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Retinal origin of phosphenes to transcranial alternating current stimulation

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ABSTRACT

Objective: To examine possible retinal contributions to cortically induced phosphenes by transcranial alternating current stimulation (tACS) involving the visual cortex.
Methods: Self-reported phosphene ratings and voltage-related potentials from the canthus, supra-orbital and sub-orbital regions of the right eye were measured to 2, 10 and 20 Hz tACS at 250 and 1000 μA intensities in healthy volunteers.
Results: Qualitatively similar, but more intense phosphenes were reported during frontalis–vertex tACS as compared to occiput–vertex tACS. In addition, voltage-related potentials were recorded at the canthus and orbit regions of the eye during frontalis–vertex, occiput–vertex and occiput-right shoulder tACS.
Conclusions: The experience of phosphenes during tACS involving the visual cortex is influenced by volume conductions effects of the scalp.
Significance: Retinal effects should be taken into account when studying the cortical modulatory effects of tACS.

1. Introduction

The modulation of natural brain oscillations with transcranial alternating current stimulation (tACS) over the primary visual cortex (V1) has recently been shown to elicit visual experiences (i.e., phosphenes) (Kanai et al., 2008). This fascinating observation not only stresses the vital importance of neuronal oscillations in understanding brain dynamics in relation to function, but at the same time opens new exciting possibilities in the field of non-invasive neuromodulation of the cortex by mimicking natural brain oscillations (Silvanto et al., 2008; Thut and Miniussi, 2009). However, we here provide evidence that part of the neuromodulatory effects found on V1 can be the result of retinal activation due to volume–conduction effects of the scalp.

Non-invasive stimulation techniques provide researchers with a means to influence cortical activity and study the brain–function relationship in a direct manner (Amassian et al., 1989; Hallett, 2007). For example, transcranial magnetic stimulation to V1 elicits phosphenes that result from activation of neuronal cells in response to a brief but strong magnetic pulse (Kammer, 1999). Recently, the use of weak electrical currents applied directly to the scalp has gained renewed interest among researchers as its modulatory effect on cortical excitability was convincingly demonstrated in a series of well designed studies (for a review, see Nitsche et al., 2008). In case of a resting cell, neuronal polarization of cortical tissue by transcranial current stimulation presumably modifies the neuron's resting state potentials without generating an actual action potential as the latter relies on a rapidly time-varying electric field. In case of an active cell, modification of firing rates in response to current stimulation has been demonstrated as well (Bindman et al., 1964). This knowledge was recently employed in an innovative study that applied transcranial alternating current stimulation (tACS) over the primary visual cortex (V1) to study phosphenes in healthy volunteers (Kanai et al., 2008). In this study tACS (range: 1–45 Hz) was applied using an occiput–vertex electrode montage at intensities between 250 and 1000 μA. Results showed that under conditions of light participants rated maximum phosphene intensity between 14 and 20 Hz tACS, whereas under conditions of darkness maximum phosphene intensity was reported at 10–12 Hz tACS. A potential complicating factor in this intriguing study is the well-documented presence of phosphenes in response to extracranial electric stimulation (Motokawa and Ebe, 1952; Riggs et al., 1974; Thilo et al., 2004). Unfortunately, contributions of retinal activation resulting from volume–conduction effects were

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not studied, which makes it difficult to dissociate cortical from retinal contributions. The aim of the present study was to examine the possibility of retinal influences on phosphenes in response to tACS.

2. Materials and methods

2.1. Experiment 1

2.1.1. Participants

Eight healthy volunteers (2 females) participated in the experiment. All participants were aged between 21 and 29, mean ± SD, 24.75 ± 2.96, years and a safety screening list confirmed no contra-indications for non-invasive brain stimulation (Keel et al., 2000). None of the subjects had damaged skin tissue or skin disease. Subjects had no history of psychiatric or neurological conditions and had normal or corrected-to-normal vision. Written informed consent was obtained. The participants were unaware of the aim of the study and the study was approved by the local ethical committee of Utrecht University.

