Prefrontal hemodynamic changes produced by anodal direct current stimulation

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\textbf{A B S T R A C T}

Transcranial direct current stimulation (tDCS) is a noninvasive brain stimulation technique that has been investigated for the treatment of many neurological or neuropsychiatric disorders. Its main effect is to modulate the cortical excitability depending on the polarity of the current applied. However, understanding the mechanisms by which these modulations are induced and persist is still an open question. A possible marker indicating a change in cortical activity is the subsequent variation in regional blood flow and metabolism. These variations can be effectively monitored using functional near-infrared spectroscopy (fNIRS), which offers a noninvasive and portable measure of regional blood oxygenation state in cortical tissue. We studied healthy volunteers at rest and evaluated the changes in cortical oxygenation related to tDCS using fNIRS. Subjects were tested after active stimulation (12 subjects) and sham stimulation (10 subjects). Electrodes were applied at two prefrontal locations; stimulation lasted 10 min and fNIRS data were then collected for 20 min. The anodal stimulation induced a significant increase in oxyhemoglobin (HbO\(_2\)) concentration compared to sham stimulation. Additionally, the effect of active 10-min tDCS was localized in time and lasted up to 8–10 min after the end of the stimulation. The cathodal stimulation manifested instead a negligible effect. The changes induced by tDCS on HbO\(_2\) as captured by fNIRS, agreed with the results of previous studies. Taken together, these results help clarify the mechanisms underlying the regional alterations induced by tDCS and validate the use of fNIRS as a possible noninvasive method to monitor the neuromodulation effect of tDCS.

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\textbf{Introduction}

Application of transcranial direct current stimulation (tDCS) to the cortex has been shown to shift the membrane potential of superficial neurons in a de- or hyperpolarizing direction, and to modulate spontaneous neuronal activity as well as the processing of afferent signals (Paulus, 2004; Priori, 2003). If tDCS is continuously applied for 5 min or more, it can provoke sustained changes in neuronal firing rates that last for many hours after the current is switched off (Bindman et al., 1962). Consistent with animal data, in humans these changes in excitability also persist beyond the time of stimulation if tDCS is applied for several minutes and the after-effects can remain stable for an hour or more if tDCS is given for 9 min or longer (Lang et al., 2004; Nitsche et al., 2003; Nitsche and Paulus, 2001). tDCS modulates excitability in a polarity-specific manner. In humans, anodal polarization increases excitability measures of the motor and visual cortex (Edwards et al., 1993; Paulus, 2004). Cathodal stimulation produces opposite effects: excitability is reduced and some functions worsen (Been et al., 2007).

Changes in cortical excitability are associated with changes of the underlying cortical neuronal activity and with subsequent changes in the regional cerebral blood flow (rCBF). For this reason, it is conceivable that a measurable output of the after-effects of tDCS – virtually in any cortical area – could be obtained by measuring the rCBF. This aspect is not trivial, as so far it has been possible to measure the after-effects of tDCS only in the motor and visual cortex, where stimulation can be evaluated by motor evoked potentials and phosphenes threshold to transcranial magnetic stimulation, respectively. However, it is not possible to assume that every different cortical area responds to tDCS in the same way. This is an important aspect as tDCS has been proposed as a possible neuromodulation treatment for many neurological and psychiatric disorders (Boggio et al., 2008; Monti et al., 2008; Ferrucci et al., 2008; Ohn et al., 2008; Nitsche et al., 2009). Both anodal and cathodal tDCS have been reported to increase the metabolism of the cortex underlying the stimulation electrodes (Lang et al., 2005). In the study by Lang et al. (2005), positron emission tomography (PET) was used to study brain metabolism of the cortex underlying the stimulation electrodes; however, PET is an invasive technique that cannot be repeated often in control subjects and patients.

The idea that regional cerebral blood flow could reflect neuronal activity began with the experiment by Roy and Sherrington (1890).
Investigations on the energy metabolism of the human brain were limited by the fact that the brain within the skull is not easily accessible for performing experimental procedures and that the nervous tissue is composed of many different cell populations; additionally, more recent techniques for studying the metabolic state of the brain, such as single photon emission-computed tomography (SPECT), functional magnetic resonance (fMRI) and PET, are not easily applicable in any situation.

