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Cortical hemodynamic changes during the Trier Social Stress Test: An fNIRS study

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ABSTRACT

The study of the stress response has been of great interest in the last decades due to its relationship to physical and mental health. Along with the technological progress in the neurosciences, different methods of stress induction have been developed for the special requirements regarding the acquisition of neuroimaging data. However, these paradigms often differ from ecologically valid stress inductions such as the Trier Social Stress Test (TSST) in substantial ways.

In the study at hand, we used the rather robust optical imaging method of functional Near-infrared Spectroscopy (fNIRS) to assess brain activation during the TSST and two non-stressful control conditions. Additionally, we measured other stress parameters including the cortisol response and subjective stress ratings. As expected we found significant increases in subjective and physiological stress measures during the TSST in comparison to the baseline and control conditions. We found higher activation in parts of the cognitive control network (CCN) and dorsal attention network (DAN) – comprising the dorsolateral prefrontal cortex, the inferior frontal gyrus and superior parietal cortex – during the performance of the TSST in comparison to the control conditions. Further, calculation errors during the TSST as well as subjective and physiological stress parameters correlated significantly with the activation in the CCN. Our study confirms the validity of previous neuroimaging data obtained from adapted stress procedures by providing cortical activation data during a classical stress induction paradigm (i.e., the TSST) for the first time.

Introduction

The study of stress in humans is of great relevance because high levels of stress over long durations are considered to play a key role in the development of mental and physical health problems (Allen et al., 2017; Simeon et al., 2007b). For example, stress has been associated with depression and cardiovascular disorders (Grippo and Johnson, 2009; Rao et al., 2008), anxiety disorders – like social phobia (Krämer et al., 2012; Schmitz et al., 2011), PTSD (Simeon et al., 2007a), and panic disorder (Petrowski et al., 2013, 2010) – obesity (Laessle and Schulz, 2009) and psychosis (Pruessner et al., 2013). One of the most frequently used and validated methods for the induction of acute stress in a laboratory setting is the Trier Social Stress Test (TSST) which has a high ecological validity. During the TSST, which includes an anticipation phase and a test phase, subjects have to give a free speech and perform a mental arithmetic task in front of a committee (Kirschbaum et al., 1993). Although the neuro-endocrinological pathway of the stress response – the hypothalamic-pituitary-adrenal axis (HPAA) – is well studied, until today, only a few studies have tried to adapt stressful environments in a neuroimaging setup to examine cortical activation patterns during social stress, e.g. through the use of public speech, arithmetic or other mental tasks in fMRI. One paradigm used in neuroimaging is the Montreal

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Imaging Stress Test (MIST) in which an arithmetic challenge with negative feedback is used. Studies that used this task suggest decreased activity in the orbitofrontal cortex, deactivation of the limbic system and hippocampus as well as increased activity in the frontal lobes, the somatosensory and visual areas during the stress induction (Dedovic et al., 2009b, 2009a). Moreover, comparing stress responders and non-responders in an event-related MIST revealed increased activity in prefrontal, temporal and dorsolateral areas in stress responders (Dedovic et al., 2009c). Also other MRI studies that used arithmetic challenges reported positive associations of the cerebral blood flow (CBF) in the frontal cortex and subjective stress ratings (Wang et al., 2005). Consistently similar findings have been found with other stress induction tasks. With respect to public speech challenges, Tillfors et al. (2001) reported increased rCBF in the amygdala and decreased rCBF in orbitofrontal and temporal areas in social phobic subjects in comparison to non-phobic subjects as measured with PET (Tillfors et al., 2001). Other studies tried to provoke emotional stress, e.g. through the use of negative pictures (Sinha et al., 2004; Yanagisawa et al., 2011; Yang et al., 2007). In a study that used functional near-infrared spectroscopy (fNIRS), elevated activity within the frontal cortex during the exposure to negative pictures has been observed (Yang et al., 2007). Also activity of the ventrolateral PFC as assessed with fNIRS has been shown to have a regulative function on social pain during social exclusion (Yanagisawa et al., 2011). Similarly, Lee et al. (2014) observed in an adapted TSST fMRI paradigm, that watching video-recordings of oneself performing the TSST vs. another person increases activity in the bilateral IFG and insula. Further, cortical activation in the right IFG was higher and subjective stress was lower in males than in females (Lee et al., 2014). Usually, findings showing elevated activity within prefrontal cortex areas are thought to reflect top-down control processes over subcortical affective processing (Wager et al., 2008).

However, from a methodological perspective, many studies lack the ecological validity of the TSST. Due to the methodological restraints imposed by most neuroimaging methods, many studies resulted in designs that used only parts of the TSST (e.g., only the arithmetic challenge or public speech) or changed the environment of the measure in significant ways (e.g., by showing a videotaped interview while subjects lie in the fMRI or PET scanner instead of standing in front of a committee). These adaptations are usually implemented due to the specifics of the corresponding neurophysiological method, e.g., to avoid movement (critical for fMRI) or speech-related artefacts (critical for EEG and fMRI) in the original form of the TSST. With regards to the ecological validity, these settings might significantly differ from real-life stress situations, which might be a reason why some stress neuroimaging studies fail to show significant increases in stress indices (Dedovic et al., 2009c).

In the current study, we tried to verify the usage of an optical neuroimaging method in this context that is relatively robust with regards to movement and speech artefacts: functional Near-infrared Spectroscopy (fNIRS). For fNIRS measurements, a set of optodes is used to emit light in the near-infrared spectrum into the human brain. In these wavelengths, light is capable to penetrate the human skull and brain up to 2–3 cm (Cui et al., 2011; Haeussinger et al., 2014, 2011). In the brain, this light is absorbed to different degrees by oxygenated and deoxygenated blood, which results in changes in the amount of reflected light that can be measured on the head with receiver optodes. Most importantly for our case, the method can be applied in mobile settings (Ehlis et al., 2014) and has been shown to be reliable (Plichta et al., 2006; Schecklmann et al., 2008) and valid (Huppert et al., 2006; Plichta et al., 2007) when compared to other imaging methods. Due to its relative robustness, fNIRS might be suitable to measure cortical activation in the ecologically valid TSST environment.

