EEG mean frequency changes in healthy subjects during prefrontal transcranial direct current stimulation

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EEG mean frequency changes in healthy subjects during prefrontal transcranial direct current stimulation. J Neurophysiol 112: 1367–1375, 2014. First published June 11, 2014; doi:10.1152/jn.00088.2014.—In this pilot study we evaluated electroencephalographic (EEG) mean frequency changes induced by prefrontal transcranial direct current stimulation (tDCS) and investigated whether they depended on tDCS electrode montage. Eight healthy volunteers underwent tDCS for 15 min during EEG recording. They completed six tDCS sessions, 1 wk apart, testing left and right direct current (DC) dipole directions with six different montages: four unipolar montages (one electrode on a prefrontal area, the other on the opposite wrist) and two bipolar montages (both electrodes on prefrontal areas), and a single sham session. EEG power spectra were assessed from four 1-min EEG epochs, before, during, and after tDCS. During tDCS the outcome variable, brain rate ($f_b$), changed significantly, and the changes persisted for minutes after tDCS ended. With the DC dipole directed to the left (anode on the left prefrontal area or wrist), $f_b$ increased, and with the DC dipole directed to the right (anode on the right prefrontal area or wrist), $f_b$ decreased, suggesting asymmetric prefrontal cortex functional organization in the normal human brain. Anodal and cathodal effects were opposite but equally large. Gender left these effects unchanged.

TDCS; brain stimulation; EEG; prefrontal areas; brain rate
**EEG MEAN FREQUENCY CHANGES DURING PREFRONTAL TDCS**

**Design.** In all subjects tDCS was delivered on one or both frontal lobes for 15 min during an EEG recording. Each participant underwent six tDCS sessions each testing a different electrode montage: two unipolar montages (one electrode on a frontal lobe, the other on the opposite wrist) accounting for four sessions reversing electrode polarity, and two sessions with bipolar montages (anode on the left frontal lobe and cathode on the right frontal lobe; cathode on the left and anode on the right). These six montages covered all possible configurations with at least one electrode on a frontal lobe, and the other on the opposite frontal lobe or the opposite wrist: anode on the left frontal area, cathode on the right frontal area (AK); anode on the left frontal area, cathode on the right wrist (Ak); anode on the left wrist, cathode on the right frontal area (aK); anode on the right frontal area, cathode on the left frontal area (KA); anode on the right wrist, cathode on the left frontal area (Ka); and anode on the right frontal area, cathode on the left wrist (KA). In three montages (AK, Ak, and aK), the direct current (DC) dipole was oriented laterally toward the left (anodal electrode on the left body side) and in the other three (KA, Ka, and kA) toward the right (anodal electrode on the right body side). In unipolar sessions although a minimal amount of current may cross the brainstem cardiorespiratory and autonomic centers and the heart, the danger is considered nonexistent (Vandermeeren et al. 2010; Im et al. 2012). To avoid possible long-term effects, a 1-wk interval elapsed between the tDCS sessions. All subjects also underwent a sham session (no current delivered through the electrodes) with one of the six montages randomly chosen. Sessions were conducted in random order. In each session, EEG activity was recorded for at least 5 min before tDCS started to at least 10 min after it ended. Quantitative EEG data were recorded and digitally stored for offline data analysis.

**tDCS procedure.** tDCS was delivered at 1.5 mA with 5 × 8 cm flexible (conductive rubber) electrodes applied on one (unipolar stimulation) or both (bipolar stimulation) frontal lobes between the Fp1-F3-F7 or Fp2-F4-F8 EEG electrodes for 15 min. A conductive gel was applied between the electrode rubber and the subject’s skin. tDCS electrodes and EEG electrodes were kept in place with an elastic net that allowed the electrode cables to pass through the mesh. For real stimulation, current was ramped up at the beginning and ramped down at the end for 2 s to avoid perceivable fast transients that might have enabled subjects to distinguish between real and sham stimulation when the current was switched on and off.

**EEG electrodes.** EEG electrodes were positioned according to the 10–20 System (F8, F7, F4, F3, C4, C3, T4, T3, T6, T5, P4, P3, O2, and O1). EEG signals were acquired by a digital apparatus (Micromed-Italy) with standard amplitude and filter band values (amplitude: 10 µV/div; filter band pass: 0.5–70 Hz).

