Phosphene attributes depend on frequency and intensity of retinal tACS

Eugen Kvašňák[1], Mária Orendáčová[1*], Jana Vránová[1]

[1] Department of Medical Biophysics and Medical Informatics, Third Faculty of Medicine, Charles University in Prague, Prague, Czechia

*Corresponding author:
Mária Orendáčová: maria.orendacova@lf3.cuni.cz

Short title: Phosphenes depend on tACS parameters

Summary

Phosphene is the experience of light without natural visual stimulation. It can be induced by electrical stimulation of the retina, optic nerve or cortex. Induction of phosphenes can be potentially used in assistive devices for the blind. Analysis of phosphene might be beneficial for practical reasons such as adjustment of transcranial alternating current stimulation (tACS) frequency and intensity to eliminate phosphene perception (e.g., tACS studies using verum tACS group and sham group) or, on the contrary, to maximize perception of phosphenes in order to be more able to study their dynamics. In this study, subjective reports of 50 healthy subjects exposed to different intensities of retinal tACS at 4 different frequencies (6, 10, 20 and 40 Hz) were analyzed. The effectiveness of different tACS frequencies in inducing phosphenes was at least 92%. Subject reported 41 different phosphene types; the most common were light flashes and light circles. Changing the intensity of stimulation often induced a change in phosphene attributes. Up to 9 phosphene attributes changed when the tACS intensity was changed. Significant positive correlation was observed between number of a different phosphene types and tACS frequency. Based on these findings, it can be concluded that tACS is effective in eliciting phosphenes whose type and attributes change depending on the frequency and intensity of tACS. The presented results open new questions for future research.

Keywords

Transcranial alternating current stimulation (tACS) • phosphene • electrical stimulation • tACS frequency • tACS intensity

Significance: The presented results showed that retinal tACS at frequencies of 6, 10, 20 and 40 Hz is a very effective (92%) trigger of phosphenes. Subjects reported 41 types of phosphenes. Phosphenes depended on tACS frequency and intensity, and a substantial set of phosphene attributes (up to 9 different attributes) varied with changing tACS intensity.

1. Introduction

Phosphene is the term that refers to the experience of seeing light without real natural visual stimulation [1]. Phosphenes can arise spontaneously [2] or they can be induced artificially [1]. Spontaneously occurring phosphenes can be experienced in situations such as migraines [3], deep meditation [4] and intense emotional experience [4]. Artificially induced phosphenes result from mechanical stimulation [1], chemical stimulation [5], and electromagnetic stimulation of the brain [6, 7].

Phosphene research has become scientifically and clinically crucial for the development of visual prostheses for blind [8]. The mechanisms responsible for phosphene perception are an intriguing interface at which various disciplines such as neuroscience, mathematics, biophysics, nonlinear system theory and psychology overlap [7, 9, and 10]. Phosphenes are frequently experienced in the participants undergoing transcranial alternating current stimulation (tACS) [11]. TACS belongs to the larger family of non-invasive transcranial electric stimulation (tES), an umbrella term that includes transcranial direct current stimulation (tDCS), tACS and transcranial random noise stimulation (tRNS) [12]. TES is used for clinical and experimental treatment of a various conditions such as treatment of pain [13] and cognitive improvement [14].TACS is based on stimulation by a weak alternating current passing between at least two scalp electrodes soaked in a conductive medium [15]. Mechanism of tACS is thought to consist in interfering of tACS frequency with the frequency of endogenous electroencephalographic (EEG) activity [16]. There are a number of tACS studies that find a link between tACS and its effect on EEG activity and improvement in brain functions [14, 17]. So far, several tACS studies have been dedicated to scrutinize the origin of phosphenes [6, 7]. There is still an ongoing debate regarding the origin of phosphenes. Some studies suggest that phosphenes originate in the retina [6, 18] whereas other studies suggest that the visual cortex is the site of origin of the phosphenes [19]. There are also studies which propose that phosphene originate in both retina and brain cortex [7, 11]. To the best of our knowledge, it has not been yet investigated which and how many attributes of phosphene change with increasing tACS intensity. Also, it has not been yet studied whether there is a causal relationship between tACS frequency and phosphene appearance. It has also not been analyzed whether different tACS frequencies are equally effective to induce phosphenes. We believe that investigation of phosphene dynamics in relation to different tACS frequencies and changes of tACS intensity might be beneficial for deeper understanding of the responses of brain dynamics to various tACS frequencies. Deeper understanding of the link between phosphene dynamics and tACS parameters such as frequency and intensity might be also useful for practical reasons such as adjustment of tACS frequency and intensity to eliminate phosphene perception (e.g., tACS studies using verum tACS group and sham group) or, on the contrary, to maximize perception of phosphenes in order to be more able to study their dynamics.

