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Assessment of non-BDNF neurotrophins and GDNF levels after depression treatment with sertraline and transcranial direct current stimulation in a factorial, randomized, sham-controlled trial (SELECT-TDCS): An exploratory analysis

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Abstract

The neurotrophic hypothesis of depression states that the major depressive episode is associated with lower neurotrophic factors levels, which increase with amelioration of depressive symptoms. However, this hypothesis has not been extended to investigate neurotrophic factors other than the brain-derived neurotrophic factor (BDNF). We therefore explored whether plasma levels of neurotrophins 3 (NT-3) and 4 (NT-4), nerve growth factor (NGF) and glial cell line derived neurotrophic factor (GDNF) changed after antidepressant treatment and correlated with treatment response. Seventy-three patients with moderate-to-severe, antidepressant-free unipolar depression were assigned to a pharmacological (sertraline) and a non-pharmacological (transcranial direct current stimulation, tDCS) intervention in a randomized, 2 × 2, placebo-controlled design. The plasma levels of NT-3, NT-4, NGF and GDNF were determined by enzyme-linked immunosorbent assay before and after a 6-week treatment course and analyzed according to clinical response and allocation group. We found that tDCS and sertraline (separately and combined) produced significant improvement in depressive symptoms. Plasma levels of all neurotrophic factors were similar across groups at baseline and remained significantly unchanged

regardless of the intervention and of clinical response. Also, baseline plasma levels were not associated with clinical response. To conclude, in this 6-week placebo-controlled trial, NT-3, NT-4, NGF and GDNF plasma levels did not significantly change with sertraline or tDCS. These data suggest that these neurotrophic factors are not surrogate biomarkers of treatment response or involved in the antidepressant mechanisms of tDCS.

Keywords

GDNF; Major depressive disorder; Neurotrophins; Sertraline; Transcranial direct current stimulation

1. Introduction

The neurotrophic hypothesis of depression postulates that the depressive state is associated with decreased neuroplasticity, neuronal atrophy and/or aberrant neurogenesis in key brain areas related to mood and memory (Lang and Borgwardt, 2013). In this context, successful antidepressant treatment response is associated with an increase in the expression of neurotrophic factors related to neuronal growth, survival (Duman and Monteggia, 2006) and neuroplasticity (Brunoni et al., 2008; Sen et al., 2008). These neurotrophic factors include the neurotrophin family (such as NT-3, NT-4, NGF and BDNF) and other CNS factors such as the GDNF (Jiang and Salton, 2013; Neto et al., 2011). Particularly, BDNF has been extensively investigated, with several studies showing that its blood levels are lower in depressed patients compared to healthy controls, and that its blood levels increase after successful antidepressant drug and electroconvulsive treatments (Brunoni et al., 2008, 2014a; Molendijk et al., 2014).

However, BDNF is not the only neurotrophin associated with depression and antidepressant efficacy and, in fact, its role might be less important than previously assumed (Molendijk et al., 2014). Therefore, other neurotrophic factors should be investigated as potential biomarkers of the depressive state and predictors of antidepressant response. In this context, the neurotrophins NT-3, NT-4 and NGF bind to both the TrK and the p75 neurotrophin receptors. As BDNF also binds to these receptors, non-BDNF neurotrophins may also have a role in the neurotrophic hypothesis of depression (Jiang and Salton, 2013). Moreover, recent work showed that GDNF also has an important role in the development of stress- and mood-related disorders (Miller, 2011; Uchida et al., 2011).

Indeed, recent evidence suggests that non-BDNF neurotrophins and GDNF are involved in depression pathophysiology. For instance, GDNF and NGF serum levels are reduced in late-life (Diniz et al., 2012, 2013) and adolescent depression (Pallavi et al., 2013). GDNF and NT-3 levels are also decreased in major depression (Otsuki et al., 2008). However, the effect of antidepressant treatment on the blood levels of these neurotrophic factors has been insufficiently investigated and, to the best of our knowledge, no study has systematically investigated for the effects of non-invasive brain stimulation therapies, such as tDCS, on neurotrophic factor levels and antidepressant response. This would be relevant considering the emerging evidence of tDCS – a non-invasive, affordable, easy to use technique – as a potential alternative for the treatment of major depression (Brunoni et al., 2013c; Loo et al.,

