

**Sex-specific interaction between cortisol and striato-limbic responses to psychosocial stress**

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### **Abstract**

Although women and men differ in psychological and endocrine stress responses as well as prevalence rates of stress-related disorders, knowledge on sex differences regarding stress regulation in the brain is scarce.

Therefore, we performed an in-depth analysis of data from 67 healthy participants (31 women, taking oral contraceptives), who were exposed to the ScanSTRESS paradigm in an fMRI study. Changes in cortisol, affect, heart rate, and neural activation in response to psychosocial stress were examined in women and men as well as potential sex-specific interactions between stress response domains.

Stress exposure led to significant cortisol increases with men exhibiting higher levels than women. Dependent on sex, cortisol elevations were differently associated with stress-related responses in striato-limbic structures: Higher increases were associated with activations in men but with deactivations in women. Regarding affect or heart rate responses, no sex differences emerged.

Although women and men differ in their overall stress reactivity, our findings do not support the idea of distinct neural networks as base of this difference. Instead, we found differential stress reactions for women and men in identical structures. We propose considering quantitative predictors like sex-specific cortisol increases when exploring neural response differences of women and men.

**Keywords:** Hippocampus, Amygdala, Ncl. Caudatus, Ncl. Accumbens, Thalamus, fMRI

## **1. Introduction**

On average, women and men show various differences in variables related to the central nervous system (CNS), including neuroanatomical, autonomic, and psychological variables; consistently, a sex-specific genetic architecture was found for several CNS-related phenotypes (David et al., 2018; McCarthy et al., 2017). With the advent of human brain-imaging techniques, sex differences in the brain were found, covering – among others – anatomical variables, connectivity measures, as well as neural correlates in affect and cognitive functions (Cahill, 2006; Choleris et al., 2018; Grabowska, 2017; Ingalhalikar et al., 2014a; Ritchie et al., 2018). These findings are paralleled by clear sex differences in prevalence rates for stress-related mental disorders (Bangasser & Valentino, 2014; Kudielka & Kirschbaum, 2005). On the other hand, the distribution of variables like functional connectivity (FC) or neuroanatomical variables were also reported to be overlapping in women and men (Grabowska, 2017; Joel & Fausto-Sterling, 2016; Joel et al., 2020; Joel et al., 2018; Joel et al., 2015). Therefore, it was suggested that, depending on methodological aspects, clear sex dimorphisms in neural variables are detectable or not (Chekroud et al., 2016; Del Giudice et al., 2015; Del Giudice et al., 2016; Ingalhalikar et al., 2014b; Joel & Tarrasch, 2014; Rosenblatt, 2016). This leads to the plausible and relevant question, to what extent sex differences in stress regulation and stress-related psychopathology can be attributed to differences between women and men in the brain's response to stress.

Women tend to report more perceived stress, anxiety, and tension during and after acute stress exposure than men (Kelly et al., 2008; Kirschbaum et al., 1999; Merz & Wolf, 2015). Interestingly, these self-report-based differences are consistent with findings in animal models as female rodents show more passive and stress-related behavior in response to stress (Beery & Kaufer, 2015; McEwen & Milner, 2017; Rincón-Cortés et al., 2019). Moreover, mean corticosterone stress responses are higher in female than in male rodents (Goel et al., 2014; Oyola & Handa, 2017). However, sex differences in hypothalamic-pituitary-adrenal (HPA) axis reactivity in rodents are not consistent with findings in humans,

as healthy men show significantly larger responses than women to acute psychosocial stress induction (Kudielka & Kirschbaum, 2005; Liu et al., 2017; Zänkert et al., 2019). The reasons for this inconsistency between animal and human studies are not well understood but significant differences in both stress quality and intensity could hamper the comparability of findings. While animals are often exposed to completely novel, uncontrollable, and potentially life-threatening aversive situations, human participants usually experience moderate social-evaluative threat while being fully aware that they can abort the exposure at any time. Moreover, possible explanations for this sex-specific response pattern in humans range from an impact of stressor type (social rejection challenges as typical female, achievement stressors as typical male stressor) to a modulating impact of hormonal concentration fluctuations in different menstrual cycle phases and the use of oral contraceptives (OCs) (Zänkert et al., 2019). Regarding heart rate responses to stress, findings are more inconclusive with some studies reporting sex differences (Emery et al., 2018; Seo et al., 2017) that partly depended on menstrual cycle phases (Childs et al., 2010; Kudielka et al., 2004), while others failed to find differences (Kelly et al., 2008; Kirschbaum et al., 1999).

So far, a few attempts have been made to evaluate neural sex differences by implementing distinct stress paradigms in functional magnetic resonance imaging (fMRI) environments. A perfusion-based fMRI study reported a sex-specific neural activation model featuring primarily striato-limbic activation in women and asymmetric frontal blood flow in men. Moreover, the correlation between these sex-specific activation patterns and cortisol was higher in men (Wang et al., 2007). Regarding neural stress processing, (pre)limbic structures seem to be of particular relevance since dissociations between women and men for these regions have been reported (Goldfarb et al., 2019; Kogler et al., 2015; Seo et al., 2017; Seo et al., 2011). Focusing on the amygdala, resting state FC studies emphasized sex-specific responses in limbic circuits. Furthermore, associations between FC and cortisol were also found to differ significantly between women and men (Henckens et al., 2010; Kogler et al., 2016; Vaisvaser et al., 2013; Veer et al., 2012; Vogel et al., 2015). To date, investigations into sex differences regarding neural processing of psychosocial stress remain

scarce and yielded mixed results (Noack et al., 2019). While some evidence for stress-induced neural response differences between women and men exists (Chung et al., 2016a; Dahm et al., 2017; Kogler et al., 2015; Kogler et al., 2017), no consistent sex-specific neural response-pattern emerged.