**Data analyses.** For the quantitative EEG tracing analysis, four artifact-free epochs each lasting 60 s (t0 = baseline, 5 min before stimulation; t1 = 1 min after stimulation began; t2 = 15th min after stimulation began = last minute before stimulation ended; and t3 = 10 min after it ended) were selected by two neurophysiologists blinded to subjects, montages, and sessions. On these selected epochs we measured the relative power in the various EEG frequency bands (delta = 1–4 Hz; theta = 4–8 Hz; alpha = 8–13 Hz; and beta = 13–20 Hz), calculated the EEG mean frequency and its deviations from the baseline value (fT), and ran a within-subjects factorial ANOVA to find out how time point (EEG epoch), DC dipole direction, and tDCS montage affected them. To simplify the statistical analysis, as our dependent variable we considered a single value, the brain rate dependent variable we considered a single value, the brain rate

$$f_{b} = \frac{\sum \Delta f_{b} \times \Delta f_{b}}{\sum \Delta f_{b}}$$

where the index i denotes the frequency band (for delta i = 1, for theta i = 2, etc.); fbi is the mean frequency in Hz for any band (in our arrangement, for delta fbi = 2.5, for theta fbi = 6, for alpha fbi = 10.5, and for beta fbi = 16.5); V is the corresponding mean power in any

band, drawn from the EEG recording; and V is the sum of all Vi band potentials (V = \sum Vi). This formula allowed us to transform the mean powers in the different frequency bands into an estimated overall EEG mean frequency without passing it through integral calculus. We also computed and statistically analyzed fT deviations from the baseline value (fT) as a percentage difference, \(\Delta f_{b} \%\), at t1, t2, and t3 compared with t0, \(\Delta f_{b} \% = \frac{f_{b}(t) - f_{b}(0)}{f_{b}(0)} \times 100\). This calculation removed most of the individual fT variability unrelated to tDCS, such as the variability at baseline before stimulation, so that \(\Delta f_{b} \%\) became more sensitive and more specific than fT. In ANOVA, \(\Delta f_{b} \%\) could be compared only vs. sham, not vs. the baseline value at t0, because at t0 all \(\Delta f_{b} \%\) values and variance are zero. For any given montage, AK, Ak, aK, KA, Ka, and kA, we therefore assessed \(\Delta f_{b} \%\) only at t1, t2, and t3 and compared it only vs. sham, whereas we assessed fT at t0, t1, t2, and t3 and compared it vs. sham and vs. the baseline value at t0 for that same montage.

As our independent outcome measures, we considered three variables: EEG recording time point (4 levels: t0, t1, t2, and t3); DC dipole direction (3 levels: left, i.e., anode on the left forehead or wrist and cathode on the right forehead or wrist; right, i.e., cathode on the left and anode on the right; and sham, i.e., no dipole applied, no direction); and tDCS electrode montages (6 levels: AK, Ak, aK, KA, Ka, and kA). For statistical purposes all sham montages counted as a single montage SH. Because SH was inactive, it differed from the other six montages so we investigated a total of seven montage levels. These were unequally nested within the three dipole direction levels: AK, Ak, and aK within the left direction level; KA, Ka, and kA within the right direction level; and SH within the sham direction level (no direction). Within-subjects ANOVA with Bonferroni post hoc tests were run to verify the effects and interactions among the three independent variables time, direction, and montage on fT and \(\Delta f_{b} \%\). The Bonferroni procedure was preferred over other post hoc tests to evaluate significance in a conservative way given the small sample size compared with the numerous variables and levels. For one experiment we also compared the Bonferroni P values with those for a less conservative test (Newman-Keuls test). We also conducted ANOVA tests on a fourth independent variable, hemisphere (2 levels: left and right), to verify possible differences between the left and right hemisphere responses to tDCS. To avoid increasing the degrees of freedom with four variables in a small study sample, we tested this hemisphere variable separately from the other three variables. Finally, we used a series of t-tests to verify whether gender affected any montage effect on fT and \(\Delta f_{b} \%\) at any time point. For this comparison we used the t-test instead of ANOVA because splitting the eight-subject sample into two four-subject samples according to gender resulted in samples that were too small for ANOVA.

