Brain Topography
https://doi.org/10.1007/s10548-019-00715-x

ORIGINAL PAPER

Perceptual and Physiological Consequences of Dark Adaptation: A TMS-EEG Study

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Received: 30 November 2018 / Accepted: 4 May 2019

Abstract
Existing literature on sensory deprivation suggests that short-lasting periods of dark adaptation (DA) can cause changes in visual cortex excitability. DA cortical effects have previously been assessed through phosphene perception, i.e., the ability to report visual sensations when a transcranial magnetic stimulation (TMS) pulse is delivered over the visual cortex. However, phosphenes represent an indirect measure of visual cortical excitability which relies on a subjective report. Here, we aimed at overcoming this limitation by assessing visual cortical excitability by combining subjective (i.e., TMS-induced phosphenes) and objective (i.e., TMS-evoked potentials - TEPs) measurements in a TMS-EEG protocol after 30 min of DA. DA effects were compared to a control condition, entailing 30 min of controlled light exposure. TMS was applied at 11 intensities in order to estimate the psychometric function of phosphene report and explore the relationship between TEPs and TMS intensity. Compared to light adaptation, after DA the slope of the psychometric function was significantly steeper, and the amplitude of a TEP component (P60) was lower, only for high TMS intensities. The perceptual threshold was not affected by DA. These results support the idea that DA leads to a change in the excitability of the visual cortex, accompanied by a behavioral modification of visual perception. Furthermore, this study provides a first valuable description of the relationship between TMS intensity and visual TEPs.

Keywords Dark adaptation · Phosphene perception · Visual cortex · TMS-EEG · TEPs

Introduction

Chronic visual deprivation alters functional organization and excitability of the occipital cortex, as shown by deafferentation studies in animals (Gilbert and Wiesel 1992) and by evidence from blind patients (Glass et al. 1977; Gothe et al. 2002; Kremlácek et al. 2013; Théoret et al. 2004). Existing literature suggests that even short-lasting periods of light deprivation (minutes/hours) can cause functional changes in cortical activity (Boroojerdi et al. 2000; Fierro et al. 2005; Marjerrison and Keogh 1967). For example, after 60 min of dark adaptation (DA), functional magnetic resonance imaging showed an increased response of visual cortex to incoming visual input (Boroojerdi et al. 2000), while electroencephalography (EEG) recordings revealed a slowdown of occipital alpha-band activity (Marjerrison and Keogh 1967).

In humans, modulations of cortical excitability can be assessed non-invasively through transcranial magnetic stimulation (TMS). When applied to the visual cortex, TMS can induce phosphenes, illusory visual percepts that have been proposed as an index of visual cortex excitability (Merabet et al. 2003). Previous studies suggest an increase in cortical excitability following short-lasting periods (i.e., 45 min) of DA, as shown by a reduction in phosphene threshold (Boroojerdi et al. 2000, 2001; Fierro et al. 2005). On the contrary, longer periods of DA have been associated with a reduction of cortical excitability, as indicated by an overall increase in phosphene threshold after 5 days of

Handling Editor: Gregor Thut.

This is one of several papers published together in Brain Topography on the "Special Topic: Cortical Network Analysis with EEG/MEG".

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10548-019-00715-x) contains supplementary material, which is available to authorized users.

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Published online: 10 May 2019

visual deprivation in healthy volunteers (Pitskel et al. 2007), consistently with what has been observed in blind patients (Gothe et al. 2002).

Although TMS-induced phosphenes offer a unique opportunity for testing visual cortex excitability non-invasively, this measure presents a few limitations. First, it relies on a subjective report, and it can be influenced by response criterion (i.e., the individual tendency toward a "yes" or "no" response) as well as other experimental factors, such as instructions given to participants (Mazzi et al. 2017). Furthermore, typically phosphenes can be elicited in around 60% of the tested participants (Romei et al. 2008; Taylor et al. 2010), thus limiting the investigation of cortical excitability in a broader population.

Concurrent TMS-EEG recording may overcome these limitations, by providing an objective and reliable measure of neural activity (Casarotto et al. 2010; Lioumis et al. 2009; Thut and Miniussi 2009). TMS-evoked potentials (TEPs) are indeed considered quantifiable markers of cortical excitability (Komssi and Kähkönen 2006; Miniussi et al. 2012; Miniussi and Thut 2010).

In the present study, we aimed to assess changes in visual cortex excitability as a function of environmental light by means of both phosphene perception and TMS-EEG. Unlike previous studies that focused either on measures of brain activity (e.g., BOLD activity; Boroojerdi et al. 2000) or phosphene reports, here we combined subjective report (i.e., phosphenes) with an objective measure of cortical excitability (i.e., TEPs). In addition, since our protocol entailed the application of TMS at different intensities, we decided to conduct an exploratory analysis to investigate the relationship between this parameter and the amplitude of each TEP component.