Some of the limitations encountered when using the aforementioned imaging technologies can be overcome by functional near-infrared spectroscopy (fNIRS). fNIRS is a noninvasive, repeatable method that allows for regional assessment of the oxygenation state of hemoglobin in tissue (Chance et al., 1993; Edwards et al., 1993; Paulus, 2004). fNIRS measures cerebral concentrations of oxyhemoglobin (HbO2) and deoxyhemoglobin (HHb) by observing the absorption of near-infrared light. These parameters are expressed as μM variations from the baseline. Since HbO2 and HHb have different absorption spectra in the visible and near-infrared wavelength ranges, spectroscopy techniques can be used to provide an index of blood oxygenation and hence oxygen delivery; thus, changes in HbO2 and HHb concentration, as measured by near-infrared spectroscopy, could be considered a good index of rCBF variations. fNIRS can therefore provide metabolic information without invasive intervention and it is able to detect even small changes in the cerebral hemodynamic response to functional stimulation (Obrig et al., 1996). Several reports described the capability of fNIRS to measure the hemodynamic changes related to the human brain activities, such as motor, visual, auditory, language, and other cognitive functions (Edwards et al., 1993; Franceschini et al., 2003; Jaszewski et al., 2003; Roland and Larsen, 1976; Sander et al., 1995; Tansakol et al., 2001; Villinger et al., 1993; Williamson et al., 1996). Moreover fNIRS has been used to monitor rCBF modifications in physiological and pathological conditions with good results both in adults and in children (Cope and Delpy, 1988; Mehagnoul-Schipper et al., 2002; Obrig et al., 1996; Okada et al., 1993; Wyatt, 1994).

The purpose of this study was to investigate the relationship between the rCBF and neuronal activity modulated by tDCS. For this purpose, tDCS, with electrodes overlaying prefrontal cortex, was used to induce a change in neural activity and fNIRS was used to evaluate the hemodynamic changes in the same regions. More specifically, the anode was placed over the left prefrontal cortex and the cathode over the right one. This set up offers the opportunity to evaluate simultaneously the effect of anodal and cathodal stimulation over the underlying brain structures. We also analyzed the effects of a sham stimulation to rule out possible unspecific effects or arousal changes produced by the experimental procedures.

**Methods**

**Subjects**

To evaluate whether the hemodynamic effects of anodal tDCS can be detected using fNIRS, 12 healthy volunteers (6 females and 6 males, aged 24–39 years, mean ± SD 29.5 ± 3.9) were recruited for the study. Of the 12 participants, 10 (5 males and 5 females; aged 24–39 years, mean ± SD 29.8 ± 4.3) also participated in the sham stimulation condition. The participants were screened for history of hormonal, metabolic, circulatory, psychiatric and neurological disorders, and were medication-free at the time of the study. The participants were seated comfortably in a semi-darkened room and were instructed to refrain from speaking and to remain awake while in a calm, relaxed state. All participants gave their informed consent; the procedures had the approval of the hospital ethics committee and were conducted in accordance with the Declaration of Helsinki.

**Experimental protocol**

The experimental set up is reported in Fig. 1. Subjects were tested under two conditions: real stimulation (12 subjects) and sham stimulation (10 subjects). After the end of the stimulation, the effects of the tDCS on the rCBF in the prefrontal cortex were monitored with a continuous-wave fNIRS system. The fNIRS system does not measure the absolute concentration value of HbO2 and HHb, but their deviations from a control value. In this experimental protocol, the control value was chosen to be at the end of the fNIRS data collection. The after-effects produced by the tDCS tend in fact to decay with time (Nitsche and Paulus, 2001), and fNIRS data were collected for a sufficiently long time, such that the final hemodynamic conditions could be considered with reasonable confidence as representing the baseline state.

