* Corresponding author. Department of Psychiatry and Psychotherapy, University Medical Center Freiburg, Hauptstr. 5, 79104 Freiburg, Germany. Tel.: +49 761 270 65010; fax: +49 761 270 66190.
E-mail address: christoph.nissen@uniklinik-freiburg.de (C. Nissen)

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References

[1] Steriade M, Contreras D, Curró Dossi R, Nuñez A. The slow (1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. J Neurosci 1993;8:3284–99.
[2] Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 2001;10:1899–901.
[3] Billiard M, Dauvilliers Y. Idiopathic hypersomnia. Sleep Med Rev 2001;5:349–58.
[4] Dinges DF, Powell JW. Microcomputer analysis of performance on a portable, simple visual RT task sustained operations. Behav Res Methods Instrum Comput 1985;17:652–5.
[5] Monte-Silva K, Kuo M, 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;3:424–32.
[6] Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, et al. Transcranial direct current stimulation: state of the art 2008. Brain Stimul 2008;3:206–23.
[7] McIntire LK, McKinley RA, Goodyear C, Nelson J. A comparison of the effects of transcranial direct current stimulation and caffeine on vigilance and cognitive performance during extended wakefulness. Brain Stimul 2014;4:499–507.

Conceptual and Procedural Shortcomings of the Systematic Review "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" by Horvath and Co-workers

Dear Sir,

We are writing in reply to the above-mentioned paper by Horvath and colleagues, published in Neuropsychologia recently [1]. In this article, the authors conclude, based on a systematic review of research data exploring the physiological effects of tDCS, a non-invasive brain stimulation technique that beyond an effect on motor evoked potentials, tDCS has no impact on physiological parameters. We are concerned about the validity of the conclusions for various reasons. Since this paper reviews a whole field of research and comes to debatable assumptions, it is especially important that basic quality requirements are fulfilled, which is unfortunately not the case.

First, this review suffers from numerous conceptual flaws and misunderstandings. Second, the work contains relevant design problems, several errors and many incompletely or incorrectly cited data. A complete list of the factual errors is beyond the scope of the present letter. It would require a complete re-review of all original studies, which is not possible in a reasonable time frame, also because even parts of the data supplied in the tables referring to the original studies are wrong. We will focus our reply primarily on studies from our groups.

The introduction contains some relevant shortcomings about basic concepts of tDCS. The statement that the primary effect of tDCS is a modulation of the "resting membrane potential of neuronal populations via ionic adjustment of extracellular space" is a misconception and not supported by the respective reference [2], nor by animal slice and human in vivo experiments (e.g. Refs. [3–6]). In these studies, different return electrode positions, and neuronal orientations, result in antagonistic effects of tDCS delivered via identically positioned target electrodes, which would not be the case if ionic adjustment of extracellular space would be the main driver for the effects. It is also a misconception that the alteration of resting membrane potentials lasts for some minutes after stimulation termination, and also this statement is not supported by appropriate references. The abolishment of also short-lasting after-effects via NMDA-receptor block is in accordance with a synaptic effect [7,8].

The methodological approach chosen by the authors is not well suited to explore the data base from the original studies. For example, in case of tDCS, but similar to other neuromodulatory interventions, like repetitive transcranial magnetic stimulation (rTMS), or pharmacological interventions, one main intrinsic aspect is physiologically based state-dependency and non-linearity of effects. In this connection, the diversity of intervention protocols, tasks, and subject groups, with rare availability of studies in which strict replication was performed, makes it difficult to compare studies retrospectively by pooled data. This problem could be solved if for a specific task/measure numerous studies would be available, which differ in a limited set of factors, enabling the systematic exploration of the impact of protocol variants on the results. Unfortunately, this is not the case for tDCS in many instances. Therefore results of a quantification, which lump studies together without taking *critical* protocol components (stimulation duration, intensity, target and return electrode positions) and anatomical/physiological (e.g. dominant-non-dominant sides) differences into account, can be misleading. To give some examples, antagonistic effects due to different return electrode positions in different studies will result in a null outcome, if put together in a single analysis, as shown for the impact of tDCS on visual evoked potentials [3,4]. Pooling results of 13 min and 26 min anodal tDCS on motor cortex excitability, where anodal tDCS enhances excitability in the 13 min stimulation condition, but results in an excitability diminution in case of 26 min stimulation, probably due to calcium overflow mechanisms [9], will also result in an overall zero effect. This, however, does not mean that these stimulation protocols are inefficient, but that protocol differences result in physiologically based discernable effects. Statistical heterogeneity might also apply, and can and should be tested before conducting such an analysis (Cochrane Handbook for Systematic Reviews of Interventions, 2015). Unfortunately, in this review these aspects have not constantly been taken into account. These would have likely resulted in the conclusion that the type of analysis conducted in