In the study at hand, we attempted to implement fNIRS measurements in the TSST by only changing subtle aspects of the TSST protocol to preserve its high ecological validity. We implemented two non-stressful control conditions where subjects were asked to (1) read numbers and (2) do calculations without any social or time pressure, in order to isolate the specific effects of the stress component on, e.g., the fNIRS data. Also, we changed the arithmetic task of the TSST into a block design to measure the hemodynamic response. All other aspects of the TSST remained unchanged. Stress induction was validated by subjective ratings on visual analog scales (VAS) as well as with salivary cortisol and heart rate measurements.

From the above outlined prior neuroimaging studies (Dedovic et al., 2009c; Tillfors et al., 2001; Wang et al., 2005; Yanagisawa et al., 2011), we hypothesized an increase of cortical activity in parts of the cognitive control network (CCN) and dorsal attention network (DAN) during the TSST as compared to the non-stressful control conditions. The CCN, which includes areas of the dorsolateral prefrontal cortex (dlPFC), inferior frontal gyrus (pars opercularis) (IFG) and inferior anterior parietal lobule, is thought to be a mediator between the DAN and the default mode network (Spreng et al., 2010). During stress-induction, the subjects are forced to adapt to the higher demands of the situation by using attention regulation and emotion regulation, which should be neurally accompanied by higher activation of the CCN and DAN (Kogler et al., 2015; Kohn et al., 2014). As subjects who are more stressed by the TSST – due to higher discrepancies between the resources and demands of the task – would need to activate regions of cognitive control more strongly to fulfill the task, we expected a positive relationship between activation in areas of the CCN during stress and other physiological and subjective stress indices.

Materials and methods

Participants

32 healthy and unmedicated (with the exception of contraceptive medication) participants were recruited at the University of Tübingen. This study was approved by the ethics committee at the University Hospital and University of Tübingen. All procedures were in line with the Declaration of Helsinki in its latest version and all participants gave their written informed consent. Exclusion criteria of the study comprised: A history of or an acute mental disorder as assessed by a structured clinical interview for DSM-IV (SCID), a history of or an acute neurological disorder, diabetes, kidney failure, arterial hypertension, Cushing syndrome, cortisone medication, and smoking of more than 3 cigarettes per week. The mean age of the sample was 21.44 years (SD = 2.93), 26 subjects were female of which 18 took contraceptive medication. 8 females did not take contraceptive medication, two of which were at the end of their menstrual cycle (>28 days since beginning) and one at the beginning (<7 days since beginning). Most subjects (56%) performed sports activities 1 to 2 times per week. However, subjects were told not to perform any sort of physical training at the day of the measurement, to sleep the night before as long as they usually do and not to drink alcohol the day before the measurement. Further, subjects were instructed not to drink and eat 30 min before the measurement started.

Procedures

At the day of their measurement, subjects completed the basis documentation (including demographic variables and sports habits) together with the experimenter in a separate room. Afterwards, they were led to the fNIRS laboratory where the preparation for the fNIRS measurement was done. fNIRS was assessed during all control conditions and all parts of the TSST. However, due to methodological considerations (total measurement time, possibility of controlling task performance, artificial neural activity through movement artefacts) only the TSST arithmetic task was formally analyzed with regards to the fNIRS data and compared to the control conditions (CTL1, CTL2) specifically designed for this part of the paradigm. The fNIRS laboratory was adapted for the purposes of the TSST: The fNIRS machine was positioned behind the subjects who were seated at a table. After the preparation, a 7-min resting-state measurement was performed, followed by the first salivary sample and a measure of positive and negative affect with the Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988). Two control tasks in standing positions were completed afterwards, each of 6-min duration. During the control tasks, only a friendly study nurse was present in the room that stood behind the subjects in front of the NIRS machine. To minimize social stress, the participants were not observed during the control conditions. The first task (CTL1) was a number-reading task, in which subjects read printed numbers from 1022 downwards in steps of 13 aloud from a sheet of paper. The 6-min control condition consisted of 6 blocks, each comprising 40 s of reading and 20 s of pausing. The second control task (CTL2) comprised serial subtractions without any aids in steps of 13 from different starting points with spoken responses, however, without any time or social pressure. Again, the subjects had to perform 6 blocks, each with 40 s computing and 20 s pausing. To make sure that the subjects were as relaxed as possible, they were told that they should take as much time as they like for the calculation. When subjects made errors, the study nurse said “Okay, thank you. Please count now from …”, giving them the right number, without indicating errors.

After the completion of the control tasks, the TSST started. The TSST committee members dressed in white physician coats – one male and one female – entered the room, introduced themselves and took a seat on the other side of the table. During the whole TSST, the committee members remained neutral and unresponsive to verbal (e.g., making a joking comment) and non-verbal (e.g., smiling) social interaction signs of the participant. Subjects were informed that they should imagine that they made an application for a job at the clinic and that they would have to give a speech of 5 min in which they report on their personal strengths and why they believe they are the best applicant for the position. Afterwards, subjects were given 5 min to prepare for the interview (the stress anticipation phase) in which they were allowed to make notes with paper and pencil. The anticipation phase was followed by the free speech, in which subjects had to stand up and step back from the table where the committee was still seated. They were told that they would be videotaped during the following tasks for an accurate analysis of their behavior and a video camera beside the committee members was turned on. Their notes were taken away and after a baseline measurement of 10 s, subjects started with their speech, during which the committee members kept quiet. If the subjects paused for longer than 20 s, they were told to keep on talking about their strengths. After the interview phase, an arithmetic stress task followed. Subjects were told that the following task would be independent from the prior tasks. During that task, the participants had to calculate aloud. Like in the second control task, they had to subtract 13 from different starting points in 6 blocks, each comprising 40 s calculation and 20 s pause. Starting points differed between the CTL2 condition and the TSST arithmetic condition. To maximize social stress, subjects were told to count as fast and correctly as possible. Also, they had to hold eye contact with one of the committee members. If an error occurred, the other committee member said “Stop. Start again from …”. Again, complete neutrality was held by the committee members. Before the start of block 3 and block 5, subjects were asked to work faster and better. After the task, the camera was turned off and the committee members left the room without any comment. The order of the control conditions and the TSST was fixed to prevent a carry-over effect of the stress response into the control conditions.

