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What do you feel if I apply transcranial electric stimulation? Safety, sensations and secondary induced effects

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HIGHLIGHTS

- tES is a painless and safe technique.
- tDCS induced sensations are modulated by electrode size and intensity.
- Sham stimulation might not be an effective blinding method with anodal tDCS.

ABSTRACT

Objective: The goals of this work are to report data regarding a large number of stimulation sessions and to use model analyses to explain the similarities or differences in the sensations induced by different parameters of tES application.
Methods: We analysed sensation data relative to 693 different tES sessions. In particular, we studied the effects on sensations induced by different types of current, categories of polarity and frequency, different timing, levels of current density and intensity, different electrode sizes and different electrode locations (areas).
Results: The application of random or fixed alternating current stimulation (i.e., tRNS and tACS) over the scalp induced less sensation compared with transcranial direct current stimulation (tDCS), regardless of the application parameters. Moreover, anodal tDCS induced more annoyance in comparison to other tES. Additionally, larger electrodes induced stronger sensations compared with smaller electrodes, and higher intensities were more strongly perceived. Timing of stimulation, montage and current density did not influence sensations perception. The analyses demonstrated that the induced sensations could be clustered on the basis of the type of somatosensory system activated. Finally and most important no adverse events were reported.
Conclusion: Induced sensations are modulated by electrode size and intensity and mainly pertain to the cutaneous receptor activity of the somatosensory system. Moreover, the procedure currently used to perform placebo stimulation may not be totally effective when compared with anodal tDCS.
Significance: The reported observations enrich the literature regarding the safety aspects of tES, confirming that it is a painless and safe technique.

1. Introduction

Transcranial electric stimulation (tES) has been increasingly used to date to modulate brain activity with two main focuses: To study the brain–behaviour relationship (Dayan et al., 2013; Miniussi et al., 2013; Filmer et al., 2014) and to induce beneficial effects on motor, cognitive and affective functions in healthy and

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disease states (Brunoni et al., 2012; Meinzer et al., 2013; Lüdemann-Podubecká et al., 2014). In addition to the more well-known transcranial direct current stimulation (tDCS), other very promising electrical stimulation protocols have been introduced in previous few years, i.e., transcranial alternating current stimulation (tACS) and random noise stimulation (tRNS) (Paulus, 2011; Guleyupoglu et al., 2013). The effectiveness of these protocols, their equivalence and the mechanisms of action are under investigation in many laboratories and hospitals worldwide (e.g., Fertonani et al., 2011; Stagg and Nitsche, 2011; Feurra et al., 2013; Pirulli et al., 2013, 2014; Reato et al., 2013; Bestmann et al., 2014). However, the use and comparison of these tES protocols indicate that they might have secondary induced effects, which might influence the subject/patient response, especially from a clinical perspective (see the placebo effect, Benedetti, 2014). Therefore, in this context, one aspect that should be considered in addition to the mechanisms of action and effectiveness is related to the "secondary" induced sensations, which, in general, are not the direct focus of the investigation.

Previous studies have demonstrated that the application of the tDCS protocol induces minimal discomfort sensations, which are summarised as mild tingling and itching sensations under the electrodes, predominantly in the first few seconds of the tDCS (Gandiga et al., 2006; Poreisz et al., 2007). With reference to tRNS, some studies have demonstrated that tRNS is characterised by a reduced perception of induced sensations compared with tDCS. It has been suggested that the intensity of tRNS should be approximately three times higher than tDCS to evoke the same percept (Ambrus et al., 2010, 2011). At equal intensity, the tACS evoked sensations, which are visual and cutaneous, are strictly related to the frequency of stimulation that is used (Turi et al., 2013). It should be noted that for tACS in the range of 8–20 Hz and an intensity close to 1 mA or higher, the possibility of inducing phosphene perception via retinal stimulation significantly impairs what the subject can perceive (Schwiedrzik, 2009; for a discussion, see Schutter and Hortensius, 2010; Brignani et al., 2013).

In this context, we should consider that these sensations, even if mild, might invalidate the experimental and clinical results when sham tES is used in comparison to real tES (Gandiga et al., 2006) or when different tES protocols are compared between them (e.g., tRNS vs. tDCS) to test their value.

