Effect of left anodal transcranial direct current stimulation on hypothalamic-pituitary-adrenal axis (HPAA) activity in patients with depressive episodes: a randomized, triple-blind, pilot trial

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Abstract

Background

This study's main aim was to evaluate the effect of left anodal transcranial direct current stimulation (tDCS) on hypothalamic-pituitary-adrenal axis (HPAA) activity in participants with depression. As an additional exploratory objective, we aimed to find any relationship between baseline morning cortisol concentrations, family history of depression, and somatic symptoms.

Materials and Methods

A 3-week, randomized, triple-blind, pilot trial was carried out. Participants (n = 47, drop-out: 14.89%) were allocated randomly to one of two groups: tDCS and control group (sham stimulation). Since the effector hormone of the HPAA is cortisol, salivary cortisol was used as an HPAA activity marker. The primary outcome was the effect of tDCS on the diurnal cortisol pattern (DCP). Secondary outcomes were tDCS effects on cortisol awakening response (CAR) and maximal cortisol decline (MCD), as well as the variation of cortisol concentrations between the initiation of tDCS and 2 weeks later. Intention-to-treat and per-protocol analyses were carried out.

Results

Regarding our primary outcome, we found no significant effectof tDCS on the interaction *group * time * daytime*. Concerning our secondary outcomes, tDCS had no significant effects on CAR, MCD or cortisol concentration variation between the initiation of tDCS and 2 weeks later in the tDCS group when compared with the sham stimulation group. Finally, an exploratory analysis found a significant relationship between morning cortisol, family history of depression, and somatic symptoms.

Conclusions

Our main results indicate that anodal tDCS did not affect HPAA activity in depression. More studies are needed to confirm these findings.

2. Introduction

Depression is an affective disorder characterized by permanent sadness, anhedonia, loss of perspective, and suicidal behavior [1,2]. According to the World Health Organization (WHO), about 300 million people live with depression, constituting a prevalence of 4.4% worldwide, representing high costs for regional health systems and high disability-adjusted life years[3].

Although there are many hypotheses concerning this affective disorder, the causes and pathophysiology of depression are still uncertain. However, many studies in the last decades have demonstrated a relationship between depressive episodes and inflammation[4] or high HPAA activity, reporting altered diurnal cortisol curves[5–7] and increased morning salivary cortisol[8,9], or increased cortisol awakening response[10] during depression. Furthermore, there are studies on depression which found a reduction of the HPAA activity after pharmacological treatment[11], with the modulatory effect on cortisol metabolism being a critical component for the treatment of depression.

International guidelines for treating depressive episodes mostly recommend antidepressant agents combined with psychotherapeutic treatment[12,13]. However, about 15–30% of patients with depression show no symptom reduction despite guideline-recommended therapies[14]. Novel augmentation treatments, including non-invasive procedures such as transcranial direct current stimulation (tDCS)[15–18], have been developed in the last decade to try to overcome the non-response issue.

Evidence is increasing regarding tDCS and depression but effectiveness studies of tDCS on HPAA activity in depression are lacking as depression itself alters HPAA activity[19–21]. There are still no studies which evaluate the effect of tDCS therapy on cortisol concentrations in patients with depression. In many studies, stimulation procedures, such as electroconvulsive therapy, have been correlated with cortisol reductions and clinical improvement in people with depressive episodes[22,23]. In addition, pharmacological agents, such as mirtazapine and tricyclic antidepressants, have been shown to produce significant reductions in salivary cortisol in people with depression[11,24]. Cortisol reduction might also occur with tDCS, which involves stimulating the CNS at a low intensity. To investigate this, this study's main aim was to evaluate the effect of tDCS on HPAA activity in participants with depression by conducting a randomized, triple-blind, pilot trial. In this trial we assessed the effect of tDCS on thecortisol concentration of participants with depression. We hypothesized that tDCS will show a positive effect in the treatment of depression and reduce the cortisol concentrations in the saliva of participants with depression.

Separately, as part of an exploratory analysis, we looked for relationships between baseline morning cortisol concentrations, family history of depression, somatic symptoms and psychometric characteristics (i.e., depression severity and suicidal behavior) in participants with depression, hypothesizing that morning cortisol concentration will have a significant relationship with the variables mentioned above.

3. Materials And Methods

a. Study design

The pilot trial described here was a randomized, triple-blind, trial conducted at the Central Institute of Mental Health (Mannheim, Germany). Details of the blinding are given below. In this study we compared two groups of patients with depressive episodes: an experimental group (tDCS) and a control group

(sham stimulation). In addition, we measured salivary cortisol to measure HPAA activity in both groups of participants, evaluating diurnal cortisol pattern (DCP), cortisol awakening response (CAR), maximal cortisol decline (MCD), and variation of cortisol before and after stimulation. During the trial, participants continued to receive pharmacological and psychotherapeutic treatments. The trial schedule and measurements are presented in Table 1.

All participants were informed about the study and provided written informed consent before participation. This pilot trial was conducted following the Helsinki Declaration and approved by the Ethics Committee of the Medical Faculty Mannheim of the University of Heidelberg.

b. Randomization and blinding

Study participants were randomly assigned in approximately equal numbers (1:1) to an experimental (tDCS) and a control group. Each patient was assigned to one of the four stimulation devices (A, B, C and D), depending on the order of their inclusion in the study. Two of the four devices were tDCS (A and C) and the remaining two were sham stimulation devices (B and D). To ensure compliance with the quality criterion of reliability all stimulation treatments were carried out by the same practitioner (H.S.), with the same devices, in the same rooms, and always on weekdays (Monday to Friday) between 2:00 and 4:00 PM. The same physician always collected the assessment questionnaires. During the trial, neither the patients, nor the practitioner, nor the physician were informed about whether the treatment given was real or simulated. This blinding status was extended through the evaluation of the raw data, and disclosure only took place at the beginning of the final data analysis, reducing any possible experimental bias.

c. Participants

Initially, forty-seven patients (20 female and 27 male participants; mean age: 45.30 ± 14.20) with depressive disorders were recruited from the inpatient unit "affective disorders" (Central Institute of Mental Health, Mannheim) for this pilot trial between 15th August 2016 and 20th September 2017. The characteristics of the participants who completed the trial are shown in Table 2. To be included in the study participants had to meet the DSM-V criteria, evaluated through SCID-I interviews, for a depressive episode or major depression and sign the informed consent form. The interviews were carried out by Central Institute of Mental Health clinical experts, who were not involved in the study. Included patients had a Hamilton Depression Rating Scale (HDRS) of at least 18 points with baseline stability (i.e., less than 25% improvement one week before the beginning of stimulation). Before starting the study, we checked that the participants had an ECG sinus rhythm. Therapy-naïve status was not an inclusion criterion for this pilot trial so that the study participants could continue their prescribed treatments at the inpatient unit. During the trial medication was not changed but the doses were adjusted slightly if needed. We chose a three-week trial interval (21 days) to try to avoid the occurrence of essential changes in the

pharmacological treatment. Finally, patients taking benzodiazepines as a *pro re nata* (PRN) treatment could participate with doses no greater than 1.5 mg/d of Lorazepam or equivalent, to keep additional pharmacological effects as low as possible. Participants taking sleep inducers such as Zopiclone and Zolpidem as a PRN treatment were not excluded from this pilot trial.