2.1.2. tACS

A battery-driven Eldith DC-stimulator Plus (NeuroConn GmbH, Ilmenau, Germany) was used for stimulation with conductive-rubber electrodes placed in wet sponges saturated with Parker Spectra 360 electrode gel (Parker Laboratories, Fairfield, USA). Both the stimulation and reference electrode were 5 × 7 cm and were fixed with two rubber bands. A bipolar montage was used and the electrode of interest was positioned at either Oz or Fpz in accordance with the International 10–20 EEG system. The second electrode was placed over the vertex (Cz). Stimulation was sinusoidal with a current intensity of 250 or 1000 μA. Three stimulation frequencies (i.e., 2, 10 and 20 Hz) were tested with a stimulation duration of 10 s, resulting in a total stimulation duration of 4 min. Impedance limit was set to <10 kΩ and intensity was ramped up and down for 1 s. The maximum current density was 28.57 μA/cm^2.

2.1.3. Procedure

Participants were seated in a dimly lit room and had their eyes open during stimulation. Observers were first familiarized to phosphenes by using 1000 μA tACS stimulating the visual cortex at 16 Hz for 10 s which served as the reference for the forthcoming ratings. During the experimental tACS conditions subjects were asked to rate phosphenes on a 4-point scale: 0, nothing noticeable during stimulation; 1, phosphene weaker than the standard; 2, phosphene as strong as the standard; 3, phosphene stronger than the standard. Intermediate values were allowed (see also Kanai et al., 2008). The experiment consisted of two tACS conditions: (1) occiput–vertex montage and (2) frontalis–vertex montage. In each condition there were six trials (i.e., 3 frequencies × 2 intensities) which were repeated twice. The observed internal-consistency between the two ratings as calculated by the Cronbach's alpha was 0.95. Stimulation intensities were pseudo-randomized and the frequencies were changed between the trials starting with 2 or 20 Hz, respectively. Between stimulation trials participants rated the phosphene intensity while the tACS stimulator was being adjusted for the next trial (~45 s). Upon completion of the experiment, subjects were asked to report the quality of the induced phosphenes across the different montages. Total duration of the experiment was approximately 30 min.

2.1.4. Data analysis

To examine montage-related differences in phosphene ratings between the different conditions Bonferroni corrected paired-samples *t*-tests were performed and included the following comparisons: occiput tACS (2 Hz, 250 μA) versus frontalis tACS (2 Hz, 250 μA), occiput tACS (2 Hz, 1000 μA) versus frontalis tACS (2 Hz, 1000 μA), occiput tACS (10 Hz, 250 μA) versus frontalis tACS (10 Hz, 250 μA), occiput tACS (10 Hz, 1000 μA) versus frontalis tACS (10 Hz, 1000 μA), occiput tACS (20 Hz, 250 μA) versus frontalis tACS (20 Hz, 250 μA), and occiput tACS (20 Hz, 1000 μA) versus frontalis tACS (20 Hz, 1000 μA). The alpha level of significance was set at 0.05 (two-tailed) throughout.

2.2. Experiment 2

2.2.1. Participants

Four healthy male volunteers aged between 21 and 34, mean ± SD, 27.3 ± 5.6, years participated in the second experiment. No contra-indications for non-invasive brain stimulation were found using a safety screening list (Keel et al., 2000). Subjects had no damaged skin tissue or skin disease and normal or corrected-to-normal vision. Written informed consent was obtained. The participants were unaware of the aim of the study and the study was approved by the local ethical committee of Utrecht University.

2.2.2. tACS

Stimulation was delivered by a battery-driven Eldith DC-stimulator Plus (NeuroConn GmbH, Ilmenau, Germany) with conductive-rubber electrodes placed in wet sponges saturated with Parker Spectra 360 electrode gel (Parker Laboratories, Fairfield, USA). Stimulation parameters were comparable to experiment 1. Both stimulation and reference electrode were 5 × 7 cm and were fixed with two rubber bands. Stimulation was sinusoidal with a current intensity of 250 or 1000 μA. Three bipolar montages (i.e., occiput–vertex, frontalis–vertex, and occiput-right shoulder) at stimulation frequencies 2, 10 and 20 Hz were applied for a duration of 5 s. Impedance limit was set to <10 kΩ and intensity was ramped up and down for 1 s. The maximum current density was 28.57 μA/cm^2.

