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Transcranial Electrical Stimulation Modifies the Neuronal Response to Psychosocial Stress Exposure

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Abstract: Stress is a constant characteristic of everyday life in our society, playing a role in triggering several chronic disorders. Therefore, there is an ongoing need to develop new methods in order to manage stress reactions. The regulatory function of right medial-prefrontal cortex (mPFC) is frequently reported by imaging studies during psychosocial stress situations. Here, we examined the effects of inhibitory and excitatory preconditioning stimulation via cathodal and anodal transcranial direct current stimulation (tDCS) on psychosocial stress related behavioral indicators and physiological factors, including the cortisol level in the saliva and changes in brain perfusion. Twenty minutes real or sham tDCS was applied over the right mPFC of healthy subjects before the performance of the Trier Social Stress Test (TSST). Regional cerebral blood flow (rCBF) was measured during stimulation and after TSST, using pseudo-continuous arterial spin labeling (pCASL). Comparing the effect of the different stimulation conditions, during anodal stimulation we found higher rCBF in the right mPFC, compared to the sham and in the right amygdala, superior PFC compared to the cathodal condition. Salivary cortisol levels showed a decrease in the anodal and increase in cathodal groups after completion of the TSST. The behavioral stress indicators indicated the increase of stress level, however, did not show any significant differences among groups. In this study we provide the first insights into the neuronal mechanisms mediating psychosocial stress responses by prefrontal tDCS. *Hum Brain Mapp 00:000–000, 2014.*

Key words: ASL; fMRI; PFC; psychosocial stress; tDCS; cortisol

INTRODUCTION

Over the last decades, stress has become a persistent characteristic of everyday life in Western societies [e.g., Clay, 2011]. Besides its undesirable effects on the immune and cardiovascular systems, stress has also been associated with changes in population health such as the continuous rise in the number of absent days from work due to mental illnesses. One of the main physiologic markers of stress is the activation of the hypothalamic–pituitary–adrenal (HPA) axis, which results in the secretion of

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glucocorticoids (in humans mainly cortisol) from the adrenal cortex. Animal studies have demonstrated the involvement of the PFC in the regulation of the HPA axis and the subsequent stress response and suggest an inhibitory role of the PFC [Gilabert-Juan et al., 2013; Herman et al., 2003]. Glucocorticoids, for their part, are known to play a key role in stress-related somatic and mental disorders [Chrousos and Kino, 2007]. Psychosocial stress has been identified as among the most influential types of stress in activating the HPA axis [Kirschbaum and Hellhammer, 1994]. In stress research, the Trier Social Stress Test (TSS) [Kirschbaum et al., 1993] which is comprised of an anticipatory period followed by both a public speech and a mental arithmetic task in front of an evaluating committee, has proved to be a reliable tool to evoke an activation of the HPA axis in a laboratory setting [Dickerson and Kemeny, 2004]. Dependent measures obtained in conjunction with this test usually include behavioral, physiologic or endocrinologic markers of stress [Dedovic et al., 2009; Kern et al., 2008; Pruessner et al., 2008; Wang et al., 2005]. In response to psychological stress increased cortisol secretion and according to the results of functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies, a decrease in activity of limbic system components,—including the hippocampus, hypothalamus, anterior cingulate cortex and medio-orbitofrontal cortex, was observed [e.g., Dedovic et al., 2009; Pruessner et al., 2008]. Metabolic glucose rate in the rostral medial PFC (Brodman area 9 and 10) was also negatively correlated with stress-induced salivary cortisol increase [Kern et al., 2008]. Thus, the right medial PFC (mPFC) has been implicated in having a key role in the human brain's response to stress. As this area is a crucial component of the emotion network, it has a principal position in the organization and control of behavior, and therefore in the regulation of the stress response. Furthermore, neurons that are active at the release site of a group of stress hormones and neurotransmitters, have been identified in this cortical area [e.g., Charney, 2004]. In the present study, therefore, we first addressed the question as to whether transcranial direct current stimulation (tDCS) over the right mPFC can modulate the stress response in healthy humans. tDCS is a well-established noninvasive technique for interventional use in research with potential therapeutic use in neurorehabilitation, chronic pain, focal epilepsy, and neuropsychiatric disorders [Fregni et al., 2006; Liebetanz et al., 2006; overview in Nitsche et al., 2008; Webster et al., 2006]. During tDCS, low-amplitude direct currents are applied via scalp electrodes and penetrate the skull entering the brain. These time-invariant, constant amplitude currents are ramped up and down only at the onset and at the end of stimulation, usually range in intensity from 0.5 to 2 mA and are applied from seconds to minutes. The currents do not elicit action potentials but they are able to modify the transmembrane neuronal potential and thus influence the level of cortical excitability and modulate the firing rate of individual neurons in response to other inputs [Creutzfeldt et al., 1962]. Depending on the polarity of the stimulation, tDCS can increase (anodal tDCS) or decrease (cathodal tDCS) cortical excitability in the stimulated brain regions and connected areas. The duration of the excitability shifts depends on the stimulus duration, strength and polarity, e.g. tDCS has been delivered for up to 9–13 min over the motor cortex with a 1 mA intensity inducing effects outlasting the stimulation period by up to 60 min [Nitsche and Paulus, 2000, 2001). According to our knowledge, only two previous studies have investigated the effect of tDCS on the level of cortisol in the saliva, however, the results are somewhat contradictory. Brunoni et al. [2013] found decreased cortisol level after bipolar stimulation—anodal stimulation over the left and cathodal over the right prefrontal area—while subjects were observing emotionally negative and natural pictures. In the other study using again a bipolar montage, the plasma cortisol level was significantly increased independently from the type of the stimulation [Raimundo et al., 2012]. Nevertheless, in these works a stress situation was not introduced. Therefore the aim of our present study was to evaluate the effects of tDCS applied over right mPFC (covering parts of BA 9 and 10) on acute stress by comparing cortisol levels in saliva before and after stimulation and stress exposure and in parallel, by measuring differences in rCBF in this cortical area induced by anodal or cathodal tDCS.