Patients with psychotic disorders, post-traumatic stress disorders, panic disorders, and borderline personality disorders were excluded from the study. We also excluded females with current pregnancies, participants with a conservatorship or with legal protectors, and patients who could not give their consent because of severe mental illness. Finally, we excluded patients complying with the following criteria: with metal implants or medical devices (e.g., pacemakers), illegal drug and alcohol dependency at the time of the study, acute suicide crisis, medical conditions that alter adrenal functions, use of glucocorticoids, intake of medication that changes the heart rate and its variability (e.g., beta-blockers), arrhythmias of any type, dementia syndrome (DSM-V/ICD-10 criteria), past medical history of severe cranioencephalic trauma, neurological diseases of any kind, severe decompensated medical conditions (e.g., therapy-resistant arterial hypertension, heart insufficiency, respiratory insufficiency), neoplastic diseases of any kind and infectious diseases of any kind.

Of the 47 patients included in the study, 40 completed the three-week treatment and seven participants left the trial (drop-out rate: 14.89%). We interrupted the trial for a 20-year-old participant on the third day of stimulation due to a newly identified skin rash all over the scalp. The trial had to be interrupted on the 7th day for a 78-year-old participant due to his discharge from the inpatient ward. For 3 participants (ages 48, 53 and 34) the trial was terminated before the first stimulation appointment due to new diagnostic assessments which triggered trial exclusion criteria. Similarly, one participant (age 38) left the study before the first stimulation appointment due to entering into a manic phase. Finally, a 27-year-old patient decided against further participation in the study because of side effects such as headaches which occurred during and after the first stimulation treatment.

Concerning medication, all 40 participants who completed the stimulation trial received the direct current stimulation as an add-on therapy to guideline-compliant pharmacotherapy for depression. Seven of these 40 participants received monotherapy with an antidepressant agent. Another 14 patients were treated with a combination of 2 antidepressants, and one patient was treated with a combination of 3 antidepressants. Six patients had been prescribed an antidepressant plus augmentation therapy (e.g., Aripiprazol), and five received a combination of 2 antidepressants and augmentation therapy. One patient received a quadruple combination of 3 antidepressants and quetiapine. Two received a triple combination of lithium, quetiapine and an antidepressant. Two patients received quetiapine monotherapy and two received dual therapy with lithium and quetiapine. There was no significant difference between the two groups concerning pharmacotherapy.

d. Transcranial direct current stimulation (tDCS)

Stimulation was performed using a CE-certified microprocessor-controlled device (Sooma Medical, Helsinki, Finland) which emitted a direct current with a maximum output of 40 mA. Direct current (DC) was applied via a pair of electrodes (anode and cathode) made of conductive rubber (dimensions: 5 x 7 cm, 35 cm²). Before placing the electrodes, these were wetted on sponges soaked in a 144 mol/L NaCl solution in order to lower the physiological skin resistance. Electrodes were placed based on the 10-20 international system, with the anode placement at EEG point F3 (left dorsolateral prefrontal cortex) and the cathode at EEG point Fp2 (right dorsolateral prefrontal cortex). A hood facilitated correct placement and this was adapted to the head of the participant.

The four stimulation devices (A, B, C and D) were previously programmed to perform the stimulation either in real (DC of 2.00 mA) or in sham modus (DC of 0.30 mA). In the sham stimulation modus, the devices produced an initial DC of 2.00 mA for a few seconds, ramping down to a DC of 0.30 mA and maintaining this current for a period of 30 minutes (treatment duration), giving sensations similar to real stimulation.

The stimulation was carried out according to the manufacturer's protocol, encompassing three weeks of treatment with daily 30-minute stimulation sessions. This protocol was used regardless of the device mode (real or sham stimulation). In our trial, participants received tDCS or sham stimulation until the end of week 3, from Monday to Friday, between 2:00 and 4:00 PM, with stimulation pauses at weekends.

e. Cortisol assessment

General procedure: To assess the activity of the HPAA we measured cortisol using saliva samples. The advantages of using salivary cortisol are mostly related to the practicability of the sample collection, with less invasiveness and less effort for the participant [9,25]. Moreover, many studies have demonstrated a good correlation between plasma and salivary cortisol[26,27], verifying the method's effectiveness for assessing plasma cortisol concentration. Salivary cortisol measurements were acquired as reliable indicators of total free plasma cortisol, with marginally lower concentration due to the presence of 11ß-HSD2 in saliva[28]. The advantages over blood cortisol measurements, such as laboratory independence and “stress-free” sampling, are more important for the repeated measurements used to determine the circadian rhythmicity of cortisol secretion.

Saliva collection occurred during the week when patients participated in daily ward routines. Participants received instructions on the saliva collection procedures. Immediately before the sample collection participants must not consume any nutrients or fluids, or smoke, or brush their teeth since cortisol levels in saliva may be increased by these actions. The collection was done using a kit based on cotton swabs and tubes (Salivette™, Sarstedt, Leicester). Participants were instructed to chew on the saliva collectors for 30 to 45 seconds immediately after awakening but when still in bed (A), 30 min. after awakening (B), 8 h. after awakening (C), and 14 h. (D) after awakening. The starting point for saliva sample collection was between 07:30 and 08:00 AM. After the saliva absorption, the cotton swab was removed from the

oral cavity and placed into the corresponding tube. All samples were stored at -25˚C. After thawing, samples were centrifuged for 5 min at 3000 rpm, resulting in a clear low-viscosity supernatant. As published elsewhere, the samples of supernatant were analyzed using a time-resolved immunoassay with fluorescence detection[29,30]. The lower limit of detection was 0.43 nmol/L, with inter- and intraassay coefficients of variation of less than 10% across the expected range of cortisol levels. The concentrations were calculated in nmol/L[29,30].

Saliva samples for DCP, cortisol awakening response, maximal cortisol decline, and stimulation-related cortisol levels: Two sets of DCP samples were collected during the study. Participants were asked to collect saliva four times during the day before the first stimulation (baseline) and at the end of the third week[31]. Based on samples A and B (see above), the cortisol awakening response (CAR) was calculated from the difference between the two samples (i.e., CAR = B - A). Finally, the maximal cortisol decline (MCD) was calculated from the difference between the highest morning cortisol value (i.e., sample B) and the last value (i.e., sample D). Finally, salivary cortisol was collected "immediately before stimulation" (pre), "immediately after stimulation" (post), and "2 hours after stimulation" (2h-post) in W0 and W2 (Table 1). These samples were collected to record the short-term effects of tDCS on HPAA activity.