Methods

Participants

Fifteen young healthy participants gave written informed consent to participate in the study. One-third of them could not reliably report TMS-induced phosphenes, a proportion consistent with previous literature (Romei et al. 2008; Taylor et al. 2010). Two further participants could not report phosphenes reliably during the training block (see procedure below). The remaining 8 participants (5 females, 6 right-handed, mean age ± SD: 23 ± 3 years) took part in the TMS-EEG experiment. All participants had no contraindication for TMS application (Rossi et al. 2009), they all had normal or corrected-to-normal vision and no history of neurological disorders. The study was run in accordance with the declaration of Helsinki, the TMS safety guidelines (Rossi et al. 2009) and approved by the local Ethics Committee of the IRCCS San Giovanni di Dio Fatebenefratelli, Brescia (Italy), where the experiment took place.

Experimental Procedure

Participants were comfortably seated in a sound-attenuated room. At the beginning of the experiment, the center of the TMS coil was placed 1 cm above and 2 cm lateral to the inion to target V1/V2 and it was moved in steps of 0.5 cm until the stimulation hotspot was identified. The hotspot was defined as the scalp location over the left hemisphere where TMS could reliably induce stable phosphene perception in the right visual field. Information about the individual hotspot location was not stored. A training block, identical to phosphene perception assessment (see next section), was run to familiarize participants with the task and to assess their reliability in reporting phosphenes. To confirm that participants authentically perceived phosphenes, we checked on two criteria (Mazzi et al. 2017): the dependence of phosphene perception on the stimulated hemisphere (i.e., phosphenes must be localized in the right visual field when stimulating the left hemisphere) and on the stimulation intensity (i.e., the number of perceived phosphenes must increase as a function of TMS intensity).

After the training block, participants underwent two conditions (30 min each) in counterbalanced order (Fig. 1a). During the DA condition, participants were blindfolded using eye patches with an adhesive edge. Once participants wore the patches and had their eyes closed, we switched off the main light in the room and turned on a low light lamp (this was used by the experimenter to read the questionnaire, see below). Participants were asked whether they could detect any change (lamp on/off) in the environmental light, to ensure that they were carefully blindfolded (de Graaf et al. 2017). None of the participants was able to report any difference. Conversely, during the control condition, hereafter referred to as light adaptation (LA), participants had their eyes open and the environmental light was kept constant at 390 lx, as in Boroojerdi et al. (2000). During both adaptation periods, participants were asked to relax and to answer the *Temperament and Character Inventory* (Cloninger 1994; Fossati et al. 2007), whose items were read aloud by the experimenter. This was done to avoid mind-wandering or drowsiness. Data from the inventory were not stored nor analysed. At the end of both adaptation periods, phosphene perception was assessed concurrently to EEG recording (see the following section for details).

Phosphene Perception Assessment

During the assessment of phosphene perception ("MOCS & TMS-EEG" blocks in Fig. 1a), participants were blindfolded with their eyes closed (de Graaf et al. 2017), using

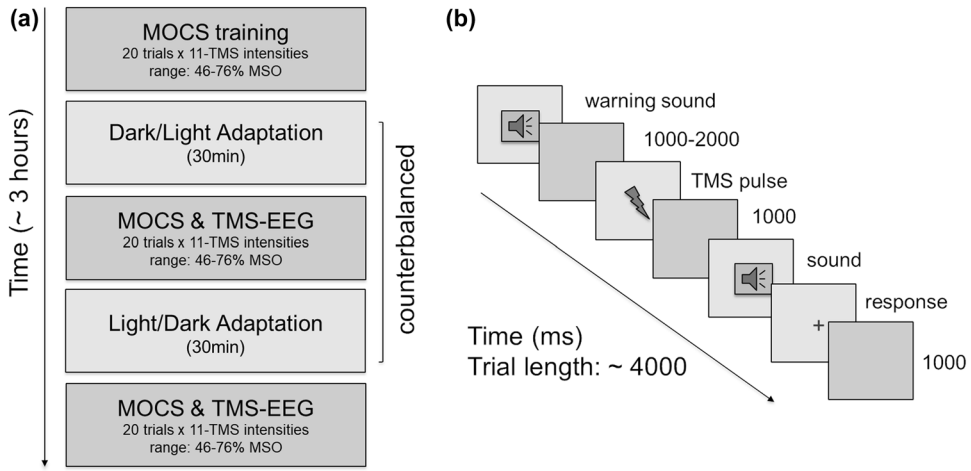


Fig. 1 Experimental procedure. **a** Experimental session. **b** Trial structure

the same procedure as in the DA, and were asked to fixate an imaginary point in front of them. Each trial started with a warning tone (1000 Hz; 150 ms), followed (at a variable interval between 1 and 2 s) by a TMS pulse. One second following the TMS pulse, a second sound, identical to the previous one, indicated participants to report whether they perceived a phosphene or not, by pressing one of two buttons on a conventional computer keyboard with the index and middle finger of the right hand (Fig. 1b).