For that reason, we decided to analyze these domains based on subjective written descriptions of phosphene dynamics obtained from 50 participants undergoing anodal and cathodal tDCS and 6, 10, 20 and 40 Hz tACS. Particular data used in this analysis come from our previous studies which focused on scrutinizing sensation thresholds, pain thresholds and phosphene thresholds in anodal tDCS, cathodal tDCS, 6, 10, 20 and 40 Hz tACS [20, 21]. While tDCS did not induce phosphene sensations in any of the participants, we observed that the vast majority of the participants reported the presence of phosphenes during tACS. Data on tACS and tDCS data including phosphene perception thresholds and sensation and pain thresholds are available in our older analyses [20, 21]. We observed the trend for more detailed descriptions of phosphenes including greater number of various phosphene types for 20 and 40 Hz tACS frequencies than for 6 and 10 Hz tACS frequencies (unpublished data from [20]). Based on these

preliminary observations, we decided to investigate whether there is positive correlation between the number of perceived phosphene types and tACS frequency or not. Apart from this domain of our interest, we were also interested whether there are some common phosphene types occurring under multiple tACS frequencies across the participants/within the same participant. Also, the effectiveness of 6, 10, 20 and 40 Hz tACS frequencies in their capability of inducing phosphenes was studied too. Last but not least, we investigated how many phosphene attributes (e.g size, brightness, type) were reported altogether for each specific tACS frequency. The following questions summarize our interest domains as well as the rationale for their investigation:

1. Do specific retinal tACS frequencies (6, 10, 20, 40 Hz) significantly differ in their effectiveness in elicitation of phosphenes?
Hypothesis 1: Retinal tACS frequencies 6, 10, 20, 40 Hz) will be comparably effective in inducing phosphenes.
Rationale for the investigation: TACS frequencies between 2-75 Hz were found to be effective in eliciting phosphene perception [11, 22] in stimulation of left motor cortex [22] and prefrontal and occipital regions [11] 6, 10, 20, 40 Hz tACS was found to be significantly effective in eliciting phosphene [22] . Since retinal tACS is characterized by low threshold of phosphene perception [11, 23], we hypothesized that 6, 10, 20, 40 Hz frequencies used for retinal tACS will be comparably effective for induction of phosphenes across the participants.

2. Is there a relationship between tACS frequency and the number of perceived phosphenes types?
Rationale for the investigation: In comparison to lower tACS frequencies, higher tACS frequencies, such as 20 and 40 Hz, were found to be associated with lower phosphene threshold [11, 23]. Furthermore, 20 and 40 Hz correspond to high frequency EEG activity, namely to beta and gamma EEG activity, which are connected with higher brain arousal compared to lower EEG frequencies such as theta and alpha [24]. For that reason we hypothesized, that higher tACS frequencies will be associated with greater brain arousal and greater activation of stimulated retina and adjacent prefrontal brain areas leading to enhanced ability of brain to generate more different phosphene types.

3. Does a particular frequency of tACS elicit any common phosphene type in more participants?
Rationale for the investigation: In complex phosphene shapes, there was found strong frequency specificity during periodic light-flickering stimulation of brain [25]. Therefore we hypothesized; perception of common phosphene types will be associated with specific retinal tACS frequencies in more participants.