Implausible morning cortisol values (i.e., values of samples A or B ≤ 3 nmol/L) were replaced with half of the minimum morning cortisol value (in this case, 1.50 nmol/L), as suggested elsewhere[32].

f. Outcomes

The primary outcome of this pilot study was any change in the DCP from the baseline to week 3 (W3) in the tDCS group in comparison with the control group. Additionally, with the data on the DCPs, the area under the curve with respect to the ground (AUCg) was calculated, using the formulae of Pruessner and colleagues[33]. Secondary outcomes included any change in the CAR from the baseline to W3, any change in the cortisol morning decrease from the baseline to W3, and any change in stimulation-related cortisol levels between W0 and W2.

Finally, we included in our analysis any variations of the MADRS scores through the trial period in both groups. In this case, as suggested elsewhere[34], we defined clinical response based on MADRS scores if the MADRS total values showed a reduction to ≥ 50% of the initial value with a final score of ≤ 9 points. Finally, also as described elsewhere[35], we defined partial response if the MADRS total values showed a reduction of ≥ 25% of the initial value.

g. Statistical analysis

Numeric variables that fitted a parametric distribution are specified in the text as mean (standard deviation). Numeric variables with a non-parametric distribution (i.e., median ≠ mean) are shown as median (interquartile range, 3rd quantile – 1st quantile). Category variables and count data are specified as numbers or fractions. Data with more than two decimals were rounded. Values smaller than 0.005 are presented as < 0.005 and values greater than 1 million are expressed in scientific notation[4]. Descriptive data in the text are presented in tables. To evaluate the significance of differences between groups, t-tests were used for continuous parametrically distributed data and the U-test for continuous non-parametrically distributed data. We used the χ^2 or the exact Fisher test for the category and count data. For these differences, p values were calculated. This study defined statistical significance whenever the two-tail-p-value was less than or equal to 0.05.

Primary and secondary outcomes and MADRS scores were analyzed blindly by one of the authors who did not know the assignment of interventions to the trial groups (B.P.P). Intention-to-treat (ITT) and per-protocol analyses (PP) were performed for both primary and secondary outcomes using linear mixed models (LMM). We calculated the interaction *time* * *daytime* * *group* for the primary outcome. For the secondary outcome we calculated *time* * *group* (CAR and MCD) and *time* * *daytime* * *group* (stimulation-related cortisol levels). The interactions were corrected for *gender*, *age,* and *weight* confounding factors. We carried out multiple realizations with linear interactions and a maximum of 1000 iterations in case of missing values. For both trial groups, CAR, DCP, MCD, and stimulation-related cortisol levels were estimated in the multiple realizations using the variables gender and age of the participants. In the LMM, fixed effect omnibus tests were carried out to define the main effects of the variables in the model and to evaluate the variable in the model against the null model. The results of the ITT analysis were reported graphically, and the ITT and PP analyses' results are described in the text using 95% confidence intervals (95CI). Cohen's d was computed for ITT and PP analyses for the sample size effect. Post-hoc tests were carried out for the LMM when differences between the *time* * *group* (MCD, CAR) and the *time* * *daytime* * *group* (DCP and stimulation-related cortisol levels) were significant.

For the statistical analyses and the descriptive data, the SPSS software (International Business Machines Corporation, New York, United States of America), version 26.0, was used. For the LMM, we used the R-based software jamovi 2.0.0[36] and the toolbox GAMLj[37]. Finally, we generated the graphs using Prism 8 GraphPad (GraphPad Software Inc., California, United States of America).

h. Exploratory analysis

In addition to our principal objectives in this trial, we carried out an exploratory analysis with the baseline information of this trial to search for relationships between morning salivary cortisol, somatic symptoms, family history of depression and psychometric data (MADRS item 10 "suicide thoughts" and HRDS score). For that purpose, a generalized linear model was computed using the daytime B salivary cortisol concentrations (i.e., from samples collected between 8:00 and 8:30 AM). In the model, we included the

dichotomic variables “family history of depression” and “somatic symptoms” as factors. Finally, we included “age”, “weight”, “BDI scores”, “HRDS scores” and “MADRS item 10” as covariates. For this analysis, we used the R-based software jamovi 2.0.0 [36] and the toolbox GAMLj [37], and defined statistical significance whenever the two-tail-p-value was lesser than or equal to 0.05.

4. Results

a. MADRS scores during the trial

The ITT analysis for MADRS scores revealed, regardless of the trial group (tDCS and control group), a significant time variation of the total MADRS score, with a decrease of 10.68 points (reduction of 34.26% with respect to the baseline scores) in 3 weeks (95CI [-12.92; -8.44]; *Cohen's d* = -1.65). Although reductions occurred in this study, no significant interaction *time* * *group* results were found (Figure 1). Finally, the PP analysis also showed no significant reductions for the interaction *time* * *group* but significant changes in the MADRS values during the trial time.

b. Adverse effects

Adverse effects triggered by tDCS or by sham stimulation were recorded using a self-assessment questionnaire at W0. Adverse effects were recorded, such as itching, tingling, burning, cutaneous rash, pain, concentration deficit, acute mood changes, and visual perception disorders. A statistically significant difference between the two stimulation groups could only be observed for the side effect of tingling ($\chi^2_{1,40}$ = 4.96, p = 0.026). While only 6 of 13 patients in the sham group reported tingling as a side effect, this side effect occurred in 15 of the 21 in the tDCS group. The side effects of itching and burning were also reported more frequently by the tDCS group; however, the differences between the two groups concerning itching and burning were not significant. Four patients treated with tDCS and one with sham stimulation reported a cutaneous rash after stimulation; here, too, differences between the groups were not significant. Finally, occurrence of a manic clinical condition was not observed in any test person, neither under sham stimulation nor under tDCS.

c. Primary outcome

The ITT analysis revealed, independent of the trial group (tDCS and sham stimulation) and stimulation protocol time (baseline and W3), a significant daytime variation of salivary cortisol concentration with a decrease of 7.92 nmol/L cortisol in 24 hours (95CI [-10.73; -5.12]; *Cohen's d* = -0.64). However, we found no significant results for group (tDCS and sham stimulation), for the interaction effect *daytime* * *time* (baseline and W3), or for the interaction *group* * *daytime* * *time* (Figure 2), so that tDCS did not result in

significant variations of DCP. Also, in the ITT analysis, AUCg did not show significant differences between tDCS and sham stimulation (95CI [-34.23; 82.65]; *Cohen's d* = 0.24).

