret H. Systematic assessment of duration and intensity of anodal transcranial direct current stimulation on primary motor cortex excitability. Eur J Neurosci 2016;44:2184—90.

[29] Wiethoff S, Hamada M, Rothwell JC. Variability in response to transcranial direct current stimulation of the motor cortex. Brain Stimul 2014;7:468—75.

[30] Zappasodi F, Musumeci G, Navarra R, Di Lazzaro V, Caulo M, Uncini A. Safety and effects on motor cortex excitability of five cathodal transcranial direct current stimulation sessions in 25 hours. Neurophysiol Clin 2018;48:77—87.