Transcranial electrical stimulation over visual cortex evokes phosphenes with a retinal origin

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Kar K, Krekelberg B. Transcranial electrical stimulation over visual cortex evokes phosphenes with a retinal origin. J Neurophysiol 108: 2173–2178, 2012. First published August 1, 2012; doi:10.1152/jn.00505.2012.—Transcranial electrical stimulation (tES) is a promising therapeutic tool for a range of neurological diseases. Understanding how the small currents used in tES spread across the scalp and penetrate the brain will be important for the rational design of tES therapies. Alternating currents applied transcranially above visual cortex induce the perception of flashes of light (phosphenes). This makes the visual system a useful model to study tES. One hypothesis is that tES generates phosphenes by direct stimulation of the cortex underneath the transcranial electrode. Here, we provide evidence for the alternative hypothesis that phosphenes are generated in the retina by current spread from the occipital electrode. Building on the existing literature, we first confirm that phosphenes are induced at lower currents when electrodes are placed farther away from visual cortex and closer to the eye. Second, we explain the temporal frequency tuning of phosphenes based on the well-known response properties of primate retinal ganglion cells. Third, we show that there is no difference in the time it takes to evoke phosphenes in the retina or by stimulation above visual cortex. Together, these findings suggest that phosphenes induced by tES over visual cortex originate in the retina. From this, we infer that tES currents spread well beyond the area of stimulation and are unlikely to lead to focal neural activation. Novel stimulation protocols that optimize current distributions are needed to overcome these limitations of tES.

Although this is an intriguing hypothesis, a number of objections have been raised to this interpretation (Schwiedrzik 2009). First, there is convincing evidence that phosphenes can be induced by electrical stimulation of the retina. Brindley (1955) passed alternating current (0.03–0.7 mA) through bipolar electrodes placed directly on the conjunctiva, which reliably evoked the perception of phosphenes. Pressure blinding of the eye caused temporary blindness to both visual stimuli and electrically induced phosphenes. Brindley (1955) concluded that voltage changes induced directly in the photoreceptors were the most likely explanation of these phosphenes. Schutter and Hortensius (2010) proposed that the supposed cortically evoked phosphenes reported by Kanai et al. (2008) could be due to volume conduction from the occipital electrode to the retina, evident in the voltage changes measured near the eye. Paulus (2010) has argued that those voltage changes were too small to evoke phosphenes.

Resolving this long-standing debate (Rohracher 1935) and determining the origin of tES phosphenes has considerable implications for the ability of tES to modulate specific brain areas (or rhythms) in clinical and basic research. The cortical entrainment hypothesis claims that neural activity below a tES electrode can be modulated relatively easily. The retinal hypothesis, on the other hand, claims that although it is indeed easy to generate phosphenes with tES, these phosphenes do not result from the direct modulation of neural activity below the electrode.

We combined experimental and computational methods to investigate the origin of tES-induced phosphenes. We first use a novel paradigm to confirm that current thresholds for phosphenes decrease when electrodes are placed away from visual cortex and closer to the eye. This is expected for a retinal origin of phosphenes. Second, we show that the frequency tuning of these phosphenes can be understood from the basic response properties of retinal ganglion cells (RGCs; Benardete and Kaplan 1999). Finally, we show that there is no difference in the time it takes to evoke phosphenes by a single current pulse through an electrode near the eye and near visual cortex. This is inconsistent with the cortical hypothesis because phosphenes generated directly in cortex should be faster than retinal phosphenes by the typical response latency of V1 neurons. Taken together, our data strongly support the view that the origin of tES-induced phosphenes is retinal.

MATERIALS AND METHODS

Subjects. Six subjects (5 naive and 1 experimenter; 5 subjects for the phosphene detection task and 4 subjects for the double-pulse detection task) participated in the study. Subjects gave written informed consent, and all had normal or corrected-to-normal vision. This study was conducted according to the principles expressed in the...
Declarations of Helsinki and approved by the Institutional Review Board of Rutgers University. S-4 is the nonnaïve subject (experimenter K. Kar).