In the PP analysis we found significant results regarding the daytime variation of salivary cortisol concentrations, with a decrease of 8.85 nmol/L cortisol in 24 hours (95CI [-11.72; -5.98]; *Cohen's d* = -0.72). Similarly with the ITT analysis; we found no significant results regarding group, nor for the interactions *group* * *daytime* * *time* and *daytime* * *time*. Here too AUCg showed no significant effects for the interaction *group* * *time*.

d. Secondary outcomes

Regarding the secondary outcomes, in the ITT analysis we found no significant results for the interaction *group* * *time* and CAR (Estimate = 0.15, 95CI [-5.14; 5.43]; *Cohen's d* = 0.02; Figure 3). MCD values also showed no significant results in the ITT analysis for the interaction *group* * *time* (Estimate = 2.47, 95CI [-3.33; 8.26]; *Cohen's d* = 0.26; Figure 3). Finally, we performed an ITT analysis for the stimulation-related cortisol levels. In this case, we considered in the model the time of the stimulation (i.e., for W0 and W2) and the point of time (immediately before the stimulation, immediately after the stimulation, and two hours after the stimulation). Regarding the interaction *group* * *trial time* * *stimulation time*, we did not find any significant effects in the analysis (Estimate = - 2.33, 95CI [-5.83; 1.18]; *Cohen's d* = -0.17 Figure 3).

Concerning the PP analysis for CAR and MCD, we found no significant results for the *group* variable and the interaction *group* * *time*. In the PP analysis of stimulation-related cortisol concentrations, we also found no significant results regarding group, nor for the interactions *group* * *stimulation time* * *trial time* or *stimulation time* * *trial time*.

e. Exploratory analysis

An exploratory analysis was conducted to search for relationships between morning cortisol concentrations (prior to stimulation), somatic symptoms in people with depression, and family history of depression. For that purpose, a generalized linear model was computed.

The results of this exploratory analysis showed a significant interaction *somatic symptoms* * *family history* (OR = 5.26×10^{-11}; 95CI [1.58×10^{-16}; 1.74×10^{-5}]; $p < 0.005$). A post hoc analysis was carried out, obtaining significant differences in morning cortisol concentrations between depressive participants with and without a family history, with both groups having no somatic symptoms (M_D = -16.58; p = 0.0025). There were also significant differences between depressive participants with and without somatic symptoms, with both groups having no family history of depression (M_D = -14.44, p = 0.015). These results are presented in Figure 4.

Moreover, we found significant effects on morning cortisol concentrations of the MADRS item 10 “suicide” (OR = 0.024; 95CI [0.0012; 0.46]; p = 0.012), and the HDRS score (OR = 2.17; 95CI [1.08; 4.35]; p = 0.036). Other interactions considered in this exploratory analysis showed no significant effects.

5. Discussion

To the best of our knowledge, this study is the first to report results regarding tDCS trials and HPAA activity in patients with depression[38].

Concerning our primary outcome, our main results showed that tDCS does not significantly affect the DCP in depression in comparison with sham stimulation. In addition, we found no significant differences between the tDCS and the sham stimulation groups regarding diurnal cortisol curves, including AUCg, as the interaction *group* * *daytime* * *trial time* did not show any significant effect.

There are no clinical studies evaluating the effects of tDCS on HPAA activity in participants with depression and the current evidence is inconclusive. On the one hand, previous studies with healthy humans reported similarly negative results regarding tDCS and HPAA activity[39,40]. Moreover, two studies with participants with Sjogren’s disease[41] and with osteoarthritis of the knee[42] showed no significant effects of tDCS on HPAA activity. On the other hand, previous literature studies, such as the review by Castelo-Branco and Fregni based on two studies with left anodal neurostimulation in healthy subjects[44,45], suggested that the neurostimulation of the left dorsolateral prefrontal cortex (DLPFC) causes changes in HPAA activity by regulating the cortisol concentrations[43].

Although our study protocol included anodal stimulation of the left DLPFC, we observed no significant effects of tDCS on HPAA activity in participants with depression, unlike with other stimulation procedures, e.g., repetitive transcranial magnetic stimulation (rTMS), with which changes in cortisol concentration were seen[45,46]. Our results suggest that, in contrast to rTMS, tDCS does not have characteristics enabling it to modify HPAA activity in depression.

Concerning CAR and MCD as secondary outcomes, we found no significant effects of tDCS on awakening response and maximal decline. Similarly to the DCP, this is the first study that has reported the effects of tDCS on CAR and MCD in depression[38,47]. Considering other evidence for an effect of tDCS on CAR, one study of healthy subjects reported negative results[48]. Other stimulation procedures, such as rTMS, have shown significant changes in the CAR of depressive patients, which suggests that as with DCP the characteristics of tDCS are not appropriate for it to change the CAR significantly in people with depression. CAR depends on other variables (e.g., age, gender, BMI)[48–50], however, our results were corrected for possible confounding factors (participants’ ages, gender and weight) in both analyses, so the probability of their interaction in the analysis is very low. Finally, since the tDCS in our sample of depressive participants did not significantly affect the DCP, it is also to be expected that the tDCS did not modify the MCD, as its value is obtained as a subtraction of the last value from the second value of the diurnal cortisol curve.

Regarding stimulation-related cortisol levels as a secondary outcome of this tDCS trial, we did not find significant effects for the interaction *group * trial time * stimulation time* in patients with depression. Similarly negative findings were also reported in a study with healthy subjects, showing no modification by tDCS of stimulation-related cortisol concentrations[48]. However, the group given tDCS showed increased cortisol concentration (Fig. 3). One explanation for the increase in cortisol levels could be the differences in adverse effects (tingling) between tDCS and sham stimulation and participants' perceived excitement levels before and during the stimulation.

Concerning the therapy response (MADRS total scores), during the study we observed a reduction of the MADRS score, irrespective of the trial groups. Here, we could see an effect of the treatment as usual (i.e., pharmacological treatment) but none from the add-on stimulation treatment since neither group, tDCS or sham stimulation, showed significant differences. Similar findings are reported elsewhere in the literature[51].

Finally, in an exploratory analysis, we found that cortisol concentrations between 8:00 and 8:30 AM showed significant effects when considered with the variables of family history of depressive disorders and somatic symptoms. Moreover, within this interaction, we found that, in the absence of somatic symptoms, a positive family history of depression is related to higher cortisol concentrations, showing similar results to previous studies[52,53]. A possible explanation for the link between family history of depression and cortisol concentrations relates to the association between family risk and higher cortisol concentration[52,54] and to the stress-vulnerability model for depression[55]. On the other hand, among those with a positive family history of depression, our results showed no significant differences in cortisol concentrations between depressive participants with and without somatic symptoms (Fig. 4). Furthermore, among depressed people with somatic symptoms, there were also no significant differences in cortisol concentrations between depressive participants with and without a family history of depression. Although no previous studies describe similar results, these findings suggest that the presence of somatic symptoms does not explain cortisol variations in people with depression, with or without a family history of depression.

When there is no family history of depression, cortisol concentrations are higher in depressed people with somatic symptoms than in those with none. Some previous studies have shown a positive correlation between depression, the presence of somatic symptoms, and cortisol concentrations in humans[56–58]. However, other studies reported negative correlations between somatic symptoms and depression diagnosis[59,60], making this evidence inconclusive. Nevertheless, our results support those studies which indicate positive correlations, which may be explained by the sickness behavior theory in depression, and by inflammatory and neuroendocrine dysregulation response mechanisms[52,61–63].