2012). TDCS involves the placement of two electrodes – the anode and the cathode – over the head, inducing polarity-dependent changes in cortical excitability (Brunoni et al., 2012b; Nitsche and Paulus, 2000; Nitsche et al., 2008). When anodal tDCS is applied daily for several days over the left DLPFC, this procedure might reverse the hypoactivity observed in this area and, in turn, improve depression symptoms (Brunoni et al., 2012a). Nevertheless, tDCS mechanisms of action in depression are still elusive, and, although some animal studies have suggested that it might involve BDNF-dependent synaptic plasticity (Fritsch et al., 2010), results in human studies have been inconclusive regarding its mechanisms of action (Brunoni et al., 2014b, 2014c; Palm et al., 2013).

Herein we explored whether a pharmacological (sertraline) and a non-pharmacological (tDCS) antidepressant treatment would change plasma levels of some non-BDNF neurotrophins, namely NT-3, NT-4 and NGF; and GDNF. We used data from the SELECT-TDCS, a randomized, sham-controlled trial in which 120 depressed patients were randomly allocated to four treatment groups: (1) sham-tDCS/placebo-pill (further referred as *placebo*); (2) sham-tDCS/sertraline-pill (*sertraline-only*); (3) active-tDCS/placebo-pill (*tDCS-only*); (4) active-tDCS/ sertraline-pill (*combined treatment*).

Our goal was to investigate whether the plasma levels of these neurotrophic factors would change across the trial, according to the allocation group and clinical response. We also examined whether baseline neurotrophic factor levels would predict antidepressant response. The importance of our study is both mechanistic and clinical, as it can shed light into the pathophysiological mechanisms involved in pharmacological and non-pharmacological antidepressant response, as well as provide putative peripheral biomarkers of clinical outcome.

2. Methods

2.1. Design

The present study was approved by the Local and National Ethics Committee and took place at the University Hospital (University of São Paulo, São Paulo, Brazil) between March 2010 and September 2011. Its methodology (Brunoni et al., 2011) and results (Brunoni et al., 2013c) were described elsewhere. All participants provided written, informed consent. The study was registered in clinicaltrials.gov (NCT01033084).

The duration of the trial was 6 weeks, divided into an acute treatment phase, in which ten daily tDCS sessions were delivered consecutively for 10 weekdays, and two tDCS sessions delivered once in a fortnight. Sertraline in a fixed dose of 50 mg/day was also administered for 6 weeks. Sertraline treatment started simultaneously with tDCS and was chosen due to its efficacy and low rate of adverse effects (Cipriani et al., 2009), and because tDCS effects are enhanced in combination with serotonergic drugs (Nitsche et al., 2009).

2.2. Subjects

Participants in an acute major depressive episode with the diagnosis of major depressive disorder were enrolled. Anxiety disorders as comorbidities were allowed. The diagnosis was

performed by clinical interview and confirmed with the Portuguese-validated version of the MINI (Amorim, 2000). Only patients with a HDRS-17 score ≥ 17 were included.

Exclusion criteria were: diagnosis of other psychiatric conditions, such as bipolar disorder, substance abuse disorder, schizophrenia and others; acute suicidal ideation; previous sertraline non-response or current sertraline use; presence of any medical disorder, as determined by physical and neurological examination, review of systems and laboratory tests and; and pregnancy/breastfeeding. Participants were also excluded if they were not in good physical condition.

Non-drug-naïve patients were gradually tapered of any antidepressant medication. Those previously taking a benzodiazepine had the dose tapered down to 20 mg/day (or less) of diazepam-equivalents, which remained constant throughout the entire study. Non-drug-naïve participants were antidepressant-free for at least five half-lives of the medication(s); which corresponded to a median washout time period of 18 days (Brunoni et al., 2013c).

The primary outcome measure of SELECT-TDCS was the score change of the MADRS (Gorenstein and Andrade, 2000). Clinical response ($\geq 50\%$ of MADRS improvement from baseline to endpoint) was also evaluated.