**RESULTS.** All the recruited subjects completed the study. None of them reported being able to distinguish in any sessions whether they were actually receiving current or undergoing a sham session. We found that delivering DC stimulation and concomitantly recording EEG posed no electric problem, because electric artifacts, if any, appeared only in the first 2 s after switching the current on and off and practically appeared only in the EEG channels closer to the tDCS electrode(s). After steady-state tDCS, the 0.5- to 70-Hz band pass EEG filter effectively removed all artifacts related to constant tDCS current, so that during stimulation, apart from occasional brief artifacts recorded when current was switched on or off, none of the EEG recordings on any channels contained detectable artifacts related to steady-state tDCS. Placing the tDCS electrode(s) between the EEG electrodes, especially by using flexible (conductive rubber) tDCS electrodes, raised no technical problems.
In all montages with electrical stimulation applied with the DC dipole oriented toward the left (anode on the left forehead or wrist and cathode on the right), the EEG mean frequency $f_b$ increased in all the subjects tested. Conversely, in unipolar (Ka and ka) montages with the dipole oriented toward the right (cathode on the left forehead or wrist and anode on the right), $f_b$ decreased. In the bipolar montage with the dipole oriented toward the right (KA), and in sham $f_b$ remained essentially unchanged at all EEG recording time points $t_0$, $t_1$, $t_2$, and $t_3$ (Fig. 1, A and B). ANOVA on $f_b$ showed a significant effect for dipole direction $[F(2,14) = 5.1; P = 0.021785]$ and montage $[F(4,28) = 2.4; P = 0.048836]$, with a significant interaction between direction and time $[F(6,42) = 20.8; P < 0.000001]$. Left direction almost significantly increased $f_b$ compared with sham (10.20 vs. 9.95 Hz; $P = 0.005906$), and right direction nonsignificantly decreased it (9.89 vs. 9.95 Hz; $P = 1$), with a significant difference between left and right direction (10.20 vs. 9.89 Hz; $P = 0.033326$). When $\Delta f_b%$ was analyzed instead of $f_b$, all these effects became more evident, and significance increased for dipole direction $[F(2,14) = 42.02; P = 0.000001]$, montage $[F(2,14) = 5.12; P = 0.021469]$, interaction between direction and time $[F(4,28) = 5.4; P = 0.002412]$, difference between left and right direction (+0.21 vs. −0.13%; $P = 0.000001$), and between left direction and sham (+0.21 vs. −0.01%; $P = 0.00014$). When considering $\Delta f_b%$, the difference between right direction and sham also became significant (−0.13 vs. −0.01%; $P = 0.017153$).

When the seven montages (AK, Ak, aK, KA, Ka, kA, and SH) were analyzed individually, ANOVA showed a significant main effect of montage $[F(6,42) = 3.0; P = 0.015390]$ and a significant interaction between montage and time $[F(18,126) = 8.8; P < 0.000001]$. Post hoc tests (Table 1) showed no significant differences in $f_b$ between $t_0$, $t_1$, $t_2$, and $t_3$ in sham or between $t_0$ for nonsham montages and the other sham time points. All three montages with the dipole oriented toward the left significantly increased $f_b$ ($\Delta f_b% > 0$) at $t_1$ and $t_2$, and the significant increase lasted up to $t_3$ in the AK montage. Of the three montages with the dipole oriented toward the right, the bipolar montage KA induced no significant EEG frequency change at any time, whereas the two unipolar montages Ka and kA significantly decreased $f_b$ ($\Delta f_b% < 0$) at $t_1$ and $t_2$, and the significant decrease lasted up to $t_3$ in kA. Even if the montage inducing the strongest decrease in $f_b$ was KA (most negative $\Delta f_b%$) at $t_1$, $t_2$, and $t_3$ (Fig. 1B), its $f_b$ values failed to reach significance vs. sham because its $f_b$ value at baseline ($t_b$) well exceeded that for sham, so that decreasing $f_b$ at $t_1$, $t_2$, and $t_3$ induced $f_b$ values too close to sham at the same time points to reach significance (Fig. 1A). Despite this drawback, the significant deviation in $f_b$ at $t_1$, $t_2$, and $t_3$ from $t_b$ (Table 1) and the significant differences in $\Delta f_b%$ compared with sham disclosed the significant effect of the kA montage (Fig. 1B and Tables 1 and 2). Of the three montages with the DC dipole oriented toward the right (KA, Ka, and kA), the montage that induced the largest and longest-lasting effect was therefore KA.