Phosphene perception was assessed through the Method Of Constant Stimuli – MOCS (Kammer et al. 2001; Mazzi et al. 2017), by applying single TMS pulses over the left visual cortex at 11 different intensities (from 46 to 76% of the maximal stimulator output - MSO, in steps of 3%; 20 trials for each TMS intensity). The intensity was randomly selected in each trial and both intensity and pulse delivery were controlled automatically through a Matlab program (The MathWorks; Abrahamyan et al. 2011). The stimulation was delivered by means of a figure-of-eight 70 mm winding coil connected to a bi-phasic Magstim Rapid Stimulator (Magstim Company, Whitland, Dyfed, UK). The coil was positioned such that the handle pointed upwards and was parallel to the subject's spine. The coil position was monitored by a stereotaxic neuronavigation system (SofTaxic, Electro Medical Systems, Bologna, Italy) throughout the experiment.

EEG

During the MOCS procedure, EEG was continuously recorded (sampling rate: 5 kHz; online bandpass filter: between 0.1 and 1 kHz) from 30 electrodes (Fp1, Fp2, F7, F3, Fz, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, PO9, PO7, PO3, POz, PO4, PO8, PO10, O1, Oz, O2, I1, Iz, I2) using a TMS-compatible EEG system (BrainAmp 32MR plus, Brain Products). The ground was placed on AFz and all channels were referenced online to the right mastoid. To monitor eye movements, electrooculogram was recorded in a bipolar montage, by placing two additional electrodes above and below the lateral canthus of the left and the right eye, respectively. Skin/electrode impedance was below 5 kΩ.

Analysis

Phosphenes Report

Phosphene perception across TMS intensities was assessed by fitting a Weibull function (lapse rate at 4%) for each condition (DA and LA) and participant, by using the maximum likelihood procedure implemented in Palamedes toolbox (Prins and Kingdom 2009) in Matlab (The MathWorks). From the individual psychometric function, we extracted the threshold (i.e., the TMS intensity at which participants reported phosphenes in 50% of the trials) and the slope (i.e., a parameter indicating the steepness of the function). In order to investigate the effect of different adaptation conditions (DA and LA) on phosphene report, we ran a two-tailed Student's paired *t* test on the phosphene threshold and slope separately (statistical significance set at $p < 0.05$). The normality of the distributions was confirmed by the Kolmogorov–Smirnov test (Statistica for Windows, version 10, StatSoft).

TMS-Evoked Potentials

EEG data analysis was performed using BrainVision Analyzer 2 (Brain Products GmbH, Munich, Germany). The EEG signal was re-referenced offline to the average of the two mastoids and high-pass filtered at 2 Hz (Butterworth zero phase filter; 12 dB/oct). The TMS-induced artifact was removed by interpolating the signal from 2 ms before to 10 ms after the pulse. Independent component analysis (ICA) was applied to identify and remove components reflecting eye movements and residual TMS-related

artifacts (ICA algorithm: infomax). After visual inspection, signal from corrupted electrodes (range 0–3; mean ± SE: 0.94 ± 1.12) was interpolated. Line noise was removed (50 Hz notch filter) and the signal low-pass filtered at 40 Hz (Butterworth zero phase filter; 12 dB/oct). The EEG signal was segmented into epochs ranging from 105 ms before to 1000 ms after the TMS pulse and baseline corrected for the 100 ms preceding the TMS pulse. Epochs were rejected if the signal amplitude was higher than ± 70 μV in any channel or if eye movements and/or muscle artifacts were detected by visual inspection (range 3–28%; mean ± SD of rejected epochs: 22 ± 8%). The grand-average across trials (regardless of TMS intensity and condition) and participants was used to identify TEP components. Components within the first 60 ms were considered as measure of visual cortex excitability, whereas later components (N100, P150 and P280) were not considered for further analyses since they could be likely contaminated by auditory and somatosensory processing (Herring et al. 2015; Nikouline et al. 1999; but see Conde et al. 2019; Gordon et al. 2018). TEP amplitude was measured by pooling the signal recorded from 8 electrodes (POz, PO3, PO7, PO9, Oz, O1, Iz, I1) which covered the stimulated visual cortex, over a fixed time-window of 5 ms around the peak of each component.

Dark Versus Light Adaptation First, we aimed at testing the effects of adaptation conditions on TEP amplitude and their interaction with TMS intensity. Specifically, we were interested in evaluating if the effects of adaptation, if any, could be intensity-dependent. To this aim, we averaged TEPs based on three levels of intensities: low (46–52% MSO), medium (58–64% MSO) and high (70–76% MSO). Low and high intensities were below and above the average phosphene threshold, respectively (averaged threshold: 62.98 ± 1.54% MSO). Then, a separate repeated-measures analysis of variance (rm-ANOVA) was performed for each component with Condition (DA, LA) and Intensity (low, medium, high) as factors. The Kolmogorov–Smirnov test confirmed the normality of the distributions; the Tukey honest significant difference was applied in order to correct for multiple comparisons (Statistica for Windows, version 10, StatSoft).