Secondly, cortisol concentrations between 8:00 and 8:30 AM showed significant linkage with item 10, "suicidal thoughts", which indicates an indirect relationship between these variables. A previous meta-analysis reported a negative association between suicidal behavior and cortisol concentration in studies with a mean participant age of 40, which is compatible with our study[64]. Finally, we found significant

linkage between baseline cortisol concentration between 8:00 and 8:30 AM and baseline HDRS scores. In this case the relationship confirms findings of previous studies with cortisol concentrations and depression severity scores [65,66].

This trial is the first study that reported data regarding tDCS and HPAA in participants with depression, and it did not find significant effects of tDCS on DCP, CAR, maximal decline, and stimulation-related cortisol concentrations. These are negative results[67] but one must consider the limitations of this pilot trial. Firstly, there were initially only 47 participants (from whom only 40 completed the trial). More extensive studies would be necessary to be able to make reliable statements concerning patients with depression. Despite this, the scope of our study was within the usual range for studies with tDCS. Secondly, there was heterogeneity in the participants included in this study. However, the two groups did not differ with respect to demographic and clinical characteristics, as shown by the similar clinical and demographic parameters. Third was the inclusion of patients with bipolar depression in our study. As in similar studies, patients with unipolar and bipolar depression as the main diagnosis[68,69] were included. In our case, the included bipolar patients did not differ from the unipolar patients with respect to demographic or clinical characteristics. Fourthly, there was the inclusion of participants receiving other therapy in parallel during the trial, as it has been demonstrated that concomitant antidepressant medication may influence tDCS[70,71]. However, in our study, the inclusion of the variable "psychopharmacological treatment" did not have a significant effect as a confounding factor in the analysis and did not modify the ITT and PP analysis results. Fifthly is the assessment of cortisol by using saliva samples. Although reports indicate slightly reduced concentrations in salivary cortisol compared to plasma cortisol, many studies have demonstrated the robustness of this method in assessing cortisol concentrations[26,27]. Sixth is the restricted stimulation positioning for the tDCS. Generally, standardized protocols recommend stimulation in the left or right DLPFC. Our study was designed with a left anodal stimulation of the DLPFC, and did not show significant effects on the HPAA activity in depression. As noted in the study of Carnevalli and colleagues, it would be interesting to see whether the stimulation of other brain areas (e.g., ventromedial PFC) that are related to stress could affect the HPAA activity in depression[48]. Seventh, the characteristics of tDCS in comparison with other neurostimulation methods, such as rTMS. A tDCS device is usually set up to 1–2 mA, which in most cases is considered a weak stimulation procedure and this could explain the lack of difference found between the two study groups in this pilot trial. In the future, researchers may consider other neurostimulation procedures to investigate cortisol variations in depression, such as rTMS, which is regarded as high intensity[72] since it results in high magnetic impulses (1 Tesla) in one brain region. Finally, the inclusion of a follow-up period in future studies could allow the investigation of any posterior effects of the tDCS on HPAA activity in depression.

In conclusion, this pilot trial did not demonstrate that a 3-week regime of tDCS significantly affects DCP, cortisol awakening profile and MCD in participants with depression. Moreover, tDCS, in this trial, did not result in demonstrated changes in cortisol concentrations related to stimulation time in comparison with sham stimulation.

The exploratory analysis which was undertaken revealed that cortisol concentrations between 8:00 and 8:30 AM are correlated negatively with suicide behavior and positively with baseline HRDS scores. Moreover, in the absence of somatic symptoms, a family history of depression is related to higher cortisol concentrations. Finally, in the absence of “family history of depression”, morning cortisol concentrations are higher in people with depression with somatic symptoms in comparison with those with depression and no somatic symptoms.

Future similar studies should have a larger number of participants with depression, possibly therapy-naïve, and compare other neurostimulation methods. Regarding the results of the exploratory analysis, future studies should expand this line of research, mainly to study more profoundly the interactions between cortisol concentrations, family history of depression and somatic symptoms in depression.

Declarations

Declarations: Author Contributions/Conflict of Interests/Additional Information

- **Ethics approval and consent to participate**

Each participant or their legally authorized representative was fully informed about the objectives and procedures of the study, as well as the potential adverse effects, and gave their written consent to participate. The study protocol and all study procedures were reviewed and approved by the ethics committee of the Medical Faculty Mannheim. Additionally, this pilot trial was carried out according to the Helsinki Declaration.

- **Consent for publication**

Not applicable

- **Availability of data and materials**

The data sets generated and analyzed during the study are not publicly accessible due to the applicable data protection law of the State of Baden-Württemberg but they are available from the corresponding author on justified request.

- **Author Contributions**

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Contribution: MD developed the idea for the study, supervised the data collection, the methods and the analyses, and worked on proofreading of the manuscript.

- **Competing Interests**

Non-financial competing interests: The authors declare that they have no competing interests.

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Table

		Stimulation trial			
Weeks (W)	**Baseline**	**W0**	**W1**	**W2**	**W3**
Informed consent and study information	X				
Proof of inclusion and exclusion criteria	X				
Stimulation (tDCS/sham stimulation)		X	X	X	X
Hamilton Depression Rating Scale (HDRS)	X				
Antidepressant Treatment History Form (ATHF)	X				
Salivary cortisol, diurnal cortisol pattern (DCP) (4 Samples: A/B/C/D)	X				X
Salivary cortisol immediately before stimulation		X		X	
Salivary cortisol immediately after stimulation		X		X	
Salivary cortisol 2 hours after stimulation		X		X	
Montgomery-Åsberg Depression Rating Scale (MADRS)	X	X	X	X	X
Beck Depression Inventory Version II – 21 Items (BDI - II)	X				

Table 1 – Trial schedule and measurements (trial period = 3 weeks = 21 days). Abbreviations: tDCS = transcranial direct current stimulation; W0 = first stimulation; W1 = one week after first stimulation; W2 = two weeks after first stimulation; W3 = three weeks after first stimulation; A = salivary cortisol measured between 7:30 and 8:00 AM; B = salivary cortisol measured between 8:00 and 8:30 AM; C = salivary cortisol measured between 3:30 and 4:00 PM; D = salivary cortisol measured between 9:30 and 10:00 PM.