MATERIALS AND METHODS

Subjects

The study involved altogether 61 healthy male volunteers (mean age, 25 ± 6 years; age range, 21–32 years). One subject refused his participation during the TSST, therefore 60 subjects completed the whole study. The subjects were naive with regard to the purpose of the study (e.g., the possible effect of tDCS on stress or cortical activity). They were informed about the experimental procedure and all gave written informed consent. None of the subjects suffered from any neurological or psychological disorders, had metallic implants/implanted electric devices, nor took any medication regularly, and none of them took any medication in the 2 weeks prior their participation in the experiment. We conformed to the Declaration of Helsinki, and the experimental protocol was approved by the Ethics Committee of the University of Göttingen.

Transcranial Direct Current Stimulation (tDCS)

Direct current was administered via a pair of square rubber electrodes (7×5 cm^2), manufactured to be compatible with the MR-scanner environment. The electrodes were equipped with 5.6 kOhm resistors in each wire to avoid sudden temperature increases due to induction voltages from radio frequency pulses. They were connected to a specially developed battery-driven stimulator (NeuroConn

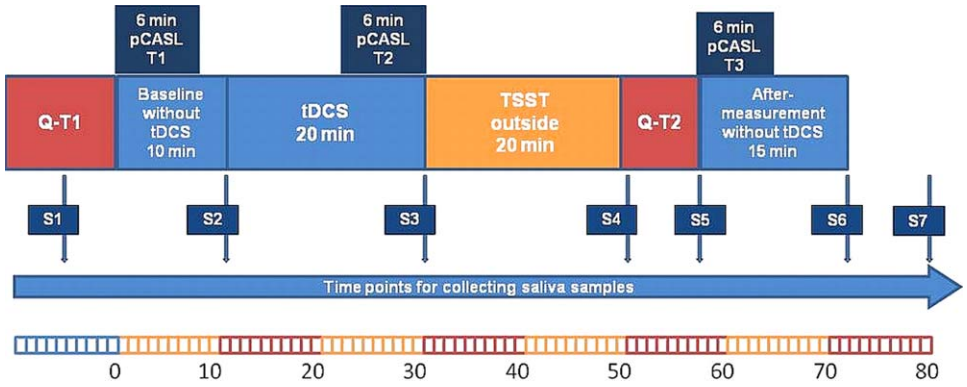

Figure 1.

Experimental procedure: After the first saliva sample was taken, the tDCS electrodes were fixed onto the scalp and the subject was placed inside the scanner. Following the first pCASL sequence a saliva sample was collected again and the stimulation started. During the last six minutes of the stimulation the pCASL sequence was repeated. After the subject was taken out from the scanner, the third saliva sample was collected. After the TSST, the fourth and fifth saliva samples were collected before and after the subject filled out the relevant questionnaires. The subject was then placed once again inside the scanner and the pCASL measurement was repeated. After the measurements the subject was removed from the scanner and two saliva samples were collected, with an interval of 10 min between each sample.

GmbH, Ilmenau, Germany) outside the magnet room via a cable running through a radio frequency filter tube in the cabin wall [for details see Antal et al., 2011].

Subjects were randomized using a computer generated order to get anodal, cathodal or sham stimulation (20 participants/group). Before subjects entered the MR scanner, for cathodal tDCS the electrodes were placed atop the F2-Fpz area (stimulating electrode), according to the 10–20 system, covering the right side of the PFC and the right medial frontal gyrus, and above the O2-P4 area (return electrode) using conventional electrode gel. For anodal tDCS the direction of the electric flux was reversed. The stimulation intensity was 1 mA, and the duration of the stimulation was 20 min in the case of active and 30 s in the case of sham stimulation. The current was ramped up and down at the beginning and end of the stimulation sessions (10 s).