TEP Amplitude as a Function of TMS Intensity To investigate if our results could be explained by a change in the stimulus–response relationship, we considered the amplitude of each TEP component as a function of TMS intensity and ran a trend analysis for the two adaptation conditions, separately. This analysis allowed us to explore the relationship between TMS intensity and TEP amplitude, extending findings on intensity-amplitude relationship previously described within the motor cortex (Komssi et al. 2004) to the visual system. Because of the exploratory nature of this analysis, we adopted a data-driven approach to look at the relationship between TMS intensity and TEP amplitude.

Despite expecting TEP amplitude to increase as a function of TMS intensity (Komssi et al. 2004; Kähkönen et al. 2005), we did not have any a priori hypotheses about the parametric function which would best describe this positive relationship (e.g., linear, quadratic, etc.). For this reason, we performed a two-step analysis. In the first step, the amplitude of each TEP component from all participants was submitted to a non-parametric smoothing spline analysis, a data-driven procedure that facilitates the detection of the type of relationship between variables without any a priori assumptions (Ramsay and Silverman 2005; Pellicciari et al. 2016; R, version 3.3.1 - R Core Team 2016; Fig. 2a). The degree of smoothing in the spline analysis is defined by the *span* parameter (*span* range 0–1; the higher the value, the smoother the fitted curve). The *span* value was selected using the Akaike Information Criterion (AIC) method, which allows models comparison on the basis of their maximum-likelihood fit to the data, taking into account model complexity (Burnham and Anderson 2002, 2004). In the second step of the trend analysis, aimed at identifying the best parametric function from step one, we fitted a set of parametric functions of increasing complexity (i.e., increasing number of parameters: linear, logarithmic, exponential, quadratic, cubic) to the spline results (Fig. 2b). For components in which TEP amplitude was negative, data were linearly transformed to

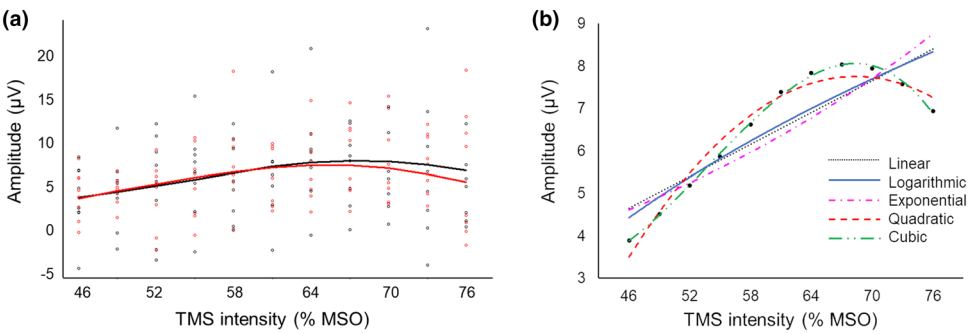


Fig. 2 Two-step trend analysis: example on P25. **a** Step1: non-parametric smoothing spline analysis on single-subject data, after LA (red) and DA (black). **b** Step2: parametric fitting on spline values; comparison among a set of functions for DA

positive values in order to apply the exponential fitting. The coefficient of determination R^2 was used to evaluate the goodness of fit of each function to the spline results. In this step, we always selected the function with a lower number of parameters that fitted the spline results. A function with a higher number of parameters was selected only if R^2 increased by 10%. This second step allows making predictions about TEP amplitude beyond the range of tested TMS intensities (i.e., 46–76% of MSO).

Results

Phosphene Report

The slope of the psychometric function was significantly higher after DA compared to LA (DA: 11.56 ± 1.74; LA: 9.35 ± 1.64; $t_{(14)}$=2.61, p = 0.035; Fig. 3a, b), indicating a greater visual sensory reliability (i.e., the steeper the function, the lower the variability around threshold; Parker and Newsome 1998). The estimated threshold for phosphene perception did not change between conditions (DA: 62.77 ± 2.24% MSO; LA: 63.20 ± 2.25% MSO; $t_{(14)}$=0.44, p = 0.675), suggesting that DA did not modulate visual cortex sensitivity (Fig. 3a–c).

TMS-Evoked Potentials

From the grand-average across TMS intensities, we identified five main components within the first 60 ms peaking over the parieto-occipital electrodes (group-averaged peak latency indicated in brackets): P25 (23 ms), N35 (34 ms), P40 (43 ms), N50 (52 ms), and P60 (66 ms), see Fig. 4.

Dark Versus Light Adaptation

The rm-ANOVA performed for each component with factors Condition (DA, LA) and Intensity (low, medium, high) revealed a main effect of Intensity for N35 and P40 (N35: $F_{(2, 14)}$ = 13.77, p < 0.001, η_p^2 = 0.66; P40: $F_{(2, 14)}$ = 7.48, p = 0.006, η_p^2 = 0.52). This effect was explained by an increase in peak amplitude as a function of TMS intensity (N35: low < medium, low < high, $p \leq 0.017$; P40: low < high, p = 0.005). For the remaining TEP components, we observed a trend towards significance in the same direction, which may be due to the low sample size (P25: $F_{(2, 14)}$ = 3.57, p =0.056, η_p^2 = 0.34; N50: $F_{(2, 14)}$ = 3.51, p =0.058; P60: $F_{(2, 14)}$ = 3.52, p = 0.058, η_p^2 = 0.33). Furthermore, the rm-ANOVA revealed a significant interaction between Condition and Intensity for P60 ($F_{(2, 14)}$ = 6.19, p =0.012, η_p^2 = 0.47). Post-hoc comparisons showed a significant difference between DA and LA at high intensities ($t_{(14)}$=2.25; p = 0.030), with the P60 amplitude after DA being significantly lower compared to LA (DA = 5.12 ± 1.08 µV; LA = 8.65 ± 1.67 µV; Fig. 5). For completeness, supplementary Fig. S1 shows the effect of TMS intensity on later TEP components.