The TSST was followed by the second salivary sample and a 7-min resting phase measurement. Afterwards, four other salivary samples were taken every 15 min. In the post-stress phase, subjects completed the following questionnaires: PANAS, a qualitative and quantitative resting-state questionnaire about the second resting-state measurement and the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B) (Lehrl, 2005). Subjective ratings of the perceived stress on a scale from 0% to 100% were assessed before and after the first resting-state measure, after both of the control tasks, and together with each salivary sample following the TSST. After the last salivary sample, subjects were debriefed (see Fig. 1).

Cortisol sampling and assays

To measure salivary cortisol, saliva was collected in salivettes (Sarstedt AG & Co., REF 51.1534.500) and stored at −20 °C until further analysis. Salivettes were then thawed and centrifuged for 2 min at 1000 to collect saliva. Cortisol levels were analyzed via enzyme immunoassay (IBL International, Cortisol ELISA, REF RE52611) according to the manufacturer's instructions. Samples were run in duplicates and average intra-assay coefficient of variation was below 10%. Due to different daytimes of cortisol assessments and the circadian rhythms of individual cortisol levels, daytime was regressed out of the cortisol concentrations. Further, cortisol concentrations were log-transformed.

Heart rate

The heart rate was assessed with a one channel electrocardiogram. For this procedure, two standard Ag/AgCl EEG ring electrodes of 8 mm diameter were attached using a conductive EEG paste to the abraded skin above the left and right collarbone. A reference electrode was placed according to the 10/20 system above FPz. The signal was recorded using the BrainAmp ExG amplifier and Brain Vision Recorder software (Brain Products, Munich, Germany). The sampling rate of the signal was 1000 Hz. Heart rate assessments were recorded during resting phase measurements, control tasks, the stress anticipation phase, the free speech and the mental arithmetic task.

Data was preprocessed and analyzed using self-written MATLAB R2017a routines (MathWorks Inc, Natick, USA). Preprocessing included band-pass filtering (0.25–50 Hz). The data of one subject was highly contaminated by power line artifacts. Hence, an additional 50 Hz notch filter was applied. Thereafter, we calculated the mean interval between subsequent R peaks for each recorded phase separately and inferred thereof the average number of heart beats per minute (BPM).

fNIRS

Cortical activation was measured with fNIRS. We used a continuous wave, multichannel fNIRS system (ETG-4000 Optical Topography System; Hitachi Medical Co., Japan) with a temporal resolution of 10 Hz. To measure frontal as well as parietal cortex areas we used an optode placement with three probesets: two frontal probesets – with reference points at F7 and F8 – and one parietal probeset – with Pz as reference point. Optodes were positioned on a combined electrode Easycap (see Table 1). Since our measurement implied movements of the participants, we additionally fixated each optode with a sponge ring that was glued on the Easycap. The sponge rings had a height of 1.2 cm, an outer diameter of 3.2 cm and an inner diameter of 0.9 cm. To allow an adaptive placement of the optode depth on the Easycap and an optimal cable routing, the rubber rings were cut sagittally (see Supplemental Fig. S1). The system consisted of 46 channels. We defined 5 regions of interest, including the left and right dlPFC, left and right IFG and the somatosensory association cortex (SAC) (see Fig. S2).

Data analysis

fNIRS preprocessing

Data were processed and analyzed using MATLAB R2015b (MathWorks Inc, Natick, USA). Furthermore, PASW (Version 22) was used for data analysis. Data preprocessing included: A first bandpass filtering (0.1-0.001 Hz) to minimize high- and low-frequency noise, movement artefact reduction by the algorithm of Cui et al. (Brigadoi et al., 2014; Cui et al., 2010), and a first interpolation of single artificial channels, as well as an ICA based reduction of teeth-clenching artefacts to reduce high amplitude noise. Afterwards, a second bandpass filtering (0.1-0.01 Hz) and a global signal reduction was performed with a spatial gaussian kernel filter (Zhang et al., 2016) with a standard deviation of $\sigma = 40$.