Apart from the ineffective KA montage, no other montage differed significantly in absolute EEG effect size at $t_1$ and $t_2$. The montages with the longest-lasting effect, i.e., the largest effect size at $t_3$, were AK and KA. The difference in effect size at $t_3$ between each of these montages and the other two montages with the same dipole direction (AK vs. Ak and aK; kA vs. KA and Ka) was significant only for AK vs. aK ($P = 0.017571$) and KA vs. kA ($P = 0.003942$) (Table 2), but when verified with the Newman-Keuls, a less conservative test than the Bonferroni test, they all became significant (AK vs. aK: $P = 0.005906$; AK vs. Ka: $P = 0.002140$; KA vs. kA: $P = 0.000476$; kA vs. Ka: $P = 0.015451$).

ANOVA on $\Delta f_b%$ showed no significant effect for the variable hemisphere $[F(1,7) = 0.007; P = 0.94]$. The $t$-test showed no significant gender-related differences in any montages at any time points.

**DISCUSSION**

This neurophysiological pilot study provides new evidence showing that DCS applied to the prefrontal areas in healthy subjects induces rapid and significant changes in the EEG.
Table 1. Changes in EEG mean frequency, $f_b$, and $\Delta f_b\%$, recorded with the tested stimulating electrode montages at different time points

<table>
<thead>
<tr>
<th>Montages</th>
<th>$f_b$</th>
<th>$t_1$</th>
<th>$t_2$</th>
<th>$t_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AK, Ak, aK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, same montage</td>
<td>$10.04 \pm 0.49$</td>
<td>$10.28 \pm 0.52$</td>
<td>$10.33 \pm 0.52$</td>
<td>$10.31 \pm 0.51$</td>
</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, sham, same time point</td>
<td>$P &gt; 0.0011\dagger$</td>
<td>$P &lt; 0.0001\ddagger$</td>
<td>$P &lt; 0.0001\ddagger$</td>
<td>$P &lt; 0.0001\ddagger$</td>
</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, same montage</td>
<td>$10.09 \pm 0.44$</td>
<td>$10.27 \pm 0.62$</td>
<td>$10.29 \pm 0.62$</td>
<td>$10.19 \pm 0.51$</td>
</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, sham, same time point</td>
<td>$P &lt; 0.0001\dagger$</td>
<td>$P &lt; 0.0001\ddagger$</td>
<td>$P &lt; 0.0001\ddagger$</td>
<td>$P &lt; 0.0001\ddagger$</td>
</tr>
<tr>
<td>AK, aK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, same montage</td>
<td>$10.00 \pm 0.30$</td>
<td>$10.25 \pm 0.39$</td>
<td>$10.25 \pm 0.36$</td>
<td>$10.08 \pm 0.35$</td>
</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, sham, same time point</td>
<td>$P = 0.0006\ddagger$</td>
<td>$P = 0.0005\ddagger$</td>
<td>$P = 1.0000$</td>
<td>$P = 1.0000$</td>
</tr>
<tr>
<td>aK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, same montage</td>
<td>$9.93 \pm 0.39$</td>
<td>$9.73 \pm 0.40$</td>
<td>$9.72 \pm 0.46$</td>
<td>$9.85 \pm 0.42$</td>
</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, sham, same time point</td>
<td>$P = 0.0368*$</td>
<td>$P = 0.0112*$</td>
<td>$P = 0.0005\dagger$</td>
<td>$P = 0.0005\dagger$</td>
</tr>
<tr>
<td>Ka</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, same montage</td>
<td>$10.05 \pm 0.18$</td>
<td>$9.78 \pm 0.23$</td>
<td>$9.83 \pm 0.23$</td>
<td>$9.84 \pm 0.25$</td>
</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, sham, same time point</td>
<td>$P = 0.0002\dagger$</td>
<td>$P = 0.0077\dagger$</td>
<td>$P = 0.0117\dagger$</td>
<td>$P = 0.0099\dagger$</td>
</tr>
<tr>
<td>kA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, same montage</td>
<td>$9.99 \pm 0.34$</td>
<td>$9.85 \pm 0.38$</td>
<td>$9.84 \pm 0.39$</td>
<td>$9.90 \pm 0.37$</td>
</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, sham, same time point</td>
<td>$P = 1.0000$</td>
<td>$P = 1.0000$</td>
<td>$P = 1.0000$</td>
<td>$P = 1.0000$</td>
</tr>
<tr>
<td>SH</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$P_{\text{v.s.}}$ $f_b$, same montage</td>
<td>$9.96 \pm 0.15$</td>
<td>$9.94 \pm 0.15$</td>
<td>$9.94 \pm 0.16$</td>
<td>$9.98 \pm 0.14$</td>
</tr>
</tbody>
</table>