2.2.3. Voltage recordings

Voltage-related potentials were recorded from the canthus, supra-orbital and sub-orbital regions of the right eye using Ag–AgCl-tipped electrodes filled with Parker Spectra 360 electrode gel (Parker Laboratories, Fairfield, USA). Recordings were made with the ActiveTwo system (BioSemi, Amsterdam, The Netherlands) relative to the common mode sense (CMS). Sampling rate was set at 256 Hz (bandwidth (3 dB): 52 Hz). The ground was attached to the forehead and consisted of the active CMS active and passive driven right leg (DRL) electrode that form a feedback loop driving the subject's average potential as close as possible to the analog-to-digital converter (i.e., the amplifier "zero") reference voltage in the A/D-box (http://www.biosemi.com).

2.2.4. Procedure

Participants were seated in a comfortable dentist chair in a dimly lit room and eyes were closed during stimulation. Prior and during the 5 s tACS electric activity from the electrodes was recorded. In addition to the occiput–vertex and frontalis–vertex montage (see Experiment 1), we included the additional occiput-right shoulder montage to explore the effect of a non-cephalic electrode position. Montage, frequencies and intensities were pseudo-randomized. Total duration of the experiment was approximately 45 min.

2.2.5. Data analysis

Raw activity was high-pass filtered at 0.1 Hz (24 dB/oct) filter and mean activity was collapsed across the three electrodes. Average tACS-evoked activity was calculated by averaging the peak-to-

Table 1
Means and standard errors of the phosphene ratings.

Montage	Intensity (μA)	Frequency (Hz)		
		2	10	20
Oz–Cz	250	0.06 ± 0.04	0.05 ± 0.04	0.10 ± 0.07
Fpz–Cz		0 ± 0	1.02 ± 0.25	1.37 ± 0.30
Oz–Cz	1000	0.04 ± 0.03	1.15 ± 0.17	1.13 ± 0.22
Fpz–Cz		0.43 ± 0.20	2.24 ± 0.20	2.86 ± 0.06

tACS montage; Oz–Cz: occiput–vertex, Fpz–Cz: frontalis–vertex.

peak amplitude of the signal across subjects. Baseline activity prior to tACS onset and during tACS were visually inspected across experimental conditions.

3. Results

3.1. Experiment 1

Participants reported qualitatively similar oscillating phosphenes in the 10 and 20 Hz occiput–vertex tACS montage at 1000 μA as demonstrated in the previous study by Kanai et al. (2008) (see Table 1). Moreover, observers reported peripheral and central visual field effects to frontalis–vertex tACS and phosphenes were reported as more intense by the observers during the 10 and 20 Hz frontalis–vertex tACS montage as compared to the 10 Hz ($t(7) = 5.3$, $p = 0.006$) and 20 Hz occiput–vertex tACS montage at 1000 μA ($t(7) = 9.3$, $p < 0.006$). Frontalis–vertex 10 Hz tACS at 250 μA yielded higher phosphene ratings as compared to occiput–vertex 10 Hz tACS at 250 μA ($t(7) = 3.6$, $p = 0.048$). The frontalis–vertex 20 Hz tACS at 250 μA resulted in higher ratings as compared to the occiput–vertex 20 Hz tACS at 250 μA ($t(7) = 4.1$, $p = 0.03$). No significant differences in phosphene intensity were seen between occiput–vertex and frontalis–vertex 2 Hz tACS at 250 and 1000 μA intensity (both p-values $\geqslant 0.1$). The main results are shown in Table 2 and Fig. 1.

3.2. Experiment 2

Increases in voltage-related potentials were observed during all tACS conditions as compared to baseline (Fig. 2). Even though the occiput-right shoulder tACS caused the smallest increase, the use of a non-cephalic electrode position did not eliminate volume–conduction effects.

In Table 2 an overview of the peak-to-peak amplitudes of the voltage-related potentials during tACS is presented.

4. Discussion

In the first experiment we applied 2, 10 and 20 Hz tACS at 250 and 1000 μA intensities for a duration of 10 s using two bipolar electrode montages in a pseudo-randomized order. Results showed

Table 2
Means and standard errors of the peak-to-peak amplitude of tACS-evoked potentials in μV.

Montage	Intensity (μA)	Frequency (Hz)		
		2	10	20
Fpz–Cz	250	4828 ± 1304	4728 ± 1245	4585 ± 1180
Oz–Cz		818 ± 52	762 ± 34	718 ± 37
Oz–Sh		592 ± 82	571 ± 81	528 ± 72
Fpz–Cz	1000	19662 ± 5116	19342 ± 5101	18502 ± 4837
Oz–Cz		3167 ± 157	3057 ± 158	2923 ± 181
Oz–Sh		2368 ± 340	2279 ± 326	2171 ± 325

tACS montage; Fpz–Cz: frontalis–vertex, Oz–Cz: occiput–vertex, Oz–Sh: occiput–right shoulder.