The heterogeneity of previous findings can probably – at least in part – be explained by methodological disparities resulting from different stress induction paradigms. Studies varied in dependent variables (endocrine, subjective, cardiovascular) as well as in stress intensity, and thereby in the magnitude of stress responses (Noack et al., 2019). A recent study by our group (Henze et al., 2020) aimed at elucidating the interaction of distinct stress activation systems in response to an improved psychosocial fMRI stress protocol (Streit et al., 2014). We found significant cortisol, subjective, heart rate, and neural reactions in response to ScanSTRESS (Henze et al., 2020). Moreover, neural stress reactions in (pre)limbic structures were associated with individual changes in cortisol and negative affect ratings. Given the robust cortisol responses and the consistent interactions between different stress response domains within a relatively large cohort, it appeared promising to further elaborate the role of sex within the same sample. Therefore, the objective of the present study was a detailed and comprehensive analysis of sex-related stress response differences in distinct response domains including neural and cortisol responses as well as changes in heart rate and affect. Instead of merely contrasting female and male responses dichotomously, as most of the studies on sex differences in neural stress processing did (Chung et al., 2016a; Dahm et al., 2017; Kogler et al., 2015; Kogler et al., 2017), we applied a statistical model including individual cortisol increases as a continuous predictor to examine stress-related sex differences in the brain (Grabowska, 2017; Joel & Fausto-Sterling, 2016; Joel et al., 2018). This model assumed that sex differences in neural stress processing might emerge when sex-related cortisol response differences are considered.

As an exploratory analysis, we also studied whether dynamic changes in neural stress responses, previously reported for (pre)limbic structures (Henze et al., 2020), differ between

women and men. Assuming that this exposure-time effect may indicate sensitization processes of ongoing stress exposure, sex differences might corroborate to a better understanding of interindividual differences related to stress vulnerability. For instance, in animals, chronic stress was found to cause damages to the hippocampus in male rats and monkeys but less, if at all, in females (McEwen, 2000).

## 2. Material and methods

### 2.1 Participants

Sixty-seven young, healthy, scanner-naïve university students (mean age  $23.06 \pm 3.14$  years) participated in the present study. Stress-induced cortisol, affect, heart rate, and neural responses of the present sample have been previously reported (Henze et al., 2020). It consisted of 31 women (mean age  $22.10 \pm 2.12$  years) and 36 men (mean age  $23.89 \pm 3.64$  years). Owing to HPA axis activity differences depending on menstrual cycle phase and OC-use (Kudielka & Kirschbaum, 2005; Zänker et al., 2019), only women using OCs were tested. Participants were recruited via flyers and social media internet platforms. Individuals who met any of the following criteria were excluded: self-reported history of or current psychiatric, neurological, or endocrine disorders; treatment with psychotropic medications or other medication affecting CNS or endocrine functions; daily tobacco or alcohol use; incompatibility with fMRI scanning (e.g., metal parts, pregnancy); regular night-shift work; undergoing a current stressful episode (e.g., major exams or emotional stress due to separation from partner or serious illness/death of a family member). All participants provided written informed consent and received a monetary compensation. The study was approved by the local ethics committee of the University of Regensburg.

### 2.2 General procedure and statistical analysis of cortisol, affect, and heart rate data

To induce psychosocial stress in the fMRI environment, the ScanSTRESS paradigm was applied (Streit et al., 2014); see Supplemental Methods A.1 and Figure A.1. Briefly, ScanSTRESS is composed of an alternating block design in a fixed order, presented in two runs, containing two conditions (stress vs. control) prompting the participants to perform

arithmetic and rotation tasks while a feedback-giving observation panel is presented via live video stream providing disapproving feedback. Moreover, between the two runs, the participants are notified that their performance was below average and they have to improve in the second run. For the present study, the protocol was slightly modified without changing the paradigm itself, in particular to improve cortisol responses to stress induction in the fMRI, as previous results were not always fully convincing (Noack et al., 2019; Streit et al., 2014). First, we implemented a prolonged (45 minutes) relaxing phase prior to stress to create sufficient baseline conditions (i.e., low cortisol levels). Moreover, we provided a detailed description and comprehensive clarification about the general scanning procedure (before the testing sessions took place) to minimize concerns prior to scanning that may confound with the response to the paradigm itself (McGlynn et al., 2007; Thorpe et al., 2008). Second, we administered a sugary drink (75 g glucose in 200 ml herbal tea) as it proved to facilitate cortisol reactivity (Gonzalez-Bono et al., 2002; Zänkert et al., 2020). The underlying mechanisms are still unknown but an influence of hunger and saturation regulating neuropeptides was discussed (Rohleder & Kirschbaum, 2007). Third, we achieved a more abrupt passage (< 10 minutes) from relaxation to stress exposure (details see Henze et al. (2020)). Figure 1 provides an overview of the improved procedure. Test sessions took place between 1 and 6 PM.

[Figure 1 about here]

Saliva samples for cortisol assessment were collected at ten time points (-75, -15, -1, +15, +30, +50, +65, +80, +95, +110 minutes) using 'Cortisol Salivettes' (Sarstedt, Nuembrecht, Germany). To collect samples at minutes -1 to +65, the experimenter, wearing medical gloves, gave the Salivette swab to the participant lying in the scanner. Mood state was compiled at the same ten time points using the German version of the Positive and Negative Affect Schedule scales (Watson et al., 1988). Saliva samples were stored at -20°C until



analysis. Samples were assayed in duplicate using a time-resolved fluorescence immunoassay with fluorometric end-point detection (DELFA [dissociation-enhanced lanthanide fluorescence immunoassay]) at the biochemical laboratory of the University of Trier (Dressendörfer et al., 1992); see Supplemental Methods A.2 for details). The intra-assay coefficient of variation was between 4.0% and 6.7%; inter-assay coefficients of variation were between 7.1% and 9.0%. During ScanSTRESS, heart rate recordings were obtained with an MRI-compatible finger oximeter (Model 7500 FO; Nonin Medical, Plymouth, MN) on the index finger, with a sampling rate of the highest heart beat within four seconds.

Data were analyzed in IBM SPSS Statistics version 25 (IBM, Corp., Armonk, NY) using repeated-measures analyses of variance (ANOVAs) regarding cortisol (nmol/l), positive and negative affect (test score), and heart rate (beats/min) with *time* as within-subjects factor and *sex* as between-subjects factor. A cortisol increase was defined as the difference between the individual cortisol peak (sample +30, +50, +65) and the pre-stress cortisol level (sample -1). Cortisol responder rates were computed with an increase of at least 1.5 nmol/l rise being defined as response (Miller et al., 2013). We used untransformed values for our analyses, as e.g., log-transformations were not necessarily appropriate for each measurement point. Therefore, we inspected skewness and kurtosis of time points individually (see Supplemental Methods A.3 for details) and found that they did not exceed acceptable values (Keele, 2008; Miles & Shevlin, 2001). Mean heart rates were calculated separately for each control and stress block. Greenhouse-Geisser corrections were applied where appropriate, and only adjusted results are reported.