MR Image Acquisition and Analysis

fMRI studies were conducted at 3 Tesla (Magnetom TIM Trio, Siemens Healthcare, Erlangen, Germany) using a standard eight-channel phased array head coil. Subjects were placed in a supine position inside the magnet bore and wore headphones for noise protection. Vital functions were monitored throughout the experiment. Initially, anatomic images based on a T1-weighted 3D turbo fast low angle shot (FLASH) MRI sequence at 1 mm^3 isotropic resolution were recorded (repetition time (TR) = 2,250 ms, inversion time: 900 ms, echo time (TE) = 3.26 ms, flip angle: 9°). This was then followed by pCASL acquisition [5 min, 32 s; Wu et al., 2007] before, during tDCS and after stress exposure. Subjects kept their eyes closed during all the pCASL acquisitions. pCASL images were acquired with following parameters: labelling time, 1,856 ms; postlabeling delay, 1,054 ms; TR, 4,150 ms; FOV, 22 cm; flip angle, 90°; TE, 18 ms; 26 slices, slice thickness, 5 mm; interslice spacing 25%.

Perfusion fMRI data were analyzed off line by using parts of the ASL-toolbox [Wang et al., 2008] and SPM8 software packages (Wellcome Department of Cognitive Neurology, Institute of Neurology, London). MR image series were first realigned to correct for head movements, coregistered with each subject's anatomical MRI, and smoothed in space with a 3D, 8-mm full-width at half-maximum Gaussian kernel. Perfusion-weighted image series were generated by pair wise subtraction of the label and control images, followed by conversion to absolute CBF image series. Voxel-wise analyses of the CBF data were conducted for each subject by using a general linear model (GLM) (first-level analysis). Three contrasts were defined in the GLM analysis, namely the CBF difference between the baseline and stimulation condition, between the baseline and stress condition and between the stimulation and the stress condition. Group comparisons were done (second level analysis) by using a GLM separately for each first level contrast. The nature of our study is more exploratory, therefore the results of the statistical analysis were first thresholded with an uncorrected voxel-wise threshold of $P < 0.001$ and a minimal cluster size of 35 pixels. For analysis of covariance a one sample t test with first level contrasts of interests and percentage of cortisol increase as a covariate was chosen.

Experimental Procedure

Figure 1 summarizes the experimental procedure. After the first saliva sample was taken, the tDCS electrodes were

fixed onto the scalp and the subject was placed inside the scanner. Following the first pCASL sequence of about 6 min, a saliva sample was collected again and the stimulation started. During the last 6 min of the stimulation the pCASL sequence was repeated. After the subject was taken out from the scanner, the third saliva sample was collected. The TSST was performed in a neighboring room, about 15 m distance from the scanner. After the TSST, the fourth and fifth saliva samples were collected before and after the subject filled out the relevant questionnaires. The subject was then placed once again inside the scanner; localiser, anatomical images and the pCASL measurement were repeated. After the measurements the subject was removed from the scanner and two saliva samples were collected, with an interval of 10 min between each sample. During the pCASL and the stimulation subjects were reminded not to fall asleep and to keep the eyes closed. Testing took place in the afternoon, between 15:00 and 21:00 to minimize influences of the diurnal rhythm of cortisol.

Trier Social Stress Test

The trier social stress test (TSST) is a standardized protocol for the reliable induction of moderate psychosocial stress in laboratory settings [Kirschbaum et al., 1993]. It consists of a 5-min preparation and anticipation period between task instruction and testing, followed by a test period in which the subject has to deliver a brief oration by means of a fictive job interview and perform mental arithmetic in front of an evaluation committee (5-min each) [for a detailed descrition see: Kirschbaum et al., 1993]. In addition, the two persons forming the committee do not provide any verbal or nonverbal feedback on the participant's performance. Within this procedure, psychosocial stress is assumed to be induced by both uncontrollability and ego-threat for the individual exposed to it [Dickerson and Kemeny, 2004]. After TSST completion, the participant was taken back to the scanning room. At the end of the whole experiment, the investigator informed the participant about the goal of the study.

Measurements of the Stress Response

Saliva samples were collected using Salivette sampling devices (Sarstedt, Nümbrecht, Germany) at seven time points (before the first scanning session, before tDCS, immediately after tDCS, immediately after TSST, 5 min after the TSST, immediately after the second scanning session and 10 min after the end of the second scanning session, see Fig. 1) for later analysis of free cortisol levels. Free cortisol levels were analyzed using a chemiluminescence immunoassay (IBL International, Hamburg, Germany). A score of percentage increase in salivary cortisol was calculated for each subject and for each sample before and after the TSST by dividing the given value by the baseline sample value (the sample before tDCS) and multiplying by 100 [Fiocco et al., 2007].