**Transcranial direct current stimulation (tDCS)**

The experimental protocol consisted of two conditions: real stimulation (12 subjects) and sham stimulation (10 subjects, see above). Sessions were separated by an interval of at least 1 week. tDCS stimulation was delivered by a battery-driven electrical stimulator (Elidith DC-Stimulator, Germany) connected to a pair of thick (0.3 cm) saline-soaked synthetic surface sponge electrodes (surface area: 35 cm² each) placed on the scalp. Electrodes were applied bilaterally at two prefrontal locations, lateral to Fp1 (anode) and Fp2 (cathode) of the 10–20 international system for EEG electrodes placement. We chose this location as it is optimal for positioning the fNIRS probe (Emir et al., 2005). The active condition (real stimulation) consisted of a 10-min anodal stimulation with a constant current of 1 mA (8-s phase in/phase out for a total stimulation time of 616 s), with total current density of 0.02857 mA/cm². We choose this duration as it produces – when applied over the motor cortex – a stable effect on most subjects for about 15 min (Nitsche and Paulus, 2001). Sham stimulation involved the same electrode placement and duration as the active condition; however, the 1 mA constant current was delivered for only 30 s, with the same phase in-phase out time (total stimulation of 46 s). This stimulation in the sham condition was used to induce the slight tingling or burning sensations that some subjects report they experience during tDCS stimulation, in order to further blind the participants as to which type of stimulation they were receiving (real or sham). As a control condition to evaluate the effects of lengthening the tDCS, in three subjects we also evaluated a tDCS duration of 15 min.

**fNIRS**

Upon the conclusion of the tDCS sessions, the effects of tDCS on rCBF in the prefrontal cortex were monitored using a continuous-wave fNIRS system first described by Chance et al. (1998) and further developed and produced at Drexel University (Philadelphia, PA). The system was composed of three modules: a flexible headpiece, which holds light sources and detectors; a control box for hardware management; and a computer that runs the data acquisition. The headpiece was 17.5 cm long and 6.5 cm wide, holding four light sources and 10 photodetectors.

The headpiece contained a specially-designed double-sided adhesive tape for fastening to the forehead and was further held securely in place with an elastic strap. The fNIRS headpiece used in this study consists of a flexible circuit board; this particular arrangement allowed a fast placement of all 16 optodes. The headpiece was attached immediately after real or sham tDCS stimulation concluded. The source-detector separation was 2.5 cm, providing a penetration depth of approximately 1.25 cm. The four light sources were each activated in turn: each source shone light with input intensity I0 and the four photodetectors surrounding the currently active source.
measured the intensity $I$ of the emerging light. The arrangement of sources and detectors on the headpiece and the configuration for data acquisition yielded a total of 16 active optodes and was designed to image cortical areas that correspond to the dorsal and inferior frontal cortices (Izzetoglu et al., 2005) (Fig. 1D). Each source emitted light at two different wavelengths in the near-infrared spectrum (730 ± 15 nm and 850 ± 15 nm) and measures of emerging light intensity were collected for each optode with a sampling frequency of 2 Hz. The overall data collection lasted 21 min, starting 1–2 min after the end of the stimulation. The data collection of the subjects that received 15-min real tDCS lasted 61 min.

Data processing and statistical analysis

Changes in light absorption, as measured by fNIRS at each of the two wavelengths, were converted to changes in concentration of HbO2 and HHb.

It is generally assumed (Delpy et al., 1988) that the attenuation that the shined light undergoes when travelling through the tissue reflects a linear superimposition of two processes, absorption and scattering, and can be represented as:

$$\text{OD}_\lambda = \log_{10} \frac{I_{0\lambda}}{I_{\lambda}} = A_\lambda + S_\lambda. \quad (1)$$

OD$_\lambda$ represents the light attenuation at the wavelength $\lambda$ expressed in optical density (OD) units; $I_{0\lambda}$ is the intensity of the input light at the wavelength $\lambda$; $I_{\lambda}$ is the intensity of the light at the wavelength $\lambda$ measured by the detector; $A_\lambda$ and $S_\lambda$ represent the light attenuation caused, respectively, by absorption and scattering at the wavelength $\lambda$.

In the near-infrared region, HbO2 and HHb are the two main chromophores; when taking into account only their contribution to light absorption, the term $A_\lambda$ in Eq. (1) can be written as

$$A_\lambda = \left( e_{\text{HbO2}}^{\lambda} \cdot C_{\text{HbO2}} + e_{\text{HHb}}^{\lambda} \cdot C_{\text{HHb}} \right) \cdot r_{sd} \cdot \text{DPF}_\lambda \quad (2)$$

based on the modified Beer–Lambert law (mBLL) (Delpy et al., 1988). In Eq. (2), $e_{\text{HbO2}}$ and $e_{\text{HHb}}$ are the specific absorption coefficients of, respectively, HbO2 and HHb at the wavelength $\lambda$; $C_{\text{HbO2}}$ and $C_{\text{HHb}}$ are the concentrations of HbO2 and HHb in the sampled volume of tissue; $r_{sd}$ is the physical source-detector separation; and $\text{DPF}_\lambda$ is the differential pathlength factor at the wavelength $\lambda$. The $\text{DPF}_\lambda$ corrects the $r_{sd}$ to give a better estimate of the real length of the path traveled by photons as a consequence of scattering. The values for $e_{\text{HbO2}}$, $e_{\text{HHb}}$, $r_{sd}$ and $\text{DPF}_\lambda$ are considered time-independent and spatially constant for the adult forehead (Delpy et al., 1988).