2.3. Procedures

Standard, commercial tDCS devices (Chattanooga Ionto™ Dual Channel Devices, Chattanooga Group, Hixson, TN 37343 USA) were used in SELECT-TDCS. The anode was placed over the left and the cathode over the right DLPFC. This bifrontal setup was used in accordance to previous studies (Brunoni et al., 2013a; Ferrucci et al., 2009) considering the prefrontal activation asymmetry observed in depression, i.e. hypoactivity of the left and relative hyperactivity of the right prefrontal cortex (Mayberg et al., 2000). We applied a dose current of 2 mA for 30 minutes per day (current density of 0.8 A/m^2).

For the sham procedure the tDCS device was turned on for only one minute and then remained turned off for the last 29 minutes. This method, previously described by Gandiga et al. (2006), mimics local skin effects albeit employing a very brief period of active tDCS which is too short to induce any neuromodulatory effect (Nitsche and Paulus, 2000).

Blood samples were collected by venipuncture immediately before (2–4 pm) the first and the last tDCS session—i.e. at study baseline and endpoint. Within 30 minutes of sample collection, samples were then spun at 3000 rpm for 15 minutes at 5°C and thereafter plasma aliquots were gently collected and stored at -80°C until analysis. Plasma levels of neurotrophic factors were measured by ELISA according to the procedures supplied by the manufacturer (DuoSet, R&D Systems, Minneapolis, MN, USA). All samples were assayed in duplicate. Lower detection limits were 5 pg/mL. Concentrations are expressed as pg/mL. Analyses of blood samples were performed blind to group assignment and outcome.

2.4. Statistical analysis

The software Stata 12 (Statacorp, College Station, TX, USA) was employed for all analyses, with 2-sided significance tests at the 5% significance level. We described clinical and

demographic variables across groups using one-way ANOVA, χ^2 tests or Fisher's exact tests, when necessary.

The following neurotrophic factors were used as dependent variables: NT-3, NT-4, NGF, GDNF and BDNF. BDNF results were already published elsewhere (Brunoni et al., 2014b) but are included and presented here as to compare the results obtained in BDNF and non-BDNF analyses. Few participants (<1% of the sample) presented extreme values (up to one-hundred times of the sample mean) in some neurotrophic factor levels. To handle with these extreme outliers, these values were trimmed to the mean + 3SD of the variable and, thereafter, they underwent natural logarithmic transformation to achieve Gaussian distribution.

We first performed a multivariate analysis of variance (MANOVA) using all neurotrophic factors as dependent variables. Independent variables were time (two levels: first collection and second collection), group (four levels: placebo, sertraline-only, tDCS-only and combined therapy) and clinical response. After that, we performed analyses of variance (ANOVAs, one dependent variable per model) to individually assess the changes for each neurotrophic factor according to time, group and clinical response.

We also explored whether sociodemographic characteristics (age—dichotomized in 40 years-old, gender, obesity—defined as a body mass index ≥ 30 kg/m², smoking status, physical activity—evaluated with the IPAQ questionnaire (Craig et al., 2003) and further explored in low, moderate and high physical activity for the present analysis); depression characteristics at baseline (melancholic and atypical depression—defined by the MINI questionnaire; severity—with a cutoff point of baseline MADRS ≥ 30 indicating severe depression; and refractoriness—defined as the therapeutic failure to two or more antidepressants in the current depressive episode) and benzodiazepine use were moderators of changes in the levels of the neurotrophic factors. Conceptually, “moderators” are baseline variables that influence the relative likelihood of an outcome according to the type of the clinical intervention (Papakostas and Fava, 2008). Statistically, we performed these analyses by assessing the interaction between each abovementioned variable with time and treatment group, as suggested by Kraemer and Blasey (2004). Each variable was explored separately in a distinct ANOVA or MANOVA model.

In addition, we investigated whether the neurotrophic factors were mediators of clinical response. According to Papakostas and Fava (2008), mediators of efficacy are changes that occur during the treatment that are correlated to the clinical outcome. Pearson's correlations were employed to explore the association of changes (baseline minus endpoint) in depression scores with changes in neurotrophic factors levels. Finally, the levels of neurotrophic factors at baseline were investigated as moderators of clinical response by performing several regression analyses with changes in depression scores as the dependent variable. Group and baseline neurotrophic factor levels were independent variables and their interaction was examined for determining whether the dependent variable was a moderator. Each neurotrophic factor was entered in a distinct model, and all regression models were “centered” as recommended by Kraemer and Blasey (2004).