Subjective individual stress levels were assessed using the German version of the Short Questionnaire for Current Strain (Kurzfragebogen zur aktuellen Beanspruchung—KAB) [Mueller and Basler, 1992] and the state form of the state-trait-anxiety inventory (STAI) [Laux et al., 1981; Spielberger et al., 1970]. Participants self-reported their current mental state by answering the questionnaires at two time points (before the first scanning session and after the TSST). Items are aggregated to one scale. Example items with regard to this questionnaire:

Please tick the respective box that corresponds to how you feel at this moment:

	Very	fairly	rather	rather	fairly	very	
1. Fresh	[a]	[b]	[c]	[d]	[e]	[f]	faint
2. Full of vigor	[a]	[b]	[c]	[d]	[e]	[f]	feeble

Furthermore anticipatory stress was evaluated by application of the German version of the Primary Appraisal and Secondary Appraisal Questionnaire [Gaab, 2009] during the TSST preparation period. Example items with regard to this questionnaire:

Please tick the respective box that corresponds to the degree of your approval to the listed statement: [ ] totally wrong; [ ] fairly wrong; [ ] rather wrong; [ ] rather correct; [ ] fairly correct; [ ] totally correct

1. I do not feel threatened by the situation. (subscale: threat)
2. The situation is of concern to me (relevant). (subscale: challenge)
3. I know what to do in this situation. (subscale: self-concept of skills)
4. It primarily depends on myself, whether the experts evaluate me positively. (subscale: locus of control)

Subscales are aggregated to "primary appraisal" (threat + challenge) and "secondary appraisal" (self-concept of skills + locus of control).

Statistics of the endocrine and behavioral data were calculated using the commercial software program SPSS17.

RESULTS

All of the subjects tolerated the stimulation well; none reported side-effects during or after the stimulation. Some of the subjects reported a slight itching sensation under the electrodes that was not polarity dependent.

Endocrine Data

Salivary cortisol was significantly increased after TSST (Fig. 2). The paired *t* test showed a higher cortisol concentration for the mean of all (four) cortisol samples (4–7) collected after the TSST (M = 12.185; SD = 6.253) compared

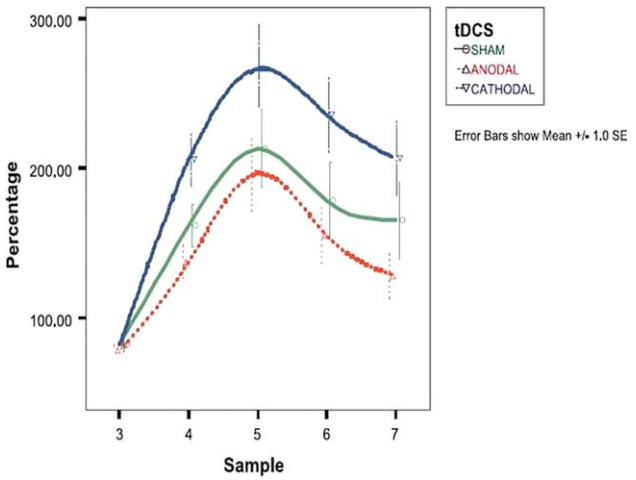

Figure 2.

Time course of percentage salivary cortisol increase after performing TSST between sample 3 and 4. A score of percentage increase in salivary cortisol was calculated for each subject and for each time point dividing the given value by the baseline sample value (the sample before tDCS; S2) and multiplying it by 100.

to the sample 2 obtained before tDCS (M = 8.185; SD = 5.839; $t(60) = 6.64$; $P \leq 0.001$).

Using the values of percentage increase of cortisol after administering the TSST (samples 4–7) the MANOVA showed a significant main effect of group, (Roy's largest root = 0.209, $F(4, 56) = 2.933$; $P = 0.028$, eta^2 = 0.173). The subsequent univariate analysis revealed significant main effects for sample 4 ($F(2, 58) = 3.974$; $P = 0.024$, eta^2 = 0.121). The effect of group was significant at the 10% level for sample 6 (F (2, 58) = 2.765; $P = 0.071$, eta^2 = 0.087) and sample 7 ($F(2, 58) = 2.967$; $P = 0.059$, eta^2 = 0.093). No effect was observed for sample 5 $F(2, 58)$ = 1.768; $P = 0.180$, eta^2 = 0.057). Significant regional pair wise differences (Tukey's HSD, $P < 0.05$) were obtained between the anodal and cathodal group in sample 4 and in sample 7. The cathodal group showed higher cortisol responses than the anodal group in both samples (Fig. 2).

The subsequent univariate analysis (mixed-effects quartic polynomial regression) revealed no significant main effects between groups. However, as hypothesized, significant interactions between time and groups [anodal vs. cathodal: $t = 3.116$, $P < 0.01$; anodal vs. sham: t = 2.015, $P < 0.05$] emerged, implying significantly altered cortisol secretion due to tDCS.