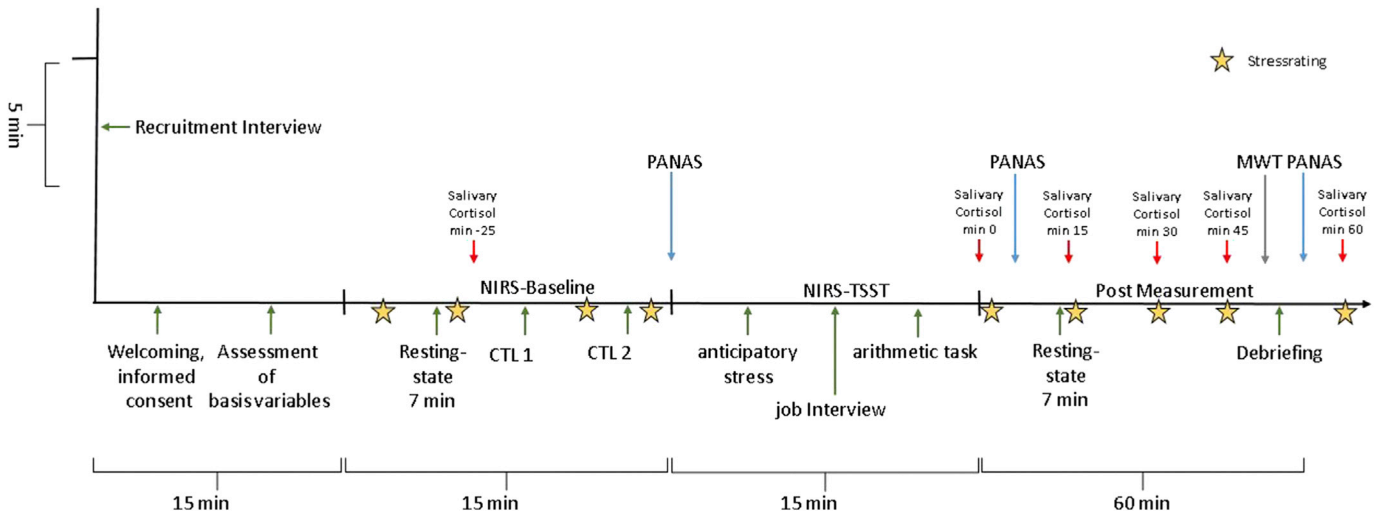

Fig. 1. Timeline of the experimental procedures.

Afterwards, all signals were visually inspected revealing local artefacts after the described pre-processing. In these cases, channels were interpolated from surrounding channels. In this sample, the maximal number of channels that had to be interpolated was 9 (<20%). After pre-processing, blocks of the control conditions and TSST arithmetic stress induction were averaged with a baseline-correction of 5 s and a linear detrending. Afterwards, mean activation values in the regions of interest (ROI) were extracted for further analysis.

Statistical analysis

As a first analysis step, within-subject comparisons of the different task conditions were performed on the stress parameters salivary cortisol, heart rate, subjective stress ratings and positive and negative affect. Changes in cortical oxygenated hemoglobin in the ROIs were also compared in the different task conditions. For these analyses, repeated measurement ANOVAs were used. Planned comparisons included differences between the control conditions and the TSST arithmetic task (CTL1 vs. TSST, CTL2 vs. TSST), relative changes from measurement point to measurement point in subjective stress and heart rate (Pre Rest1 vs. CTL1, CTL1 vs. CTL2 …) and relative changes to baseline levels in cortisol measurements (Post Rest1 vs. Post TSST, Post Rest1 vs. 15 min…).

In a last step, we analyzed correlations between the stress and performance parameters and cortical hemoglobin changes. Due to different time points during the measurement (the number of which differed between measures), different correlation approaches were used. To assess associations between the cortical activation and math performance, we used non-parametric correlation analysis (Spearman correlation coefficients), by correlating the average number of calculations and errors during the CTL2 and TSST with the corresponding cortical activation in the different ROIs. Correlations of cortical activation during the TSST and the mean cortisol stress response (mean cortisol level over all measurement points) were also calculated via non-parametric correlations.

To account for the repeated measurement of the VAS stress ratings and the heart rate measures, mixed models were computed. Due to the repeated measurement design with three measurements, we investigated the relationship between subjective stress ratings, heart rate and cortical activation within the ROIs using linear mixed models (Roy, 2006). Here, we used the data of the CTL1, CTL2 and TSST condition as data nested within subjects. To portray the co-variation due to the stress induction, we used person-centered data of the independent variables. Cortical activation was predicted by the stress ratings and heart rates as fixed factors with a random intercept with restricted maximum likelihood estimation. The mean calculation rate and error rate were used as between-subject factors in this analysis.

Since all of the assessed stress-indices measure different aspects of the same (stress) response, we only assessed unidirectional influences of cortical activity on these measurements and did not analyze mediation between them (e.g., in how far the influence of cortical activation on the cortisol response is mediated by subjective stress). For the latter analysis, other between-group study designs would be advantageous.

Table 1
Assignment of fNIRS channels to Brodmann Areas. Corresponding brain areas were extrapolated from channel positions to reference points of the probesets based on the colin27 template (Cutini et al., 2011).

Brain area	Probeset	
	Probeset A: left frontal	Probeset B: right frontal
Retrosubicular area	1	14, 16
Dorsolateral Prefrontal Cortex	5, 10, 11, 12	15, 20, 23, 24
Temporopolar Area	2	13
Subcentral Area	3	17
Pre-Motor and Supplementary Motor Cortex	8	22
Pars Opercularis	6	19
Pars Triangularis	4, 7, 9	18, 21
	Probeset C: parietal	
Somatosensory Association Cortex	25, 26, 27, 28, 30, 31, 32, 34, 35, 36, 37	
V3	38, 39, 40, 41, 43, 44, 45, 46	
Angular Gyrus	42	
Supramarginal Gyrus	29, 33	

Results

Changes in subjective and physiological stress indices

In comparison to the CTL2, mathematical performance – in terms of calculation rate, errors and calculations per error – differed significantly during the TSST arithmetical task. Subjects made more errors ($t_{(31)} = 6.857$, $p < .001$, $d = 1.21$), but also more correct calculations ($t_{(31)} = 6.160$, $p < .001$, $d = 1.09$) during the TSST. However, proportionally, their calculations per error decreased significantly ($t_{(31)} = 3.267$, $p < .01$, $d = 0.57$), indicating higher quantity but lower quality of the mathematical performance (see Table 2).

As indicated by a repeated measurement ANOVA, subjective stress ratings (see Fig. 2 A) varied significantly over time ($F_{(8,\ 240)} = 53.513$,

$p < .001$, $\eta^2 = 0.64$). Post-hoc analysis with Bonferroni-corrected repeated comparisons revealed a significant increase during the second control condition ($t_{(31)} = 5.37$, $p < .001$, $d = 0.95$), a further significant increase during the TSST ($t_{(31)} = 7.68$, $p < .001$, $d = 1.36$), and again significant decreases 15 min post TSST ($t_{(31)} = 11.47$, $p < .001$, $d = 2.03$) and 30 min post TSST ($t_{(31)} = 5.08$, $p = .001$, $d = 0.89$).