Visual stimuli. The phosphene detection and the double-pulse detection task were done in a room lit only by the background luminance (0.4 cd/m²) of a monitor (Sony FD Trinitron CRT monitor; resolution of 1,024 × 768 pixels; refresh rate of 120 Hz).

Apparatus. We used an STG4002 stimulus generator (Multi Channel Systems, Reutlingen, Germany) to deliver the tES. We used saline-soaked sponges attached to conductive rubber as electrodes (3-in. diameter). All events in a trial were synchronized and triggered using in-house software (neurostim; http://neurostim.sourceforge.net). A head-mounted EyeLink II eye tracker system (SR Research, Mississauga, Ontario, Canada) recorded eye movements by tracking the pupils of both eyes at a sampling rate of 500 Hz.

Procedures. All subjects sat in a dark room at a distance of 57 cm from the center of the monitor. Head movements were restricted by using a chin rest or a bite bar. The subjects indicated their response from the center of the monitor. Head movements were restricted by using the keyboard.

Electrode placement. We placed the stimulating electrode at three regions of interest: the occipital lobe (Oz), the temporal lobe (T5), and the frontal lobe (Fpz) according to the international 10–20 system of electrode placement. The reference electrode was always at the vertex (Cz). The maximum current density used was 0.02 mA/cm² (current intensity = 1 mA; electrode surface area = 45.6 cm²).

Phosphene detection task. This experiment was designed to determine the threshold intensity of stimulation to elicit phosphenes. The stimulation was limited to −0.5 to 0.5 mA of sinusoidal current. We used 8, 10, 15, 20, 30, and 60 Hz as the TES frequencies. The voltage was limited to 20 V. Each trial in the experiment began with a “START” sound followed by two intervals of 1,000 ms separated by a beep. Stimulation was delivered for the full duration of either of the two intervals, and the subjects reported the interval in which they saw phosphenes. We used a Bayesian adaptive threshold estimation algorithm (Kontsevich and Tyler 1999) to select the intensity of TES for each trial and defined threshold as the current at which the subjects’ performance reached 75% correct. Subjects performed a minimum of 30 trials per condition (i.e., per montage and frequency).

Double-pulse detection task. This experiment was designed to estimate the time interval required to perceive two phosphenes when two electrical pulses were applied in succession. Subjects fixedated a small dot on an otherwise dark screen. All trials in which the subjects broke fixation from a 2 × 2° window were discarded from the analysis. After a START sound to indicate the beginning of the trial, we applied two 20-ms unipolar electrical pulses and varied the onset time between the pulses (dt) over a range of 20–160 ms. One pulse was applied at the frontal electrode (Fpz), and the other at the occipital electrode (Oz). Catch trials containing only a single pulse were randomly interleaved. To create phosphenes with approximately equal perceived brightness, we adjusted the currents at the Oz and Fpz electrodes. Specifically, we determined the average (across subjects and frequencies) ratio of the threshold currents in experiment 1, chose 1 mA as the current for the Oz electrode.

We supplemented this RGC model with a front end and a back end. The front end modeled the relationship between the stimulation current and behavior. Because the retinal hypothesis predicts that the threshold delays are not different, we used a single-stimulus model. We adapted the linear cascade filter model proposed by Victor (1987). Benardete and Kaplan (1999) estimated the parameters of the model by recording from macaque retinal ganglion M cells (Benardete and Kaplan 1999). In the model, the firing rate of each RGC depends on visual contrast (c) and stimulation frequency (f).

