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RESEARCH ARTICLE

Visual evoked potentials modulation during direct current cortical polarization

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Abstract Transcranial direct current stimulation (tDCS) at low intensity induces changes in cortical excitability that persist after polarization ends. The effects of anodal and cathodal polarization remain controversial. We studied changes in visual evoked potentials (VEPs) during and after anodal and cathodal tDCS by applying, in healthy volunteers, 1 mA polarization through surface electrodes placed over the occipital scalp (polarizing) and over the anterior or posterior neck-base (reference). We compared tDCS applied at two durations, 3 and 10 min and both polarities. We assessed VEP-P100 latencies and amplitudes in response to pattern-reversal checkerboard stimuli before, during, and after polarization. Anodal polarization reduced VEP-P100 amplitude whereas cathodal polarization significantly increased amplitude but both polarities left latency statistically unchanged. These changes persisted for some minutes after polarization ended depending on the duration of tDCS and on the contrast level of visual stimuli. tDCS-induced changes in VEPs seem to depend on the duration of polarization and type of visual stimuli used. The effects induced on visual cortical neurones during polarization are more consistent than the aftereffects. Studying these changes during polarization may therefore improve our understanding of these phenomena.

Keywords VEP · Modulation · Cortical polarization · TDCS

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Introduction

Numerous publications in recent years confirm that electrical polarization of the cerebral cortex with transcranial direct current stimulation (tDCS) at low intensity (0.5–1.5 mA) through wide surface electrodes 30–50 cm^2 placed on the scalp, modulates cortical excitability. The polarizing effects of tDCS have been evaluated with various neurophysiological techniques including motor evoked potentials (MEPs), somatosensory evoked potentials (SEPs) and behavioural tests (Fregni et al. 2005, Marshall et al. 2005, Iyer et al. 2005, Rogalewski et al. 2004, Quartarone et al. 2004, Priori et al. 2003). These studies nevertheless report conflicting findings because no clear correlation emerges between increased or diminished cortical excitability and tDCS polarity. In particular, studies using SEPs report increased excitability after anodal focused polarization (Matsunaga et al. 2004) whereas those investigating MEPs (Priori et al. 1998, Nitsche and Paulus 2000, Lang et al. 2004a, b; Ardolino et al. 2005, Nitsche et al. 2005, Lang et al. 2004a, b; Uy and Ridding 2003) report contradictory effects and recently also some studies on polarization of the visual cortex (Antal et al. 2006, Antal et al. 2004a, b, c) reported contrasting results for two VEP components (N70 and P100) and for other visual perception modalities. These conflicting results may reflect neurophysiological differences among the different cortical areas studied and the different geometry of the polarizing dipole used. Most studies also applied tDCS at various durations and evaluated only its aftereffects, neglecting possible effects during stimulation.

In this study, to assess better the excitatory and inhibitory effects and aftereffects of low-intensity anodal and

cathodal tDCS we investigated changes in VEP-P100 in response to black-and-white checkerboard pattern-reversal stimuli at two contrast levels in healthy subjects during, as well as after scalp polarization lasting 3 and 10 min. To distinguish better the effects of anodal and cathodal scalp tDCS and avoid interference from cortical areas other than the visual areas studied we used a non-cephalic reference polarizing electrode.

Materials and methods

We studied 20 healthy volunteers all of whom had normal visual acuity and whose ages ranged from 26 to 50 years (12 men and 8 women). Experiments were conducted in accordance with the Declaration of Helsinki and the study procedures were approved by the hospital ethics committee. All participants gave written informed consent to the study.