A widely held opinion is that the current density is linearly linked with the perceived sensations; consequently, for the same current intensity, the feeling would be greater with a smaller electrode compared with a larger one (Ambrus et al., 2011). Nevertheless, an interesting recent paper by Turi et al. (2014) suggests that this is not the case and describes a counter-intuitive relationship between the electrode size and the perceived discomfort. Specifically, a larger electrode is associated with a stronger perceived sensation. In general, these works have led to important knowledge regarding tES-induced sensations, but with some limitations. Most of these works include small subject samples; in some reports, the stimulation is of a few seconds and does not mimic the longer stimulation periods typically applied in experimental protocols. Moreover, the parameters of stimulation adopted are often well beyond the values currently applied in the literature; thus, it is important to explore the ways in which several different parameters might influence the perception of these tES-induced sensations.

A parallel issue is that put forward by Brunoni et al. (2011), which highlights the urgent need to collect data regarding the adverse effects associated with tES and suggests the use of structured questionnaires in all protocols that use tES. Indeed, it is important to collect data regarding the adverse effects, safety and tolerability of different interventions and to know the sensations elicited by tES. Their call has been followed by two more recent ecological reports (Kessler et al., 2012; Russo et al., 2013) that have addressed previously identified limitations. In these papers, the numbers of investigated subjects were high (approximately 150 subjects per paper), but none of the studies investigated whether the perceptions sensations were different at distinct levels of current intensity, on different scalp areas, or for different subject states. Furthermore, an important issue related to tES-induced perception is blinding adequacy in the sham condition. Following Gandiga et al.'s, 2006 important work, most researchers have adopted a sham method that consists of ramping the stimulation up and down in the firsts 10–30 s of stimulation. Nevertheless, recent papers (Kessler et al., 2012; O'Connell et al., 2012) have emphasized the inadequacy of this method with 2 mA tDCS, which suggests that at such intensity, the subjects can easily distinguish real from sham stimulation. In the clinical context in the last few years, there has been a steady increase in the stimulation intensity used with tDCS; thus, it would also be important to clarify this issue.

Here, we analyse data from 693 different stimulation sessions, which were performed on 531 subjects in our laboratory during the previous five years. In this work, we systematically investigate the impact of the type of tES protocol (i.e., tDCS, tACS and tRNS) on the perception of induced discomfort considering the polarity, current intensity, electrode size, density, stimulated area, reference site, frequency of application, moment of application, and duration, as well as on the reports of adverse events.

2. Materials and methods

We administered a published questionnaire (Fertonani et al., 2010) to 531 different subjects (271 males, 260 females, 512 young: mean age ± standard deviation 22.4 ± 3.0 years; 19 elderly: 66.8 ± 5.4 years) who came to our lab to participate in several tES experiments. All subjects were neurologically healthy. Some experimental designs were within subjects; thus, the participants evaluated the sensations perceived in 693 stimulation sessions. The sessions included 434 tDCS sessions (184 anodal, 131 cathodal, and 119 placebo), 109 tACS sessions (25 at 6 Hz, 27 at 10 Hz, 28 at 25 Hz, and 29 placebo) and 150 tRNS sessions (72 at high frequency – HF, 14 at low frequency – LF, and 54 placebo). The stimulations were performed before the execution of an experimental task (offline) or during the experimental task (online). Moreover, each experiment was characterised by different current intensities, density levels, electrode sizes and stimulated areas. tES was delivered by battery-driven stimulators (BrainStim, EMS, Bologna, Italy; Eldith-Plus NeuroConn, Ilmenau, Germany) through a pair of electrodes. The electrodes were inserted in sponges soaked in saline solution, moreover an electroconductive gel was applied under the sponges before the montage to reduce skin impedance. At the end of each experimental session, we asked the participants to complete a sensation questionnaire, which describes seven different sensations they may have experienced during the different stimulations, as well as other sources of discomfort or problems they may have experienced during the stimulation (Fertonani et al., 2010).

The Ethics Committee of the IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy, approved all studies, and informed consent was obtained from all participants prior to the initiation of the experiments.

2.1. Data analysis

Sensations gathered by questionnaires refer to seven different perceptions of discomfort: itching, pain, burning, heat, pinching, iron taste, and fatigue. For each perception, the participants were

asked to express a value of perception strength that ranged from 0 (absence) to 4 (strong). In order to provide an evaluation of the general perceived discomfort induced by tES, a new aggregate variable (referred to as *discomfort*) was computed as the summation of the strength score recorded for each single sensation, so that the discomfort variable ranged from 0 (absence of discomfort) to 28 (maximum discomfort).

For investigating which factors contributed to perceived discomfort, generalised linear models (GLM, with Poisson distribution for dependent variable and log-link) were adopted. We evaluate the effects of different factors: type of current (tDCS, tACS, tRNS), polarity/frequency (anodal, cathodal, 6 Hz, 10 Hz, 25 Hz, HF, LF, placebo), timing (online, offline), density (the real range was from 0.040 to 0.167 mA/cm^2, but for analyse purpose they were grouped in four congruent levels), intensity (0.75–1.00 to 1.50–2.00 mA), electrode size (9, 16, 22.9, 25, 35 cm^2), reference (cephalic vs. extracephalic) and electrode area (frontal, central, occipital) on discomfort variable. Sidak corrections were adopted for all adjustments of post hoc analyses.