4. Are there any common phosphene types perceived during different retinal tACS frequencies within the same participant?
Rationale for the investigation: So far, there were some common repeated phosphene patterns, known as Kluver's forms, documented in other modalities of brain stimulation than tACS [26, 9].This may possibly indicate there are some universal phosphene patterns occurring when using different protocols and modalities of brain stimulation. For that reason, we hypothesized some common repeated phosphene patterns might occur in the same participant during the stimulation of different retinal tACS frequencies.

5. How many attributes of phosphenes change when tACS intensity changes?
Rationale for the investigation: So far, multiple attributes of phosphene attributes, including phosphene brightness, size as well as the position of phosphene in the field of view, were reported to be modulated by tACS frequency and intensity [6, 11, and 27]. However, to the best of our knowledge, other possible attributes of phosphene appearance elicited by tACS has been not systematically studied yet. For that reason, we decided to study what other phosphene attributes were reported to be modulated by tACS frequency and intensity.

2. Methods

Based on sample size estimation, 90 participants was target number of sample size for statistical nalysis of investigation of data of our previous study from which phosphene-related data comes from for our present study [20]. However, finally, 62 healthy participants (both males and females, age 18-21 years, mean age: 19 years) was the total number of participants who met inclusion criteria and participated in this study. To be included in this study, participants had to be at least 18 years old, psychostimulants and medication free, without neurological problems, having normal or corrected-to-normal vision and no epilepsy history. All exclusion criteria were presented via online Google-form questionnaire submitted at the day of experiment right before the beginning of the experiment. Participants were recruited by flyers at school and by viral ways among university students of Third Faculty Medicine, Charles University of Prague. Participants were stimulated with anodal tDCS, cathodal tDCS, 6 Hz tACS, 10 Hz tACS, 20 Hz and 40 Hz tACS. Our study was blended crossover with randomized trials that were not blinded. Each subject receives all treatments (anodal tDCS, cathodal tDCS, 6 Hz tACS, 10 Hz tACS, 20 Hz and 40 Hz tACS) in a random order. The study was done in within-subject design; all participants received all 6 treatments. Participants were aware of receiving verum tDCS and tACS. Sham condition (within-subject design) in our experiment was not included in order to minimize possible unwanted effects of too long experimental procedure such as irritation and/or tiredness of the participants. Control group (sham, between-subject design) was not included due to considering total sample size (62 participants) too small sample for dividing it between verum stimulation (6 different tested treatments) and control group (no stimulation).

Stimulation intensity for each of the 6 stimulation protocols ranged between 0.2-2.0 mA with increments of 0.1 mA. Each electrical stimulus of equal intensity lasted 10 s (ramp time 1 s) and was separated by a 6 s inter-stimulus period. Total period of electrical stimulation was 18 minutes. Circular sponge electrodes (5 cm diameter) were placed on AF7 and AF8 according to International 10-20 system. 5 cm diameter circular sponge electrodes were chosen to decrease the current density and consequently reduce level of subjective unpleasant effects of transcranial electrical stimulation. AF7 and AF8 were selected as target brain areas due to keeping good level of electrical conductance (electrodes placed at the naked forehead) during the stimulation period. Also, AF8 and AF7 are sufficiently far from each other so that the risk of a potential short-circuit is eliminated. Electrical stimuli were generated by a STARSTIM device (Neuroelectrics, Ltd, Spain) [20]. Participants were instructed to record (in a prepared Excel spreadsheet) the electrical intensity values that corresponded to their perception threshold, pain threshold, phosphene threshold, and to describe in as much detail as possible the subjective appearance of phosphene that was eventually perceived during each stimulation setting [20]. To be included in our analysis, it was necessary to describe at least 1 attribute of phosphene appearance (e.g., size, brightness, type) for at least 1 tACS frequency. The experiments took place in the afternoon and evening hours and were carried out in

Department of Medical Biophysics and Medical Informatics in Third Medical Faculty of Charles University in Prague. The experiment was approved by the Ethics committee of the Third Medical Faculty of Charles University in Prague and was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki for Human Experiments).