To test whether threshold delays were significantly different in the two conditions, we performed a permutation test of the null hypothesis that the Oz-Fpz and Fpz-Oz data belonged to the same distribution (Efron and Tibshirani 1994). In this procedure, we used MATLAB's fmincon, varied the four free

P = g[ξ, θ, R(ω, c)]

where P is the predicted fraction correct, g is a Weibull function, and θ and ξ are the threshold and slope of the sigmoid psychometric function, respectively. Note that none of these assumptions about the front or back end of the model introduces frequency tuning; all frequency tuning in the model arises from the properties of the RGCs.

The model predicted the fraction correct for any stimulation current and frequency. Using MATLAB's fmincon, we varied the four free
model parameters ($\beta$, $\delta$, $\xi$, and $\theta$) and the constrained RGC parameters to obtain the best fit of the complete psychometric function (3 montages, 4 temporal frequencies, and a large number of current intensities, not just the threshold) for each subject. The values of the RGC model parameters were constrained to fall within the range estimated for primate ganglion cells by Benardete and Kaplan (1999).

RESULTS

When stimulated with a weak alternating current through a primary electrode placed on Oz and a reference electrode on Cz, subjects report perceiving a widely distributed peripheral flicker for which temporal frequency varies with stimulation frequency (Brindley 1955; Kanai et al. 2008; Rohracher 1935). The subjective brightness of this flicker increases when the first electrode is placed more anterior. This property of tACS-induced phosphenes has been known for some time (Rohracher 1935) and is inconsistent with the cortical hypothesis but strongly suggests a retinal origin.

Phosphene thresholds. To measure these phenomena in an objective manner, we used a two-interval, forced-choice task (Fig. 1) and an adaptive method to determine current levels required to evoke phosphene reliably from electrodes placed over Oz, T5, or Fpz, each with a reference on Cz. Figure 2 shows data from all subjects. The current thresholds decreased for electrodes closer to the eye and therefore further away from the visual cortex. This supports the view that the phosphene originates in the eye and not from direct stimulation of the cortex.

In other words, each model contained RGCs for which properties were within the measured range of macaque ganglion cells.
**Frequency tuning.** Figure 2 also shows that the phosphenes thresholds were tuned for the temporal frequency of stimulation. This feature has previously been attributed to the entrainment of cortical oscillations (Kanai et al. 2008). We investigated whether this temporal frequency tuning could instead be interpreted in terms of retinal response properties. Qualitatively, this interpretation is supported by the fact that RGCs have a temporal frequency tuning with peak sensitivity in the α-range in the dark; see, for instance, Fig. 1 in Benardete and Kaplan (1999). Moreover, the peak sensitivity of RGCs shifts toward the β-frequency band in the light (Purpura et al. 1990), which matches the shift in the optimal stimulation frequency for phosphenes in a lit room (Kanai et al. 2008).

To provide quantitative support for the match between phosphenes thresholds and RGC properties, we used a well-established model for RGCs (Victor 1987). This model provides a quantitative description of the relation between visual contrast and the firing rate of RGCs, including their temporal frequency tuning.

We developed a simple, descriptive front end to this model to relate tES current (the independent variable in our study) to the effective visual contrast generated at the retina (the input to the RGC model). Specifically, we assumed that the effective visual contrast decreased with the electrotonic distance between the electrode and the eye. The parameters describing this decrease were estimated from the data. Similarly, we added a descriptive back end to the RGC model that described how firing rate was related to behavioral performance (see MATERIALS AND METHODS). The parameters of the front and back end were unconstrained and determined by optimization to produce the best fit to the individual performance curves. The parameters of the RGC model, however, were constrained to lie within the ranges measured in the primate retina (Benardete and Kaplan 1999).

The curves in Fig. 2 show the best fits of the model to the individual subject data (for details, see MATERIALS AND METHODS). We could not estimate the thresholds at stimulation frequencies of 30 Hz and higher as they were above the predetermined range of our current stimulation amplitude (0.5 mA); we did not use those data for the model fits. Note however, that this implies that thresholds >30 Hz were at least 0.5 mA, hence all subjects had U-shaped response curves, as expected qualitatively from the RGC properties.