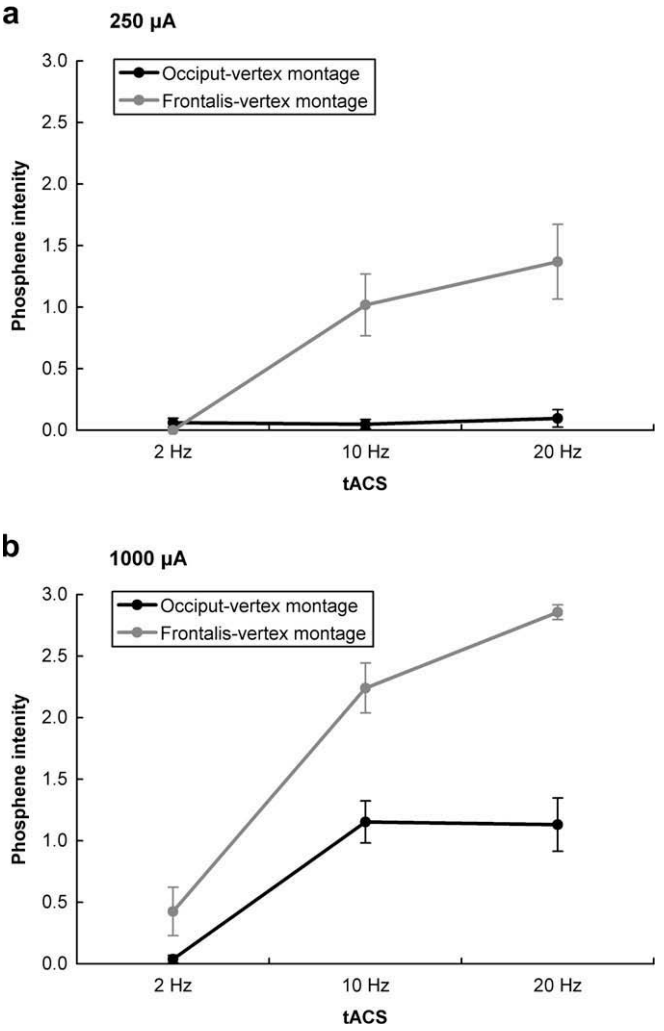

Fig. 1. Means and standard errors of the phosphene ratings of occiput–vertex and frontalis–vertex 2, 10 and 20 Hz tACS at 250 (a) and 1000 μA intensity (b).

that qualitatively similar phosphenes can be observed during various tACS montages at different intensities across the scalp, indicative for a different mechanism underlying the generation of phosphenes. Based on the initial observation that phosphenes were more intense during frontal tACS, we hypothesized that as a result of volume–conduction effects of the scalp electricity may have leaked to the retina of the eyes. To examine this possibility, we performed a second experiment in which voltage-related potentials from electrodes placed over the canthus, supra-orbital and sub-orbital regions of the right eye were recorded during 2, 10 and 20 Hz occiput–vertex, frontalis–vertex, and occiput-right shoulder tACS montage at 250 and 1000 μA. All three montages demonstrated, albeit varying in intensity, increases in voltage-related potentials during tACS (Fig. 2). This finding adds further support for possible retinal activation due to volume–conduction effects of the scalp as suggested by the first experiment. The results from our first experiment showing that the frontalis–vertex tACS montages yield similar yet more intense phosphene ratings suggest that volume–conduction effects may have contributed to the phosphenes elicited by the modulation of cortical oscillations. Possible retinal origin of phosphenes to tACS concurs with the induction of phosphenes by extracranial electric stimulation (Motokawa and Ebe, 1952; Riggs et al., 1974; Thilo et al., 2004) and increases in voltage-related potentials around the eye during tACS found in the present study. Moreover, recent simulations of current density distributions on a standard spherical head model during transcra-