For statistical analysis of the relationship between tACS frequency and the number of perceived phosphenes, Spearman's correlation test with alpha error at 95% probability was used. P value below 0.05 was considered significant. To investigate whether all 4 tACS frequencies elicited phosphenes, data were visually analyzed for each tACS frequency, respectively. To examine whether there were participants who reported phosphenes for 4, 3, 2, or 1 tACS frequency, the data were visually inspected in within-subject fashion. To examine whether there are common phosphene types experienced by multiple participants, our data were analyzed in a cross-subject manner for the reported phosphene types for each tACS frequency used, respectively. To examine whether there is an individual preference for one phosphene type induced by multiple tACS frequencies in a particular participant, the data were analyzed within-subject for the reported phosphene types for each tACS frequency used. In order to determine how many attributes of phosphene appearance change with changing tACS frequency, our data were analyzed across participants for each tACS frequency, respectively.

3. Results

At the end of recording part of experiment, we obtained data from 62 participants but 12 participants were excluded from final analysis because their results did not fulfill rigorous results requirements (like describing at least 1 attribute of phosphene appearance, e.g., size, brightness, type for at least 1 tACS frequency).A total of 50 participants were included in analysis as they gave description of at least one phosphene attribute for at least one tACS frequency.

3.1 Effectiveness of specific tACS frequency to induce phosphenes

6 Hz tACS did not induce phosphenes in 4 persons. 10 Hz tACS did not induce phosphenes in 2 persons. 10 Hz tACS did not induce phosphenes in 1 participant. 40 Hz tACS did not induce phosphenes in 1 person. There was a massive preponderance of participants (45 of 50) who saw phosphenes under all 4 tACS frequencies. In 4 subjects, phosphenes appeared during 3 tACS frequencies, whereas the experience of phosphenes during 2 tACS frequencies was not reported. Only 1 participant experienced phosphenes during just 1 tACS frequency. All tACS frequencies we tested (6, 10, 20, 40 Hz) elicited phosphenes. Phosphene incidence by particular tACS is depicted in the following Tab.1.

	Specific tACS			
	6 Hz	10 Hz	20 Hz	40 Hz
Participants reporting phosphenes under specific tACS frequency	92%	96%	98%	98%

Tab. 1: Number of participants reporting phosphenes under specific tACS frequency.

3.2 Frequency of tACS ➡ Number of phosphene types

Different frequencies of tACS induced various phosphene types in different participants. 6 Hz tACS elicited 12, 10 Hz tACS elicited 14, 20 Hz tACS elicited 21 and 40 Hz tACS elicited 28 phosphene types (Tab. 2).

tACS frequency (Hz)	Number of phosphene types
6	12
10	14
20	21
40	28

Tab. 2: Number of different phosphene types perceived during the particular tACS frequency

The correlation between tACS frequency and the number of phosphene types was analyzed using Spearman's correlation coefficient with alpha error set at the 95% confidence level (Tab. 3).

Variable	**Correlation between tACS frequency and number of phosphene types (Spearman Correlation Coefficient)**	
	tACS frequency (N=4)	Number of phosphene types
tACS frequency	1.0000	0.9866
	P=0.013	P=0.013
Number(N) of perceived phosphene types	0.9866	1.0000
	P=0.013 (Spearman Correl. Coef.)	P=0.013 (Spearman Correl. Coef.)

Tab. 3: Correlation between tACS frequency and number of perceived phosphene types (Marked correlations are significant at significance level p <0.05000; N=4)

3.3 Frequency of tACS ➡ Preferred phosphene types

Participants were stimulated by 4 different frequencies of tACS under. All together, they reported 41 different phosphene types. Phosphene types reported by more than 4% participants are shown in Tab. 4.

Phosphene types (> 4% participants)	**tACS**			
	6 Hz	**10**	**20**	**40**
Light flashes	44%	46%	40%	34%
Circles	8%	8%	6%	14%

Tab. 4: Occurrence of particular phosphene types induced by specific tACS frequencies.