TEP Amplitude as a Function of TMS Intensity

As expected, the relationship between TEP amplitude and TMS intensity was positive (Fig. 6). The first step of the trend analysis (non-parametric spline analysis), performed to identify the degree of smoothing (AIC method), showed a consistent span value across TEP components (span: 0.86 ± 0.01). Following the span calculation, we identified the best parametric fitting. The goodness of fit we observed in the selected function was higher than 97% for all components (R^2 range 97–100%). Figure 6 shows the best model fitting for each component in both conditions (DA and LA).

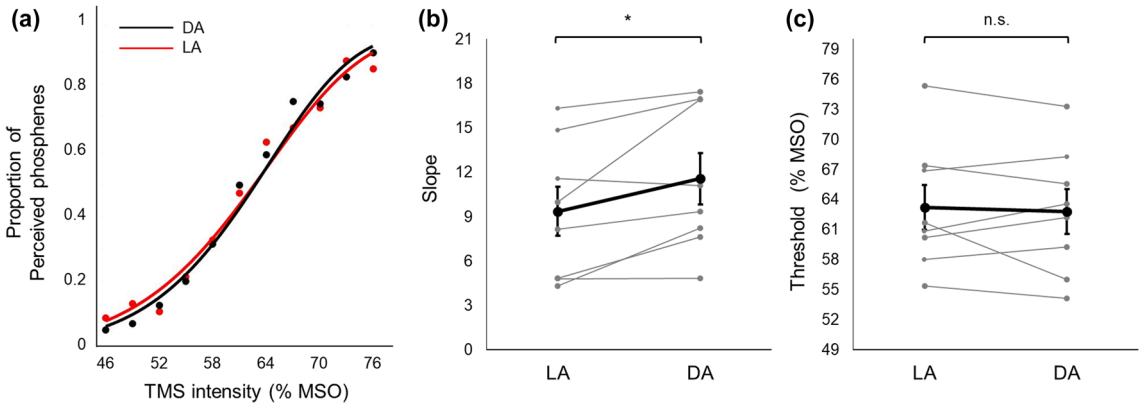
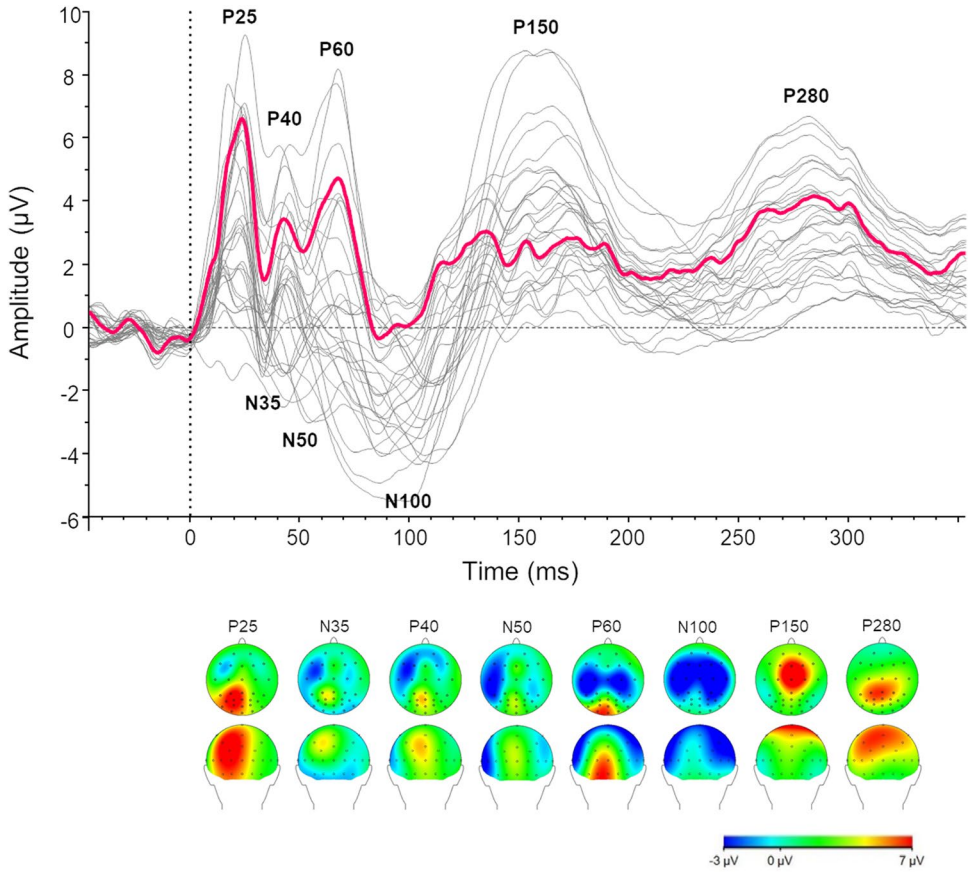