$S_\lambda$ is generally considered a constant factor, dependent on the geometry (Obrig and Villringer, 2003). This allows the differential representation of the mBLL:

$$\Delta\text{OD}_\lambda(t) = \text{OD}_\lambda(t) - \text{OD}_{\lambda,\text{control}} = \log_{10} \frac{I_{\lambda,\text{control}}}{I_{\lambda}(t)} =$$

$$= (A_\lambda(t) + S_\lambda) - (A_{\lambda,\text{control}} + S_\lambda) = A_\lambda(t) - A_{\lambda,\text{control}} =$$

$$= \left( e_{\text{HbO2}}^{\lambda} \cdot \Delta C_{\text{HbO2}}(t) + e_{\text{HHb}}^{\lambda} \cdot \Delta C_{\text{HHb}}(t) \right) \cdot r_{sd} \cdot \text{DPF}_\lambda \quad (3)$$

with $\Delta C_{\text{HbO2}}(t)$ and $\Delta C_{\text{HHb}}(t)$ representing the differences in the HbO2 and HHb concentrations between the instant in time $t$ and the control condition.

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**Fig. 1.** Schematic representation of the experimental setup. (A) The tDCS electrodes were placed bilaterally at two prefrontal locations, with the anode lateral to Fp1 and cathode lateral to Fp2. (B) After the tDCS stimulation, the NIRS probe was placed on the prefrontal area so as to cover the two stimulated locations. (C) The arrangement of the 4 light sources and 10 photodetectors on the NIRS probe and the configuration used for data acquisition yielded 16 active optodes, each monitoring different areas of the prefrontal cortex. (D) The configuration used for data acquisition yielded 16 active optodes, each monitoring different areas of the prefrontal cortex. (D) The arrangement of the 4 light sources and 10 photodetectors on the NIRS probe and the configuration used for data acquisition yielded 16 active optodes, each monitoring different areas of the prefrontal cortex.
In continuous-wave fNIRS, the differential mBLL is used and light attenuation is measured at two (or more) wavelengths in order to calculate $\Delta CHbO2(t)$ and $\Delta CHhb(t)$. The continuous-time fNIRS system used in this study measured light attenuation at two wavelengths (namely, 730 and 850 nm); therefore $\Delta CHbO2(t)$ and $\Delta CHhb(t)$ were computed through the solution of the two equations:

$$
\begin{align*}
\Delta OD_{730 nm}(t) &= \left( \frac{\epsilon_{CHbO2,730 nm} + \epsilon_{CHhb,730 nm} \cdot \Delta CHbO2(t)}{r_{ad} \cdot DPF_{730 nm}} \right) \left( \frac{1}{\epsilon_{CHbO2,730 nm} - \epsilon_{CHhb,730 nm}} \right) \\
\Delta OD_{850 nm}(t) &= \left( \frac{\epsilon_{CHbO2,850 nm} + \epsilon_{CHhb,850 nm} \cdot \Delta CHhb(t)}{r_{ad} \cdot DPF_{850 nm}} \right) \left( \frac{1}{\epsilon_{CHbO2,850 nm} - \epsilon_{CHhb,850 nm}} \right)
\end{align*}
$$

(4)

thus leading to:

$$
\begin{align*}
\Delta CHbO2(t) &= \epsilon_{CHbO2,730 nm} \cdot \Delta OD_{850 nm}(t) \cdot \frac{1}{r_{ad} \cdot DPF_{850 nm}} \\
&\quad - \epsilon_{CHbO2,850 nm} \cdot \Delta OD_{730 nm}(t) \cdot \frac{1}{r_{ad} \cdot DPF_{730 nm}} \\
&\quad \div \epsilon_{CHbO2,730 nm} \div \epsilon_{CHbO2,850 nm} - \epsilon_{CHbO2,730 nm} \div \epsilon_{CHbO2,850 nm}
\end{align*}
$$

(5)

Overall, the two hemodynamic variables, $\Delta CHbO2(t)$ and $\Delta CHhb(t)$ were measured for each of the 16 optodes.