this paper is not adequate. This does not mean that any review or pooled meta-analysis is meaningless, but calls for more sophisticated approaches suited to unravel the impact of various factors on the neurophysiological effects. Given the current state of the field, including rare availability of replication studies in strict sense, balanced scholarly reviews might be more appropriate to give an overview about the state of the field, and suggest future directions of research. This would, however, include a meaningful discussion of heterogeneous study results, which also takes into account presumably discernable physiological effects of experimental protocol differences.

For errors in the methods and results sections, main problems are erroneous data inclusion/exclusion, description, extraction, aggregation, and pooling. In the following, we provide some examples for studies involving the authors of this letter.

The in-text citations with regard to the tables and figures refer to those published by Horvath et al.

For the MEP results, Nitsche and Paulus [5] measured MEP amplitudes with 0.1, and not 0.25 Hz in the very short stimulation duration condition. Furthermore, as described in this publication, for very short stimulation, sham stimulation was inevitably mixed with real stimulation, and thus a sham stimulation condition is available in contrast to the information supplied in Table 1. In the same paper, 7 min tDCS (as stated in Table 1) was not conducted. Also for the very short stimulation condition, comparing studies conducted in different years is tricky due to the criteria established by the authors, because only one study was conducted between 2005 and 2009. From this study [10], only one condition resulted in an excitability enhancement of about 10%, as suggested by Fig. 2b. All other conditions with the electrode placed over the target area resulted in larger excitability enhancements. It seems that these were not included in the analysis, which results in description of an erroneous small effect for the relevant measure. In the specific condition seemingly included in the analysis, target electrode size was diminished in comparison to the other experiments, which could have reduced efficacy of stimulation [11]. Thus, the conclusion that tDCS effects in this condition diminished over years seems not well justified. For the other analyses exploring tDCS effects on MEPs dependent from the year of conduction of the respective studies, similar problems apply. Stimulation durations longer than 7 min induce wide-range durations of after-effects (from 10 min to over 1 h), and dependent on exact stimulation duration and intensity, can induce antagonistic effects due to physiological reasons [9,12]. Therefore it is not appropriate to agglomerate the respective data, as performed in the paper under consideration. The same applies for pooling together of distal and proximal upper extremity, and lower extremity target muscle data, which are known also from other plasticity induction techniques to result in heterogeneous effects. Beyond these conceptual problems, although it is understandable in case of such an extensive review that it cannot provide all study details, Table 1 does not fully allow tracking back single studies in case of multiple publications from the same group and year, thus correctness of the respective data cannot be followed back easily. Furthermore, it should be mentioned that a couple of studies and experiments are not included in Table 1 for different reasons, e.g. 5, and 11 min anodal tDCS conducted in the paper by Nitsche and Paulus 2001. Thirugnanasambandam et al., 2011, and Monte-Silva et al., 2010 are missing [13,14]. A high number of studies were excluded from analysis as "they did not supply numerical data." It is an open question, whether a clearer picture would have emerged if Horvath et al. had asked the authors for the raw data and included them into the analysis. For some studies, reasons supplied for exclusion are not correct. The studies from Caparelli-Daquer et al., 2012, and Kuo et al., 2013 were not conducted by the same group, they share just some co-authors [15,16]. Nitsche and Paulus 2001 [17] supply standard error of means, contrary to the respective statement of Horvath and co-workers. In contrast to the statement of the authors, furthermore Monte-Silva et al. [18] did not include data reported in a prior report in the respective study. In some instances, results from studies were included, which fulfill exclusion criteria of the authors (e.g. [19] included over 50 year old humans). These mistakes compromise the reliability of the MEP after-effect analysis.