Behavioral Data

For both behavioral stress indicators (STAI-State, KAB) the MANOVA for repeated measurements showed a significant main effect of time (Roy's largest root = 0.231, $F(2, 53) = 6.133$; $P = 0.004$, eta^2 = 0.188). There were no differences in anticipation of TSST between the groups and no effects of interaction between time and tDCS were found (Roy's largest root = 0.088, $F(2,54) = 2.367$; $P = 0.103$, eta^2 = 0.081) (Fig. 3A,B). The Greenhouse–Geisser corrected values of the subsequent univariate analysis showed that both stress indicators increased significantly after TSST (KAB: $F(1, 54) = 9.338$; $P = 0.004$, eta^2 = 0.147; STAI-State: $F(1, 54) = 11.440$; $P = 0.001$, eta^2 = 0.175).

Imaging Data

Main and group effects

Generally, the main effect of the stimulation, compared to the baseline condition, was an increased rCBF in the

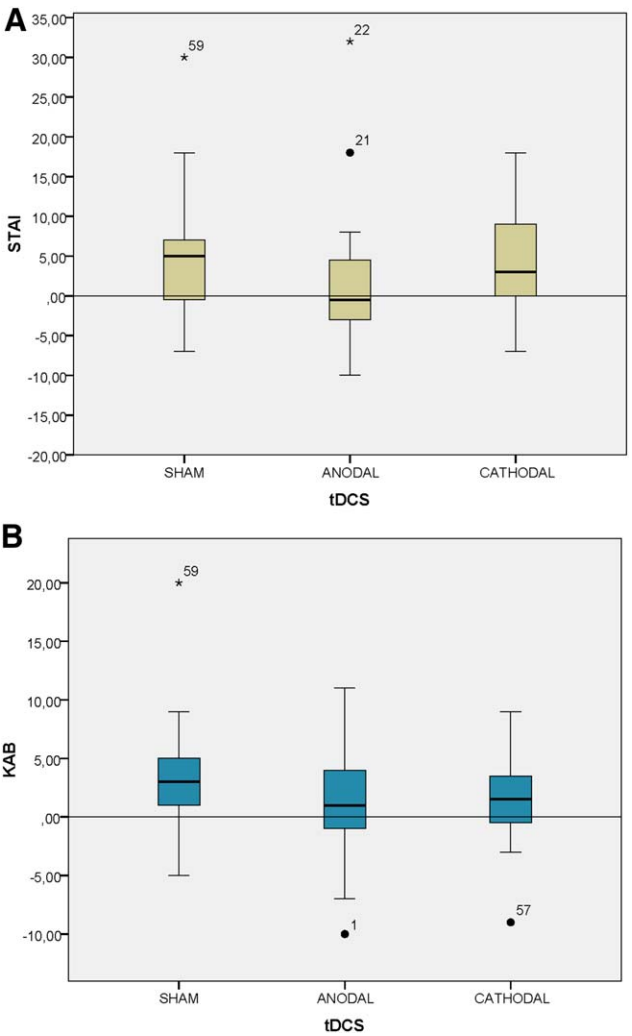

Figure 3.

The results of the behavioral stress indicators (after TSST—baseline; **A**: STAI-State; **B**: KAB). There was no effect of anodal or cathodal tDCS on the subjective stress measurements. Error bars represent SDs, dots: outliers with more than 1 SD; stars: outliers with more than 2 SDs.

TABLE I. Regions of significant contrasts conditions

2nd level contrast	Anatomical region	MNI coordinates			Peak Z-score	Cluster size
		x	*y*	*z*		
Stimulation > baseline	Medial frontal right	22	−14	46	4.58	434
	Medial frontal left	−26	14	36	4.15	747
Baseline > stimulation	Superior frontal left	−28	58	32	4.53	2,119
	Precentral right (BA 3)	52	−14	30	4.42	1,025
	Inferior temporal left	−60	−10	−38	4.27	582
	Paracentral lobe	−2	−34	46	4.45	374
	Middle temporal left	−62	−66	6	4.16	129
	Middle temporal right	64	−40	−26	4.81	70
	Inferior parietal right	66	−34	46	3.84	138
	Inferior parietal left	−56	−48	54	4.35	134
	Fusiform gyrus left	−30	−62	−30	3.64	108
	Lingual gyrus right	10	−90	0	3.98	112
	Precuneus	−4	−86	42	3.68	62
Baseline > post TSST	Insula right	50	−28	22	6.44	4,519
	Insula left	−40	−8	10	6.22	5,000
	Cingulate right	16	−36	40	4.27	512
	Cingulate left	−10	2	28	3.68	46
	Mid cingulate right	16	0	30	4.00	285
	Temporal cortex left	−48	−82	26	4.37	165
	Precuneus left	−28	−46	40	4.10	149
	Medial frontal right	36	0	40	3.73	131
	Inferior parietal right	60	−40	26	3.81	62
	Inferior temporal left	−62	−66	−10	3.60	89
Baseline < post TSST	Medial frontal left	−8	50	20	3.95	159
Stimulation > post TSST	Insula left	−34	0	6	5.18	2,181
	Cingulate right	10	12	32	4.69	2,600
	Postcentral right (BA 3)	20	−36	64	4.49	621
	Superior temporal right	44	−10	−6	3.91	500
	Inferior temporal right	50	−32	16	3.68	90
	Precentral left (BA 4)	−26	−28	64	3.94	297
	Superior frontal left	−14	−15	66	3.94	105
Post TSST > stimulation	Medial frontal	0	50	46	4.15	708
	Medial frontal right	52	20	40	3.54	57
	Inferior temporal right	52	−10	−30	3.70	92