Baseline characteristics	tDCS (n = 24)	Sham stimulation (n = 23)	p
Gender (f/m)	11/13	9/14	0.239
Age (in years)	48.29 (15.21)	42.13 (12.65)	0.181
Weight (in kg)	86.05 (17.96)	81.42 (18.07)	0.378
Edinburgh Laterality Coefficient	78.15 (41.12)	91.29 (23.98)	0.234
Number of depressive episodes (including actual episode)	3 (1.64)	3.42 (2.06)	0.478
Age at disease beginning (in years)	36.38 (13.00)	30.41 (10.61)	0.136
ATHF scores	5.89 (2.47)	5.81 (3.64)	0.931
Somatic Symptoms (y/n) *	12/9	13/6	0.462
Family history of unipolar depression (y/n) *	10/11	8/11	0.726
Main diagnosis according to DSM-IV (unipolar/bipolar depression) *	19/2	16/3	0.451
First depressive episode (first/recurrent) *	4/17	3/16	0.786
Duration of current episode (months) *	4.05 (1.27)	4.05 (1.69)	0.992
Comorbid dysthymia (yes/no) *	5/16	2/17	0.412
Comorbid generalized anxiety disorder (yes/no) *	5/16	2/17	0.412
Comorbid personality disorder, other than borderline personality disorder (yes/no) *	3/18	3/16	1.000
BDI-II Scores	31.90 (12.32)	29.53 (10.52)	0.597
HDRS Scores	22.24 (4.38)	20.11 (5.21)	0.157
Item 10 – MADRS “suicide thoughts” **	1.95 (1.32)	1.84 (1.21)	0.855
Total MADRS Scores			
W0 **	33.46 (6.32)	29.30 (6.48)	1.000
W1 **	29.08 (6.04)	28.48 (4.31)	1.000

W2 **	26.13 (8.49)	24.22 (7.14)	1.000
W3 **	21.79 (7.39)	19.00 (7.81)	1.000
DCP (nmol/L)			
Baseline – (between 7:30 and 8:00 AM) **	11.19 (8.59)	16.42 (15.20)	1.000
Baseline – (between 8:00 and 8:30 AM) **	13.94 (7.54)	20.36 (10.19)	1.000
Baseline – (between 3:30 and 4:00 PM) **	5.32 (4.84)	5.14 (1.97)	1.000
Baseline – (between 9:30 and 10:00 PM) **	7.64 (19.46)	7.46 (19.94)	1.000
W3 – (between 7:30 and 8:00 AM) **	12.63 (5.41)	13.15 (8.35)	1.000
W3 – (between 8:00 and 8:30 AM) **	15.68 (8.34)	15.60 (9.81)	1.000
W3 – (between 3:30 and 4:00 PM) **	7.10 (5.51)	8.51 (4.58)	1.000
W3 – (between 9:30 and 10:00 PM) **	3.24 (3.73)	2.79 (4.20)	1.000
Stimulation-related cortisol concentrations (nmol/L)			
W0 – pre **	5.42 (3.11)	5.97 (3.66)	1.000
W0 – post **	3.87 (3.19)	4.93 (3.11)	1.000
W0 – 2h-post **	3.66 (2.42)	3.27 (2.33)	1.000
W2 – pre **	6.20 (3.21)	3.33 (3.53)	0.307
W2 – post **	5.37 (2.65)	2.77 (3.19)	0.822
W2 – pre **	6.33 (5.19)	4.36 (2.83)	1.000

Table 2 – Characteristics of the participant groups and experimental concentrations of cortisol. Values are expressed as mean (standard deviation)

** Of the 47 patients included in the study, 40 completed the three-week treatment, and seven dropped out of the study before finishing the trial. Hence, data of 4 participants of the sham stimulation group and 3 of the tDCS are missing.*

Abbreviations: W0 = first stimulation; W1 = one week after first stimulation; W2 = two weeks after first stimulation; W3 = three weeks after first stimulation; ATHF = Antidepressant treatment history form; BDI-II = Beck depression inventory II; HDRS = Hamilton depression rating scale; MADRS = Montgomery–Åsberg depression rating scale; tDCS = Transcranial direct current stimulation; pre = immediately before stimulation; post = immediately after stimulation; 2h-post = 2 hours after stimulation.

*** P-values are Bonferroni-corrected*

Figures

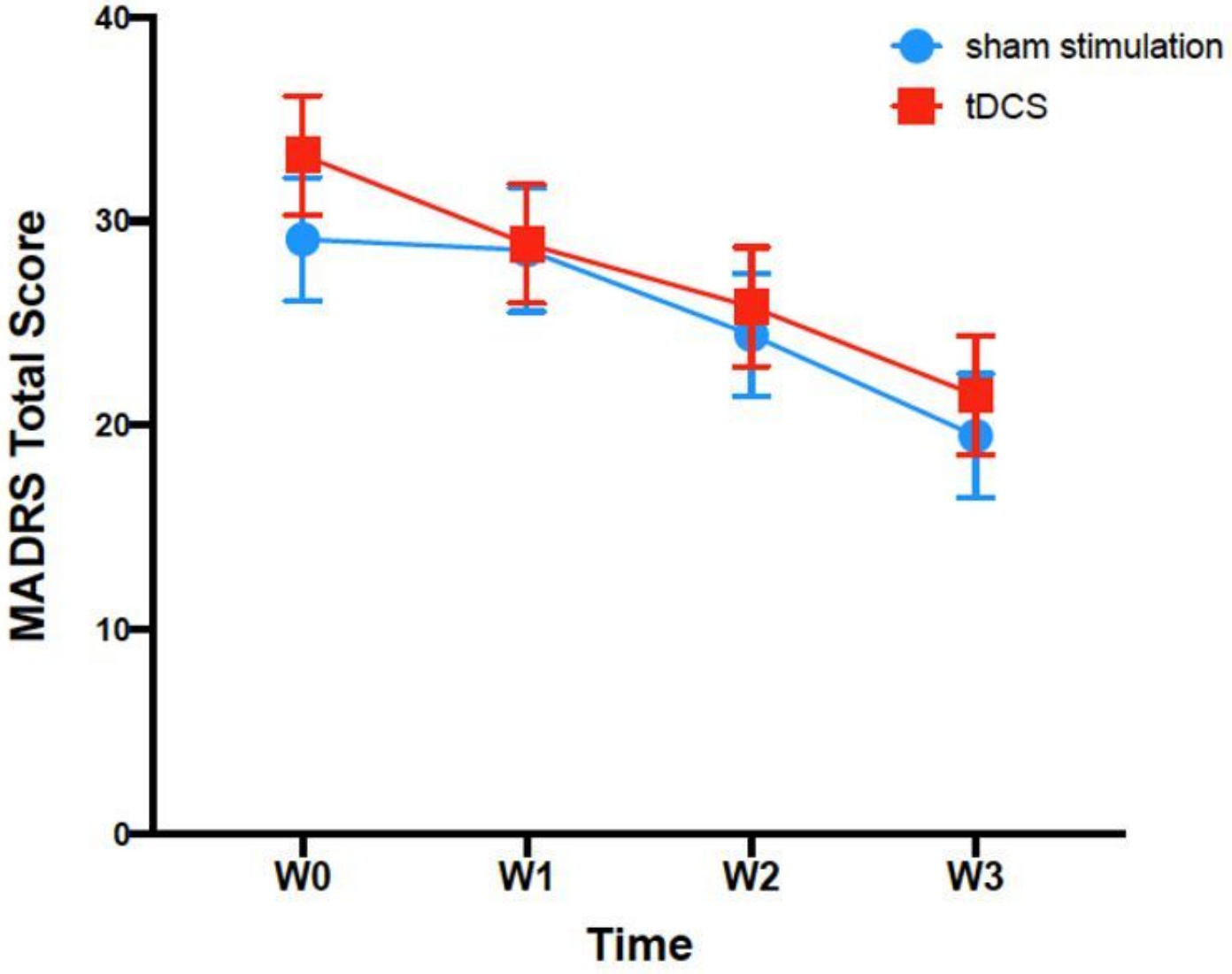

Figure 1

MADRS total score as response variable; estimated marginal means for MADRS scores for tDCS and sham stimulation groups.