The control condition used for the mBLL was the average attenuation value recorded during the last minute of data acquisition. The values for $\Delta CHbO2(t)$ and $\Delta CHhb(t)$ were then calculated for the other 20 min, yielding a total of 2400 points for each optode and each variable. Since the variations in concentration of HbO2 and HHb induced by the tDCS are expected to occur on a much slower time scale, we reduced the number of points per optode and per variable to 20: each point represented the average over 1 min.

Additionally, the fNIRS probe held two rows of optodes: the bottom row closer to the orbitofrontal area and the top row covering more caudal regions of the prefrontal cortex, but both spanning the entire length of the fNIRS probe. Given the position of the tDCS electrodes, there was no reason to expect the optodes in the top and bottom row to differ. Therefore, in order further to reduce the dimensionality of the data, we averaged together the signals from pairs of optodes that shared the same degree of laterality but belonged to different rows. This operation led to a total of eight channels. Going from the one at the far left to the one to the far right, the channels were named L4, L3, L2, L1, R1, R2, R3 and R4 (these channels were obtained by averaging, respectively, optodes 1–2, 3–4, 5–6, 7–8, 9–10, 11–12, 13–14 and 15–16).

After real tDCS, the measurements of $\Delta CHbO2$ and $\Delta CHhb$ from the eight channels were analyzed as independent variables with an initial two-way MANOVA whose factors were

- Hemodynamic variable, with two levels (“$\Delta CHbO2$” and “$\Delta CHhb$”) and
- Time, with 20 levels (one for each minute of the recording).

The effects of tDCS were observed only for $\Delta CHbO2$ and not for $\Delta CHhb$, so this last variable was no further analyzed (see Results).

The measurements of $\Delta CHbO2$ from the eight channels were then analyzed as independent variables with a two-way MANOVA whose factors were

- Stimulation condition, with two levels (“real stimulation” and “sham stimulation”) and
- Time, with 20 levels (one for each minute of the recording).

This was followed by a series of eight univariate two-way repeated-measure ANOVAs, with Stimulation condition as between-subject factor (with two levels) and Time as within-subject factor (with 20 levels).

All post-hoc comparisons were performed with Tukey’s honest significance test. All results were considered significant at $p<0.05$.

**Results**

Overall, real tDCS stimulation (with anode over the left prefrontal cortex) was performed on a total of 12 subjects. The stimulation produced an increase in $\Delta CHbO2$, more markedly in the areas under the anode. $\Delta CHbO2$ reached a peak in about 3–6 min after the start of the recording and then slowly decaying, reaching again a baseline level after the effect of the stimulation vanished. On the right prefrontal area, where the cathode was placed, the time course appeared similar, though the amplitude of the increase was more limited, probably denoting a border effect (Fig. 2A). In contrast to the marked increases of $\Delta CHbO2$, the stimulation produced negligible changes in $\Delta CHhb$ (Fig. 2B). These qualitative observations were confirmed by the subsequent statistical analyses. MANOVA revealed a significant effect for the eight latent variables ($\Delta CHbO2$ and $\Delta CHhb$ values obtained from the eight channels) as a group in relation to the Hemodynamic variable (Roy’s largest root = 0.461; $F(8, 593) = 34.15, p<0.0001$) and the Time factor (Roy’s largest root = 0.424; $F(19, 599) = 13.38, p<0.0001$). Also, the interaction between the two factors was significant (Roy’s largest root = 0.122; $F(38, 599) = 1.92, p = 0.001$), which is consistent with the observed differences in the Time factor being due to $\Delta CHbO2$.

A control condition was also tested on 10 subjects who received sham stimulation. In these subjects, the spatiotemporal course of $\Delta CHbO2$ obtained for the real stimulation sessions (Fig. 3A) was compared with the course obtained for the sham sessions (Fig. 3B). The sham stimulation produced no detectable changes in $\Delta CHbO2$.