3. Results

3.1. Overview

Of 120 patients initially enrolled, 103 completed the original study. Of those, 73 (71%) had their baseline and endpoint NT-3, NT-4, NGF, GDNF and BDNF plasma levels analyzed. The remaining samples were not collected for technical reasons and patient refusal. The clinical and demographic characteristics of these patients did not differ from the completers of the original SELECT-TDCS trial. Also, these characteristics did not differ across groups at baseline. In addition, the results observed in SELECT-TDCS (clinical improvement of tDCS-only and sertraline-only over placebo, and of the combined treatment in comparison to all groups) were reproduced in this subsample (Table 1).

3.2. Changes of neurotrophic factor plasma levels over time, group and clinical response

The values of neurotrophic factor levels across groups are displayed in Table 2. The MANOVA showed no main effects of time and no interactions between time with group, clinical response and group and clinical response. Similar results were observed for each repeated-measures ANOVA (Table 2). This means that the plasma levels of neurotrophic factors remained unchanged throughout the trial, regardless of clinical response and/or allocation group (Table 3).

In our ANOVA and MANOVA models, we also did not observe influence of any clinical and demographic variable, such as age, gender, obesity, smoking status, physical activity, melancholic depression, atypical depression, severity and benzodiazepine use on neurotrophic changes over time. (Table 4)

3.3. Neurotrophin plasma levels and depression

Changes in depression scores were not correlated with changes in any neurotrophin plasma levels in the total sample (p 's > 0.22). Also, no correlation was observed when examining each group separately (p 's higher than 0.3, 0.46, 0.16 and 0.31 for the placebo, sertraline-only, tDCS-only and combined treatment groups, respectively). According to the regression models, baseline plasma levels of NT-3 ($p = 0.16$), NT-4 ($p = 0.57$), NGF ($p = 0.18$), GDNF ($p = 0.54$) and BDNF ($p = 0.88$) did not moderate depression improvement—i.e., the plasma levels of neurotrophins at baseline were not associated with depression improvement.

4. Discussion

In the present study, we used data from SELECT-TDCS, a factorial, randomized, sham-controlled trial to examine changes in neurotrophic factors in 73 depressed patients before and after a 6-week treatment course of tDCS, sertraline and the combined treatment. Our main finding was that, similarly to BDNF, the plasma levels of GDNF and the neurotrophins NT-3, NT-4 and NGF did not significantly change over time, regardless of treatment response or allocation group. In addition, the baseline plasma levels of these neurotrophic factors did not predict clinical response. To the best of our knowledge, no other clinical non-invasive neuromodulation study has investigated the impact of a non-invasive brain stimulation treatment on the plasma levels of non-BDNF neurotrophic factors (Fidalgo et al.,

2014). In fact, we were only able to identify one animal study that examined the effects of low-frequency rTMS on NGF hippocampal levels of rats (a NGF increase was observed after low-frequency rTMS) (Tan et al., 2013) and of high-frequency rTMS on GDNF expression levels in the *substantia nigra pars compacta* of rats subjected to an experimental model of Parkinson's disease (also showing an increase of GDNF levels).

Although our study was the first to clinically evaluate the effect of a non-invasive brain stimulation treatment on NT-3, NT-4, NGF and GDNF plasma levels in a sample of depressed patients, our findings are in line with previous tDCS reports examining BDNF plasma levels, from Palm et al. (2013) and our group (in fact, using the same subsample that was here investigated) (Brunoni et al., 2014b). Taken together, these studies suggest that peripheral levels of BDNF and non-BDNF neurotrophic factors do not appear to be surrogate biomarkers of tDCS antidepressant response. This does not necessarily mean that tDCS does not induce neuroplastic effects, as shown, for instance, in an animal study (Fritsch et al., 2010) but rather that such effects do not reflect on peripheral blood levels.