**Double-pulse detection task.** When a single 20-ms pulse of 1 mA was applied to Oz, subjects reliably reported seeing a brief flash of peripheral light. On the one hand, this shows that entrainment of cortical oscillations is not necessary to evoke phosphenes from an electrode on the occipital lobe. However, more importantly, it provides us with a tool to determine whether currents injected at the occipital lobe spread to the eye.

A single pulse through a frontal electrode (Fpz) also evokes a phosphenes, and it is generally accepted that these phosphenes have a retinal origin (Brindley 1955; Rohracher 1935). We asked whether the phosphenes evoked by occipital stimulation could have a direct cortical origin instead. The distinguishing feature we use is that retinal stimulation, unlike direct cortical stimulation, requires some time to reach visual cortex. We call this latency $\Delta_{V1}$, and we estimate it as ~60 ms based on EEG data (Jeffreys and Axford 1972). In the experiment, we applied one frontal pulse (Fpz) and one occipital pulse (Oz) and asked the subjects to report whether they saw one or two pulses.

To perceive two phosphenes requires a minimum time ($\delta_T$) between the two corresponding bursts of neural activity in visual cortex. Under the cortical hypothesis, the current pulses in an Fpz-Oz sequence (Fig. 3) would have to be apart by $\delta_{FO} = \delta_T - \Delta_{V1}$ to create V1 activity separated by $\delta_T$. In an Oz-Fpz sequence, however, the Oz pulse gets a head start, hence the current pulses would only have to be $\delta_{OF} = \delta_T - \Delta_{V1}$ apart. Under the retinal hypothesis, there is no difference between the conditions because both phosphenes originate at the retina with negligible time delays due to electrical conduction from Oz to Fpz.

Figure 4 shows the sensitivity to detect two pulses using the signal detection measure $'d'$. As expected, sensitivity to detect two pulses increased with the delay between the pulses. Importantly, sensitivity for Oz-Fpz sequences was indistinguishable from sensitivity for Fpz-Oz sequences. Statistically, none of the subjects had a significant difference between the minimal delay needed to distinguish reliably ($'d' = 1.5$) two pulses in the Oz-Fpz and Fpz-Oz sequences. This is consistent with the retinal hypothesis. The cortical hypothesis, on the other hand, predicts that these curves should be shifted laterally by $2 \times \Delta_{V1} \approx 120$ ms. We used this estimate of the expected effect to determine the statistical power of our test (see MATERIALS AND METHODS); the power was >0.8 for each subject. Hence, our data do not allow us to reject the null hypothesis that the phosphenes have the same source, and this is not likely to be due to a lack of power in the statistical analysis. We infer that Oz and Fpz stimulation have the same source. Because Fpz stimulation is generally agreed to have a retinal origin (Brindley 1955; Rohracher 1935), we conclude that Oz stimulation does, too.

**DISCUSSION**

We investigated the origins of phosphenes induced by tES. Consistent with the previous literature (Rohracher 1935; Schut-
and Hortensius 2010), we found that phosphene thresholds increased with the distance between the electrodes and the eye. Furthermore, we showed that the temporal frequency tuning of current thresholds is consistent with the known properties of RGCs. Finally, we found no difference in the time it takes to elicit a phosphene by stimulating over visual cortex compared with frontal cortex even though a direct cortical effect of electrical stimulation predicts a time difference as large as 120 ms. Taken together, these data strongly argue that tACS-induced phosphenes originate in or before RGCs and are unlikely to be caused by the entrainment of cortical oscillations (Kanai et al. 2008).

We will first discuss the possible mechanisms underlying phosphene generation in the retina and then the implications of our findings for the use of tES in clinical applications and basic research.