To select the best GLM (in terms of goodness of fit and factor significance), a series of models, accounting for all factors and their interactions, were computed by considering both Akaike information criterion (AIC) and Bayesian information criterion (BIC) indexes. These indexes combine the absolute contribution of the fit with model parsimony, so that the model with the lowest index was selected (Yang, 2005).

Additionally, a comparison of perceived discomfort between 20 young and 19 elderly subjects (intensity 2 mA, electrode size 35 cm^2) was conducted using a generalised estimating equation (GEE, with Poisson distribution and log-link) model. This comparison was applied with discomfort as a dependent variable, subject-age as a between factor, and condition as a within factor (three repeated evaluations: placebo, anodal online, anodal offline).

Finally, a comprehensive investigation of the sensations in terms of their correlation and mutual variability through a factor analysis was performed to identify specific relationship patterns in the perceived sensations. This approach (i.e., the multivariate analysis) allowed us to provide a precise interpretation on the type of perceived sensations, and therefore cluster some of these sensations within a specific "sensory" system. Separate analyses were computed for the paired young and elderly subjects previously analysed by GEE. A factor analysis was conducted through a principal component analysis method for factor extraction and via application of the varimax rotation to simplify the factor interpretation.

Statistical analyses were performed using SPSS software (v. 21.0 IBM Statistics, IBM Corp) and R language and environment (v.3.0.3 R Development Core Team). Statistical significance was set at $p < 0.05$.

3. Results

In general, among the total stimulation sessions, discomfort was felt in at least one sensation in 76% of the cases; however, only 5% of the cases perceived sensations as more than one and/or with a strength score >2. The discomfort (aggregate sensation variable) mean was 2.62 (SD = 2.66; range: 0–16). The highest incidences were for pinching, itching and burning (62, 46 and 28% on the total stimulations), with means equal to 0.93 (SD = 0.95), 0.68 (SD = 0.90) and 0.39 (SD = 0.72), respectively. All data are reported in Table 1. Of the subjects who reported any sensations, the perceptions were predominately confined to the beginning of the stimulation (72%); however, some subjects reported sensations towards the middle (14%) or the end (10%) of the stimulation or both at the beginning and end of the stimulation (3%), whereas some subjects provided no responses (1%). Importantly, apart from 4 cases of mild transient skin irritation in the electrode area, none of the subjects reported adverse events, such as dizziness or headaches.

3.1. Factor effect on discomfort variable

The best GLM obtained from the model selection procedure (AIC = 2514, BIC = 2617) comprised the factors type of current, polarity/frequency, intensity, electrode size and the two-way interactions: polarity/frequency × intensity, polarity/frequency × electrode size. The factor timing, density, reference, and electrode area were excluded because of their poor contribution (assessed by higher AIC = 2530 and BIC = 2660 than those of the best model above) in explaining the total discomfort variability. The main effects of the estimated GLM were all significant (p-values ranged from 0.032 for current to $p < 0.001$ for polarity/frequency), whereas only the interaction polarity/frequency × electrode size remained significant ($p = 0.001$). In particular, regarding the type of current, tDCS was more perceivable than tACS ($p = 0.066$)—even if the value was a trend—and tRNS ($p = 0.004$), with mean discomfort values equal to 2.62 (standard error SE = 0.29), 1.57 (SE = 0.35) and 1.25 (SE = 0.26), respectively. With respect to the polarity/frequency factor, anodal and cathodal stimulation induced more discomfort (discomfort variable mean equal to 2.98, SE = 0.35 for anodal; 2.60, SE = 0.30 for cathodal) compared with the other categories (which had mean values of discomfort that ranged from 0.94 to 2.16).

A more focused analysis on the data gathered in the tDCS setting of the effect of polarity/frequency, intensity and electrode size, was carried out by performing the same previously described GLM (Table 2). Anodal was confirmed as the most bothersome among the polarities, with a discomfort mean equal to 3.83 (SE = 0.24) and was almost significantly higher than placebo ($p = 0.056$), which had a discomfort mean equal to 3.08 (SE = 0.21). Regarding the intensity factor, the means of the discomfort variable increased significantly (from 2.67 to 3.74, $p = 0.013$) with the enhancement from 1 to 2 mA. This trend was also confirmed within each polarity category in which the larger discomfort (mean 4.79, SE = 0.40) was observed for the 2 mA intensity in anodal polarity. Similarly, an increase in discomfort was recorded with increasing electrode size: 25 and 35 cm^2 electrode sizes (discomfort mean equal to 3.87, SE = 0.19) were statistically less comfortable ($p = 0.033$) than electrode sizes of 16 cm^2 or smaller (discomfort mean less than 2.94, SE = 0.21).