Fig. 3 Phosphene perception assessment. **a** Weibull function fitted to subjective report of perceived phosphenes averaged across subjects as a function of TMS intensity, after LA (red) and DA (black). **b** The slope of the psychometric function after DA is significantly steeper than after LA (p = 0.035). **c** No significant difference in phosphene threshold between DA and LA



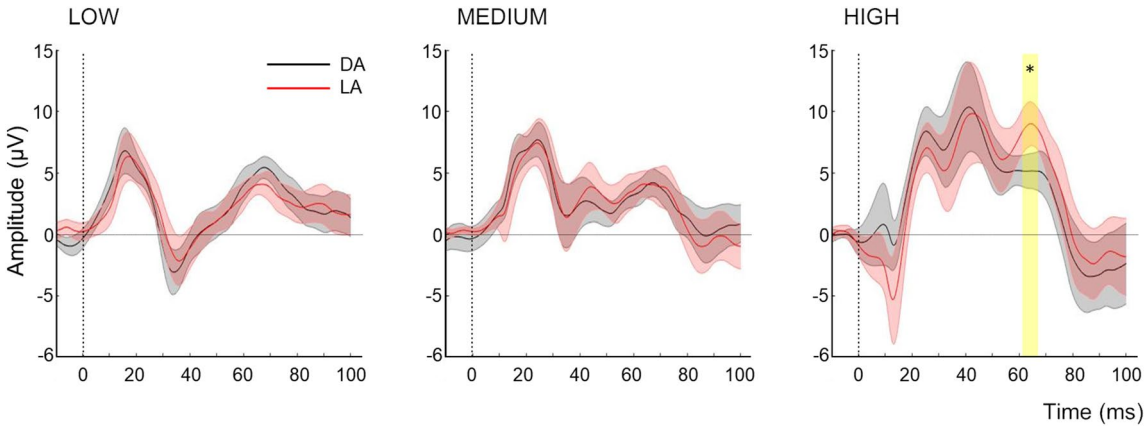


Fig. 4 Grand-average TEP after DA regardless of TMS intensity (data after LA are comparable). Signal from parieto-occipital electrodes pooling in thick-colored line. Topographical maps of main components (upper row: all electrodes; lower row: occipital view); amplitude range as shown in colorbar

Fig. 5 Interaction between Intensity (low, medium and high) and Condition (LA, red; DA, black): in high TMS intensities, P60 after DA is significantly lower compared to LA ($p = 0.030$). Shaded areas represent the SE

Importantly, for each component, the selected parametric function was consistent between adaptation conditions. The relationship between TEP amplitude and TMS intensity was linear only for N35 (DA and LA: $R^2 = 0.98$). We observed a quadratic trend with the parabola opening downwards (i.e., negative *a* parameter) for P25 (DA: $R^2 = 0.97$, LA: $R^2 = 0.98$), and an exponential trend for P40 (DA: $R^2 = 0.98$, LA: $R^2 = 1$). Finally, both N50 (DA and LA: $R^2 = 0.98$) and P60 (DA and LA: $R^2 = 0.99$) best fitted to a quadratic function with the parabola opening upwards (i.e., positive *a* parameter). Corroborating findings from the rm-ANOVA (i.e., a significant interaction between Intensity and Condition for P60), confidence intervals calculated for spline values showed that the only case in which DA and LA did not overlap was represented by the amplitude of P60 at highest TMS intensities (Fig. 6).