### **2.3 fMRI acquisition and data analysis**

Participants were scanned in a Siemens MAGNETOM Prisma 3T MRI (Siemens Healthcare, Erlangen, Germany) equipped with a 64-channel head coil. A series of blood-oxygenation-level-dependent gradient echo-planar-imaging (EPI) images was acquired with the following parameters: repetition time 2000 ms, echo time 30 ms, 90° flip angle, 64 x 64 matrix, 192-mm field of view, 37 3-mm axial slices with 1-mm gap. Data were analyzed using FSL 6.0

(FMRIB Software Library, Oxford, United Kingdom). The first five EPI volumes were discarded to allow for T1 equilibration. fMRI data processing was carried out using FEAT version 6.0 (see Supplemental Methods A.4 for details). The  $z$  (Gaussianized  $t/F$ ) statistic images were thresholded nonparametrically using clusters determined by either  $z > 3.1$  or  $z > 2.3$ .

For each subject, general linear models (GLMs) were defined containing regressors for control and stress conditions and the respective announcement phases. In sum, twelve regressors resulted: six conditions (stress arithmetic subtraction, stress figure rotation, control numbers, control figures, announcement of stress, announcement of control) and six motion regressors. GLMs were carried out on three levels: for each subject, one GLM was computed for each run (first level,  $z > 3.1$ ) to account for scanner drifting. Subsequently, a fixed effects analysis (second level,  $z > 3.1$ ) was obtained to measure mean responses. On a third level, unpaired two-group analyses (mixed effects,  $z > 2.3$ ) were conducted to study sex differences: First, an unpaired two-group difference analysis was conducted to study sex-specific (men > women, women > men) responses for the main task effects, namely stress > control and control > stress. Secondly, we performed an unpaired two-group analysis with continuous covariate interaction (grand mean centered) to examine if the linear relationships between neural responses and cortisol increases (continuous covariate) differ between women and men (men > women, women > men). This model considers mean cortisol response differences between women and men. In addition, this model enabled to evaluate the interaction of cortisol and neural responses in the two subsamples separately. Corrections were performed over the whole brain with each contrast (stress > control, control > stress) thresholded at familywise error (FWE)  $p < .025$  (two-tailed combined test, FWE  $p < .05$ ).

Association analysis with cortisol increase were computed within *a priori*-defined striato- limbic anatomical regions of interest (ROIs) using masks from the Harvard-Oxford Atlas. We included the following eight masks, as the respective regions have been reported to respond

to stress in a sex-specific manner (Chung et al., 2016a; Dahm et al., 2017; Goldfarb et al., 2019; Kogler et al., 2015; Kogler et al., 2017; Noack et al., 2019; Seo et al., 2017; Seo et al., 2011; Wang et al., 2007): hippocampus, parahippocampal gyrus, amygdala, cingulate cortex, thalamus, ncl. caudatus, ncl. accumbens, and putamen. We applied Benjamini-Hochberg corrections (Benjamini & Hochberg, 1995; Nichols et al., 2017) to account for increases in false discovery rate and report uncorrected  $p$ -values as well as corrected significance level. ROI-analyses were performed using `fslmaths` and `featquery`. We created masks in which each voxel was assigned a 1 that had a  $\geq 50.0\%$  chance of belonging to the specific ROI and then we binarized these masks. Subsequently, mean beta values (extracted from second level analysis) were exported to SPSS and *post hoc* one-way ANOVAs were computed with sex as fixed factor and *cortisol increase* as covariate.

### 3. Results

#### 3.1 Sex differences in cortisol, psychological, and heart rate responses

For cortisol measures, we detected a significant *time* x *sex* interaction ( $F_{3,162} = 3.33$ ,  $p = .028$ ,  $\eta^2 = .045$ ; see Figure 2) as well as significant main effects for *time* ( $F_{3,162} = 9.85$ ,  $p \leq .001$ ,  $\eta^2 = .132$ ) and *sex* ( $F_{1,65} = 6.69$ ,  $p = .012$ ,  $\eta^2 = .093$ ). While men showed significantly higher cortisol levels than women briefly after entering the lab (-75 minutes), levels subsequently decreased and both groups showed similar cortisol concentrations immediately prior to stress onset (-15 minutes). In response to stress exposure, men showed significantly higher cortisol responses than women. Results of calculated *post hoc*  $t$ -tests regarding each time point and cortisol increases are shown in Supplemental Results Table B.1. When analyzing the two subsamples separately, the main effect *time* reached significance in women ( $F_{2,73} = 3.47$ ,  $p = .028$ ,  $\eta^2 = .104$ ) and men ( $F_{2,82} = 8.01$ ,  $p \leq .001$ ,  $\eta^2 = .188$ ). We detected 32.3% responders in women (all using OCs; 67.7% non-responders) and 77.8% responders in men (22.2% non-responders). Moreover, the timing of the individual cortisol peak was not significantly different between women and men ( $\chi^2(2) = 1.021$ ,  $p = .600$ ).

[Figure 2 about here]

Consistent to our previous analysis (Henze et al., 2020), we found significant main effects for *time* in affect measures and mean heart rate levels ( $ps \leq .001$ ,  $\eta^2 > .299$ ). Positive affect scores decreased and negative affect scores increased during ScanSTRESS. Participants showed elevated heart rates during the stress blocks compared to the control blocks in both runs. We detected neither significant interactions of *time* x *sex* nor main effects *sex* regarding affect or heart rate measures ( $ps \geq .153$ ,  $\eta^2 < .020$ ). Given the absence of sex differences in affect and heart rate reactions, no sex-specific associations with neural responses were analyzed.

### 3.2 Sex-specific associations of cortisol and neural responses

A whole-brain unpaired two-group difference analysis revealed no significant sex-specific cluster for activations (stress > control) nor deactivations (control > stress); two-tailed combined FWE-corrected  $p < .05$ . However, when cortisol increases were used as covariate in a whole-brain unpaired two-group difference analysis with continuous covariate interaction (grand mean centered, two-tailed combined FWE-corrected  $p < .05$ ), a sex-specific cluster reached significance (see Figure 3). In detail, we detected a sex-specific relationship between cortisol increases and neural responses within a cluster comprising the bilateral hippocampus, parahippocampal gyrus, (anterior and posterior) cingulate cortex, thalamus, and ncl. caudatus. When men were compared to women in the total sample, higher cortisol increases were found to be related to more activation within this cluster (see Figure 3A). In the female subsample alone, higher cortisol increases were associated with more deactivation in cingulate cortex, thalamus, and ncl. caudatus (see Figure 3B), while in the male subsample higher cortisol increases were associated with more activation in hippocampus, parahippocampal gyrus, amygdala, cingulate cortex, prefrontal areas, ncl. caudatus, and ncl. accumbens (see Figure 3C). Peak voxels within the cluster are reported in the Supplemental Results Tables B.2-B.4.