Regarding the comparison of discomfort between the young and elderly subjects evaluated by the GEE model, only the subject-age factor was significant ($p < 0.001$), with mean discomfort

Table 1
Sensations values. Mean value (±standard deviation) and median of each reported sensation and the general sensation index (discomfort) for the total sessions of stimulation (693 sessions). The last row indicates the percentage of subjects who experienced a particular sensation.

	Itchiness	Pain	Burning	Heat	Pinching	Iron taste	Fatigue	Discomfort
Mean ± SD	.68 ± .90	.12 ± .40	.39 ± .72	.21 ± .47	.93 ± .95	.11 ± .43	.18 ± .51	2.62 ± 2.66
Median	.00	.00	.00	.00	1.00	.00	.00	2.00
Subjects (%)	46	10	28	19	62	7	14	76

Table 2
Results. Post-hoc comparisons of the main effects of the GLM performed on the tDCS data. Only significant comparisons were reported. SE: standard error.

Factors/ predictors	Categories	Mean	SE	Significant comparison	Sidak *p* value
Polarity	Anodal	3.83	0.24	Anodal vs. placebo	0.056
	Cathodal	3.17	0.25	–	–
	Placebo	3.08	0.21	–	–
Intensity (mA)	0.75	3.20	0.59	–	–
	1.0	2.67	0.29	1.0 vs. 1.5	0.054
	1.5	3.55	0.17	–	–
	2.0	3.74	0.27	2.0 vs. 1.0	0.013
Size (cm^2)	9	2.88	0.35	9 vs. 25	0.033
	16	2.94	0.21	9 vs. 35	0.033
	25	3.87	0.19	16 vs. 25	0.011
	35	3.87	0.19	16 vs. 35	0.011

equal to 1.68 (SE = 0.20) and 4.64 (SE = 0.59) for the elderly and young subjects, respectively. In a descriptive way, in this particular paired sample, the more perceived sensations were pinching (mean intensity = 1.6), itching (1.3) and burning (0.8) in the young subjects and pinching (0.8), burning (0.4) and itching (0.3) in the elderly group. Thus, the elderly participants perceived less sensations than the young participants.

3.2. Sensation analysis

A preliminary analysis of relations among sensations was evaluated via a Pearson linear correlation coefficient, *r*. In general, sensations were significantly correlated with each other. In particular, itching, pinching and burning were the most correlated with each other (itching–pinching: $r = 0.60$, $p < 0.001$; pinching–burning: $r = 0.44$, $p < 0.001$; and itching–burning: $r = 0.33$, $p < 0.001$) and with the aggregate discomfort variable (itching–discomfort: $r = 0.74$; burning-discomfort: $r = 0.67$; and pinching-discomfort: $r = 0.83$; $p < 0.001$ for all correlations).

Prior to the factor analysis, a Kaiser–Meyer–Olkin measure (equal to 0.70) and Bartlett test ($p < 0.001$) were computed to ensure the factor analysis applicability to our data. The estimated number of retained factors was two, which explained a large amount (49.5%) of the total variability, providing a meaningful visual representation of the relations between different sensations. The varimax rotation of the factor loadings allowed the detection of five sensations (itching, pinching, pain, heat and burning), with a high weight on the first factor (Factor 1), which were well distinguished from the other two sensations, iron taste and fatigue, that had a high weight on the second factor (Factor 2) (Fig. 1). In particular, itching, pinching and burning, appeared to be the most "close" to each other; conversely, iron taste and fatigue had different behaviours.

Fig. 1 shows a bi-plot graph (where both subjects and variables are displayed) of all perceived sensations in the whole sample of subjects. Itching, pinching, heat, burning and pain had similar response profile (i.e., direction of the arrows) represented by Factor 1, that could be interpreted as the activation (with different degrees proportional to the length of each arrow) of the cutaneous receptors in the somatosensory system. In particular, itching and pinching are close together, and the same is true for heat and burning, as highlighted in Fig. 2. Moreover, as also showed in Fig. 1, iron taste and fatigue contribute to explain a different dynamic (Factor 2), and their representation going in opposite directions means that who perceive iron taste, does not perceive fatigue and vice versa.