For EEG alterations, Keeser and co-workers [20] (note that the wrong paper is mentioned in the reference section) report reduced Delta activity in the resting state induced by prefrontal anodal tDCS, in contrast to the statement by Horvath and co-workers, who attribute this effect to task performance in the main text body. In Table 19 it is stated that *increased* Delta was found in this paper, which is also not correct. With regard to task-related EEG alterations induced by tDCS, Horvath et al. state for the same study that "this group reported no significant change in P300 amplitude or latency at Pz (although they reported some attenuation at other electrodes, Pz was the only electrode explored in the second included study)" in a working memory task. However, Keeser and co-workers [20] actually describe a significant effect for the P2, and P3 ERP components at the electrode Fz as well as reduced P2 latency in the 2-back condition of this task. Notably, other resting state EEG studies, which show similar effects, are ignored [21,22]. These studies slightly differ methodologically, but in all studies the anodal electrode was placed over the DLPFC, and all studies describe reduced frontal low frequency EEG oscillations in the Delta or Theta band accomplished by anodal tDCS. Unfortunately, this effect is ignored by the authors of this review.

Horvath and colleagues furthermore excluded studies using functional MRI connectivity (fcMRI) measures with the argument that "data acquisition, analyses and reporting conducted by each are dissimilar …" At least two studies used exactly the same tDCS applications (anode placed over the left DLPFC, cathode placed over the supraorbital region, stimulation intensity: 2 mA, stimulation duration: 20 min) and found corresponding results: increased functional MRI connectivity (fluctuations of the BOLD signal less than or equal to 0.1 Hz) within frontal and parietal cortices after active stimulation [23,24].

For the analysis of tDCS combined with laser-evoked potentials, each of the single studies included in the respective analysis [25–27] resulted in a significant reduction of the N2 component by cathodal tDCS, thus it is unclear what caused the non-significant result in the analysis conducted by Horvath and co-workers. One explanation might be that data might have been pooled together independent from electrode position, and stimulated hemisphere, which would, however, be inappropriate, given the presumed regional effects of tDCS and the physiology of pain perception. Furthermore, with regard to [27] (Table 13), anodal and sham stimulation conditions are mentioned, which were not part of the respective study. A similar problem applies for the visual evoked potential measures, where in all 3 included studies the return electrode was placed at different positions, which because of the dependency of tDCS effects from current flow direction likely resulted in stimulation of different neuronal populations, or antagonistic stimulation of the same neuronal populations (see also above). Thus, pooling these data makes little sense, and the resulting null effect does not argue against efficacy of tDCS.

With regard to other sources, in Table S2, the information supplied about the studies by Polania et al., 2012 [28,29], is wrong, even with respect to the physiological methods. Tracking back to original studies is also not possible for the studies gathered in

Table S1, where only the number of excluded studies is supplied, without naming them or giving specific reasons for exclusion. Here more transparency with regard to the respective decision processes would have been needed. Thus, for the examples given, data inclusion, and analyses are faulty, and interpretation of data debatable on various grounds.

For the discussion, with regard to MEP suitability to monitor tDCS-induced excitability alterations, unfortunately statements about clinical and research applications of TMS, which follow different criteria and outcome parameters, are mixed. Furthermore, the recommendations for MEP determination by the authors are not in accordance with present consensus statements from expert groups [30], and the arguments are inconsistent with available data. The abolishment of tDCS effects on MEPs by glutamatergic block, and the enhancement of tDCS effects by glutamatergic agonists [8,31], as monitored by TMS intensities resulting in 1 mV MEP at baseline, are in contrast with the statement of the authors that this intensity should not be appropriate to monitor glutamatergic plasticity.