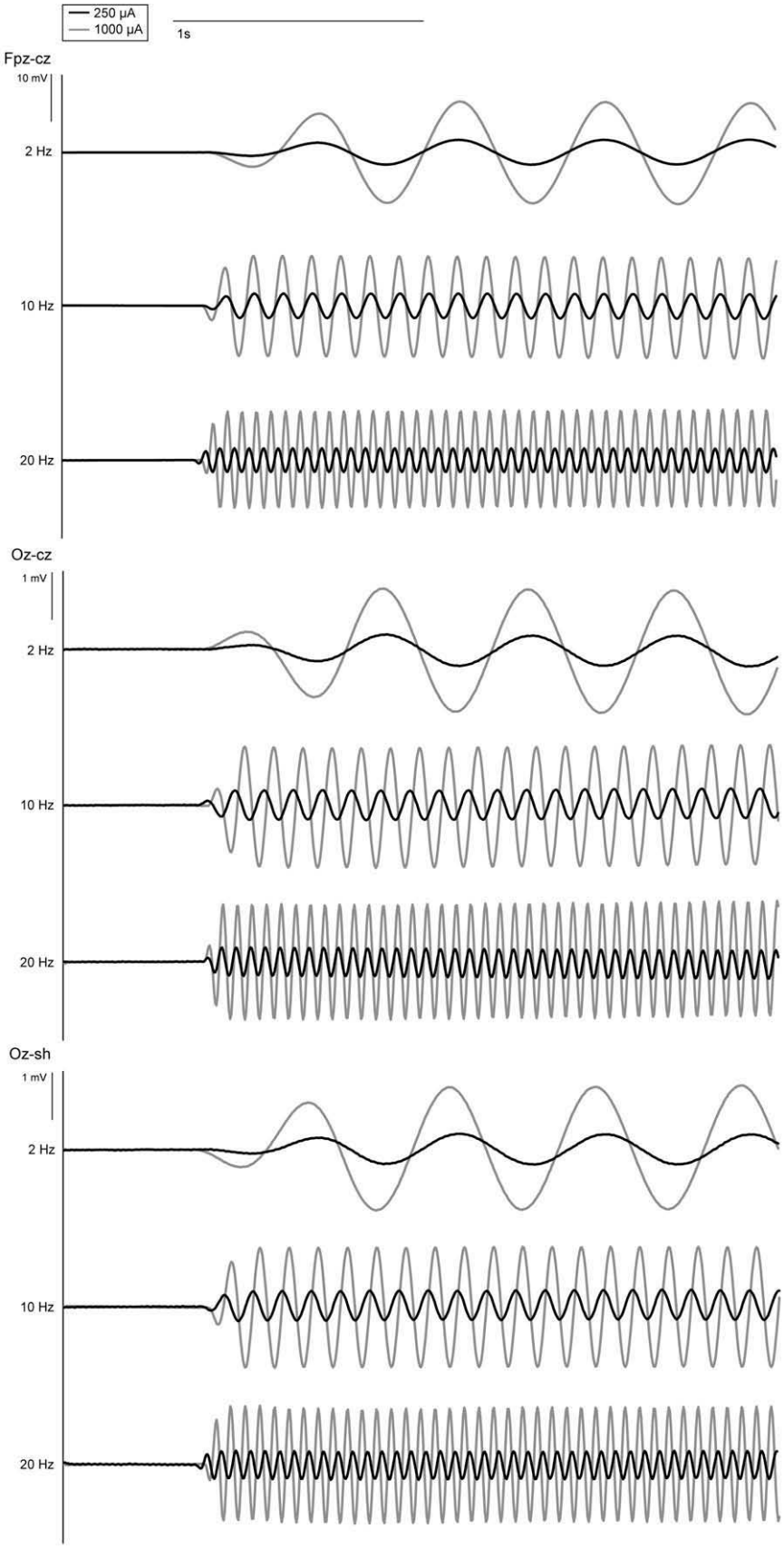

Fig. 2. Voltage-related potentials recorded around the right eye during 2, 10 and 20 Hz tACS with an intensity of 250 (black trace) and 1000 µA (gray trace) for the frontalis–vertex (Fpz–Cz), occiput–vertex (Oz–Cz), and occiput-right shoulder montage (Oz–Sh).

nial electric current stimulation (Miranda et al., 2009) have demonstrated the importance of electric currents on the scalp with respect to retinal effects (Roth, 2009). Together with the fact that frequency-dependent effects of tACS found by Kanai et al. (2008) could also reflect properties of retinal ganglion cell firing rates and excitability levels (Attwell, 2003) or an effect of dark adaptation (Schwiedrzik, 2009), the additional activation of retinal cells, either by propagation of electrical currents reaching retinal cells and/ or inducing subtle rhythmic muscle contractions in the eyes (Bennett and Rabbetts, 1998), makes it difficult to separate cortical from retinal sources of activation. Changing the size of the stimulation electrodes has been proposed to overcome any volume–conduction effects (Nitsche et al., 2008). Even though, no phosphene tACS studies are yet available that have systematically looked at this issue, a recent study by Antal et al. (2008) reported phosphene perception in a subset of participants during left motor cortex-contralateral orbit tACS that remained present even when the electrode size was increased. This observation suggests that changing electrode size may not be sufficient to eliminate possible volume–conduction effects (Rohracher, 1935).