right and left mPFC and a bilateral decrease in rCBF in several brain regions, including parts of the temporal and parietal cortices and the left superior fontal areas (Table I). Comparing the effect of the different stimulation conditions, during anodal stimulation we found higher rCBF in the right mPFC compared to the sham and in the right amygdala and right superior PFC compared to the cathodal condition (Table II) (Fig. 4). Table I represents the main effects of stimulation conditions and TSST, while Table II summarizes the brain regions of significant contrasts between different stimulation groups and experimental conditions.

The main effect of TSST compared to stimulation condition was an increased rCBF observed in the medial frontal areas just under the stimulating electrode and in the temporal areas (Table I). Furthermore, a general decrease of rCBF in the temporal and parietal areas, in the insula and in the cingulate cortex. Comparing the effect of the different stimulation conditions, rCBF decreased in the cathodal group compared to anodal in the posterior insula and compared to sham in subcortical areas, such as thalamus and right hippocampal gyrus (Table II) (Fig. 5).

DISCUSSION

Stress plays a role in triggering or worsening many disorders, including depression and cardiovascular diseases. Therefore, there is an ongoing need to develop new methods and treatments in order to manage stress reactions. There are a variety of methods available to control acute and chronic stress, including exercise, and pharmacological treatments. The administration of repetitive transcranial magnetic stimulation (rTMS), a noninvasive technique for directly stimulating cortical neurons over the PFC was successfully applied in the treatment of posttraumatic

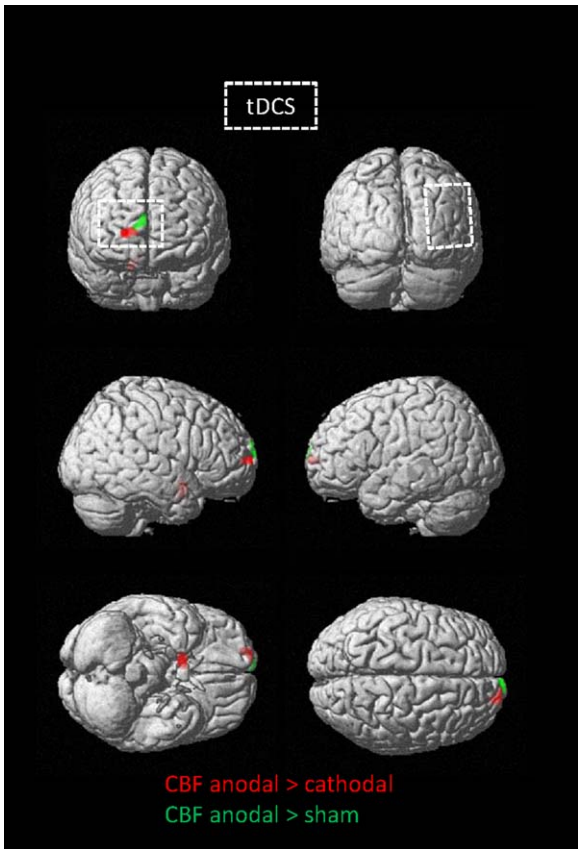

Figure 4.
Regions of higher rCBF ($P < 0.001$ uncorr.) during anodal tDCS compared to cathodal (red) and to sham (green). The positions of the electrodes are marked with dotted line.

stress disorder [Cohen et al., 2004]. In this article we provide the first insights into the mechanisms mediating the stress response after application of tDCS to the mPFC, combining three different methods: functional imaging, endocrinological measurements, and psychophysics. The major finding of our study is that tDCS applied over the mPFC before performing the TSST, modified baseline rCBF: under the electrode anodal stimulation increased it compared to sham and cathodal stimulation. Furthermore, tDCS mediated cortisol release in a polarity dependent way: cathodal stimulation increased, whilst anodal stimulation decreased cortisol response, measured in the saliva of participants. Our results are in agreement with previous data, concerning the inhibitory effect of anodal tDCS on cortisol levels [Brunoni et al., 2013]. However, in this study left frontal and not right frontal anodal tDCS induced a decrease in cortisol response previous (right-left PFC in the Brunoni et al. study vs. right mPFC and O2-P4 area in the present study). Furthermore, the experimental setups in the two studies (the presentation of emotionally negative and neutral pictures in the previous vs. psychosocial stress situation in the present study) were also different.