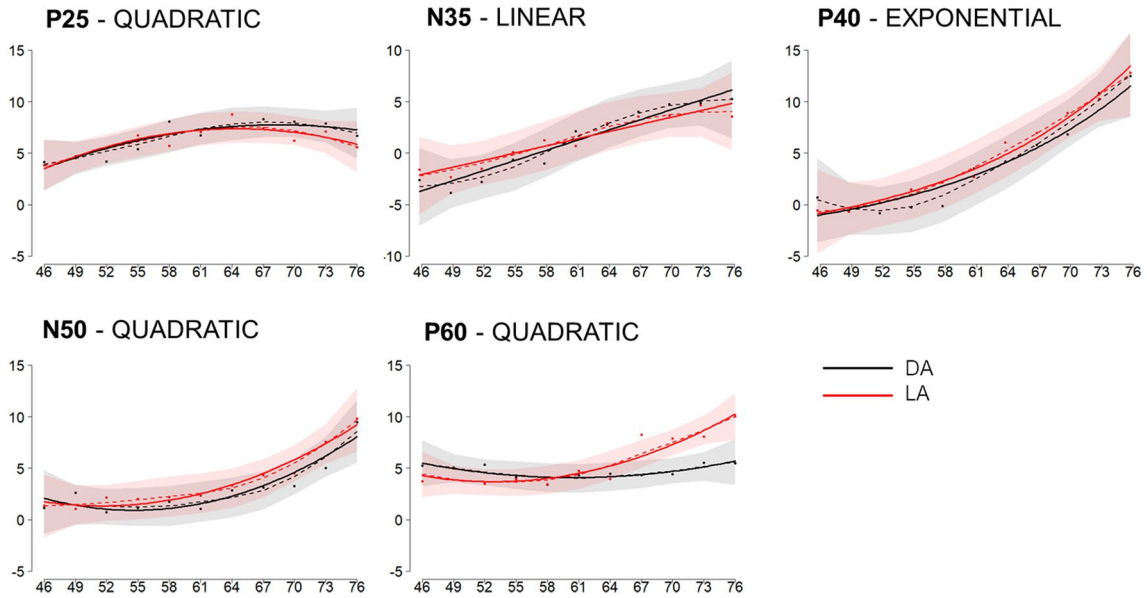


Fig. 6 Best model fitting (continuous line) to spline results (dashed line), for each component separately (dots: original TEPs amplitude averaged across subjects), after LA (red) and DA (black). X-axis: TMS intensity (% MSO); y-axis: TEP amplitude (μV); shaded areas represent confidence intervals

Discussion

In the present study, we investigated the effects of DA on visual cortex excitability using TMS-induced phosphenes and simultaneous TMS-EEG recording. After 30 min of DA, the psychometric function describing the relationship between the number of perceived phosphenes and TMS intensity was steeper (i.e., higher slope) compared to the control condition (Fig. 3a, b). Such modulation in phosphene perception was accompanied by a lower amplitude of a positive TEP component peaking around 60 ms after TMS pulse, only for high TMS intensities (Fig. 5). The interaction between adaptation condition and TMS intensity could not be explained by an effect of DA on the type of relationship between TEP amplitude and TMS intensity, which was linear for N35, quadratic for P25, N50, P60 and exponential for P40, in both adaptation conditions (Fig. 6).

At behavioral level, the slope of a psychometric function provides information about the rate of perceived phosphenes as a function of TMS intensity. A higher slope suggests a greater visual sensory reliability, i.e., the variability around the threshold is reduced, and participants are more likely to report the presence of a phosphene at high TMS intensities, and, conversely, less likely to report phosphenes at lower intensities (Parker and Newsome 1998). A previous finding about the DA effects on phosphene perception found a positive relation between TMS intensity and perceived phosphenes, which was steeper after 180 min of DA (Boroojerdi et al. 2000). However, the authors did not perform a statistical test for this observation, because the low number of tested TMS intensities prevented a function fitting, from which the slope parameter can be obtained. Conversely, the parametric function fitting applied here to phosphene perception rate enabled us to test slope modulation statistically.

Previous studies reported an increase in visual cortex excitability after DA, indexed by a reduction in phosphene threshold (Boroojerdi et al. 2000; Fierro et al. 2005), which we did not observe here. Nonetheless, the studies mentioned above (Boroojerdi et al. 2000; Fierro et al. 2005) tested longer periods of DA (i.e., a minimum of 45 min), therefore it might be that 30 min of DA, as used in the present study, are insufficient to affect phosphene threshold. Moreover, it should be noted that in those studies (Boroojerdi et al. 2000; Fierro et al. 2005) phosphene threshold has been estimated differently. Here, phosphene threshold was calculated by using the MOCS, a reliable procedure commonly used in psychophysics (Kammer et al. 2001; Mazzi et al. 2017), whereas in previous studies (Boroojerdi et al. 2000; Fierro et al. 2005), the threshold was defined less systematically as the minimum intensity able to elicit a phosphene in 3 out of 5 trials (Boroojerdi et al. 2000; Fierro et al. 2005).

At neurophysiological level, we found a decrease in P60 amplitude after DA compared to LA. A recent study within the motor cortex reported an increase of P60 amplitude as a consequence of low cortical excitability, obtained by applying an inhibitory low-frequency repetitive-TMS protocol (Casula et al. 2014). The P60 increase described by Casula et al. (2014) has been linked to the involvement of inhibitory mechanisms underlying this component, likely modulated by slow $GABA_B$-(gamma-aminobutyric