Our study was also one of the first to examine changes on NT-3, NT-4, NGF and GDNF plasma levels after a pharmacological treatment. The few clinical studies investigating the impact of an antidepressant treatment on these neurotrophic factors yielded mixed findings. For instance, Sun et al. (2013) observed that both electroacupuncture and fluoxetine increased GDNF levels in 75 depressed patients. In addition, Zhang et al. (2010) found that pharmacological treatment in patients with bipolar depression significantly increased GDNF levels, reaching similar levels to the non-depressed control group. Regarding NGF, the findings are contradictory, with one study (Hellweg et al., 2008) showing that NGF levels remained unchanged after paroxetine or amitriptyline treatment whereas another trial with duloxetine observed a decrease in NGF serum levels over the course of a 12-week treatment, with clinical response directly associated with a NGF decrease (Martino et al., 2013). Rybakowski et al. (2013) evaluated the effects of a single ketamine infusion in bipolar depression in NT-3, NT-4, NGF, GDNF and BDNF. Although response and remission rates were around 50% and BDNF decreased in non-responders, the authors observed no changes in the serum levels of the other neurotrophic factors. Finally, Gronli et al. (2009) examined NT-3 and NGF serum levels in depressed patients after electroconvulsive treatment, observing no changes regardless of treatment response. Importantly, none of these studies had a placebo arm.

In this study, we investigated and compared the antidepressant effects on peripheral biological markers of sertraline (a SSRI) and tDCS (a non-pharmacological, NIBS intervention). Pharmacotherapies primarily decrease over-activity of limbic structures (Quide et al., 2012) and, subsequently, increase hypoactivity of cortical structures (Mayberg et al., 2000). Pharmacotherapies are also associated with several systemic effects (e.g., anti-inflammatory activity, increase of peripheral BDNF levels, normalization of the HPA axis), although it is unclear whether this is indicative of a direct effect of antidepressant drugs in non-brain areas or, conversely, an effect secondary to depression improvement (Kupfer et al., 2012; Nemeroff and Goldschmidt-Clermont, 2012). Conversely, the antidepressant mechanisms of action of tDCS are much more unclear, although studies performed hitherto indicated that tDCS does not exert effects on heart rate variability (Brunoni et al., 2013b)

and its effects of cytokine plasma levels are non-specific, as a decrease of inflammatory proteins was also observed in the placebo group (Brunoni et al., 2014c). Conversely, tDCS improved central motor neuroplasticity and EEG prefrontal activity in depressed patients (Player et al., 2014; Powell et al., 2014) as well as cognitive control, working memory and emotional processing (Boggio et al., 2007; Brunoni et al., 2014d; Oliveira et al., 2013; Wolkenstein and Plewnia, 2013). Thus, our results are in line with current evidence that tDCS antidepressant effects might be more associated with changes in brain activity rather than systemic effects, in contrast to pharmacotherapies in which systemic effects are also observed.

Some strengths and limitations of our study should be underscored. Strengths include (1) the enrollment of a relatively large ($n = 73$) and homogeneous sample size of antidepressant-free patients with moderate to severe depression and (2) the use of a placebo control group, which corroborated our findings as we were therefore able to assess the stability of our measures over time. Limitations comprise (1) the relatively low dose of sertraline (50 mg/day)—however, although it is possible that higher drug doses would have led to an increase in the neurotrophic factor plasma levels, there is no evidence of such a “dose–response” relationship in literature and, moreover, the combined treatment group did not yield an increase in neurotrophic factors as well; (2) the assessment of peripheral blood levels, which might not necessarily reflect the expression of the assessed molecules in the CNS i.e., whether these levels are peripheral surrogate markers of neuroplasticity or, conversely, epiphenomena of the depressive episode—although there is evidence that the blood–brain barrier is permeable to neurotrophins (Poduslo and Curran, 1996). Therefore, further investigations should be integrated with a multimodal approach including genetic analysis of these neurotrophic factors (polymorphisms and RNA expression, which were not investigated in the present study), proteomics/metabolomics and neuroimaging (Niciu et al., 2014). Another possibility, although difficult for technical and ethical reasons, is to directly assess the levels of these neurotrophic factors in the cerebrospinal fluid. Finally, (3) our results are not generalizable to other peripheral (serum or whole blood) blood analysis, as we analyzed plasma levels. For instance, although serum and plasma BDNF levels seem to be both lower in depressed patients (Bocchio-Chiavetto et al., 2010), some studies also yielded divergent findings when comparing plasma vs. serum BDNF levels in depressed patients (Bocchio-Chiavetto et al., 2010; Piccinni et al., 2008). This occurs because serum analyses also take in account the BDNF released by platelets, which is an important source of BDNF storage. In fact, plasma BDNF may reflect the steady-state condition of bioavailable protein, as compared to platelet BDNF (Munkholm et al., 2014). Therefore, further studies should also investigate whether serum non-BDNF levels change after pharmacological and non-pharmacological treatments.