Conversely, for the elderly subjects (results not displayed), only burning and pinching appeared to have different behaviours with respect to the other sensations and contributed to the definition of the first and second factors, respectively. Thus, the elderly subjects perceived less than the young subjects (as reported in the GEE model output previously described) and specifically, they felt only burning and pinching sensations.

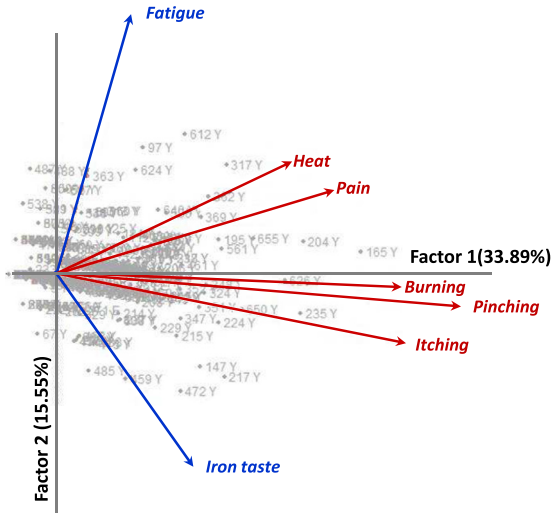

Fig. 1. Factorial analysis output for the total sample. Bi-plot graph of the first two factors: The first factor (Factor 1) is on the horizontal plane and includes all somatosensations perceived (itching, pinching, heat, burning and pain). The second factor (Factor 2) is on the vertical plane. The subjects are represented in light grey: the numbers represent each subject number, and the letters represent Y = young and E = elderly. The variables are represented in red. Each sensation is depicted by an arrow and the intensity of the somatosensations perceived is proportional to the length of each arrow. When two sensations are reported together and at the same intensity, the closer to each other the arrows are. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

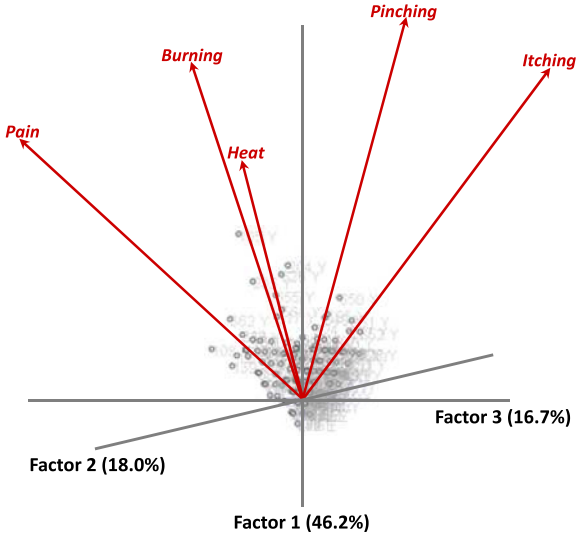

Fig. 2. Bi-plot graph in three-dimensions of sensations (itching, pinching, heat, burning and pain) having higher weights on Factor 1. The subjects are represented in light grey, and the variables are represented in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion and conclusion

The analysis of 693 tES sessions indicated that tES is a painless and safe technique. With our wide range of observations, we enrich the literature regarding the safety aspects of tES (Brunoni et al.,

2011). An important point is that in our experiments, tES was applied on different scalp areas, with a wide range of experimental parameters (e.g., an intensity from 0.75 to 2.00 mA, a current density from 0.040 to 0.167 mA/cm^2, and a duration from 4 to 22 min); in no case was the stimulation rated as painful or particularly annoying, and no safety concerns arose because of adverse events. The cases of mild transient skin irritation, which is an effect reported in literature (Brunoni et al., 2011), may occur because of the action of tES on vasomotion or increased skin temperature, which induces vasodilatation on the skin surface under the electrode; nevertheless, this adverse effect can be considered to be minor. Our results highlight that tES application was associated with only minor remarks of unpleasantness; only 5% of the participants perceived more than one sensation or rated the intensity in one sensation greater than 1 (on a five point scale, from 0 to 4). Consistent with previous reports in the literature (Poreisz et al., 2007; Russo et al., 2013), the most reported sensations are itching, pinching and burning. In agreement with previous observations, the sensations identified here were reported with all types of stimulation. The co-occurrence of these three sensations may also be because of a partial semantic overlap of their definitions, even if each of these terms is characterised by different nuances of meaning and thus, they are not interchangeable.