In conclusion, tACS may be able evoke phosphenes through the modulation of brain oscillations to non-invasive frequency-dependent electric stimulation of V1. However, the present study provides evidence of retinal contributions to the effects of cortical modulation that could lead to misinterpretations of the results. Therefore, inclusion of multiple montages and extra control experiments in future electrical current stimulation studies may improve signal-to-noise ratios and the ability to dissociate cortical from retinal contributions to phosphene experience. In sum, tACS may offer new ways to directly tap into the cortical circuitry by mimicking natural brain oscillations and set the stage for an exciting new research field of frequency-dependent cortical modulation.

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References

Amassian VE, Cracco RQ, Maccabee PJ, Cracco JB, Rudell A, Eberle L. Suppression of visual perception by magnetic coil stimulation of human occipital cortex. Electroencephalogr Clin Neurophysiol 1989;74:458–62.

Antal A, Boros K, Poreisz C, Chaieb L, Terney D, Paulus W. Comparatively weak after-effects of transcranial alternating current stimulation (tACS) on cortical excitability in humans. Brain Stimul 2008;1:97–105.

Attwell D. Interaction of low frequency electric fields with the nervous system: the retina as a model system. Radiat Prot Dosimetry 2003;106:341–8.

Bennett AG, Rabbetts RB. Clinical visual optics, 3rd edition. Oxford: Butterworth-Heinemann; 1998.

Bindman LJ, Lippold OC, Redfearn JW. The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long-lasting after-effects. J Physiol 1964;172:369–82.

Hallett M. Transcranial magnetic stimulation: a primer. Neuron 2007;55:187–99.

Kammer T. Phosphenes and transient scotomas induced by magnetic stimulation of the occipital lobe: their topographic relationship. Neuropsychologia 1999;37:191–8.

Kanai R, Chaieb L, Antal A, Walsh V, Paulus W. Frequency-dependent electrical stimulation of the visual cortex. Curr Biol 2008;18:1839–43.

Keel JC, Smith MJ, Wassermann EM. A safety screening questionnaire for transcranial magnetic stimulation. Clin Neurophysiol 2000;112:720.

Miranda PC, Faria P, Hallett M. What does the ratio of injected current to electrode area tell us about the current density in the brain during tDCS? Clin Neurophysiol 2009;120:1183–7.

Motokawa K, Ebe M. Selective stimulation of color receptors with alternating currents. Science 1952;116:92–4.

Nitsche MA, Cohen LG, Wassermann EM, Priori A, Lang N, Antal A, Paulus W, Hummel F, Boggio PS, Fregni F, Pascual Leone A. Transcranial direct current stimulation: state of the art 2008. Brain Stimul 2008;1:206–23.

Riggs LA, Merton PA, Morton HB. Suppression of visual phosphenes during saccadic eye movements. Vision Res 1974;14:997–1011.

Rohracher H. Über subjektive Lichterscheinungen bei Reizung mit Wechselströmen. Z Sinnesphysiol 1935;66:164–81.

Roth BJ. What does the ratio of injected current to electrode area not tell us about tDCS?. Clin Neurophysiol 2009;120(6):1037–8.

Schwiedrzik CM. Retina of visual cortex? The site of phosphene induction by transcranial alternating current stimulation. Front Integr Neurosci 2009;3:1.

Silvanto J, Muggleton N, Walsh V. State-dependency in brain stimulation studies of perception and cognition. Trends Cogn Sci 2008;12:447–54.

Thilo KV, Santoro L, Walsh V, Blakemore C. The site of saccadic suppression. Nat Neurosci 2004;7:13–4.

Thut G, Miniussi C. New insights into rhythmic brain activity from TMS-EEG studies. Trends Cogn Sci 2009;13:182–9.