Some other specific phosphenes as “horizontal lines”, “vertical lines”, “stripes”, “lines with unspecific orientation”, “TV grains”, “Dots”, “round lights”, “waves”, “dots”, “tunnel”, “spot”, “round light”, “twinkles”, “semicircle”, “blinking bulb”, “checkerboard”, “sparkles”, “lightening”, “grid”, “bracket”, “bubble”, “rectangle”, “spiral”, “snowing”, “waterfall”, “light in water surface”, “carousel”, “concentric”,

"stripes", "water surface", "waves", "rhombus", "aura around things", "curves, "gazing at the lamp", "light fan", "scattered lines", "pixels", "mixture of colors", "comb", "lines perpendicular to each other", "stellar shapes", "oblique lines with the impression of profundity" were reported less than 4% participants.

3.4 Participant's preference for a particular type of phosphene regardless tACS frequency

There were some specific phosphene types occurring repeatedly under different frequencies of tACS within the same participant. The most frequent phosphene types repeatedly reported by the same participant at different tACS frequencies are shown in Tab. 5.

Number of tACS frequencies during which the same phosphene type has been reported	The particular phosphene type
2	Light flashes (6%); circles (4%);
3	Light flashes (6%); circles (4%);
4	Light flashes (14%);

Tab. 5: Phosphene types which repeatedly occurred under more than one tACS frequency. The numbers in the brackets represent the percentage of the participants reporting the particular phosphene type.

3.5 tACS intensity change ➡ Phosphene attributes changes

When tACS intensity changed, the participants often referred change of at least 1 attribute in the following fashion: For 6 Hz tACS it was 33 (of 50) participants, for 10 for tACS it was 34 (of 50) participants, for 20 Hz tACS it was 40 (of 50) participants, and for 40 Hz tACS it was 33 (of 50) participants. The change of phosphene brightness and phosphene type during increasing tACS intensity were two the most frequent reported phosphene attributes (Figure 1). Interestingly, tACS frequency was positively associated with higher percentage of participants referring phosphene type change induced by intensity change (27% for 6 Hz, 35% for 10 Hz, 35% for 20 Hz, 52% for 40 Hz). On the other hand, phosphene color was mentioned to change only in 0 % for 6 Hz tACS, 3% for 10 Hz tACS, 3 % for 20 Hz tACS and 0 % for 40 Hz tACS. In total, the following 9 different phosphene attributes were observed: 1. Phosphene type, 2. Occurrence of simultaneous phosphenes of a different type, 3. Frequency of phosphene occurrence per stimulation period, 4. Size of phosphene, 5. Colour of phosphene, 6. Brightness of phosphene, 7. Type of phosphene movement (phosphenes were reported to move in a visual field), 8. Speed of phosphene movement, 9. Trajectory of phosphene movement. Figure 1 graphically displays relations between tACS frequencies and percentage of the participants reporting the change of the particular phosphene attribute associated with increasing tACS intensity.