The perceived sensations were rated as significantly less intense in the tRNS experiments compared with the tDCS experiments; remarkably, with tACS, the perceptions appeared to be lower in level compared with tDCS. The particularly low level of discomfort that characterises tRNS has previously been emphasized by Ambrus et al. (2010), in which the authors verified that the "50% perception threshold" of tRNS was 1.2 mA relative to the approximately 0.4 mA of the tDCS. Our broad data set confirms their observations. Regarding tACS perception, the only paper in the literature (Turi et al., 2013) reports different perceptions according to the frequency of stimulation. In our case, we did not identify significant differences between 6, 10 and 25 Hz. However, at a descriptive level, the 10 Hz values (mean 2.16) were higher than the 6 and 25 Hz (means of 1.51 and 1.27, respectively), therefore the 10 Hz value is a plausible reason for the lack of a significant difference between tDCS and tACS ($p = 0.066$). Therefore, our results appear to confirm, in part, those of Turi et al. (2013), with a different location (electrodes placed on the parietal cortex and vertex) and current density (1 mA/16 cm^2). This difference between tDCS and other tES may be a result of the current discharge modality. With tDCS, the intensity of the current is constant and continuously excites the cutaneous receptors (i.e., fibres) of the somatosensory system, whereas with tACS and tRNS, the current varies continuously and only larger myelinated fibres of the tactile system may be activated. Nevertheless, for the same reason, a potentially relevant factor is that for a given current intensity n, with tDCS, the level ranges from 0 to n mA, whereas with alternating currents, it ranges from $-n/2$ to $n/2$, which results in a smaller level of polarisation.

Interesting data obtained from our analyses indicate that both the area of the electrode and the intensity of stimulation significantly influenced the perception of somatosensations. The role of intensity is intuitive: increasing its level increases the perceived discomfort. In contrast, the influence of the electrode area is counter-intuitive: a smaller stimulating electrode (higher current density) is associated with a weaker evoked sensation, and a larger electrode (lower current density) is associated with a stronger sensation. This finding appears in contrast with the idea that at the same current intensity in a small electrode, there is an increased current density compared with a large electrode, and the higher current density should induce more discomfort. Nevertheless, Turi et al. (2014) have systematically investigated the role of the electrode area and current intensity in a methodological experiment and reached the same conclusion. The explanations that they propose refer to the work by Martinsen et al. (2004), which suggests that the perception of direct current is stronger with larger electrodes because of a spatial summation phenomenon (more cutaneous receptors are stimulated).

Regarding the sham stimulation, we have verified that for tRNS, tACS and cathodal tDCS, placebo-induced perception is not different from real induced perception. Nevertheless, anodal tDCS is different from sham with a marginally significant value of $p = 0.056$, and this finding was irrespective of the intensity. If we examine the mean values of the two conditions, they occur at the same order of magnitude (3.83 anodal vs. 3.08 sham, the variable has a range from 0 to 28); thus, the perceptions are quite similar. Nevertheless, a difference, although minimal, exists and is close to significance. Moreover, in the model obtained, there is no interaction between the tDCS polarity and stimulation intensity; thus, this datum is not modulated by the intensity of the stimulation. This finding contrasts with previously reported data (Kessler et al., 2012; O'Connell et al., 2012), which suggest that subjects can easily distinguish real from sham stimulations at 2 mA tDCS intensity. Here, we emphasize that anodal tDCS (and not cathodal) can be disentangled from sham tDCS irrespective of the intensity levels; nevertheless, this difference is minimal. We did not consider the difference between the evaluations of naïve vs. experienced participants, which could differ (Ambrus et al., 2010). Nevertheless, we may merely hypothesise that for naïve subjects, blinding may be efficacious, whereas for experienced subjects, who know the presence of a placebo condition and have expectations regarding the induced sensations, blinding may not be appropriate (Ambrus et al., 2012). In this regard, given that sham stimulation sometime might not be an effective blinding method when compared to anodal tDCS, we strongly propose as a standard procedure to ask subjects, at the end of the experiment/treatment, if they think to have received real/s or placebo/s stimulation/s (see the revised questionnaire in Appendix A). It has been suggested that in some situations in which it is relevant to grantee the blinding procedure, it is possible to topically apply local anaesthetics (Nitsche et al., 2008; McFadden et al., 2011).

An interesting result that, to our knowledge, has not been previously described is the difference in the levels of reported sensations between young and elderly individuals. In two experiments with identical experimental parameters, the elderly subjects reported a lower rating of the sensations. This finding may has multiple possible causes. It may be that in physiological aging, there is a lower perception of sensation because of changes in skin conductance; however, recent work by Kemp et al. (2014) excludes this hypothesis. Instead, the higher threshold of perception appears to be more attributable to dysfunctions of the peripheral and/or central nervous systems. Another hypothesis, which may complement rather than exclude the physiological hypothesis, is more psychological and involves a lower propensity to complain in elderly individuals (Petrini, 2014). Moreover, they may be more accustomed to feeling mild discomfort, which leads to an underestimation of the tES sensations.