[Figure 3 about here]

Table 1 depicts the results from *post hoc* ROI-analyses including uncorrected *p*-values and Benjamini-Hochberg corrected significance thresholds. We found significant interactions of *sex x cortisol increase* for amygdala, ncl. caudatus, and ncl. accumbens. Men showed positive associations between beta values and cortisol increases while women showed negative associations (see Figure 4). Main effects of *sex* were found for hippocampus, parahippocampal gyrus, amygdala, cingulate cortex, ncl. caudatus, ncl. accumbens, and putamen. *Post hoc* analyses in the two subsamples separately are displayed in Table 2. In women, we found negative associations between cortisol increases and beta values for thalamus and ncl. caudatus. In men, positive associations emerged for hippocampus, parahippocampal gyrus, amygdala, cingulate cortex, ncl. caudatus, and ncl. accumbens. It should be noted that these correlations remained significant after exploratory exclusion of a male participant showing a pronounced (but endocrinologically plausible) cortisol increase of 22.36 nmol/l (see Figure 4).

[Table 1 about here]

[Figure 4 about here]

[Table 2 about here]

### **3.3 Explorative analysis of sex differences in exposure-time effects**

As the research questions of the present study arose from findings of our aforementioned study (Henze et al., 2020), we also addressed the question if women and men show distinct neural reactions in response to the two runs of ScanSTRESS. A whole-brain two-way mixed

effects ANOVA (two groups, two runs per subject,  $z > 3.1$ , FWE-corrected  $p < .05$ ) did not reveal a significant *run* x *group* interaction. We found similar clusters in women and men comprising hippocampus, parahippocampal gyrus, amygdala, prefrontal cortex, and cingulate cortex to respond differently to the two runs. Figure 5 illustrates these activation changes in women and men; peak voxels are reported in the Supplemental Results Tables B.5 and B.6. Consistent to our previous analysis (Henze et al., 2020), *post hoc* ROI-analyses (repeated measures ANOVAs, *run* as within-subjects factor, *sex* as between-subjects factor) revealed main effects of *run* for hippocampus, parahippocampal gyrus, and amygdala ( $ps \leq .001$ ,  $\eta^2 > .160$ ). While we did not find significant interactions of *run* x *sex* ( $ps \geq .367$ ,  $\eta^2 < .014$ ), for thalamus, a significant main effect *sex* ( $F_{1,61} = 5.22$ ,  $p = .026$ ,  $\eta^2 = .079$ ) was detected, indicating mean response differences of women and men in the first run (women:  $M = -.01$ ,  $SD = .22$ ; men:  $M = -.10$ ,  $SD = .26$ ) compared to the second run (women:  $M = .01$ ,  $SD = .21$ ; men:  $M = -.15$ ,  $SD = .27$ ).

[Figure 5 about here]

## 4. Discussion

### 4.1 Sex-specific associations of cortisol and neural responses

The present data confirmed the well-known sex-specific cortisol stress response pattern, with men exhibiting higher responses than women (Goel et al., 2014; Kudielka & Kirschbaum, 2005; Liu et al., 2017; Nicolson et al., 1997; Seeman et al., 2001; Zänkert et al., 2019). Higher cortisol levels in men occurred already 75 minutes prior to stress onset, suggesting a more pronounced anticipation response (Kirschbaum et al., 1992; Kudielka & Kirschbaum, 2005). After the relaxation phase, women and men reached similar mean levels (see Figure 2). It should be noted again that all female participants used OCs and that women tested in the luteal phase of their menstrual cycles were repeatedly found to show higher cortisol

responses to stress (Kirschbaum et al., 1999; Rohleder et al., 2003; Rohleder et al., 2001; Uhart et al., 2006; Wolf et al., 2001). In addition, it appears likely that the block design of ScanSTRESS, with frequent interruptions of the stress induction by control blocks, might have hampered even higher cortisol responses. However, not using an alternating block design would cause other fMRI-related problems (e.g., limited detection power) (Noack et al., 2019; Quaedflieg et al., 2013; Sandner et al., 2020).

While empirical evidence for consistent interactions of distinct stress domains remains scarce (Campbell & Ehlert, 2012; Cohen et al., 2000; Henze et al., 2020), this is the first study focusing on sex-related differences between associations of cortisol and task-related neural responses to psychosocial stress. Whereas previous findings on sex-specific neural stress responses suggest pronounced striato-limbic activation in women and stronger frontal activation in men (Goldfarb et al., 2019; Kogler et al., 2015; Seo et al., 2017; Seo et al., 2011; Wang et al., 2007), our data revealed no such clear functional distinction for particular neural structures. Instead, we found different associations for women and men between cortisol reactions and responses of identical striato-limbic structures. A whole-brain analysis in the total sample documented higher cortisol increases in men to be associated with more activation in hippocampus, parahippocampal gyrus, cingulate cortex, thalamus, and ncl. caudatus compared to women. In the female subsample, higher cortisol increases were related to deactivations in these structures whereas in the male subsample, higher cortisol increases were related to activations. In contrast to previous findings, proposing a small degree of overlap between the stress networks of women and men (Kogler et al., 2017; Seo et al., 2017; Seo et al., 2011; Wang et al., 2007), our data corroborate differential responses for women and men in identical structures (Goldfarb et al., 2019; Kogler et al., 2016). ROI-analyses confirmed interactions of *sex x cortisol increase* for amygdala, ncl. caudatus, and ncl. accumbens, underpinning positive associations in men and negative associations in women, with the latter describing lower cortisol increases to be associated with more activation. Thus far, only resting state data exists, including two male-only samples (Veer et

al., 2011; Vogel et al., 2015) and one mixed sample (Kogler et al., 2016), reporting a positive effect pattern for men and a negative one for women.