Again, these qualitative observations were confirmed by the subsequent statistical analyses. The initial MANOVA revealed a significant effect for the eight latent variables ($\Delta CHbO2$ values obtained from the

![Fig. 2. Spatio-temporal representation of $\Delta CHbO2$ (A) and $\Delta CHhb$ (B) in 12 subjects that received real tDCS. The x-axis represents the time (in minutes) of the fNIRS data collection and the y-axis represents the distribution of the eight channels across the forehead. The $\Delta CHbO2$ and $\Delta CHhb$ values are color-coded and the scale is defined by the colorbar at the right of the graphs.](image-url)
time (in minutes) of the fNIRS data collection and the sham stimulation have been included in these graphs. The stimulation (A) and sham stimulation (B). Only the 10 subjects that received both real stimulation condition and the time factor (Roy’s largest root=0.230; F(1,8)=5.64, p=0.010), confirming the differential effect of the stimulation on the time course of ΔCHbO2. Tukey’s post hoc tests revealed that during the real stimulation session the ΔCHbO2 for the minutes 2–8 was consistently higher than the concentration measured at the end of the recordings and higher than the concentration measured during sham stimulation. In channel L4, the other location for which the stimulation condition was significant, the time factor revealed a statistically significant difference (F(19,10)=3.57, p<0.0001), with again a ΔCHbO2 peak occurring at minutes 4–6. Fig. 3C shows the mean ΔCHbO2 and its 95% confidence interval obtained for the real stimulation and the sham session when considering only the channels for which the stimulation effect was significant.

In a subgroup of three subjects, real tDCS was also delivered for 15 min. As expected, the hemodynamic effects of 15-min tDCS lasted longer compared to 10-min tDCS. Fig. 4 shows the time course of ΔCHbO2 after the 15-min tDCS.

Discussion

The main result of this study is that weak anodal tDCS produces a local increase of the concentration of HbO2 in the underlying brain tissue. Moreover we demonstrated that this effect is relatively focal. In fact, the difference between the stimulation and sham condition on ΔCHbO2 was strongly significant only for a few channels. In particular, it was significant at the channels on the left side (L3 and L4). These channels correspond to the area stimulated with anodal tDCS, thus suggesting that mainly anodal stimulation acts on ΔCHbO2. The presence of significant variations over time confirms that the effects of the anodal stimulation are localized in time and, with 10-min tDCS, last up to 8–10 min (6–8 min from the beginning of the recording) after the end of the stimulation, with a peak effect at 6 min after the end of stimulation. Moreover, longer stimulation session (15 min) produced longer effect on the hemodynamic response.

This effect is unlikely to be due to a general alteration in arousal of subjects or other non-specific causes for several reasons: (1) sham stimulation would have produced a similar effect, (2) an effect of general arousal would have been expected to involve both anodal (left) and cathodal (right) sites of stimulation, and (3) similar effects would have been expected at all channels and not in a focal manner (see below). The effect must have been cerebral in origin because the stimulation settings for the tDCS have been proven to alter the cortical excitability (Marshall et al., 2005; Nitsche and Paulus, 2001); additionally, the optode spacing used in the fNIRS device ensured readings sensitive to the hemodynamic activity in the first millimeters of cortical gray matter (Chance et al., 1998; Firbank et al., 1998).

In this study we observed that cathodal stimulation has a negligible effect. This could simply be due to a different threshold of cathodal stimulation on the hemodynamic response. Lang et al. (2005) reported that both anodal and cathodal tDCS increased the metabolism of the cortex underlying the stimulation electrodes, with cathodal stimulation producing a much less effective increase of the metabolic response under the electrodes. In any case it is well known that cathodal and anodal tDCS affect cortex in a different manner (Lang et al., 2004; Nitsche et al., 2003). Another plausible explanation is that we evaluated the brain hemodynamic response at rest (the subjects were not involved in any task and were completely relaxed).

As a follow-up, a repeated-measure ANOVA was conducted on each single channel. Findings revealed markedly significant differences between the real stimulation and the sham condition in channels L3 (F(1,9)=7.89, p=0.020) and L4 (F(1,8)=5.64, p=0.044). Channel L3 showed a significant interaction between the stimulation condition and the time factor (F(1,19)=2.01, p=0.010), confirming the differential effect of the stimulation on the time course of ΔCHbO2. Tukey’s post hoc tests revealed that during the real stimulation session the ΔCHbO2 for the minutes 2–8 was consistently higher than the concentration measured at the end of the recordings and higher than the concentration measured during sham stimulation. In channel L4, the other location for which the stimulation condition was significant, the time factor revealed a statistically significant difference (F(19,10)=3.57, p<0.0001), with again a ΔCHbO2 peak occurring at minutes 4–6.