We suggest that our data offer valuable indications for ameliorating the comfort in tES application. To date, there are no published safety guidelines for the selection of stimulation parameters, and the first safety recommendations (Nitsche et al., 2003a; Poreisz et al., 2007) are commonly overridden. Therefore, the currently adopted criterion is to observe the parameters applied in similar studies in the literature and not exceed them in terms of intensity, density and duration of stimulation, and making sure that tES is administered by trained personnel. One important observation is that, at least in the motor system, the effects of tDCS appear to be dose dependent for currents of approximately 1 mA and a duration not longer than 13 min (Nitsche and Paulus,

2001; Nitsche et al., 2003b). When we modify these parameters by increasing the intensity (Batsikadze et al., 2013; Pirulli et al., 2014), interleaving pauses (Fricke et al., 2011), prolonging the stimulation duration (Monte-Silva et al., 2013) or combining protocols (Bortoletto et al., 2014), the results may be quite different. These data suggest that changing parameters might not be a matter of increasing perception but more of changing the induced effects.

However, to perform these protocols while avoiding confounding factors, the most important thing is to obtain the best possible interface (i.e., low impedance) between the electrodes and skin to diminish the voltage required to perform stimulation (note that stimulation should not be conducted directly over lesions). To this aim, the electrodes are typically inserted in sponges soaked with saline solution (see Dundas et al., 2007). It has been noted that the relationship between the salinity of the solution and comfort/discomfort (i.e., requested voltage) of the stimulation is very important. Dundas et al. (2007) suggested that the ideal should be a NaCl solution concentration between 15 and 140 mM. An inappropriate solution can increase the potential to perceive discomfort from the stimulation (Dundas et al., 2007). However, if caution is not used, the physiological solution can leak from the sponges, which modifies the features of the contact area. To improve scalp contact, especially when the participants have dense hair, it may be useful to apply an electro-conductive gel under the surface of the electrode/sponge to make the contact area and, therefore, the current distribution uniform. Indeed, the presence of dense hair may significantly influence the outcome of the stimulation (Horvath et al., 2014). In our lab, we routinely use gel to minimise impedance in all experiments; we have found that it is very useful, especially in combination with sponges. Nevertheless, not all types of electro-conductive gel are equivalent; we have verified that the application of some more viscous gels caused more pronounced unpleasant sensations in volunteers and were difficult to uniformly spread to cover the electrode. Another important consideration to minimise impedance is to obtain better adherence between the electrode and scalp. To fix the electrodes, rubber bands are typically provided in tDCS kits. Nevertheless, with such bands, the contact may be sub-optimal, especially at the electrode corners, and the wings of the electrode may be raised. Thus, not all electrode surfaces are in contact with the head, and the current density subsequently increases in an uncontrolled manner. In our laboratory, we have verified that it is better to use a tubular net-shaped elastic bandage in mesh tissue for electrode fixation. These bandages are very easy to use and maintain perfect adherence of the whole electrodes, which provides a uniform electrode–skin contact.

In the work by Ambrus et al. (2011), it has also been demonstrated that electrode shape does not influence the induced perception; standard rectangle electrodes induce the same type of sensations as circle shape electrodes, providing that they have the same surface.

Considering the results relative to Factor 1, which explains the main induced sensation, we can clearly see in Fig. 2 that the clustering of these sensations are congruent with the different somatosensory systems: touch, thermoception, and nociception. Therefore, such sensations are congruent with the distribution of these systems over the skin. In regard to the second factor that explained our data (Factor 2), there were two reported percepts that exhibited opposite trends, metallic taste and fatigue. The perception of metallic taste was reported after electric stimuli were applied to the tongue and after weak currents that stimulated the trigeminal nerve (Lawless et al., 2005; Hettinger and Frank, 2009). Therefore, metallic taste perception, which was reported only by 7% of the subjects, might be strictly related to the action on gustatory nerves, and it is quite likely that the electrode configuration in relation to the trigeminal nerve is the relevant factor. With fatigue, it is more difficult to provide an explanation in strict relation with the stimulation because it was reported at a very mild level (0.18; 14% of the subjects, Table 1) and it is a complex percept. This report is most likely related to the general state of the subject who was requested to perform a task in his/her best conditions and who may experience slight fatigue at the end of the experiment.