In the same way, heart rate measures (see Fig. 2 B) significantly varied over time ($F_{(6, 180)} = 57.676$, $p < .001$, $\eta^2 = 0.66$). Post-hoc analysis revealed a significant increase in heart rate from resting state 1 to CTL1 ($t_{(30)} = 8.76$, $p < .001$, $d = 1.6$), from CTL1 to CTL2 ($t_{(30)} = 3.1$, $p < .01$, $d = 0.56$) from the stress anticipation to the interview ($t_{(30)} = 9.49$, $p < .001$, $d = 1.7$) and a significant decrease from TSST to resting state 2 ($t_{(30)} = 10.71$, $p < .001$, $d = 1.9$).

For salivary cortisol concentrations, we also found a significant variation over time ($F_{(5, 155)} = 12.218$, $p < .001$, $\eta^2 = 0.28$) (see Fig. 2 C). Through the adapted TSST version, cortisol was elevated from baseline scores directly after the TSST ($t_{(31)} = 3.0$, $p < .01$, $d = 0.54$), at 15 min post TSST ($t_{(31)} = 4.12$, $p < .001$, $d = 0.72$), at 30 min post TSST ($t_{(31)} = 3.82$, $p < .001$, $d = 0.67$) and at 45 min post TSST ($t_{(31)} = 2.67$, $p < .05$, $d = 0.47$). 40% of the subjects showed an increase higher than 2.5 nmol/l and 59.4% of the subjects showed an increase higher than 1.25 nmol/l and could be defined as responders (Miller et al., 2013).

Regarding positive and negative affect, a 2 (negative vs. positive affect) by 3 (before TSST vs. post TSST vs. 45 min post TSST) ANOVA revealed a significant interaction ($F_{(2,60)} = 6.519$, $p < .01$, $\eta^2 = 0.18$). Post-hoc analysis with repeated comparisons revealed no significant change in positive affect, but a significant increase from pre- to post TSST ($t_{(31)} = 5.4$, $p < .001$, $d = 0.95$) and a significant decrease from post TSST to 45 min post TSST ($t_{(31)} = 5.9$, $p < .001$, $d = 1.06$) in negative affect.

Changes in cortical activation

We analyzed differences between the cortical activation during the arithmetic TSST and the control conditions (CTL1 and CTL2) within the 5 ROIs (see Fig. 3). A repeated measurement ANOVA with the factors "condition" and "ROI" revealed significant main effects for the experimental condition ($F_{(2, 62)} = 6.314$, $p < .01$, $\eta^2 = 0.17$) and ROI ($F_{(4, 124)} = 3.14$, $p < .05$, $\eta^2 = 0.09$). Post hoc analysis (see Table 3) of the experimental conditions was separately done for each ROI and p-values were adjusted with the procedure of Armitage-Parmar (Sankoh et al., 1997). In comparison to the CTL1, subjects showed higher activation during the performance of the TSST arithmetic challenge in the bilateral dlPFC ($d = 0.49$ to 0.57), the left IFG ($d = 0.57$) and the SAC ($d = 0.55$). Also in comparison to the CTL2, a similar pattern of activation was found, however, effect sizes were smaller and only significant in the left IFG ($d = 0.48$) and SAC ($d = 0.44$).

We further explored the data for relationships between cortical activation and behavioral indices of the TSST. To this end, we investigated two issues: Relationships between cortical activation and mathematical performance and relationships between cortical activation and stress-indices.

Our analysis revealed significant positive associations between left and right dlPFC activation and error rates during the TSST ($r_{(30)left} = 0.412$, $p < .05$; $r_{(31)right} = 0.417$, $p < .05$) and during the CTL2 ($r_{(30)left} = 0.414$, $p < .05$; $r_{(30)right} = 0.392$, $p < .05$). Also, increases in error rates from CTL2 to TSST were positively associated with increases in activation from CTL2 to TSST in the left dlPFC ($r_{(30)left} = 0.45$, $p < .01$) and the left IFG ($r_{(30)left} = 0.44$, $p < .05$). Moreover, during CTL2, activation in the left and right dlPFC ($r_{(30)left} = -0.407$, $p < .05$; $r_{(30)right} = -0.408$, $p < .05$) and the SAC ($r_{(30)left} = -0.383$, $p < .05$) were negatively correlated with calculation rate.

Regarding cortisol responses, we found positive correlations between activation within the right dlPFC during the arithmetic TSST challenge and the stress-induced cortisol response ($r_{(30)} = 0.40$, $p < .05$). Similar to error rates, increases in activation within the left IFG from the CTL1 to TSST were associated with increases in the individual cortisol level ($r_{(30)} = 0.43$, $p < .05$).

In the same way, subjective stress ratings and heart rate measures were associated with brain activation (see Table 4). Positive associations were found between stress ratings and bilateral dlPFC activity, left IFG activity and SAC activity (all $p < .01$). Similar effects were found between heart rate and bilateral dlPFC activity and left IFG activity, respectively ($p < .05$ to $p < .001$). Importantly, both subjective stress and heart rate showed significant associations, when controlled for average calculation errors. Also, results for subjective stress and heart rate stayed stable, when controlled for calculation rate.

Table 2
Performance of the study sample (N = 32). The number of errors and calculations were averaged over blocks (40 s) and afterwards over subjects.