VEP recording

In all subjects VEPs were obtained with black-and-white pattern-reversal checkerboards (two cycles per degree), at two levels of contrast: CH = 100 cd/m^2, contrast 100/1 and CL = 50 cd/m^2, contrast 50/1. To avoid habituation owing to a constant checkerboard-reversal rate, patterns were reversed at a frequency of 2 Hz ± 20%. Signals were recorded through silver-chloride surface electrodes with the reference electrode placed on the vertex and the recording electrode 1 cm above the inion. The acquisition system automatically rejected artifacts and bandpass filtered between 2 and 100 Hz. VEPs were recorded by averaging 60 traces, so that each recording lasted about 45 s. Because the widest amplitude and most stable component in response to this kind of stimulation is P100 we evaluated this component alone.

Transcranial direct current stimulation

The occipital cortex was polarized through the scalp with a battery-powered (24 V) constant current unit adjustable between 0 and 2 mA, through wide rectangular electrodes (5 × 8 cm in size) made of saline-soaked synthetic sponge. Polarizing electrodes were applied with an elastic net head-cap. The scalp polarizing sponge electrode (5 × 8 cm) was placed on the occipital region upon the round, metal VEP recording electrode (1 cm in diameter) and isolated from it with a 1 mm-thick plastic disk measuring 3 cm in diameter. The reference polarizing sponge electrode was placed randomly over the anterior neck base (ten subjects) or on the back over C7 (ten subjects). For each subject, the position of the reference electrode was maintained constant throughout the study. A preliminary study showed that the position of the reference electrodes over the neck (anterior or posterior) had no influence on the effects of tDCS (data not shown). Stimulation intensity was pre-set at 1 mA, a strength that preliminary tests showed was below the subjects' cutaneous sensory threshold. At higher intensities (around 1.3–1.5 mA) most subjects felt a tingling sensation under the electrodes. To avoid causing the subjects discomfort and to exclude arousal effects that might alter VEPs, during the study experiments we therefore kept the current intensity just below this threshold. To comply with safety requirements and to exclude functional alterations of the brainstem, body temperature, arterial pressure and heart rate were non invasively monitored in all subjects during tDCS and for at least 20 min afterwards.

Experiment 1: short-duration polarization

Ten subjects underwent two tDCS sessions including two recordings (anodal and cathodal) each lasting 3 min and performed on different days. VEPs were elicited by high contrast black-and-white checkerboards in the first session, and by low contrast black-and-white checkerboards in the second session. In each session, anodal and cathodal polarizations were randomly delivered. Subjects underwent nine VEP recordings (three baseline before, three during and three after polarization) with a 1-min interval between one recording and the next. When the VEP reached baseline values and remained stable for 60 min a second identical sequence of recordings was obtained to test the opposite polarity.

Experiment 2: long-duration polarization

The other ten subjects underwent four 10 min tDCS sessions on four different days. In each stimulation session anodal or cathodal polarization were randomly delivered. VEPs were elicited by high contrast black-and-white checkerboards in the first and second session, and by low contrast black-and-white checkerboards in the third and fourth session.

Subjects underwent 12 VEP recordings (3 baseline before, 3 during and 6 after polarization). VEPs were recorded at 1 min intervals for baseline, at 3 min intervals during polarization up to 10 min after polarization, and at 10 min intervals thereafter, so that the last recording was obtained 30 min after polarization ended. On another day, a second identical sequence of recordings was obtained to test the opposite polarity.

In all experiments, we assessed latencies and amplitudes of VEP-P100 defined as the highest positive deflection in the trace. To normalize the data among subjects, we then calculated the mean P100 latency and amplitude of VEPs in the three baselines traces acquired for each subject and the percentage changes from baseline of VEPs recorded during and after polarizations. To compare the effectiveness of cathodal and anodal polarization in modulating VEP, the "absolute" percentage variations in VEPs during different polarizations (i.e. the absolute percentage value of the increase or decrease in P100 amplitude from baseline) were also subjected to statistical analysis.