The performed analyses indicated that four features do not influence the perceived sensations. The first of these factors is the timing of the stimulation: our data indicate that the application of stimulation during or before task execution does not modify the induced sensations. We have reasoned that the application when the subject is not performing a task could lead to stronger perceptions because the subject's attention is entirely focused on the perceived sensations; however, this is not the case. The second feature that was not significant is the stimulated area: stimulating the frontal vs. central vs. occipital area does not modify the induced sensations, and the same finding is true irrespective of the montage used. The third factor is the positioning of the reference in a cephalic vs. extracephalic position. Even the positioning of two electrodes on the head with a cephalic montage does not appear to be relevant to increase the level of perceived sensations. Finally, the last factor was, surprisingly, the current density. Nevertheless, it should be noted that we have included both the current intensity and the electrode size in the model, which better explained the variations in induced perceptions. However, we must remember that in our model, there may be some confounding factors. For example, we did not include the factor that accounts for repetition of the stimulation over the same area in the model because all data were collected from normal subjects, and these data were not available. This factor, which is relevant to clinical trials, could influence the perception of sensation in a different way. In this regard and regarding additional aspects, the collection of large data samples over different laboratories in the future will be necessary to implement models, including all potentially confounding factors. To improve the questionnaire used to collect the data presented in this work (Fertonani et al., 2010), we suggest a revised form (see Appendix A). In the revised form of the questionnaire, we have also included a choice between real and placebo stimulations to be indicated at the end of the entire experiment for each session, as well as a more codified safety report of adverse events.

In this study, we have demonstrated that alternating currents appear less perceivable than tDCS, regardless of the other parameters of application. Of the different types of tES, anodal tDCS is the type that induces more perceptions, which are stronger than the ones perceived with sham stimulation. Moreover, the perception of induced sensation is directly linked to the electrode size; thus, the bigger the electrode, the stronger the sensation. Additionally, we report that older participants perceive less tDCS-induced sensation compared with young participants. Finally, based on the data reported in the present study, tES is a painless and safe technique.

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Appendix A. (revised questionnaire, English version)

Subject code: _____ **Date:** __/__/_____

Experiment: _____

Did you experience any discomfort or annoyance during the electrical stimulation? Please answer the following questions regarding the different sensations and indicate the degree of intensity of your discomfort according to the following scale:

- ***None*** **= I did not feel the described sensation (0)**
- ***Mild*** **= I mildly felt the described sensation (1)**
- ***Moderate*** **= I felt the described sensation (2)**
- ***Considerable*** **= I felt the described sensation to a considerable degree (3)**
- ***Strong*** **= I strongly felt the described sensation (4)**

In the first stimulation block

Itching:	☐ None	☐ Mild	☐ Moderate	☐ Considerable	☐ Strong
Pain:	☐ None	☐ Mild	☐ Moderate	☐ Considerable	☐ Strong
Burning:	☐ None	☐ Mild	☐ Moderate	☐ Considerable	☐ Strong
Warmth/Heat:	☐ None	☐ Mild	☐ Moderate	☐ Considerable	☐ Strong
Pinching:	☐ None	☐ Mild	☐ Moderate	☐ Considerable	☐ Strong
Metallic/Iron taste:	☐ None	☐ Mild	☐ Moderate	☐ Considerable	☐ Strong
Fatigue:	☐ None	☐ Mild	☐ Moderate	☐ Considerable	☐ Strong
Other_____:	☐ None	☐ Mild	☐ Moderate	☐ Considerable	☐ Strong

When did the discomfort begin?

☐ At the beginning of the block ☐ At approximately the middle of the block ☐ Towards the end of the block

How long did it last?

☐ It stopped quickly ☐ It stopped in the middle of the block ☐ It stopped at the end of the block

How much did these sensations affect your performance?

☐ Not at all ☐ Slightly ☐ Considerably ☐ Much ☐ Very much

Identify whether these sensations were located over the head or in a different location

☐ On the head _____ ☐ Other _____

In the second stimulation block

. . ..(if there is more than one condition, repeat the list above here based on the block numbers)

If you would like to provide more details, please briefly describe the experimented sensations in relation to the 'Other' or "Fatigue" response:

To be administered at the end of the entire experiment

Do you believe that you received a real or placebo stimulation?

In the first stimulation block/day/week:	☐ real	☐ placebo	☐ I don't know
In the second stimulation block/day/week:	☐ real	☐ placebo	☐ I don't know

. . ..

For the researcher/clinician:

Please report any adverse event/problem (e.g., skin irritation, headache, scalp pain, dizziness, or others, please specify) that occurred and rate the event/problem on a scale from 1 to 4 as previously described.

Additional comments: .....

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