Variable	mean	SD
Number of errors (Control condition)	0.87	1.35
Calculation (Control condition)	7.84	3.22
Ratio Calculation/Error (Control condition)	22.37	24.29
Number of errors (TSST)	1.84	1.96
Calculation (TSST)	9.75	3.63
Ratio Calculation/Error (TSST)	9.05	11.09

Discussion

The aim of this study was to answer the so far unexplored question of whether the results from stress induction tasks as adapted for neuroimaging experiments (e.g., the MIST) can actually be generalized to ecologically more valid stress induction paradigms such as the TSST. To this end, we used a rather robust optical imaging method – fNIRS – to assess brain activation in a relatively ecologically valid stress situation. Our stress parameters showed significant subjective, physiological and endocrinological change through the stress induction via the TSST. As expected, we observed significant increases to and significant peaks of our subjective and physiological stress parameters during the stressful TSST speech and arithmetic task in comparison to the resting and control conditions. Further, we observed an expected cortisol response to the stress induction, which implies that the stress induction was successful.

On a cortical level, we found higher activation in the dlPFC, IFG and SAC during the performance in the TSST when compared to a simple number reading control condition (CTL1). Similar effects were found, when we contrasted the TSST activation to the CTL2. However, for the latter contrast, significant effects were restricted to the left IFG and the SAC. This result is in line with meta-analytic data from fMRI studies, which shows reliable IFG activation through physiological and psychosocial stress induction (Kogler et al., 2015). The IFG is part of the cognitive control network (CCN) (Spreng et al., 2010) and is associated with the regulation of negative affect, as the inhibition of emotional memory and behavior (Aron et al., 2004; Depue et al., 2007; Wang et al., 2005). Interestingly, in an adapted TSST task for fMRI, Lee and colleagues observed gender differences in the relationship of IFG activation and stress ratings (Lee et al., 2014). In this task, subjects were measured with fMRI while watching video clips of themselves performing the TSST vs. same sex others that didn't seem to be stressed though the task. While there was a significant increase in the BOLD response during the self vs. other contrast in the bilateral IFG and insula, an interaction of gender by condition was also observed. Male subjects reported significantly less subjective stress and showed stronger activation in the right IFG than female subjects. This result somehow contrasts our own results in which we found a positive relationship between subjective stress and cortical activation in frontal areas. However, we analyzed within-subject correlations, while the result of the study by Lee et al. (2014) is based on between-subject (gender) differences. Further, there might be a difference between the neural correlates during the stress task in comparison to watching oneself in a video after the TSST completion. In our study, we hypothesized that higher stress in the acute stress phase would be accompanied by higher activity in the CCN due to compensational

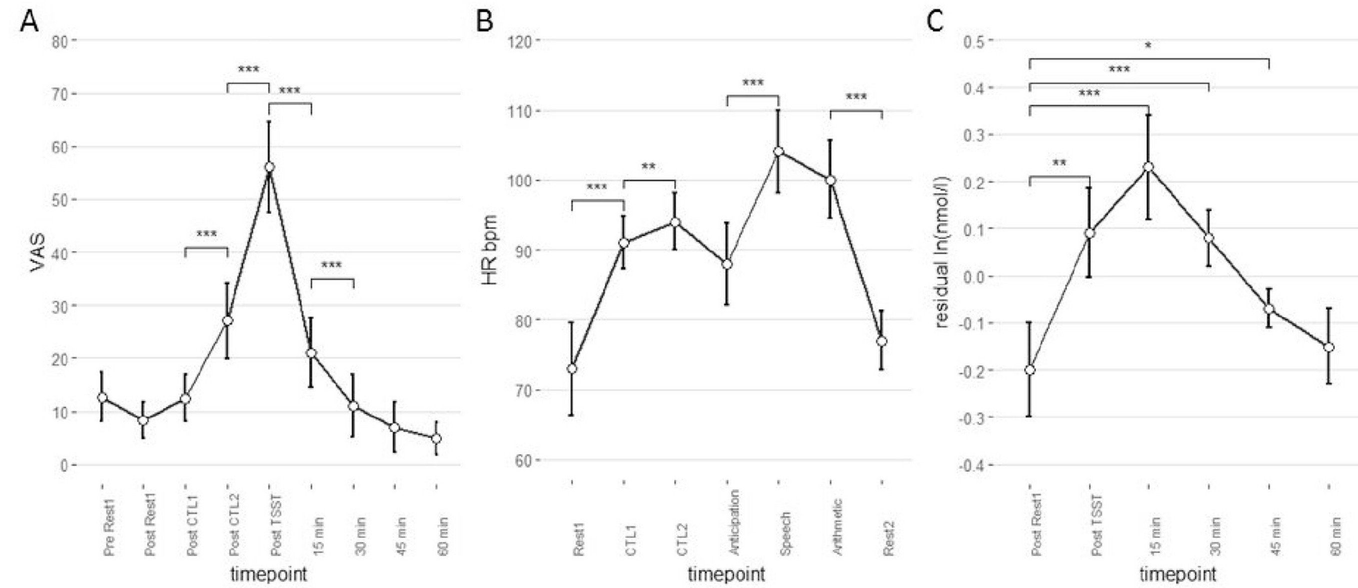

Fig. 2. Response of the TSST on the subjective and physiological stress indices. **A)** Trajectory of subjective stress over the different experimental conditions. The time-points "15 min, 30 min …" refer to the time since the completion of the TSST. **B)** Changes in heart rate in beats per minute over the different experimental conditions. **C)** The induced cortisol response. The graph shows the log-transformed and daytime-corrected cortisol values. Negative values are due to the covariate-correction for daytime. *p < .05, **p < .01, ***p < .001.