Statistics

Student's *t* test was used to compare the mean of the three baseline absolute values of P100 amplitude and latency for each subject during a single session and the mean variation in these variables (expressed in absolute terms) measured during cathodal and anodal polarizations. A "between conditions" analysis of variation (ANOVA) was used to study the percentage variations in P100 latencies and amplitudes from baseline and the variations in these variables during and after polarizations with the between-factor polarization (anodal vs. cathodal) and with repeated-measure factors contrast (high and low), and time (pre-, during, post-polarization recordings: total 9 recordings in experiment 1 and 12 recordings in experiment 2). Tukey's honest significant difference test was used for post hoc analysis. *P* values less than 0.05 were considered significant for all tests.

Results

In all subjects we obtained reproducible, artefact free VEP recordings during and after tDCS. Placing the reference electrode in a different non-cephalic site (anterior or posterior neck base) had no influence on the tDCS-induced changes in P100.

In all subjects vital signs (body temperature, heart rate and arterial pressure) remained stable throughout the polarization procedures.

P100 amplitude

Experiment 1 (short-duration polarization)

The two polarization protocols yielded a similar amplitude P100 (cathodal, 5.0 ± 1.5 μV and anodal, 4.8 ± 1.6 μV; *t* test: $P = 0.89$). The amplitude of P100 was slightly lower after low-contrast than after high-contrast stimuli but the difference was not significant (4.4 ± 1.7 and 5.0 ± 1.5 μV, *t* test: $P = 0.36$).

Analysis of variance detected a significant effect on P100 amplitude of the between-group factor polarization ($F_{1,36} = 112.78$, $P < 0.001$). The between-group factor polarization showed an interaction with the repeated-measure factors contrast ($F_{1,36} = 4.34$, $P < 0.05$) and time ($F_{8,288} = 62.14$, $P < 0.001$). ANOVA also detected a poorly significant interaction of the between-group factors polarization with the repeated-measure factors contrast and time ($F_{8,288} = 2.76$; $P = 0.051$).

Post hoc comparison showed that P100 amplitudes significantly increased (by about 40% for high-contrast stimuli and 50% for low-contrast stimuli from baseline, $P < 0.001$) during cathodal polarization and decreased (by about 30% for high-contrast and 40% for low-contrast stimuli, $P < 0.001$) during anodal polarization. After cathodal polarization, the increase in P100 amplitude persisted for 1 min, ($P < 0.01$) with high-contrast stimuli and for 2 min with low-contrast stimuli ($P < 0.01$) (Figs. 1, 2). Conversely, the amplitude of P100 recorded shortly after anodal polarization remained unchanged from the pre-polarization recordings both with high-contrast and with low-contrast stimuli. After cathodal and anodal polarization, recordings obtained before polarization and at 3 min after polarization showed a similar amplitude P100.

During polarizations, no significant difference was found in the mean absolute variations (absolute value

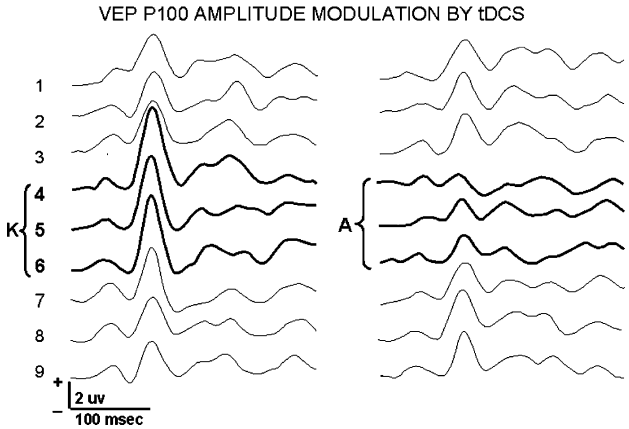

Fig. 1 Visual evoked potential (*VEP*) P100 amplitude modulation in a representative subject: each trace is an average of 60 visual stimuli sampled sequentially a minute. Positivity upward. *Left*: *1*, *2*, *3* baseline condition; *4*, *5*, *6* amplitude increase during 3 min cathodal transcranial direct current (*tDCS*) polarization (K); *7*, *8* aftereffect; *9* return to baseline condition. *Right*: *1*, *2*, *3* baseline condition; *4*, *5*, *6* amplitude decrease during 3 min anodal polarization (*A*); *7*, *8* aftereffect; *9* return to baseline condition