Effect of Stimulation at Rest

First, our data also support the results of previous studies showing that simultaneous non-invasive electrical stimulation and blood flow imaging in the MRI environment is technically feasible and safe [Stagg et al., 2013; Zheng et al., 2011]. Furthermore, we also showed that tDCS not only modulated activity directly under the stimulating electrode but also in a network of brain regions that are functionally connected to the stimulated area (e.g., amygdala, cingulate cortex). In line with previous findings [Stagg et al., 2013; Zheng et al., 2011] we observed an increase in rCBF under the anode compared to the sham and cathodal conditions. However, we did not find a significant change during the cathodal condition. One can speculate that the difference between ours

TABLE II. Regions of significant contrasts between groups

2nd level contrast	Anatomical region	MNI coordinates			Peak Z-score	Cluster size
		x	*y*	*z*		
Stimulation-baseline						
Anodal > cathodal	Amygdala right	12	6	−16	4.02	106
	Superior frontal right	16	66	8	3.84	74
Anodal > sham	Medial frontal right	6	70	16	3.45	49
Post TSST-baseline						
Anodal > cathodal	Anterior cingulate left	−6	12	26	3.48	50
Anodal > sham	Postcentral right	46	−34	66	4.49	43
	Superior parietal left	−20	−66	66	3.92	71
	Superior parietal right	32	−70	54	3.53	37
Post TSST-stimulation						
Anodal > cathodal	Posterior insula right	28	−32	30	4.08	436
Sham > cathodal	Midbrain left, limbic lobe, cingulate cortex	−10	−14	−30	3.69	118
	Thalamus and right parahippocampal gyrus	18	−28	28	3.64	70

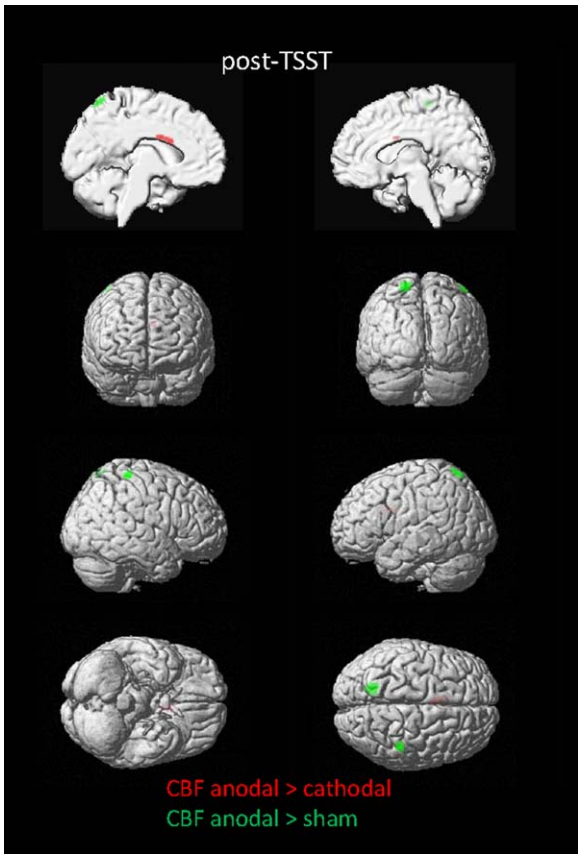

Figure 5.
Regions of higher rCBF after TSST in the anodal group compared to cathodal (red) and to sham (green) ($P < 0.001$ uncorr).

and the above mentioned studies might be related to the variation in methodological parameters, e.g. the distance and orientation of intra-cortical and cortico-cortical axons, with respect to the site of stimulation, may result in a specific predilection to excite different neuronal populations. It should also be considered that besides the focal effects tDCS can also induce remote activity and excitability changes, resulting in higher between-subject variabilities due to anatomical differences and non-specific stimulation's effects. Furthermore, the tendency for cathodal stimulation to induce a small or even no change in rCBF compared to anodal tDCS might be explained by the activation of a different number of excitatory and inhibitory synapses in the cortex [e.g., Megias et al., 2001].