5. Conclusion

In this randomized, placebo-controlled trial assessing the changes of NT-3, NT-4, NGF and GDNF plasma levels after a 6-week treatment course for major depression, we found no changes in the blood levels of these neurotrophic factors over time, regardless of treatment group and clinical response. We therefore suggest that these neurotrophins and GDNF in peripheral blood are not putative peripheral biomarkers of antidepressant response for

pharmacological and non-pharmacological interventions. Nonetheless, considering that this is one of the first studies in the field, our findings need to be replicated in randomized, controlled trials.

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Abbreviations

NT-3	neurotrophin-3
NT-4	neurotrophin-4
NGF	nerve growth factor
BDNF	brain-derived neurotrophic factor
CNS	central nervous system
GDNF	glial cell derived neurotrophic factor
TrK	tyrosine kinase
tDCS	transcranial direct current stimulation
DLPFC	dorsolateral prefrontal cortex
SELECT-TDCS	Sertraline vs. Electric Current Therapy for Treating Depression Clinical Study
MINI	Mini International Neuropsychiatric Interview
HDRS	Hamilton Depression Rating Score
MADRS	Montgomery–Asberg Depression Rating Scale
ELISA	enzyme-linked immunosorbent assay
ANOVA	analysis of variance
SD	standard deviation
ANCOVA	analysis of covariance
SSRI	selective serotonin reuptake inhibitor

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Table 1

Clinical and demographic characteristics of the sample.

	Placebo	Sertraline-only	tDCS-only	Combined treatment	p	Total	Total from the original study	p
Sample size, n	19	18	15	21	0.79	73	120	–
Age, years (SD)	50 (12)	41 (1)	41 (12)	41 (13)	0.1	41 (12)	42 (12)	0.58
Female, n (%)	12 (63)	11 (61)	10 (66)	18 (85)	0.3	51 (70)	82 (68)	0.77
On benzodiazepines (%)	2 (10)	2 (11)	1 (7)	5 (23)	0.53	10 (14)	23 (20)	0.3
Severe depression, n (%)	12 (63)	11 (61)	11 (73)	12 (57)	0.8	46 (63)	70 (58)	0.49
MADRS, baseline, mean (SD)	31.5 (6)	31 (7)	32 (6)	31 (6)	0.92	31 (6)	31 (6)	0.5
MADRS endpoint, mean (SD)	24 (9)	19 (13)	19 (12)	10 (6)	<0.01	18 (11)	19 (11)	0.38
Clinical response	4 (21)	7 (39)	7 (46)	16 (76)	<0.01	34 (46)	47 (39)	0.52

tDCS, transcranial direct current stimulation; MADRS, Montgomery–Asberg depression rating scale; SD, standard deviation. Severe Depression: MADRS 30. Significant *p* values (<0.05) are highlighted in bold.

Table 2

Neurotrophin plasma levels at baseline and endpoint according to treatment group.

	Placebo	Sertraline-only	tDCS-only	Combined treatment	All groups	
NT-3 (pg/mL)	Baseline	68 (50–126)	68 (45–550)	50 (39–151)	66 (41–114)	64 (42–151)
	Endpoint	68 (42–189)	103 (45–459)	140 (41–757)	79 (35–241)	91 (39–261)
NT-4 (pg/mL)	Baseline	377 (334–424)	363 (315–529)	377 (353–480)	412 (339–464)	377 (339–485)
	Endpoint	367 (334–438)	400 (358–534)	407 (377–526)	387 (339–452)	391 (339–461)
NGF (pg/mL)	Baseline	87 (51–166)	76 (51–116)	65 (44–170)	72 (54–121)	75 (51–127)
	Endpoint	80 (40–107)	75 (49–138)	88 (62–198)	84 (57–121)	83 (54–143)
GDNF (pg/mL)	Baseline	52 (19–140)	68 (28–546)	40 (11–339)	53 (20–143)	56 (20–184)
	Endpoint	50 (11–157)	69 (27–498)	111 (20–754)	92 (18–140)	65 (18–174)
BDNF (ng/mL)	Baseline	18 (11–20)	13 (7–20)	15 (14–17)	15 (11–18)	15 (11–20)
	Endpoint	15 (11–20)	17 (13–21)	16 (13–19)	15 (11–19)	16 (11–19)