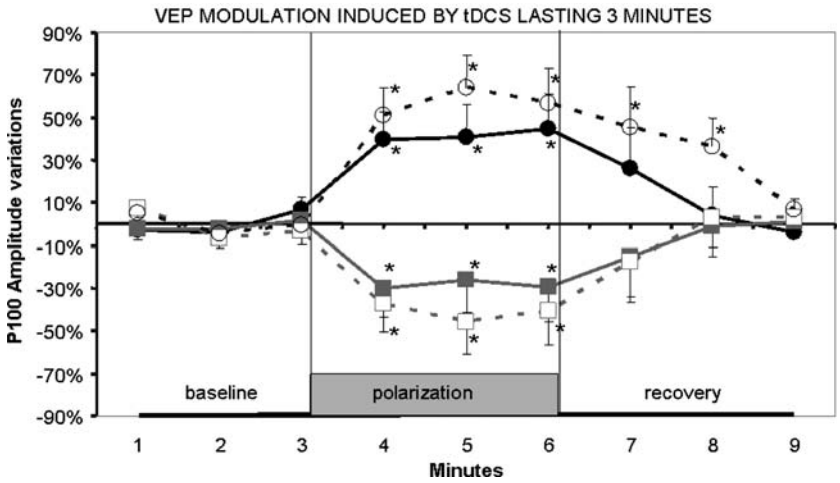

Fig. 2 Visual evoked potential P100 amplitude modulation induced by short-duration tDCS polarization (mean ± SD values of all subjects). *Black lines* cathodal polarization, *grey lines* anodal polarization, *thick lines* high-contrast VEPs, *dotted lines* low contrast VEPs. *$P < 0.01$

of the percentage increase or decrease) in the amplitude of the VEP P100 elicited with high-contrast and low-contrast stimuli after cathodal and anodal stimulations (t test: $P = 0.67$). Although absolute variations in the P100 amplitude during cathodal and anodal polarizations appeared more pronounced with low-contrast than with high-contrast stimuli the difference was not significant (t test: $P = 0.23$).

Experiment 2: long-duration polarization

The two polarization protocols yielded a similar amplitude P100 (cathodal, 5.2 ± 1.3 μV and anodal, 4.9 ± 1.4 μV; t test: $P = 0.82$). The amplitude of P100 was slightly smaller after low-contrast than after high-contrast stimuli but the difference was not significant (4.3 ± 1.9 and 5.1 ± 1.3 μV, t test: $P = 0.32$).

Analysis of variance detected a significant effect on P100 amplitude of the between-group factor polarization ($F_{1,36} = 41.98$; $P < 0.001$). The between-group factor polarization showed an interaction with the repeated-measure factors contrast ($F_{1,36} = 4.21$; $P < 0.05$) and time ($F_{11,396} = 39.94$; $P < 0.001$). ANOVA also detected a poorly significant interaction of the between-group factors polarization with the repeated-measure factors contrast and time ($F_{11,396} = 2.65$; $P < 0.057$).

Post hoc comparison showed that the amplitudes of the VEP P100 elicited by high-contrast stimuli significantly increased (for low-contrast stimuli by about 30% and for high-contrast stimuli by about 40% from baseline, $P < 0.001$) during cathodal polarization and decreased (by about 40% for low-contrast and 50% for high-contrast stimuli, $P < 0.001$) during anodal polarization. After cathodal polarization, the increase in P100 amplitude persisted longer after low-contrast ($P < 0.01$) than after high-contrast stimuli ($P < 0.05$) (20 vs. 4 min) (Fig. 3). With high-contrast stimuli, no difference was found in the amplitude of the P100 recorded before and shortly after anodal polarization, whereas with low-contrast stimuli the P100 amplitude decrease persisted for one minute after polarization ended.