The only structure that reached significance in whole-brain as well as every ROI-based correlation analysis, also in both subsamples separately, is the ncl. caudatus. As part of the striatum, this area showed sex-specific FC patterns partly depending on menstrual cycle phase (Hidalgo-Lopez et al., 2020; Yoest et al., 2018). Moreover, evidence for larger gray matter volumes in female brains within this structure exists (Luders et al., 2009). Another stress-relevant area within the striatum is the ncl. accumbens (McEwen et al., 2016). As reward-related area, an expressed desire of revenge in men was found to be correlated with increased activity while in women this was associated with deactivations (Dumais et al., 2018; Singer et al., 2006). In this context, two aspects should be considered: first, when applying ScanSTRESS, participants are instructed to show maximal effort, consistent with the cover story that the study aims at investigating brain activations during maximal mental performance. Second, after the first run of ScanSTRESS, participants are exposed to a standardized negative feedback regarding their performance combined with the urgent request to try harder. Therefore, these factors of psychosocial stress might have led to pronounced reactions of the ncl. accumbens in particular and to an overall striatal response. Moreover, another ScanSTRESS study that found associations between striatal activation and perceived group discrimination in ethnic minority individuals strengthens the evidence for an involvement of the striatum and inherent structures (Akdeniz et al., 2014). While this view is also supported by a recent study that used a psychosocial stress paradigm (Kogler et al., 2015), another study did not report sex differences in putamen responses during stress perception (Wang et al., 2007). Therefore, it is tempting to speculate that the striatal network modulates sex-specific interactions in response to the repeated experience of failure and social-evaluative threat as induced by ScanSTRESS. The finding of altered left amygdala FC to striatal regions correlating positively with cortisol in men but negatively in women (Kogler et al., 2016) emphasizes this hypothesis. Moreover, a previously reported analysis of our present data on the association between cortisol and neural stress responses, independent



of sex, revealed no significance for striatal structures (Henze et al., 2020). This is consistent with the assumption of a sex-specific modulating striatal effect on stress responses.

Among others, the hippocampus has been considered as decisive HPA axis related structure ever since (Herman et al., 2005; Hermans et al., 2014; Jankord & Herman, 2008) and one of the most prominent findings describes deactivations in response to psychosocial stress along with negative associations with cortisol (Pruessner et al., 2008). However, data exists showing the opposite (Henze et al., 2020; Noack et al., 2019). Here, we found a positive correlation of activations and cortisol in men confirming sex-related differences after stress induction for the hippocampus (Seo et al., 2011; Yagi & Galea, 2019). Moreover, our data showed a comparable pattern regarding the parahippocampal gyrus for men, while in women no significant association with cortisol emerged for hippocampus and parahippocampal gyrus, respectively.

Concerning the amygdala and cingulate cortex, the dissociation between women and men is more obvious. Especially for the amygdala, its activating impact on HPA axis responses to stress has been reported frequently (Henze et al., 2020; Herman et al., 2005; Jankord & Herman, 2008; Noack et al., 2019). Previous work showed an association between stress and amygdala activation only in women (Kogler et al., 2015; Wang et al., 2007). We found that activations were associated with higher cortisol increases in men and lower values in women, confirming a sex-specific effect pattern regarding amygdala FC in association with cortisol (Kogler et al., 2016). Moreover, this study also revealed a negative association of altered left amygdala FC to the anterior cingulate cortex in women while the opposite was reported in a male-only sample (Veer et al., 2012).

A negative association with cortisol was found for the thalamus in women while in men no significant relationship emerged, confirming previous findings (Wang et al., 2007). The thalamus is thought to actively and dynamically gate salient inputs, minimizing the importance of currently irrelevant ones (Wolff & Vann, 2019). A recent study showed altered thalamic network centrality in response to acute psychosocial stress within a male-only

sample (Reinelt et al., 2019). Moreover, previous studies have shown stress-driven changes in thalamic activation in women and men (Noack et al., 2019). With reference to the aforementioned hypothesis on striatal involvement in sex-specific cortisol responses, the thalamus as adjacent structure may act as additional coordinator. Nevertheless, there exists just as much evidence for pronounced thalamo-striatal stress reactions in women (Wang et al., 2007) as in men (Seo et al., 2017; Seo et al., 2011).

In sum, we found a dissociation between women and men regarding the association between neural and cortisol responses to acute psychosocial stress induction. Currently, it can only be speculated why these associations point in opposite directions. It appears conceivable that our results are consistent with the idea that sex-specific differences in the brain may indicate compensation mechanisms aimed at maintaining comparable abilities or preventing maladaptive differences (Grabowska, 2017). Hence, men might show a positive association between cortisol and striato-limbic responses and women a negative association to maintain similar and adaptive outcomes, e.g., comparable subjective and heart rate responses.

#### **4.2 Explorative analysis of sex differences in exposure-time effects**

Although there is evidence that women and men respond differently to ongoing stress exposure (Goldfarb et al., 2019; McEwen & Milner, 2017), we did not detect any significant sex-specific changes when comparing responses of the first with those of the second run of ScanSTRESS. While increasing deactivations emerged for both subsamples, our results may, on a descriptive level, suggest different extents for women and men regarding the targeted clusters (see Figure 5).

#### **4.3 Heart rate and psychological responses**

As previously reported (Henze et al., 2020), we found a significant decline in positive affect ratings and an increase in reported negative affect. However, we did not detect significant sex differences. The fact that other studies reported different affect responses in women and

men may be explainable by different affect measurements (e.g., visual analogue scales) (Goldfarb et al., 2019; Kelly et al., 2006; Kelly et al., 2008). Moreover, women and men exhibited similar heart rate responses during stress. In this regard, again, the composition of the present sample has to be considered. Earlier research supports the idea of a pronounced impact of menstrual cycle phases and/or OC-use on the presence or absence of sex differences (Hidalgo-Lopez et al., 2020; Sharma et al., 2020; Yoest et al., 2018), especially regarding psychological measures (Albert et al., 2015; Childs et al., 2010; Lewis et al., 2019). Moreover, we generally assume that basic characteristics of an fMRI block design – as aforementioned – interfere with even more pronounced responses. Hence, the overall lower stress intensity, achievable by scanner paradigms compared to laboratory stressors, has to be considered. Furthermore, the absence of sex differences in a particular outcome should not lead to the misconception that the neural substrates underlying these mechanisms are necessarily identical for women and men (Cahill, 2006; Goldfarb et al., 2019).