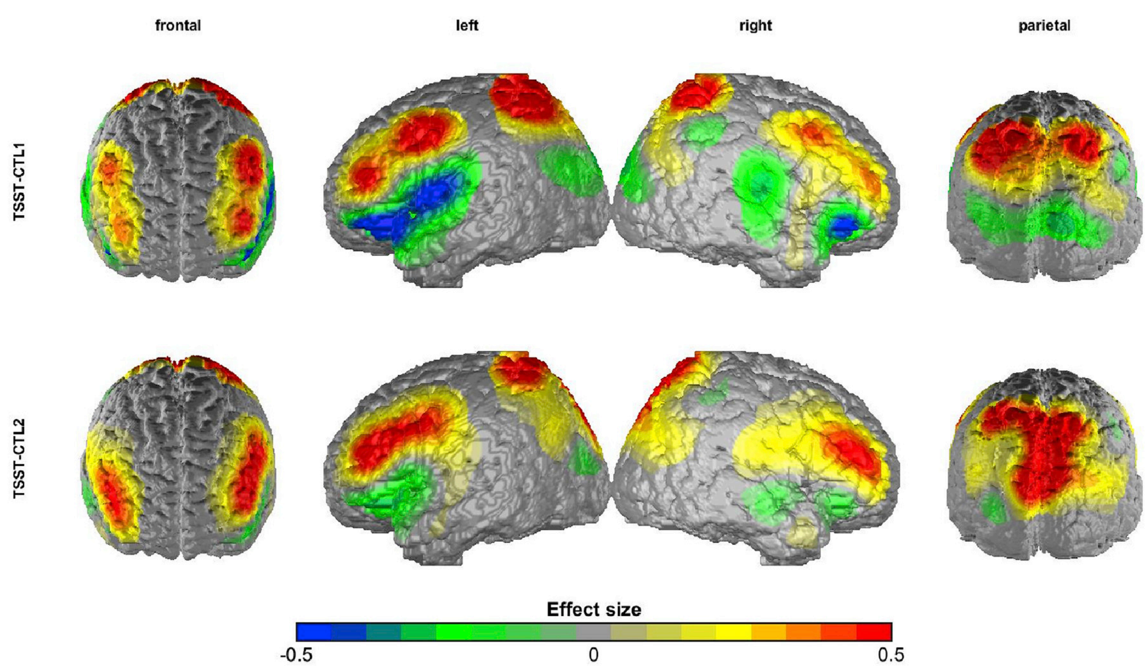

Fig. 3. Brain maps of the contrasts between the experimental conditions (TSST arithmetic task vs. control condition 1 (CTL1) and 2 (CTL2)). Effect sizes in "d" are plotted; warm colors indicate higher activation during the TSST arithmetic task in comparison to the relevant control condition.

mechanisms. However, it could also be hypothesized that higher CCN activity during the recovery phase of the stress response leads to faster recovery of stress symptoms. Such a double dissociation could explain the diverging findings of Lee et al. (2014) and our own results.

Also, higher activation in the SAC during the TSST was consistently found in comparison to the two control conditions. The SAC, which is located in the superior parietal lobule (Brodmann Area 7), is part of the dorsal attention system. However, it also includes medially the precuneus and reaches laterally into the inferior parietal lobule, which is part of the CCN and default mode network (DMN) (Vincent et al., 2008). The SAC is included in a variety of higher order cognitive functions including spatial orientation (Iwamura, 2003), visuo-spatial planning (Spreng et al., 2010), manipulation of working memory (Koenigs et al., 2009) and attention regulation (Downar et al., 2000). Also, activation in the superior parietal lobule has been associated with self-relevant information in adapted fMRI stress tasks (Lee et al., 2014), with stressful mental arithmetic tasks (Dedovic et al., 2005) and was found to be present in cortisol non-responders that performed the MIST (Dedovic et al., 2009c).

Table 3
Post-hoc comparisons between the TSST arithmetic task and the two control conditions within each ROI. * significant after adjustment for multiple comparisons with Armitage-Parmar correction.

ROI	Mean difference	SD	t	p	d
TSST vs. Control Condition 1					
Left dlPFC	.030	.052	3.25	<.001	**.57***
Left IFG	.027	.048	3.24	<.001	**.57***
Right dlPFC	.023	.048	2.75	<.01	**.49***
Right IFG	.011	.057	1.10	>.1	.20
SAC	.031	.056	3.13	<.01	**.55***
TSST vs. Control Condition 2					
Left dlPFC	.017	.059	1.68	<.1	.30
Left IFG	.024	.052	2.70	<.01	**.48***
Right dlPFC	.017	.048	2.00	<.05	.35
Right IFG	.011	.041	1.58	<.1	.28
SAC	.022	.051	2.47	<.01	**.44***

Table 4
Results for the mixed models, displaying the association between individual stress as measured by VAS, and heart rate and brain activation, when controlled for mean calculation errors.

ROI	Stress Rating	Calculation Errors	Heart Rate	Calculation Errors
Left dlPFC	$t_{(63)} = 3.28$ ***p < .01*** $\beta = .0006$	$t_{(30)} = 1.40$ $p > .1$	$t_{(61)} = 2.48$ ***p < .05*** $\beta = .002$	$t_{(29)} = 1.38$ $p > .1$
Left IFG	$t_{(63)} = 2.672$ ***p < .01*** $\beta = .001$	$t_{(30)} = 0.005$ $p > .1$	$t_{(61)} = 3.33$ ***p < .001*** $\beta = .002$	$t_{(29)} = 0.01$ $p > .1$
Right dlPFC	$t_{(63)} = 2.98$ ***p < .01*** $\beta = .0005$	$t_{(30)} = 1.83$ $p < .1$ $\beta = .025$	$t_{(61)} = 2.22$ ***p < .05*** $\beta = .001$	$t_{(29)} = 1.80$ $p < .1$ $\beta = .026$
Right IFG	$t_{(63)} = 1.02$ $p > .1$	$t_{(30)} = 1.19$ $p > .1$	$t_{(61)} = 0.02$ $p > .1$	$t_{(29)} = 1.16$ $p > .1$
SAC	$t_{(63)} = 3.02$ ***p < .01*** $\beta = .02$	$t_{(30)} = 1.6$ $p < .1$	$t_{(61)} = 1.79$ $p < .1$ $\beta = .001$	$t_{(29)} = 1.71$ $p < .1$ $\beta = .021$