During polarizations, the mean absolute variations in the amplitude of the P100 VEPs elicited either with

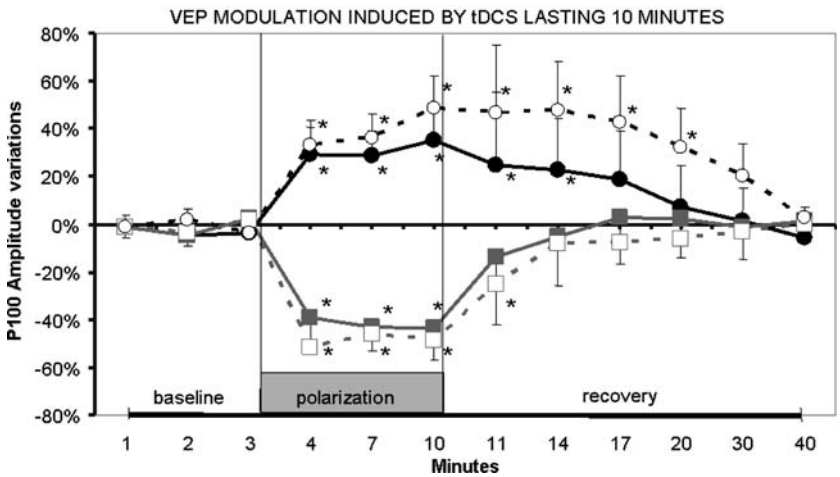

Fig. 3 Visual evoked potential P100 amplitude modulation induced by long-duration tDCS polarization (mean ± SD values of all subjects). The first VEP recording during tDCS started 1 min after polarization began. *Black lines* cathodal polarization, *grey lines* anodal polarization, *thick lines* high-contrast VEPs, *dotted lines* low contrast VEPs. *$P < 0.01$

high-contrast and low-contrast stimuli was not significantly different for cathodal and anodal stimulations (t test: $P = 0.67$).

The absolute variations in the P100 amplitude during polarizations appeared more pronounced with low-contrast than with high-contrast stimuli both during cathodal and anodal stimulations, but the difference was not significant (t test: $P = 0.26$).

P100 latency

The P100 latencies of VEPs recorded before polarization were comparable in both the experiments (Experiment 1: mean values 98.6 ± 4.6 ms for high-contrast stimuli and 112.8 ± 6.3 ms for low-contrast VEPs, Experiment 2: mean values 99.2 ± 4.9 ms for high-contrast $P = 0.98$, and 113.2 ± 6.1 ms for low-contrast VEPs $P = 0.95$).

In both experiments, ANOVA disclosed a main effect only of the between group factor contrast ($F_{1,36} = 34.63$; $P < 0.001$ for short-duration polarization and $F_{1,36} = 49.32$; $P < 0.001$ for long-duration polarization experiments). Post hoc comparison showed that the latency of VEPs obtained with low-contrast stimuli was greater than the latency of high-contrast VEPs ($P < 0.001$).

Discussion

In the healthy individuals we studied here, low-intensity (1 mA) anodal and cathodal scalp tDCS polarization induced distinct amplitude changes in VEP-P100, but left latencies unchanged. Delivering 1 mA anodal and cathodal tDCS on the occipital scalp with a non-cephalic reference electrode (neck base) enabled us to record artifact-free VEPs during and after scalp polarization. These findings clearly indicate that tDCS modulates the excitability of the visual cortical neurones.