Fig. 3. Spatio-temporal representation of ΔCHbO2 obtained for the two conditions: real stimulation (A) and sham stimulation (B). Only the 10 subjects that received both real and sham stimulation have been included in these graphs. The x-axis represents the time (in minutes) of the fNIRS data collection and the y-axis represents the distribution of the eight channels across the forehead. The ΔCHbO2 values are color-coded and the scale is defined by the colorbar at the right of the graphs. The bottom graph (C) shows the evolution of ΔCHbO2 following the real and the sham stimulation (respectively the thick solid red line and the thick solid blue line). The time courses were obtained considering only the two channels (L3 and L4) that showed a significant difference between the two stimulation conditions. The thinner dotted lines represent instead the 95% confidence intervals for ΔCHbO2.

Fig. 4. The graph shows the time course of ΔCHbO2 following 15-min tDCS. This experiment was conducted in three subjects. Time courses were obtained considering only the channels L3 and L4. Longer tDCS produce longer and more stable increase of ΔCHbO2. The thinner dotted lines represent instead the 95% confidence intervals for ΔCHbO2.
It is possible that the lack of effect of cathodal stimulation is due to a “floor effect,” and that it is not possible to further reduce the oxygen availability – determined by the hemodynamic link between neural activity and regional blood flow – in a rest condition. We cannot exclude also that there are side differences as we applied cathodal stimulation always on the right prefrontal cortex and anodal on the left prefrontal cortex. However, the experience of similar excitability findings in right and left motor cortex makes this possible difference unlikely.

How does tDCS modulate hemodynamic response?

The human brain is virtually completely dependent on glucose for energy metabolism and most of this energy comes from mitochondria. Glucose utilization reflects neuronal activity and most nerve cells are not only exclusively dependent on glucose but also lack the ability to store glycogen. This is why nervous tissue, when activated, needs an increase of oxygen and glucose availability. The increased request is supported by an increased CBF. PET and SPECT allow to monitor the increased consumption of glucose while transcranial Doppler monitors the CBF variation. fNIRS allows to monitor noninvasively oxygen availability over time.

We have not a unique explanation of how anodal tDCS can increase the oxygen availability after the end of stimulation. A first hypothesis is that anodal tDCS increases neuron excitability and this extra “work” needs to be supplied by extra oxygen delivery. The after-effect of tDCS on cortical excitability is supposed to have mechanisms similar to long-term potentiation and depression (LTP-like and LTD-like). Several studies – using tDCS or other transcranial stimulation method for inducing excitability changes – reported a delayed peak in the effect (minutes after the end of stimulation) (Huang et al., 2005; Nitsche et al., 2006; Kuo et al., 2007, 2008). Delayed LTP and LTD are unlikely.

exclude also that there are side differences as we applied cathodal effect of tDCS on cortical excitability is supposed to have mechanisms to increase the oxygen availability after the end of stimulation. A

tors the CBF variation. fNIRS allows to monitor noninvasively oxygen increase of oxygen and glucose availability. The increased request is store glycogen. This is why nervous tissue, when activated, needs an

monitor these effects by fNIRS can have very interesting clinical and applications. Change in the rCBF can be used as a measurable after-effect in the human brain.

In conclusion, the present results provide direct evidence that the hemodynamic response of the brain is substantially different when anodal or cathodal tDCS were used. The possibility to induce hemodynamic changes in the brain using tDCS and the possibility to monitor these effects by fNIRS can have very interesting clinical and scientific applications. Change in the rCBF can be used as a measurable output when cortices without any measurable output are modulated by tDCS and other neuromodulation techniques. Moreover, the monitoring of the effects on rCBF can be used to calculate the “normal” hemodynamic response to manipulation of the cortical excitability (by tDCS or other neuromodulation techniques). This would be useful, for example, in chronic cerebrovascular disorders, to identify patients with a reduced capability to increase the blood flow on demand. The fact that tDCS can modulate focially the rCBF can be used to increase the oxygen availability or to facilitate the elimination of “neurotoxic” substances in stroke patients and in degenerative disorders. In summary, these findings open new avenues to new diagnostic and therapeutic options of tDCS and fNIRS.

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