NT-3, neurotrophin 3; NT-4, neurotrophin 4; NGF, nerve growth factor; GDNF, glial cell derived neurotrophic factor; BDNF, brain-derived neurotrophic factor; tDCS, transcranial direct current stimulation. Values represent median and 25–75 interquartile range.

Table 3
Statistical values of the repeated-measures ANOVAs and MANOVA of neurotrophin plasma levels.

	Time			Time ± group			Time ± response			Time ± group ± response		
	F	d.f.	p	F	d.f.	p	F	d.f.	p	F	d.f.	p
NT-3	0.95	1,153	0.33	0.76	3,153	0.52	0.8	1,153	0.37	1.24	3,153	0.3
NT-4	1.41	1,153	0.24	0.32	3,153	0.81	0.47	1,153	0.5	0.67	3,153	0.57
NGF	0.16	1,153	0.69	0.71	3,153	0.55	0.15	1,153	0.7	0.64	3,153	0.6
GDNF	0.05	1,153	0.82	0.75	3,153	0.52	0.3	1,153	0.58	0.24	3,153	0.86
BDNF	0.32	1,153	0.58	0.47	3,153	0.7	0.35	1,153	0.55	0.2	3,153	0.89
MANOVA	0.81	5,94	0.87	0.78	30,378	0.78	1.2	10,188	0.29	0.58	30,378	0.96

The table displays the results from the repeated-measures analyses of variance (ANOVAs) and multivariate analysis of variance (MANOVA) performed in our study. *F* represents the *F*-values, d.f., the degrees of freedom. NT-3, neurotrophin 3; NT-4, neurotrophin 4; NGF, nerve growth factor; GDNF, glial cell derived neurotrophic factor; BDNF, brain-derived neurotrophic factor; iDCS, transcranial direct current stimulation.

Table 4

Influence of clinical and demographic variables on neurotrophin changes over time.

	NT-3		NT-4		NGF		GDNF		BDNF		MANOVA	
	F	p	F	p	F	p	F	p	F	p	F	p
Age	1.75	0.19	0.01	0.93	0.6	0.44	0.69	0.41	0.05	0.83	0.84	0.82
Gender	0.3	0.58	0.7	0.38	0.2	0.65	2.4	0.12	<0.01	0.95	0.93	0.62
Obesity	0.01	0.91	0.46	0.5	0.15	0.7	0.01	0.98	0.01	0.93	0.66	0.95
Smoking status	2.75	0.1	0.42	0.53	0.1	0.75	0.13	0.71	0.04	0.85	1.16	0.24
Physical activity	2.15	0.12	1.55	0.21	2.66	0.07	0.72	0.49	0.41	0.63	0.87	0.79
Melancholic depression	0.01	0.93	0.82	0.37	0.01	0.99	0.33	0.56	3.22	0.07	1	0.48
Atypical depression	0.92	0.34	1.25	0.27	0.02	0.89	0.42	0.52	3.21	0.07	1.3	0.06
Severe depression	0.2	0.65	4.4	0.06	1.31	0.25	0.25	0.61	2.59	0.11	1.04	0.39
Benzodiazepine use	0.05	0.81	1.72	0.19	0.36	0.55	0.01	0.96	0.01	0.91	0.77	0.87

F represents the F-values. NT-3, neurotrophin 3; NT-4, neurotrophin 4; NGF, nerve growth factor; GDNF, glial cell derived neurotrophic factor; BDNF, brain-derived neurotrophic factor. MANOVA is the multivariate analysis of variance in which all dependent variables were used.