The tDCS-induced changes in VEP-P100 amplitude appeared immediately after scalp polarization began. In particular, during cathodal polarization the P100 invariably increased in amplitude and during anodal polarization decreased, regardless of which type of polarity we tested first. In all the healthy subjects we tested, the two different polarities applied invariably elicited constant, opposite changes in the VEP-P100 amplitude. The VEP changes we observed during tDCS persisted, with greater individual variability, for some minutes after polarization ended. Their duration correlated with the duration of tDCS (3 or 10 min). These aftereffects seemed more consistent for cathodal than for anodal polarization (amplitude enhancement).

A distinctive point in this tDCS study was that we recorded VEPs in response to pattern-reversal checkerboards delivered at two contrast levels. Interestingly, cathodal and anodal tDCS seemed to have a greater effect on VEP elicited by low-contrast stimuli than on high-contrast stimuli probably because low-contrast visual stimuli only submaximally recruited cortical neurons thus allowing a more pronounced decrease or increase in neuronal recruitment by locally-induced polarization or depolarization.

Although hard to compare, these findings in part agree with a study conducted by Antal et al. (2004a) investigating tDCS aftereffects on "onset stripe VEP" and reporting that cathodal polarization induced a mild facilitatory aftereffect on P100 whereas anodal polarization induced no aftereffect, although in their study N70 behaved in the opposite way. These apparently discrepant results probably depend on the different VEP modalities used: whereas we used standard checkerboard pattern-reversal stimulation (J. Vernon Odom et al. 2004; Bodis-Wollner 1992) Antal et al used stripe pattern-onset stimulation. They may also depend on the different reference electrode position (non-cephalic in our study and cephalic that of Antal et al. 2004a).

In all subjects tested, our tDCS setup using non-cephalic reference electrodes yielded highly reproducible data during and after polarization without inducing effects related to brainstem activation. Occipital cortical polarization with a non-cephalic reference electrode left heart rate and body temperature unchanged and none of our subjects reported experiencing unpleasant sensations during or after polarization, either with short-duration or long-duration tDCS. However, we cannot definitely exclude brainstem polarization in our subjects, owing to the geometry of the dipole we used. But, the effect of the polarization seems to be proportional to density of the current, namely, higher in structures near the electrodes and minimal in the middle of the electrodes (Stratton 1941). Hence in our experiments polarization probably left inner structures, such as the brainstem, only slightly affected or unaffected. Using a non-cephalic polarizing reference electrode (anterior neck base or posterior over C7), instead of the scalp reference used in most studies, allowed us to evaluate selectively the effect of scalp polarization over a well-defined cortical area avoiding possible interference due to polarization of other cortical structures near the reference scalp electrode.

Whether the differential effects of polarity depend on the spatial arrangement of cortical neurons or on neurophysiological variables of cortical neural layers,

or on polarization variables such as intensity and orientation of the scalp dipole awaits an answer from ongoing studies. A possible explanation, also confirmed by peripheral nerve studies (Ardolino et al. 2005; Accornero et al. 1977), is that anodal polarization, in the proximity of neurons, hyperpolarizes then stabilizes neuronal membrane, whereas cathodal polarization depolarizes it. Neuronal depolarization therefore decreases the activation threshold so that the visual stimuli recruit a larger neuronal population and the signal recorded from the overlying scalp electrode increases in amplitude.

Our finding that the P100 latencies after both high-contrast and low-contrast stimuli remained unchanged during and after tDCS of both polarities suggests that scalp polarization does not interfere with retino-cortical fibre conduction.

Conclusions

Low-intensity tDCS applied to the scalp with a non-cephalic polarizing reference electrode effectively and consistently modulates human VEPs. This tDCS-induced modulation of visual neuronal excitability begins immediately after polarization starts and decreases progressively within minutes after polarization ends. The magnitude of tDCS-induced VEP changes depends on the duration of polarization and the strength of visual contrast stimuli.

Our study provides useful information on tDCS-induced modulation of cortical excitability, the effect during polarization being more consistent and stable than the aftereffect on which other studies usually focussed. Using tDCS with a non-cephalic reference electrode appears safe and may allow more selective polarization of cortical areas.

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