In summary, as shown by the examples given above, this review suffers from important flaws with regard to citing and interpreting available literature, non-transparent, and in many cases erroneous data aggregation, citation of study specifics, and discussion of the results. The conclusions made in this review are not justified by the data and state of the art knowledge and thus highly problematic. Statements like “this work raises questions concerning the mechanistic foundations and general efficacy of this device” are misleading, since the mechanistic foundation of DC stimulation has been rigorously tested over decades and continues to be explained in increasingly detailed human and animal studies.

Yours sincerely,

A. Antal*
Department of Clinical Neurophysiology
University Medical Center, Georg-August University
Göttingen, Germany

D. Keeser
Department of Psychiatry, Psychotherapy and Psychosomatics
Ludwig-Maximilian University Munich
Munich, Germany

A. Priori
Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico
Milan, Italy

Università degli Studi di Milano
Milan, Italy

F. Padberg
Department of Psychiatry, Psychotherapy and Psychosomatics
Ludwig-Maximilian University Munich
Munich, Germany

M.A. Nitsche
Department of Clinical Neurophysiology
University Medical Center, Georg-August University
Göttingen, Germany

Leibniz Research Centre for Working Environment and Human Resources
Dortmund, Germany

Department of Neurology
University Medical Hospital Bergmannsheil
Bochum, Germany

* Corresponding author. Department of Clinical Neurophysiology, Georg-August University, Robert-Koch-Str. 40, 37075 Göttingen, Germany. Tel.: +49 551 398461; fax: +49 551 398126.
E-mail address: AAntal@gwdg.de (A. Antal)

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References

[1] 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.
[2] Stagg CJ, Nitsche MA. Physiological basis of transcranial direct current stimulation. Neuroscientist 2011;17(1):37–53.
[3] Accornero N, Li Voti P, La Riccia M, Gregori B. Visual evoked potentials modulation during direct current cortical polarization. Exp Brain Res 2007;178(2):261–6.
[4] Antal A, Kincses TZ, Nitsche MA, Bartfai O, Paulus W. Excitability changes induced in the human primary visual cortex by transcranial direct current stimulation: direct electrophysiological evidence. Invest Ophthalmol Vis Sci 2004;45(2):702–7.
[5] Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 2000;527(Pt 3):633–9.
[6] Kabakov AY, Muller PA, Pascual-Leone A, Jensen FE, Rotenberg A. Contribution of axonal orientation to pathway-dependent modulation of excitatory transmission by direct current stimulation in isolated rat hippocampus. J Neurophysiol 2012;107(7):1881–9.
[7] 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 2002;125(Pt 10):2238–47.
[8] 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.
[9] 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.
[10] Nitsche MA, Doemkes S, Karaköse T, Antal A, Liebetanz D, Lang N, et al. Shaping the effects of transcranial direct current stimulation of the human motor cortex. J Neurophysiol 2007;97(4):3109–17.
[11] Miranda PC, Lomarev M, Hallett M. Modeling the current distribution during transcranial direct current stimulation. Clin Neurophysiol 2006;117(7):1623–9.
[12] Batsikadze G, Moliadze V, Paulus W, Kuo MF, Nitsche MA. Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. J Physiol 2013 Apr 1;591(Pt 7):1987–2000.
[13] Thirugnanasambandam N, Grundey J, Adam K, Drees A, Skwirba AC, Lang N, et al. Nicotinergic impact on focal and non-focal neuroplasticity induced by non-invasive brain stimulation in non-smoking humans. Neuropsychopharmacology 2011;36(4):879–86.
[14] Monte-Silva K, Liebetanz D, Grundey J, Paulus W, Nitsche MA. Dosage-dependent non-linear effect of L-dopa on human motor cortex plasticity. J Physiol 2010;588(Pt 18):3415–24.
[15] Caparelli-Daquer EM, Zimmermann TJ, Mooshagian E, Parra LC, Rice JK, Datta A, et al. A pilot study on effects of 4 × 1 high-definition tDCS on motor cortex excitability. Conf Proc IEEE Eng Med Biol Soc 2012;2012:735–8.
[16] Kuo HI, Bikson M, Datta A, Minhas P, Paulus W, Kuo MF, et al. Comparing cortical plasticity induced by conventional and high-definition 4 × 1 ring tDCS: a neurophysiological study. Brain Stimul 2013;6(4):644–8.
[17] Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 2001;57(10):1899–901.
[18] Monte-Silva K, Kuo MF, Thirugnanasambandam N, Liebetanz D, Paulus W, Nitsche MA. Dose-dependent inverted U-shaped effect of dopamine (D2-like) receptor activation on focal and nonfocal plasticity in humans. J Neurosci 2009;29(19):6124–31.
[19] Munneke MA, Stegeman DF, Hengeveld YA, Rongen JJ, Schelhaas HJ, Zwarts MJ. Transcranial direct current stimulation does not modulate motor