Values are means ± SD. Electroencephalographic (EEG) mean frequency $f_b$ values (Hz) and their percent changes $\Delta f_b\%$ at different EEG recording time points during transcranial direct current stimulation (tDCS) delivered with different tDCS electrode placements (montages) and significant $f_b$ and $\Delta f_b\%$ differences from baseline and sham (baseline and sham compared with sham because at $t_0$ all $\Delta f_b\%$ values and variance are zero; sham compared with baseline). $P$ values by Bonferroni post hoc comparisons in within-subject factor ANOVA. $t_0$, $t_1$, $t_2$, and $t_3$: EEG recording time-points ($t_0$ = baseline, 5 min before stimulation; $t_1$ = after stimulation for 1 min; $t_2$ = 15th and last minute of stimulation; $t_3$ = 10 min after stimulation ended). MONT: tDCS electrode montage (AK, anode on the left frontal area, cathode on the right frontal area; Ak, anode on the left frontal area, cathode on the right wrist; aK, anode on the left wrist, cathode on the right frontal area; KA, cathode on the left frontal area, anode on the right frontal area; Ka, cathode on the left frontal area, anode on the right wrist; kA, cathode on the left wrist, anode on the right frontal area; SH, sham, random montage, no current delivered). *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$, n.s. = not significant.

mean frequency $f_b$. These EEG changes remain evident during stimulation, progressively disappear within minutes after tDCS ends, and are montage dependent. All electrode montages with the DC dipole oriented toward the left (anode on the left side of the body) increased $f_b$, whereas those with the DC dipole oriented toward the right (anode on the right side of the body) decreased $f_b$, except the bipolar montage KA (cathode on the left prefrontal area and anode on the right), which left $f_b$ almost unchanged. Apart from this exception, all the other montages tested, unipolar and bipolar, with the dipole oriented in the same direction differed only in the intensity and duration of the $f_b$ change, not on whether $f_b$ increased or decreased (Fig. 2). These results extend current knowledge on how the prefrontal areas act to modulate the brain activity recorded in EEG in healthy subjects (Davidson 2004; Kähkönen et al. 2004; Mitchell et al. 2008). They also help to explain why in behavioral and perceptual studies cathodal stimulation fails or almost fails to inhibit prefrontal and other cognitive areas and which tDCS electrode montages most efficiently induce EEG changes, a finding that may help in developing new therapeutic applications for transcranial current stimulation.

A primary point in our design was to make sure that the so-called reference tDCS electrode induced no brain effects during unipolar stimulation. We therefore placed this electrode in an extracranial region, namely the opposite wrist. In tDCS research others have placed the extracranial electrode on the upper arm (Cogniamanian et al. 2007; Priori et al. 2008) or even the leg (Meyer-Schwickerath and Magun 1951; Lippold and...
Redfern 1964). We chose the wrist because it has two advantages. Unlike the upper arm it does not require subjects to remove their clothes, and compared with the leg it shortens the body length for the current to pass through, hence has lower resistance, considering that tDCS-induced after-effects correlate in duration and magnitude negatively with distance between the two electrodes (Moliadze et al. 2010). The increased electrical resistance related to placing the reference electrode on the wrist instead of the upper arm required a minimal increase in the current voltage to maintain the desired 1.5-mA intensity. Electric resistance for arm tissues being about 30–35 cm² mean section), in turn can evoke diffuse brain EEG changes lasting minutes to tens of minutes. Others have already shown similar changes in response to transcranial magnetic stimulation (TMS) (Coohrs et al. 1998; Jing and Takigawa 2000; Graf et al. 2001; Okamura et al. 2001; Schutter et al. 2001; Griškova et al. 2007; Barr et al. 2009, 2011; reviews in Thut and Pascual-Leone 2001; Daskalakis et al. 2012). Unfortunately, the results in the various studies are difficult to compare owing to the different stimulation protocols used. They are also difficult to compare with our EEG results, insofar as TMS delivers pulsating stimuli whereas tDCS induces constant polarization. Pulsating interference such as TMS and the constant interference induced by tDCS can be expected to affect spontaneous oscillating systems such as the brain EEG generator(s) in different ways, especially considering that a commonly used TMS frequency, 10 Hz, is close to the main spontaneous EEG frequency.