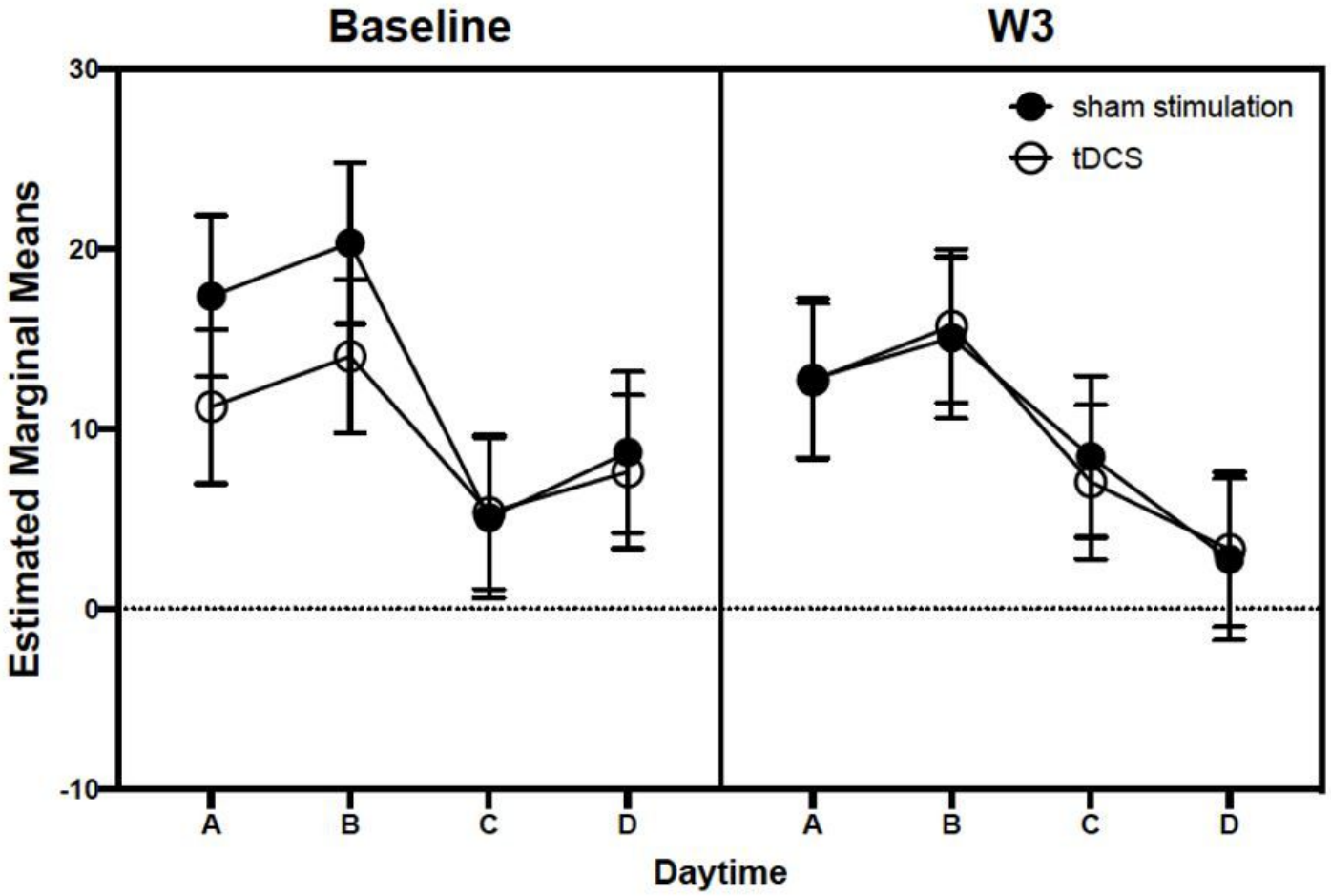

Figure 2

Estimated marginal means of diurnal cortisol profile (nmol/L) for tDCS and sham stimulation groups. Abbreviations: A = between 7:30 and 8:00 AM; B = between 8:00 and 8:30 AM; C = between 3:30 and 4:00 PM; D = between 9:30 and 10:00 PM