acid)-mediated inhibitory post-synaptic potentials (Rogasch et al. 2013). Despite the differences between the visual and motor cortex, we speculate that in our study we observed an opposite, but congruent, pattern; i.e., P60 was lower after DA compared to LA, which may suggest an increase in cortical excitability after DA, consistently with the existing literature on DA (Boroojerdi et al. 2000, 2001; Fierro et al. 2005). It is worth noting that the P60 modulation we observed after DA only occurred when TMS was applied at high intensities. Similarly, a recent TMS-EEG study on the motor cortex has shown that the effects of two different antiepileptic drugs known to alter cortical excitability as assessed by TEP amplitude (i.e., lamotrigine and levetiracetam), cannot be disentangled by applying TMS at motor threshold, but only at higher intensities (Premoli et al. 2017). Taken together, the present result and existing literature on TEPs highlight that supra-threshold TMS intensities might be a key element to effectively stimulate the cortex and detect modulations of cortical excitability (Gordon et al. 2018; Premoli et al. 2017). Our finding is also consistent with evidence that different TMS intensities may selectively activate different neural populations in the motor cortex. For example, supra-threshold and sub-threshold TMS can lead to opposite effects by inducing short-interval intracortical facilitation (SICF) or inhibition (SICI) (Ilić et al. 2002; but see also Voineskos et al. 2010). Moreover, motor-evoked potentials (MEPs) are susceptible to specific pharmacological interventions (Paulus et al. 2008) or ongoing oscillations (Schaworonkow et al. 2019) in an intensity-dependent manner. Therefore, it may be possible that intensity-specific effects revealed by TEPs may reflect the modulation of specific neural populations, although this remains a hypothesis to be tested in further studies.

TEP components recorded in the present experiment are comparable to previous findings in the visual cortex in terms of TEPs polarity and latency (Herring et al. 2015; Taylor et al. 2010; Bagattini et al. 2015). To the best of our knowledge, only one paper has described visual TEPs within the first 50 ms (Herring et al. 2015), whereas in the other two studies (Bagattini et al. 2015; Taylor et al. 2010) the signal in the first tens of milliseconds has been interpolated to remove the TMS artifact. While early components reported by Herring et al. (2015) are consistent with our findings (P20-P25, N40-N35, respectively), the authors did not observe the P40 and N50. We argue that the difference in TMS intensity between the two studies can account for this difference. Indeed, Herring et al. (2015) applied TMS intensity below the phosphene threshold (i.e., 80%). In our data, the P40-N50 complex was absent at low intensities (which on average were below phosphene threshold) consistently with Herring et al. (2015), while it emerged at medium intensities and became detectable at high intensities (Fig. 5).

TEP amplitude has been repeatedly shown to scale with TMS intensity (Casarotto et al. 2010; Kähkönen et al. 2005; Komssi et al. 2004; Rosanova et al. 2009), with previous studies indicating a non-linear and linear relationship for the motor (Komssi et al. 2004), and prefrontal cortex (Kähkönen et al. 2005), respectively. However, the limited number of TMS intensities applied in these studies (Kähkönen et al. 2005; Komssi et al. 2004) prevented a systematic investigation of the relationship between TEPs and TMS intensity. Here, we extend previous findings not only by investigating TEP amplitude as a function of TMS intensity within the visual cortex but also by exploring their relationship through a parametric function fitting, through a wide range of TMS intensities (Fig. 6). Among TEP components, P25 appeared to be the only one in which the parametric fitting (i.e., quadratic function with the parabola opening downwards) did not predict a further increment in peak amplitude at higher intensities beyond the tested range. The differences in the intensity-amplitude functions suggest that different mechanisms may underlie the generation of these components (Komssi et al. 2004), although the scarcity of studies characterizing visual TEPs makes impossible to hypothesize what these mechanisms might be.

The present study provides evidence of cortical excitability changes after short-lasting DA. We are aware of its limitations: First, out of the 15 participants tested we could analyze data only from 8 (a proportion consistent with previous phosphene literature), which may account for marginally significant results and restricts the generalization we can draw from the data. In this respect, characterizing TEPs evoked by visual cortex TMS appears of particular interest because they could become a valuable alternative to phosphenes, which can only be perceived by a population subgroup. Second, the limited number of trials for each TMS intensity prevented us from performing more detailed analyses on single-intensity TEPs (e.g., analysis on latency). However, despite these limitations, we believe the present study can be informative at least regarding two points. First, it provides a way for parametrically testing both behavioral and visual cortex excitability non-invasively. Second, the present study provides, for the first time, valuable information about the relationship between TMS intensity and visual TEPs, which can be capitalized on by future hypothesis-driven studies on cortex excitability.

In conclusion, our findings provide evidence of a cortical modulation after a short-lasting period of DA in humans, as revealed by TMS-EEG recording combined with phosphene perception assessment. Importantly, the present study highlights that testing a wide range of TMS intensities is informative both at behavioral and neurophysiological level. Indeed, the psychometric function parametrization unveiled a modulation in phosphene perception as a consequence of DA (i.e., higher slope), even in the absence of a change in

phosphene threshold. At neurophysiological level, different TMS intensities might impinge on different cortical layers and neuronal populations and might be very informative on their distinct functional roles in cortical circuits. In this respect, the application of several stimulation intensities allowed us to identify an interaction between DA and TMS intensity, with DA effects (a lower P60 amplitude) only evident when TMS was applied at high intensities, and to show that one TEP complex (P40-N50) is absent at low TMS intensities.

Acknowledgements We would like to thank Clarissa Ferrari for her valuable help and suggestions about statistics.

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