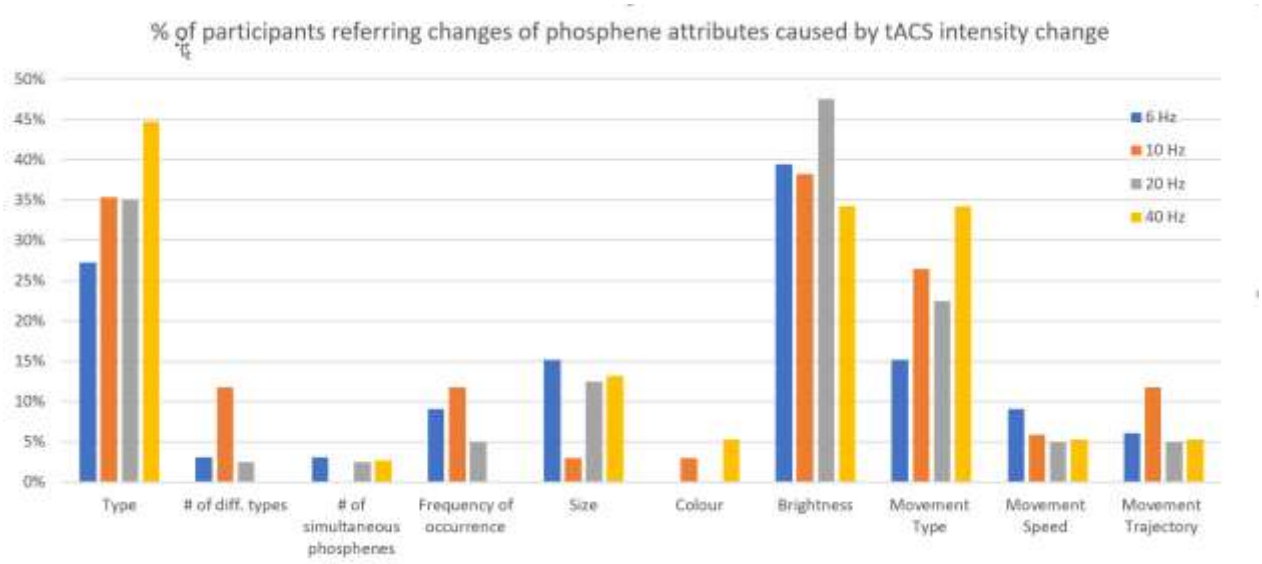

Figure 1 Change of phosphene attribute induced by change of tACS intensity *The graph display percentage of the participants (Axis Y) reporting the perception of the particular phosphene attribute (Axis X) during the stimulation by tACS. Yellow color stands for 40 Hz tACS, grey color stands for 20 Hz tACS, red color stands for 10 Hz tACS and blue color stands for 40 Hz tACS.*

4. Discussion

All four tACS frequencies (6 Hz, 10 Hz, 20 Hz and 40 Hz tACS) were found to be effective in inducing phosphenes. In the vast majority of participants, phosphenes appeared at all 4 tACS frequencies. A few had phosphenes during 3 tACS frequencies. The experience of phosphenes during 2 tACS frequencies was not recorded. Only 1 participant experienced phosphenes during only 1 tACS frequency. 1 participant did not experience phosphenes at all. Based on these results, it appears that the frequencies of 6 Hz, 10 Hz, 20 Hz, and 40 Hz are more or less comparable in their ability to elicit phosphenes in frontal regions. It is possible that retina, which is very sensitive to tACS [28] and it is considered to be importantly involved in phosphene generation [7, 18] is very sensitive in phosphene generation in multiple tACS frequencies. In relation to tACS frequencies used in this study, our results are in line with other studies which found 6, 10, 20 and 40 Hz tACS frequencies are significantly effective in eliciting phosphenes in occipital, prefrontal and motor cortex [11, 22]. Our findings seem to be at least partially congruent with other study which documented the greatest blood flow changes in retina during 2-50 Hz of periodic retinal photic stimulation [29].

We believe that future comparative studies should be done to investigate possible differences in capability of various tACS frequencies of inducing phosphenes in a various brain areas. Neuroimaging tools used for measuring brain metabolic activity, which was absent in our study, might be useful for disentangling the link between phosphene perception and its neuronal correlate when stimulating different brain regions.

Another area of interest was the number and types of phosphene attributes that may change with increasing tACS intensity. Compared to other studies reporting tACS intensity-dependent subjective hanges of phosphene size, brightness, and their position in the visual view [6, 11, 27], we identified 9 phosphene attributes that changed with increasing tACS intensity- namely, phosphene size, phosphene

brightness, phosphene type, phosphene movement type, phosphene movement trajectory, and frequency of phosphene occurrence during tACS, subjective perception of phosphene movement speed, phosphene color, and simultaneous occurrence of multiple phosphenes of different types. In total, 9 different phosphene attributes were found to change with increasing tACS intensity for 6 Hz tACS, 10 Hz tACS and 20 Hz tACS. For 40 Hz tACS, 8 different phosphene attributes were found to vary. We observed considerable differences in the frequency of reports of the change of the particular phosphene attribute across the participants. Change of phosphene brightness related to increases of tACS intensity was most frequently reported phosphene attribute per all 4 tACS frequencies. On the other hand, phosphene color and simultaneous occurrence of multiple phosphene types were the least frequent phosphenes attributes that were reported to change with increasing tACS intensity regardless tACS frequency.