## **5. Limitations and Conclusion**

First, we have to acknowledge a certain limitation of the generalizability of our findings, as only university students participated in the present study. Nevertheless, our data suggest sex-specific cortisol reactions to be differentially associated with striato-limbic responses to psychosocial stress (Seo et al., 2017). From a general perspective, we assume that detectable differences in stress responses between women and men are only partly due to biological sex (Juster et al., 2016; Rich-Edwards et al., 2018). It might be a fruitful approach to explicitly take sociocultural gender into account in future studies. Moreover, as our study sample included only OC-taking women, we have to emphasize that the present data may only contribute to a better understanding of differences in the association of neural stress responses and cortisol increases of women taking OCs versus men. It could well be appropriate to limit our conclusions to the (large) subgroup of women taking hormonal contraceptives as it was previously found in female-only studies that OC-use and menstrual cycle phase can influence the brain's response to negative stimuli (Goldstein et al., 2010;

Petersen & Cahill, 2015) and psychosocial stress (Albert et al., 2015; Chung et al., 2016b). Furthermore, at least cortisol increases are known to be modulated not only by OCs but also by menstrual cycle phases (Kirschbaum et al., 1999; Zänkert et al., 2019). However, as simple group-level analyses contrasting female and male neural responses to psychosocial stress paradigms failed to reveal consistent differences (in the present as well as in previous studies: Chung et al. (2016a); Dahm et al. (2017); Kogler et al. (2015); Kogler et al. (2017)) it appears unlikely that corresponding differences between OC-taking and naturally cycling women are extremely large. To date, studies on the impact of sex on the interaction between cortisol and neural stress responses in OC-taking women, women in luteal, and follicular phase as well as in men do not exist.

Even though women and men differ in their overall stress reactivity and regarding prevalence rates of certain stress-related pathologies, our findings do not support the view of a clear neuroanatomically differentiable 'female-typical' and 'male-typical' response to stress. Instead, our data provide further evidence for the idea that considering complex interactions and quantitative variables like individual cortisol increases is a more suitable approach to elucidate sex-related differences in central stress regulation (Shalev et al., 2020).

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**CRedit authorship contribution statement**

**Gina-Isabelle Henze:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – Original Draft, Visualization, Funding acquisition. **Julian Konzok:** Investigation, Writing – Review & Editing. **Ludwig Kreuzpointner:** Software, Writing – Review & Editing. **Christoph Bärtl:** Software, Writing – Review & Editing. **Marina Giglberger:** Investigation, Writing – Review & Editing. **Hannah Peter:** Investigation, Writing – Review & Editing. **Fabian Streit:** Writing – Review & Editing, Funding acquisition. **Brigitte M. Kudielka:** Resources, Writing – Review & Editing, Funding acquisition. **Peter Kirsch:** Writing – Review & Editing, Supervision, Funding acquisition. **Stefan Wüst:** Resources, Writing – Original Draft, Supervision, Project administration, Funding acquisition.

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**Declaration of Interest:**

The authors report no biomedical financial interests or potential conflicts of interest.

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## Figure Legends

Figure 1.

Experimental procedure including the repeated collection of cortisol samples, affect ratings, and heart rate measurements during ScanSTRESS.

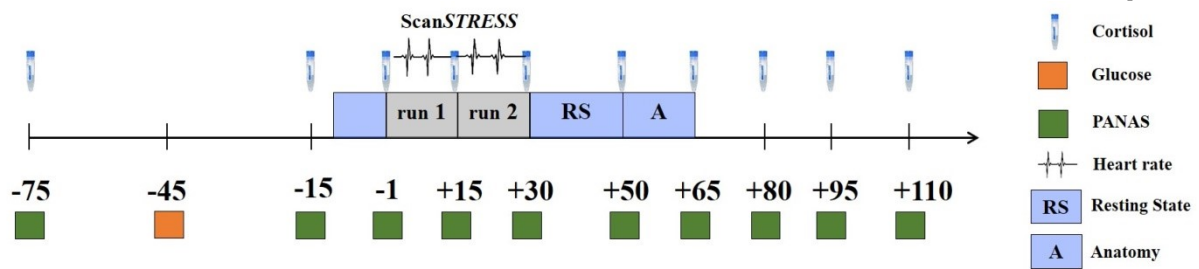
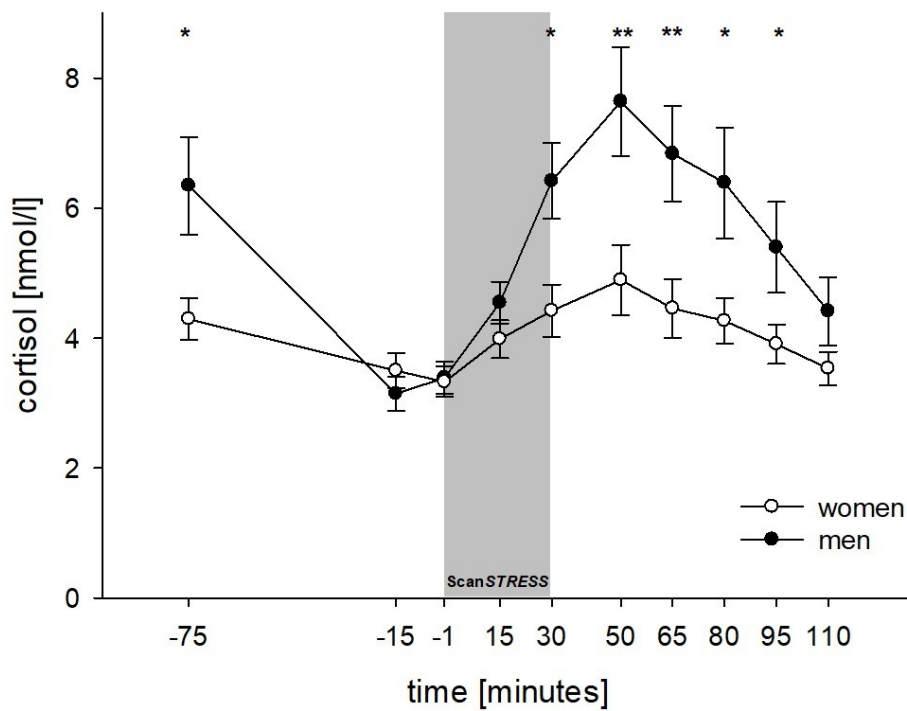