Within the retina, several mechanisms could contribute to the generation of phosphenes by the application of electrical fields (Attwell 2003). Clinical observations help to narrow down the likely locus for phosphene generation by tACS. For instance, electrical stimulation of the conjunctiva induces phosphenes even in patients with damaged photoreceptors (retinitis pigmentosa or ablatio retinae), and in these patients the frequency tuning of the phosphene threshold is preserved (Meier-Koll 1973). Frequency tuning is lost, however, in patients whose optic nerve is severed or those in which occluded blood vessels lead to the degeneration of bipolar and ganglion cells (e.g., in retinopathia diabetica; Meier-Koll 1973). Given that models of volume conduction in the human head predict that tES can generate high current densities in the optic nerve, stimulation of the axons of RGCs is a candidate mechanism. However, pressure blinding abolishes phosphenes generated by direct stimulation of the cornea (Brindley 1955). Because pressure blinding is unlikely to affect the optic nerve, Brindley (1955) concluded from this observation that electrical stimulation directly affects the photoreceptors. More recently, it has been shown that direct electrical stimulation of the optic nerve of a blind patient with severe retinitis pigmentosa (Delbeke et al. 2003) resulted in phosphenes without the typical bandpass temporal frequency tuning shown in Fig. 2. Although invasive experiments may be needed to answer this mechanistic question conclusively, these observations strongly suggest that the characteristic frequency tuning found in tES originates before the optic nerve.

Our experiments used objective measures of phosphene detection. This minimized the influence of observer bias and avoided relying on the subjective report of observers’ phenomenal experience, which we found to be variable across subjects and somewhat difficult to describe. This ephemeral quality of the phosphenes makes it difficult to compare the effect of electrical stimulation with matched visual stimuli. Informal experiments showed that most visual stimuli mask tES-induced phosphenes more effectively than tES phosphenes mask visual stimuli. However, this finding is difficult to interpret quantitatively as any visual property that was not matched between the stimuli could contribute to this asymmetry. For instance, the phosphenes extended farther into the periphery and had a subjectively weaker appearance than any visual stimulus we could present. An additional explanation for this asymmetry is that tES polarizes the membrane of photoreceptors only tran-
siently, whereas the signaling cascade started by light typically changes the membrane for a much longer period of time (Baylor et al. 1984; Schneeweis and Schnapf 1999).

Our claim is not that transcranial stimulation cannot affect the brain. Given enough current, it is inevitable that an electrode placed on the scalp will modulate brain activity. Moreover, recent studies suggest that even at low current levels tACS can entrain EEG signals (Polanía et al. 2012). This does not imply, however, that tACS entrainment of cortical oscillations plays a causal role in the induction of phosphenes. First, we have shown that even a single 1-mA pulse over visual cortex can evoke a phosphene. This shows that synchronization with ongoing oscillations is not necessary. Second, we have provided a novel interpretation of the temporal frequency tuning of phosphenes in terms of the well-known physiological properties of RGCs. The conclusion we draw from these findings is that there is no convincing evidence that tES modulates neural activity underneath the electrode enough to generate a phosphene percept.

The broader conclusion we draw is that currents induced at the occipital pole spread widely across the scalp, well beyond the location of the electrode and outside of the direct path between electrode and reference. This is consistent with predictions of detailed models that simulate the current spread in the human head during tDCS (Datta et al. 2009). Taken together, these findings imply that tES in its current form is unlikely to lead to focal neural activation. tES in a therapeutic setting would benefit from the ability to target specific areas, hence novel stimulation protocols that optimize current distributions (Dmochowski et al. 2011) are needed to overcome this limitation.

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GRANTS

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DISCLOSURES

A patent application has been filed on subject matter disclosed in this manuscript.

AUTHOR CONTRIBUTIONS

K.K. and B.K. conception and design of research; K.K. performed experiments; K.K. analyzed data; K.K. and B.K. interpreted results of experiments; K.K. prepared figures; K.K. drafted manuscript; K.K. and B.K. edited and revised manuscript; K.K. and B.K. approved final version of manuscript.

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