However, since the participants were not instructed to focus on some specific parameters of phosphene perception and they were not given a table with a predefined specific category for each phosphene attribute, but they were only asked to write as many details possible related to the appearance of the phosphene (in a further unspecified way), the further interpretation of these outcomes remains impossible. It remains unknown whether such differences in number of participants reporting the change of the particular phosphene attribute may be attributed to some universal tendency of phosphene behavior or if it is caused by the fact that the participants did not report everything about phosphene attributes simply because they were not instructed to do so and/or they simply forgot of did not consider it relevant. If the latter is true, then it might be interesting to study what factors may be responsible for the greater tendency to remember and mention the change of phosphene type (the most frequent change of phosphene attribute in this study) and for smaller tendency for remembering and/or mentioning the change of other phosphene attributes (e.g., phosphene color). In spite of these particular limitations, we hope and believe that our findings revealing the link between increasing tACS intensity and change up to 9 phosphene attributes might be relevant for future studies investigating the link between tACS and phosphene dynamics and potential mechanisms underlying phosphene dynamics.

So far, it remains unknown how many phosphene attributes really exist and how many of them change with increasing tACS intensity. It is not known whether the total number of all possible phosphene attributes changing with tACS intensity depends on the tACS frequency or whether this number is universal for all tACS frequencies. Also, it remains unknown so far whether appearance of phosphenes elicited by tACS would depend on which particular brain area is used as a target area to tACS. Regarding differences in functions of brain areas, it is possible that phosphenes, elicited by tACS applied to different brain areas, would differ in their attributes. This hypothesis might be at least partially supported by different effects of occipital and retinal tACS on phosphene attributes [6, 11, and 23]. In contrast to occipital tACS, lower phosphene perception threshold was documented for retinal tACS [11, 23]. In addition, compared to phosphenes elicited by occipital tACS, phosphenes elicited by retinal tACS were subjectively described as more intense [6] and brighter [23]. In contrast to subjectively perceived brightness and intensity of phosphenes generated by retinal and occipital tACS, one study documented no subjectively-perceived qualitative differences in their appearance [6]. By now, there is no unified agreement on the possible explanation of differences in phosphene attributes generated by retinal and occipital tACS. Some studies hypothesize that the origin of phosphene generation plays its major role. According to some studies, phosphenes are generated from retina [6, 18]. On the other hand, other studies consider occipital cortex to be responsible for generation of phosphenes [19, 30]. There are also studies proposing phosphenes may originate from both retina and cortex [7, 11] and possible differences in phosphene appearance are attributable to different functions of retina and visual cortex [11]. Also,

differences in effects of retinal and occipital tACS on phosphene characteristics were attributed to different physical properties of biological tissues which are encountered by retinal and occipital tACS [11]. Based on the current knowledge, we believe that studying differences in phosphenes in relation to target area stimulated by tACS would be relevant.

There was a significant positive correlation between the tACS frequency and number of different phosphene types reported by participants. Based on tACS capability of modulating EEG activity [17] having the same or very similar frequency to tACS and/or its harmonics [16], it is possible that tACS frequencies used in this study might have interfered with endogenous EEG oscillations in the stimulated frontal regions. There are several studies that have found a link between phosphenes perception and EEG amplitude [10], phase [31], and frequency [10, 32]. Since higher EEG frequency activity predominates in the frontal brain areas [33] and lower frequency EEG activity predominates in the posterior cortices [34], a possible tACS-induced enhancement of frontal beta and gamma EEG activity by the corresponding tACS frequencies (i.e. 20 Hz and 40 Hz) could lead to more robust cortical effects that could hypothetically be responsible for a greater number of perceived phosphenes. Moreover, lower EEG frequencies are usually associated with lower arousal, whereas higher EEG frequencies are usually associated with higher arousal [24]. Consequently, since both frontal beta as well as gamma activity are associated with higher level of vigilance [24], attention [35] and working memory processes [36], it is therefore possible that greater number of reported phosphene types perceived at these higher EEG frequencies, is due to greater activation of memory and attention systems that affect phosphene perception. However, because our study included neither EEG recordings, nor objective measures of arousal level, it remains unknown whether tACS actually modulated EEG activity and arousal level. It is also possible that positive correlation between tACS frequency and number of phosphene types is attributable to lower phosphene threshold associated with higher tACS frequencies such as 20 Hz and 40 Hz [6, 22] and linear relationship found between 2-50 Hz frequency of retinal photic stimulation and magnitude of blood flow in stimulated retina [29] posssibly indicating positive correlation between excitation level of retina and frequency of retinal stimulation.