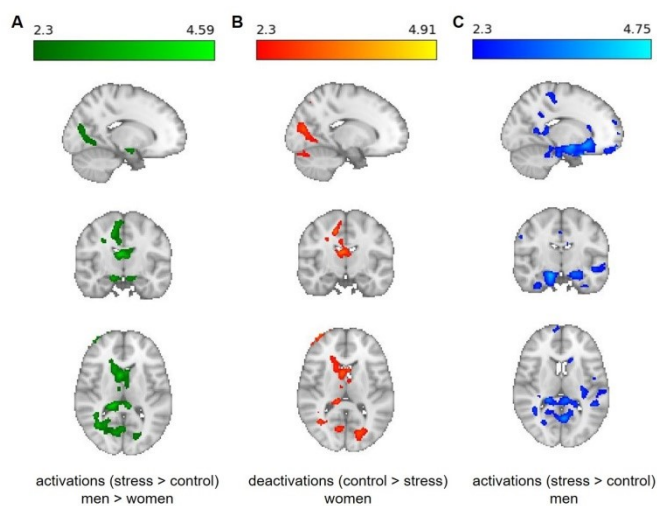
Figure 2

Salivary cortisol responses to ScanSTRESS in women and men ( $\pm$  SEM). \*\*  $p \leq .01$  and \*  $p \leq .05$  indicate significant results of *post hoc* unpaired *t*-tests for each time point.

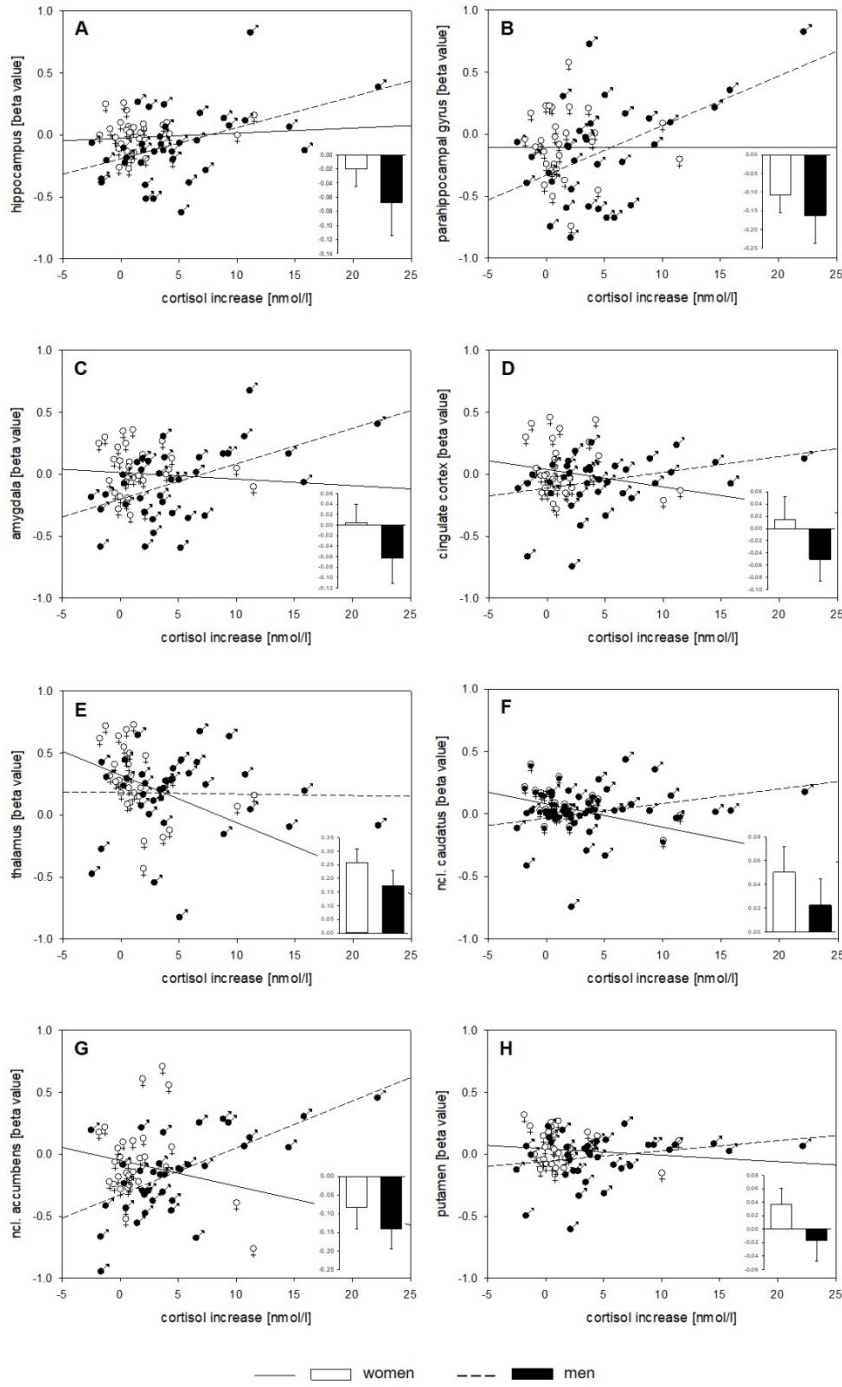


**Figure 3.**

(A) Sex-specific cluster in an unpaired two-group difference analysis with continuous covariate interaction (grand mean centered, two-tailed combined FWE-corrected  $p < .05$ ) describing a sex-specific relationship (men > women) between cortisol increases and neural responses (stress > control) in the hippocampus, parahippocampal gyrus, cingulate cortex, thalamus, and ncl. caudatus. (B) In women, higher cortisol increases were associated with more deactivation (control > stress) within cluster including the cingulate cortex, thalamus, and ncl. caudatus. (C) In men, higher cortisol increases were associated with more activation (stress > control) in a cluster comprising the hippocampus, parahippocampal gyrus, amygdala, cingulate cortex, prefrontal areas, ncl. caudatus, and ncl. accumbens.

**Figure 4.**

Sex-specific mean neural responses ( $\pm$  SEM, stress > control) and correlations of cortisol increase with beta values of the main task effect stress > control in the (A) hippocampus, (B) parahippocampal gyrus, (C) amygdala, (D) cingulate cortex, (E) thalamus, (F) ncl. caudatus, (G) ncl. accumbens, and (H) putamen derived from masks using the Harvard Oxford Atlas.