Although the complexity of the mathematical calculation was equal between CTL2 and the TSST, the calculation rate and error rate increased through the stress induction, which somehow entangles the effects of elevated stress levels and mathematical performance. Indeed, prefrontal and parietal areas have already been shown to be associated with mathematical processing (Arsalidou and Taylor, 2011). However, higher calculation performance due to social stimulation (the review board during the TSST) might be itself defined as stress because it is a result of higher load due to higher demands (McEwen, 1998). Consequently, the hemodynamic activity in die dlPFC during the performance of the TSST and CTL2 was positively correlated with error rates. It has been shown previously that the lateral prefrontal cortex – including the dlPFC and IFG – is activated during the anticipation of mathematical challenges in math anxiety and during the processing of errors (Ansari et al., 2011; Lyons and Beilock, 2012). Noteworthy, negative association between math anxiety and dlPFC activity have been reported (Artemenko et al., 2015). Also, dlPFC activity has been shown to be related to the processing of incorrect mathematical equations (Menon et al., 2002), but also to error processing in simple cognitive reaction time tasks such as the Go-Nogo task (Menon et al., 2001). Moreover, excitatory stimulation of the left dlPFC by transcranial direct current stimulation (tDCS) has been shown to reduce reaction times in arithmetic tests and reduce cortisol levels in high math anxious individuals (Sarkar et al., 2014). The role of the dlPFC in cognitive control is thought to be maintaining attentional demands of a task especially during preparatory phases, which has been supported by fMRI data of the color stroop task. Here, the dlPFC was active in the preparatory period of the difficult color naming condition, while the anterior cingulate cortex was active during the response phase (MacDonald, 2000). With respect to our own data, the correlation between dlPFC activation and error rates might reflect enhanced cognitive control via attention regulation and the preparation of a new trial start after erroneous calculations.

This interpretation is further underlined by the positive associations between cortical activity in the CCN regions and subjective stress ratings, heart rate and the cortisol response. During the TSST, subjects are exposed to a non-validating neutral reviewer board. Stress situations like public speaking activate the limbic system, including the amygdala (Dedovic et al., 2009a; Tillfors et al., 2002), which in turn triggers the primary stress response, including elevated sympathetic reactions – in our case increased heart rates – and the secondary stress response – including the HPA-axis with cortisol reactions. During the stress situation, parts of the cortex – including the CCN and DAN – are involved in the adaption to the situation and the increased behavioral demands in light of the emotional and cognitive load, which may be reflected by the activation in the dlPFC and the correlation between this activation and calculation errors. All of these factors that were inherent to the TSST situation – cognitive and emotional load through social evaluation and arithmetic error processing – are in fact stressors that lead to allostasis (McEwen, 1998). However, it is important to bear in mind that we were not able to measure subcortical activation with the fNIRS assessment.

Despite these conclusive findings, some limitations of this study need to be considered. First of all, as we outlined in the procedures, we used a fixed order of the experimental conditions to prevent carry-over effects of the stress response into the control conditions. However, from an experimental point of view, a randomized (or at least balanced) order of conditions would generally be advantageous to rule out time effects on the experimental effect. Since we did not randomize the sequence of experimental conditions, such time effects are inevitably entangled with the experimental stress induction. However, we would further argue that our results are most likely not simply due to such a time effect, since – with longer measurement durations – factors such as habituation and fatigue usually lead to a decrease in brain activation and – at the behavioral level – probably also decreased stress ratings etc. In our data, however, cortical activation as well as physiological and behavioral stress indices showed a steady increase from the simple non-stressful CTL1 to the more stressful CTL2 and (as the most demanding condition) the TSST, just as expected.

A further limitation concerns the used method of fNIRS. Since we were only able to measure the activation of the cortex in our defined probe-set regions, we could not investigate subcortical changes (due to the limited penetration depth of the near-infrared light) – including the limbic system – and regions outside the probesets. We placed our system in the best possible way to measure frontal and parietal parts of the CCN. Restricted to this region, we found stress-induced effects that correlated well with other parameters of the stress response. Finally, our results are limited to the mathematical context of the TSST arithmetic task. It is up to future studies to show, in how far other stress inducing cognitive tasks recruit the same brain regions.

Lastly, we used a relatively small sample size. Due to this limitation, the results of the correlation analysis have to be interpreted with caution. Further, the analyses of the effects of contraceptive medication and sex were not performed due to small subgroups. Future studies with larger samples will be needed to examine potential moderating effects of these variables on the cortical reactions during the TSST.

In the study at hand, we only developed control conditions for the arithmetic task since it is difficult to control for performance and mental effort in a potential control task for the free speech and preparation phase. It will be an interesting endeavor for future studies to develop appropriate and time-economical control conditions for these parts of the TSST. fNIRS may be an imaging technique that is able to measure cortical activation in such movement accompanied tasks.

In conclusion, to the best of our knowledge, this is the first study that investigates the neuronal underpinnings of the TSST in an ecologically valid environment. We found higher activation in areas of the CCN and

DAN during the TSST arithmetic task in comparison to control conditions. Critically, we found a significant response in all of our stress indices, which is not always the case in other adapted neuroimaging stress paradigms. Our data most likely reflect the adaption to the stressful arithmetic situation in terms of faster mathematical processing and higher emotional and cognitive load. The findings of the presented study are in line with results from adapted stress induction paradigms and link these studies using more adapted stress induction tasks to those found in the classic TSST. Furthermore, this is the first study that linked arithmetic errors during the TSST to brain activity. In future studies, the fNIRS-TSST paradigm might be used for the study of clinical populations.

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Authors contributions

D. R., P. H., M.T. & F.B.H. contributed in the analysis and interpretation of the data for the work and did the primary drafting. A.J.F., H.C.N., V.N., F.M. & A.-C. E. contributed in the design and acquisition of the work and revised it critically for important intellectual content.

All authors approved the final version to be published and agree to be accountable for all aspects of the work.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuroimage.2017.12.061.

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