Effect of Stimulation on Cortisol Level and rCBF after TSST

Generally, during anodal stimulation excitation is induced, and during cathodal stimulation inhibition can be observed, at least in the motor cortex [Nitsche and Paulus, 2000, 2001]. We have also found polarity dependent changes in cortisol levels after stimulation and importantly, after the execution of the TSST. However, here cathodal stimulation facilitated cortisol release while anodal stimulation decreased it. These, at first glance, seemingly paradoxical results can be enlightened by several explanations. (i) With regard to the homeostatic regulation in the human brain several studies have shown that the susceptibility of cortical neurons to change their excitability in response to presynaptic inputs can be adjusted to the level of postsynaptic activity prior to conditioning [Huang et al., 1992; Kirkwood et al., 1996; Wang and Wagner, 1999]. According to this, a "sliding modification threshold" controls the threshold for inducing synaptic plasticity [Bienenstock-Cooper-Munro model, Bienenstock et al., 1982]: a prolonged reduction in postsynaptic activity will reduce the threshold for inducing long-term potentiation (LTP) and a prolonged increase of it reduces the threshold for long-term depression (LTD). In line with this rule, in our study cathodal stimulation preconditioned the brain and made it more "susceptible" to the TSST, and therefore enhancing cortisol release. Following this argumentation, after anodal preconditioning the stress response was smaller. (ii) Another possibility includes considering what effect tDCS has in combination with other methods, e.g., with paired associative stimulation (PAS). If tDCS is applied in conjunction with PAS, its polarity dependent-effects are reversed [Nitsche et al., 2007]. Thus, if we assume that stress may be seen as a kind of synapse specific activation analogous to PAS, then the paradoxical reaction could be explained. (iii) Because of our relatively big electrode size, the stimulation may not be fully limited to the mPFC but also extend to some part of the right ventrolateral PFC that is, in addition to several other functions, responsible to inhibit affective and motor responding [Aron et al., 2004; Lieberman et al., 2007; Ochsner et al., 2012]. According to this, when this inhibitory region is stimulated (resulting in a greater inhibition), a decrease in cortisol can be observed. When it is inhibited, an increase in cortisol might occur.

In accordance with previous studies [e.g., Wang et al., 2005], we found that the TSST evoked higher rCBF in the PFC, just under the stimulating electrode as well as in the inferior temporal cortex, compared to the during stimulation conditions. Reduced rCBF after TSST was found in several regions of the parietal and temporal cortices and in the right cingulate cortex. However, polarity dependent changes after the TSST relative to baseline were found only for anodal stimulation when compared to cathodal (left ACC) and sham (postcentral gyrus and superior parietal cortex) and after TSST relative to during stimulation condition for cathodal compared to sham stimulation (left thalamus, right parahippocampal area). Remote connections between cortical areas may also have contributed to the divergent patterning of cortical network activation; Polania et al. [2011] recently described the functional coupling of cortical areas not directly implicated during DC stimulation.

In our study the activation of several anatomical regions, e.g., precuneus and the parietal areas cannot be directly related to the stress response. For explaining these fMRI changes we might have to consider the activation of resting-state networks in the brain. A large number of anatomically separate brain areas show per definition a vast amount of spontaneous neuronal activity at rest. These resting state networks include the "default mode network" that links precuneus and posterior cingulate cortex (PCC) with medial frontal regions and bilateral inferior parietal regions [e.g., Damoiseaux et al., 2006; Fox and Raichle, 2007; Greicius et al., 2003; Gusnard et al., 2001; Wang et al., 2005]. Our results could indicate that tDCS has a modulatory effect on the default network involved in behaviors that are not goal-directed. Indeed, recent study provided the first support that tDCS-induced neuroplastic alterations might be related to functional connectivity changes in the human brain in the resting state [Polania et al., 2011]. In this study after anodal stimulation the nodal connectivity degree in the left PFC, as well as in the right dorsolateral PFC significantly increased. Although in our study a different stimulation site was used, the affection of the resting state network could be demonstrated.

The questionnaires reflecting the participants' subjective stress levels confirmed an identical subjective stress-stage, however, independently from preconditioning with anodal, cathodal or sham stimulation. Thus, they were obviously less sensitive then the physiological responses. It is possible that the four to six step questionnaires are not susceptible enough to detect tDCS induced changes on the behavioral level. Furthermore, it was recently reported that the endocrinological responses do not always relate to the subjective measures of stress [Hellhammer and Schubert, 2012].

Until lately, evaluating the effect of tDCS on the brain was only possible by measuring physiological responses, e.g., the amplitude of motor evoked potentials. Combining neuroimaging techniques with concurrent tDCS allow us for a noninvasive detailed examination of stimulation-induced effects throughout the brain [Lang et al., 2005]. It was recently suggested ASL may be a more sensitive tool to investigate the effects of tDCS and its stimulation parameters on brain activity in rest than measuring BOLD activity [Zheng et al., 2011]. Indeed, during the past decade, validation studies of ASL in healthy human subjects have yielded positive results. CBF measurements with ASL perfusion MRI have been shown to agree with results from 15O-PET in healthy humans at rest [Ye et al., 2000] or during functional activation [Feng et al., 2004]. Using this methodology, here we provide first insights into the mechanisms mediating acute stress using prefrontal tDCS. We have demonstrated that tDCS can influence the neuronal mechanisms related to the stress response and can induce polarity specific cortisol release. Further studies should investigate whether this method can be used in patients suffering from chronic stress syndromes.

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