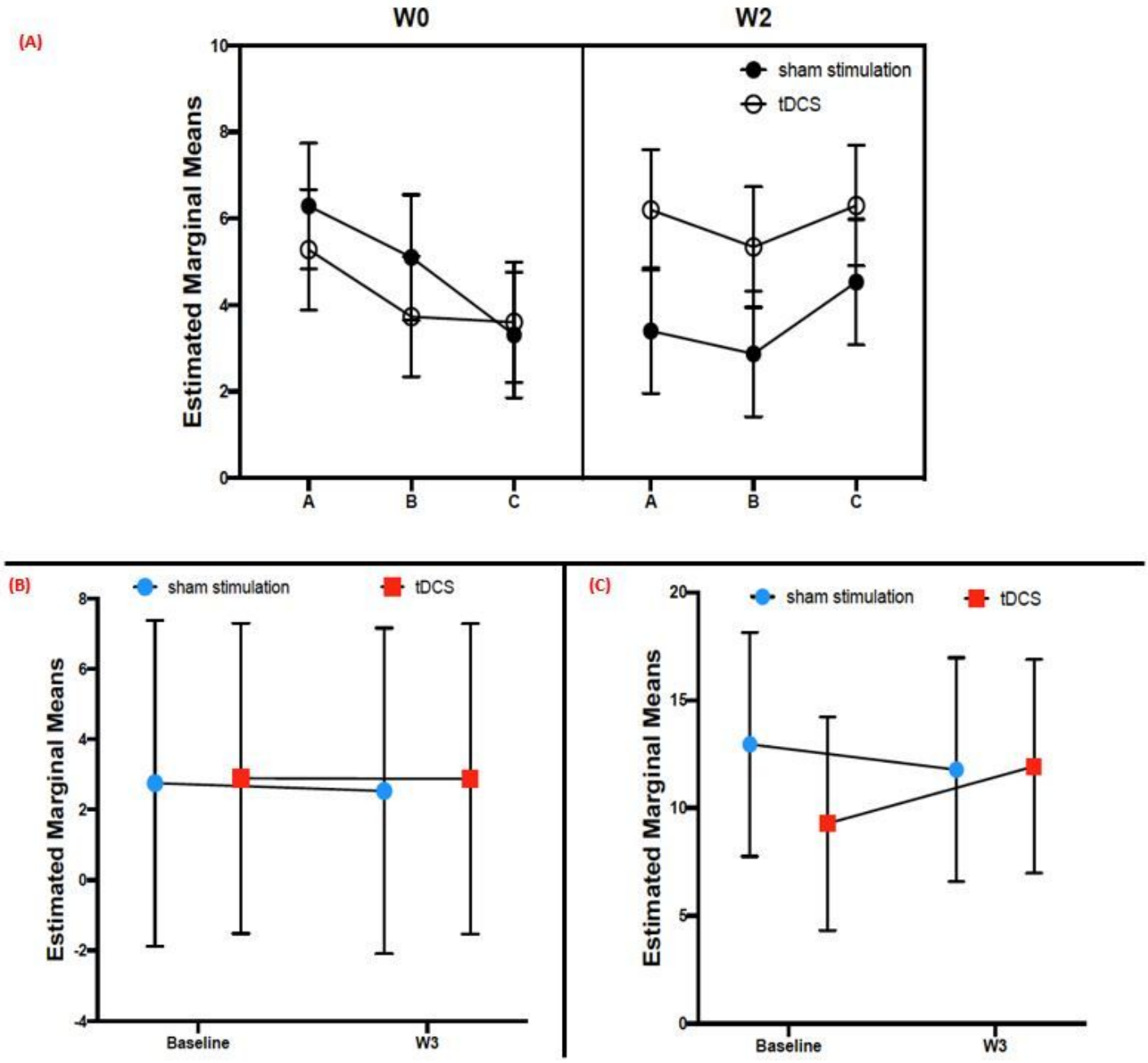

Figure 3

Secondary outcomes; estimated marginal means (cortisol concentration in nmol/L) were calculated in each model. Figure 2A presents the stimulation-related cortisol concentrations for tDCS and sham stimulation groups. Here, we present cortisol concentrations immediately before stimulation (A), immediately after stimulation (B), and two hours after stimulation (C) for W0 and W2 (table 1). Figure 2B presents the cortisol awakening response (CAR) of both tDCS and sham stimulation groups for baseline and W3. Finally, Figure 2C presents maximal cortisol decline for both tDCS and sham stimulation groups for baseline and W3

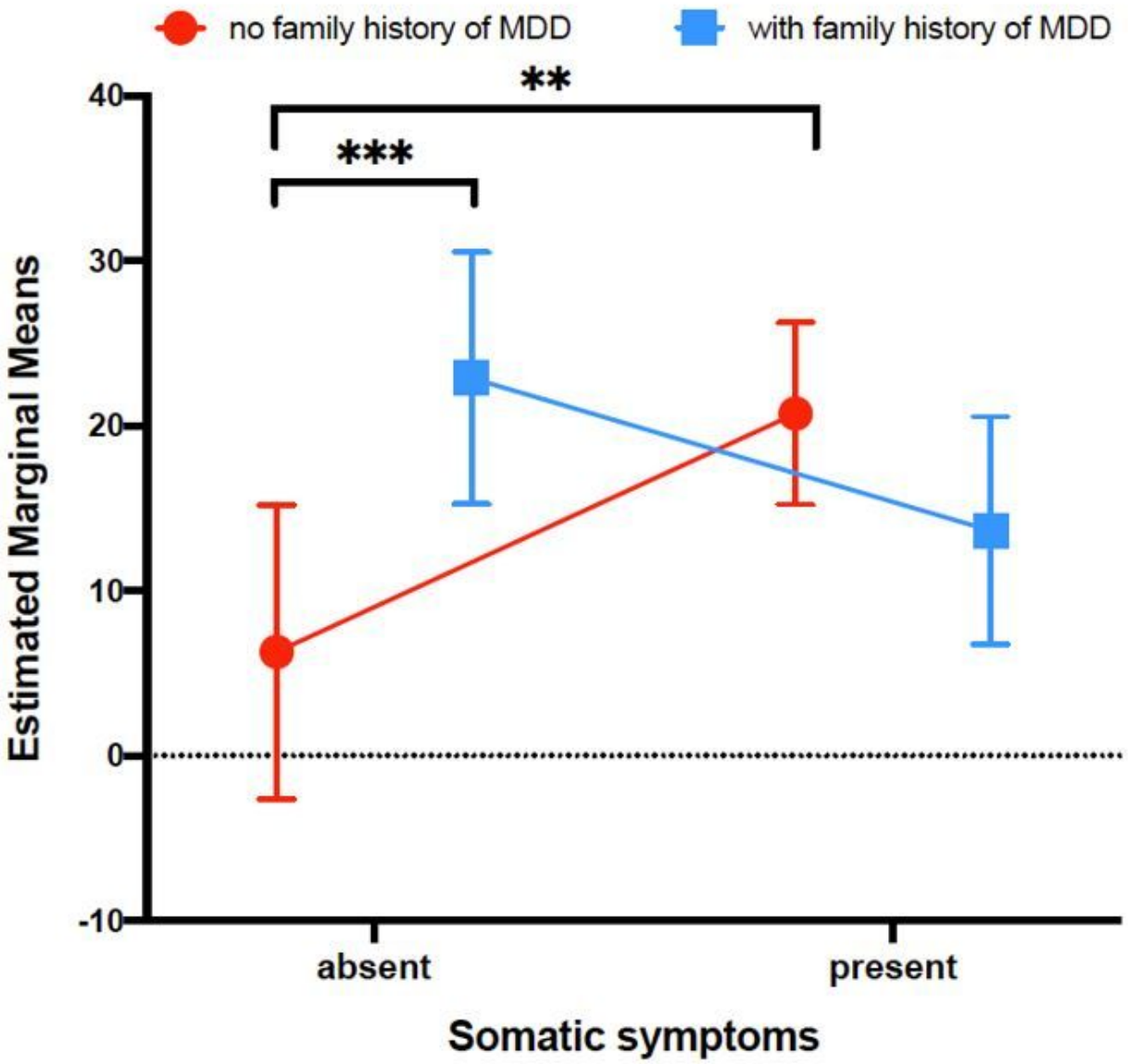

*Figure 4 - Exploratory analysis; estimated marginal means for morning salivary cortisol concentrations (nmol/L) and presence of somatic symptoms in participants with and without family history of unipolar depression. Morning salivary cortisol concentrations were extracted between 8:00 and 8:30 AM. Abbreviations: ** = $p \leq 0.01$; *** = $p \leq 0.001$; MDD = major depressive disorders.*

Figure 4

See image above for figure legend.