cortex excitability in patients with amyotrophic lateral sclerosis. Muscle Nerve 2011;44(1):109–14.
[20] Keeser D, Padberg F, Reisinger E, Pogarell O, Kirsch V, Palm U, et al. Prefrontal direct current stimulation modulates resting EEG and event-related potentials in healthy subjects: a standardized low resolution tomography (sLORETA) study. Neuroimage 2011;55(2):644–57.
[21] Jacobson L, Ezra A, Berger U, Lavidor M. Modulating oscillatory brain activity correlates of behavioral inhibition using transcranial direct current stimulation. Clin Neurophysiol 2012;123(5):979–84.
[22] Wirth M, Rahman RA, Kuenecke J, Koenig T, Horn H, Sommer W, et al. Effects of transcranial direct current stimulation (tDCS) on behaviour and electrophysiology of language production. Neuropsychologia 2011;49(14):3989–98.
[23] 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(43):15284–93.
[24] Pena-Gomez C, Sala-Lonch R, Junqué C, Clemente IC, Vidal D, Bargalló N, et al. Modulation of large-scale brain networks by transcranial direct current stimulation evidenced by resting-state functional MRI. Brain Stimul 2012;5(3):252–63.
[25] Csifcsak G, Antal A, Hillers F, Levold M, Bachmann CG, Happe S, Nitsche MA, et al. Modulatory effects of transcranial direct current stimulation on laser-evoked potentials. Pain Med 2009;10(1):122–32.
[26] Antal A, Brepohl N, Poreisz C, Boros K, Csifcsak G, Paulus W. Transcranial direct current stimulation over somatosensory cortex decreases experimentally induced acute pain perception. Clin J Pain 2008;24(1):56–63.
[27] Terney D, Bergmann I, Poreisz C, Chaieb L, Boros K, Nitsche MA, et al. Pergolide increases the efficacy of cathodal direct current stimulation to reduce the amplitude of laser-evoked potentials in humans. J Pain Symptom Manage 2008;36(1):79–91.
[28] Polania R, Paulus W, Nitsche MA. Reorganizing the intrinsic functional architecture of the human primary motor cortex during rest with non-invasive cortical stimulation. PLoS One 2012;7(1):e30971.
[29] Polania R, Paulus W, Nitsche MA. Modulating cortico-striatal and thalamo-cortical functional connectivity with transcranial direct current stimulation. Hum Brain Mapp 2012;33(10):2499–508.
[30] Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Iorio Di, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin Neurophysiol 2015;126(6):1071–107.
[31] Nitsche MA, Jaussi W, Liebetanz D, Lang N, Tergau F, Paulus W. Consolidation of human motor cortical neuroplasticity by D-cycloserine. Neuropsychopharmacology 2004;29(8):1573–8.