How these tDCS- and TMS-induced EEG changes arise remains unclear. Many data are available in the literature about prefrontal area lateralization effects on mood and cognitive functions, as well as about EEG asymmetry between the left and right prefrontal area and its role in several physiological and psychopathological conditions (commentaries and reviews in Davidson 2004; Coan and Allen 2004; Harmer et al. 2007; Demaree et al. 2005; Harmon-Jones et al. 2010; Herrington et al. 2010). Anodal stimulation to the cortex on one side may induce EEG frequency changes similar to those induced by cathodal stimulation applied to the cortex on the opposite side and vice versa. In the AeCi framework our findings suggest that the left prefrontal cortex acts to increase the EEG mean frequency, whereas the right prefrontal cortex acts to decrease it, so that by either exciting the left cortex, or inhibiting the right cortex, the EEG mean frequency increases, and vice versa.

To our knowledge this is the first study to show that tDCS to prefrontal areas can evoke diffuse brain EEG changes lasting

### Table 2. Changes in EEG mean frequency, Δf%, induced by the various stimulating electrode montages

<table>
<thead>
<tr>
<th>T.P./MONT</th>
<th>AK</th>
<th>aK</th>
<th>KA</th>
<th>ka</th>
<th>SH</th>
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<tbody>
<tr>
<td>t1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AK</td>
<td>+2.43 ± 1.20</td>
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</tr>
<tr>
<td>ka</td>
<td>+1.73 ± 1.95</td>
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<tr>
<td>aK</td>
<td>+2.49 ± 0.99</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>KA</td>
<td>+0.10 ± 0.33</td>
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<td></td>
</tr>
<tr>
<td>ka</td>
<td>−2.00 ± 2.63</td>
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<tr>
<td>aK</td>
<td>−2.61 ± 1.23</td>
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<tr>
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<td>−0.26 ± 0.72</td>
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<tr>
<td>aK</td>
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<td>ka</td>
<td>+1.96 ± 2.17</td>
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<tr>
<td>A</td>
<td>+2.50 ± 0.85</td>
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<tr>
<td>ka</td>
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<tr>
<td>ak</td>
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<tr>
<td>ak</td>
<td>+0.03 ± 0.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>−0.76 ± 3.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ak</td>
<td>−2.09 ± 1.89</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>A</td>
<td>−0.20 ± 0.71</td>
<td></td>
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</tbody>
</table>

Values are means ± SD. Comparative values for the different montages at the same time points. EEG mean frequency percentage changes Δf% from baseline (5 min before stimulation) at different EEG recording time points during tDCS delivered with different tDCS electrode placements (montages) and significant Δf% differences between different montages at the same time points. P values by Bonferroni post hoc comparisons in within-subjects factorial ANOVA. T.P., EEG recording time points. Duplicate data not shown (—). *P < 0.05; †P < 0.01; ‡P < 0.001; n.s. = not significant.

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small fraction of the axons from ventral tegmental area to prefrontal cortex end asymmetrically. The neurotransmitters for these axons are neither gamma-aminobutyric acid nor dopamine but are still unknown. This finding argues for an asymmetry in unknown prefrontal neurotransmitter content that makes for the best candidate so far to account for asymmetries found in prefrontal EEG activity, affective and cognitive functions, EEG effects, and responses to TMS and tDCS.

Our finding that unipolar tDCS elicits comparable Ae and Ci effect sizes on the induced EEG changes confirms previous suggestions that neurophysiological studies undergo less interference from external noise than do behavioral and perceptual studies (Jacobson et al. 2012a). The comparable Ae and Ci effect sizes we found in this study underline that eventual differences in behavioral and perceptual effect sizes between cathodal and anodal tDCS on cognitive areas depend mainly on the indirect and complex relation that exists between the basic neuronal mechanisms in those areas and their behavioral and perceptual effects. Hence, our findings provide no support for other hypothetical mechanisms such as a different basic neuronal activation state in cognitive vs. motor function tests (Silvanto et al. 2008), contralateral compensation, or the possibility that cathodal tDCS may actually decrease neuronal competition in cognitive areas (Antal et al. 2004), thus reducing the Ci effect.