The last domains of our interest involved investigation whether some common phosphene types occur in more participants and whether different tACS frequencies can induce the same phosphene type. Indeed, some phosphene types were perceived by at least more than one participant.

Some of perceived phosphene types reported in this study, such as circles and horizontal and vertical lines have also been reported in other studies [9, 32]. Electromagnetic and chemical brain stimulation can induce some common phosphene types [26] which are called Kluver's phosphene forms [9]. Kluver's typology includes the following four phosphene categories: 1. Lattices, gratings, honeycombs, filigrees, 2. Cobwebs, 3. tunnels, funnels, vessels ad 4. Spirals [9]. Some of these Kluver's phosphene forms were also reported in our study, e.g., lattices, tunnels and spirals. Based on these outcomes, it seems as if there are some universal phosphene types that can be elicited with higher probability than some other phosphene types, regardless of what kind of brain stimulation is used. This hypothesis could be at least partially supported by the findings of one study that found that some patterns of neuronal population are activated with greater probability than others [37]. Another supporting evidence for the possible universality of some phosphene patterns come from theoretical and mathematical studies examining the dynamics of various phosphene types [9]. Based on these outcomes, we suggest that it might be interesting to conduct future studies to examine the relationship between EEG and phosphene pattern attributes. It would be intriguing to study mechanisms responsible for changes of phosphene appearance attributes

related to changing tACS intensity and to study whether these phenomena also occur in other types of brain stimulation methods that are able to induce phosphenes. Another interesting research topic would be to investigate the link between perceived phosphene dynamics and subjective and objective measures of alertness level, working memory capacity and attention level.

5. Limitations and future perspectives

This study has several limitations that should be mentioned. The data analyzed are from experiments where, in addition to tACS, anodal and cathodal tDCS were also used (data processed in study [20]), which might have affected the perception of phosphene dynamics. Participants were not trained in systematically describing phosphenes and therefore it is possible that some participants may have perceived more attributes of the appearance of phosphene than they then described. Another limitation is the difficulty in analyzing the perception of phosphenes based on their subjective description, which may lead to data bias due to possible subtle differences between what participants actually experienced and what they described as an experience. However, this is a weakness of all experiments based on the analysis of subjective experiences. Furthermore, no control group (sham transcranial electrical stimulation) was included in order to differentiate between effects attributable to tACS and tDCS and possible effects attributable to placebo. Finally, there is a large variability in the types and protocols of electromagnetic stimulation used by the studies supporting our findings, which introduces some noise into the interpretation of the data and the discussion of possible underlying mechanisms responsible for the phenomena studied. In addition, based on the current knowledge related to retinal and occipital tACS, we propose, future studies might compare effects of retinal and occipital tACS on phosphenes attributes to investigate possible similarities and differences of these two types of tACS. Based on neuro-physiological sex-dependent differences [38, 39], we also propose it might be relevant to study whether phosphene perception somehow differ between males and females. In addition, we believe other factors such as effects of baseline stress levels on phophene perception should be taken into consideration in phosphene research due to stress-related modulatory effects on brain arousal [40] which plays an important role in phosphene perception [32]. Finally, we propose inclusion of objective measurements of brain activity, such as EEG or fMRI, should be included in order to investigate links between phosphene appearance and tACS-dependent altered patterns of brain activity.

6. Contribution of the authors:

EK contributed to the following domains: conceptualization, methodology, software, data collection, project administration and financial support for the project. MO contributed to the following domains: conceptualization, data analysis, writing original and final manuscript version. JV contributed to the work by providing statistical analysis.

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8. Conflict of interest

Declarations of interest: none.

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