Our experiments investigating which tDCS electrode montages most effectively induce EEG frequency changes provide new evidence that tDCS on prefrontal areas elicits its most pronounced and long-lasting EEG effects when delivered through the AK and kA montages (AK increases whereas kA decreases the EEG mean frequency). Even if tDCS induced only minor EEG mean frequency changes (because this variable essentially includes the alpha-band frequency power, the major EEG power spectral component, and as such is resistant to changes), the differences between montages with differing dipole orientation were highly significant at t1 and t2 in 17 out of 18 comparisons (Table 2). Our finding that the most efficient montages for inducing EEG frequency changes are AK and kA add to research on which tDCS methods most effectively modify the EEG frequency (Terney et al. 2008; Zaehle et al. 2010; Thut et al. 2011). Given that the EEG frequency correlates with clinical features such as the mental arousal level (Makeig and Jung 1995; Šušmáková and Krakovská 2007; Pop-Jordanova 2011) and mood and performance in various tasks (Klimesch 1999; Sauseng et al. 2005; Gruzelier 2009), identifying which tDCS montages and methodologies are most effective in inducing EEG frequency changes may be helpful in developing tDCS for clinical applications.

An unexpected finding, again suggesting a functional asymmetry between the left and right prefrontal cortex areas, was that the bipolar KA montage left EEG frequencies unchanged, whereas its opposite bipolar montage AK elicited the highest and longest-lasting EEG effect (Fig. 1, A and B). Given that these two montages share identical features except the DC dipole direction, we can practically exclude the hypothesis that KA, having the two electrodes close together on the forehead, left EEG frequency unchanged because it allowed some DC current to shunt through the skin on the forehead rather than reaching the brain cortex. Although this finding may depend on the small study sample, this possibility seems unlikely insofar as all the other montages induced highly significant EEG
changes. Functional asymmetry remains an interesting question for further research.

An equally interesting research direction would be to investigate whether the increased mean EEG frequency we found after anodal stimulation on the left prefrontal area might help explain the reported benefits after anodal stimulation in depression (Brunoni et al. 2012b), Alzheimer’s disease (Boggio et al. 2009), brain aging (Berryhill and Jones 2012), stroke (Jo et al. 2009), and Parkinson’s disease (Boggio et al. 2006; Pereira et al. 2013). Unfortunately, because none of these studies investigated whether EEG changes correlated with clinical changes, we cannot compare our EEG findings with their clinical results, nor did a univocal pattern emerge when we compared our findings with results from studies investigating how those conditions affect EEG frequencies, given that EEG frequencies were decreased in Alzheimer’s disease (Dauwels et al. 2010), Parkinson’s disease (Neufeld et al. 1988; Soikkeli et al. 1991; Klassen et al. 2011), and stroke (Nuwer et al. 1987; Gur et al. 1994) but increased in depression (Pollon and Schneider 1990; Knot et al. 2001) and, at least on posterior areas, in brain aging (Babiloni et al. 2006).

In conclusion, tDCS applied on the prefrontal areas in healthy subjects induces evident changes in the EEG mean frequency. Anodal stimulation to the left prefrontal area, or cathodal stimulation to the right prefrontal area, or both together (bipolar stimulation), increase the EEG mean frequency, whereas cathodal stimulation to the left prefrontal area, or anodal stimulation to the right prefrontal area, but not both together, decrease it. The EEG begins to change within minutes after tDCS starts, and depending on the montage used the changes may persist for 10 min after tDCS ends. tDCS induces its highest and longest-lasting EEG changes when delivered biologically with the anode on the left and the cathode on the right prefrontal area and unipolarly with the anode on the right prefrontal area. Anodal and cathodal unipolar tDCS elicits opposite effects with similar absolute size, thus confirming that neurophysiological studies often disclose the Ci effect.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: N.A. and O.M. conception and design of research; N.A., M.C., and L.D., and O.M. performed experiments. Data; M.C. prepared figures; M.C. and L.P. drafted manuscript; M.C. edited L.D., and O.M. approved final version of manuscript; M.C. and L.P. analyzed L.D., and O.M. approved final version of manuscript; M.C. and L.P. analyzed

REFERENCES


EEG MEAN FREQUENCY CHANGES DURING PREFRONTAL TDCS


Tadini L, El-Nazer R, Brunoni AR, Williams J, Carvallo M, Boggio P, Priori A, Pascual-Leone A, Fregni F. Cognitive, mood, and electroen-