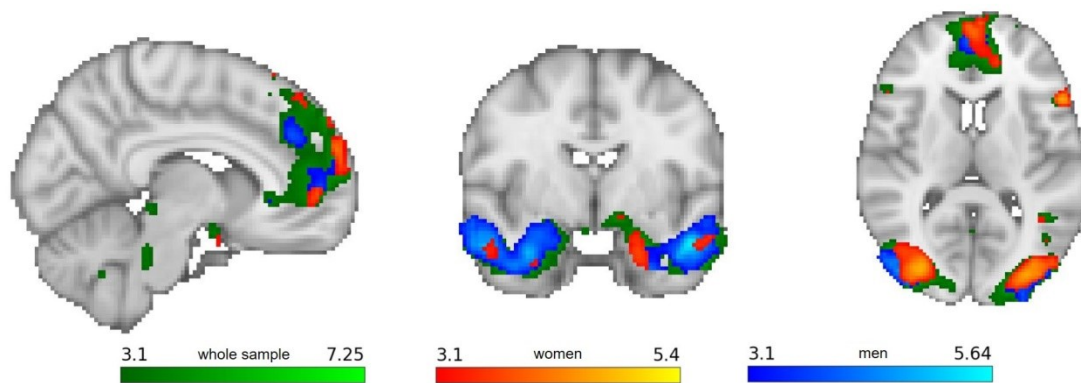


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**Figure 5.**

Activation changes over the two runs of ScanSTRESS of the female (red to yellow) and male (blue to light blue) subsample compared to the total sample as reference [green to light green; (Henze et al., 2020)].



## Table Legends

Table 1.

Results from *post hoc* one-way analyses of variances with sex as fixed factor and cortisol increase as covariate for the hippocampus, parahippocampal gyrus, amygdala, cingulate cortex, thalamus, ncl. caudatus, ncl. accumbens, and putamen including uncorrected *p*-values and Benjamini-Hochberg corrected significance thresholds ( $n = 8$  ROIs). \* indicate significant results ( $p \leq .05$ ) and values printed in boldface indicate significant results after Benjamini-Hochberg correction.

Region of Interest	Effect	Uncorrected Values				Corrected significance threshold
		df	F	<i>p</i> -Value	$\eta^2$	
Hippocampus	<i>sex x cortisol increase</i>	1, 63	2.04	.158	.031	$\leq .04375$
	<i>sex</i>	<b>1, 63</b>	<b>6.57</b>	<b>.013*</b>	<b>.094</b>	$\leq .01875$
	<i>cortisol increase</i>	1, 63	3.93	.052	.059	$\leq .00625$
Parahippocampal Gyrus	<i>sex x cortisol increase</i>	1, 63	3.10	.084	.049	$\leq .03125$
	<i>sex</i>	<b>1, 63</b>	<b>4.82</b>	<b>.032*</b>	<b>.074</b>	$\leq .0375$
	<i>cortisol increase</i>	1, 63	3.01	.088	.048	$\leq .01875$
Amygdala	<i>sex x cortisol increase</i>	1, 63	4.42	.039*	.066	$\leq .01875$
	<i>sex</i>	<b>1, 63</b>	<b>9.46</b>	<b>.003*</b>	<b>.131</b>	$\leq .0125$
	<i>cortisol increase</i>	1, 63	2.10	.152	.032	$\leq .025$
Cingulate Cortex	<i>sex x cortisol increase</i>	1, 63	3.35	.072	.051	$\leq .025$
	<i>sex</i>	<b>1, 63</b>	<b>5.52</b>	<b>.022*</b>	<b>.081</b>	$\leq .025$

		<b>63</b>					
	<i>cortisol increase</i>	1,	0.01	.924	.000	≤ .05	
		<b>63</b>					
Thalamus	<i>sex x cortisol increase</i>	1,	2.98	.089	.046	≤ .0375	
		<b>63</b>					
	<i>sex</i>	1,	2.19	.144	.034	≤ .05	
		<b>63</b>					
	<i>cortisol increase</i>	1,	3.38	.071	.052	≤ .0125	
		<b>63</b>					
Ncl. Caudatus	<i>sex x cortisol increase</i>	1,	6.55	.013*	.094	≤ .0125	
		<b>63</b>					
	<i>sex</i>	<b>1,</b>	<b>4.96</b>	<b>.030*</b>	<b>.073</b>	≤ .03125	
		<b>63</b>					
	<i>cortisol increase</i>	1,	0.35	.557	.005	≤ .0375	
		<b>63</b>					
Ncl. Accumbens	<i>sex x cortisol increase</i>	<b>1,</b>	<b>8.66</b>	<b>.005*</b>	<b>.121</b>	≤ .00625	
		<b>63</b>					
	<i>sex</i>	<b>1,</b>	<b>10.12</b>	<b>.002*</b>	<b>.138</b>	≤ .00625	
		<b>63</b>					
	<i>cortisol increase</i>	1,	.075	.391	.012	≤ .03125	
		<b>63</b>					
Putamen	<i>sex x cortisol increase</i>	1,	1.41	.240	.022	≤ .05	
		<b>63</b>					
	<i>sex</i>	1,	4.17	.045*	.062	≤ .04375	
		<b>63</b>					
	<i>cortisol increase</i>	1,	0.07	.798	.001	≤ .04375	
		<b>63</b>					

**Table 2.**

Results from *post hoc* correlation analyses between mean beta values of the hippocampus, parahippocampal gyrus, amygdala, cingulate cortex, thalamus, ncl. caudatus, ncl. accumbens, and putamen with *cortisol increase* in the two subsamples separately including uncorrected *p*-values and Benjamini-Hochberg corrected significance thresholds ( $n = 8$  ROIs). \* indicate significant results ( $p \leq .05$ ) and values printed in boldface indicate significant results after Benjamini-Hochberg correction.

Region of Interest	Correlation with <i>cortisol</i> increase				
	Women (n = 31)			Men (n = 36)	
	Uncorrected Values	Corrected significance threshold		Uncorrected Values	Corrected significance threshold
	<i>r</i>	<i>p</i> -Value		<i>r</i>	<i>p</i> -Value
Hippocampus	.087	.321	≤ .0375	<b>.466</b>	<b>.002*</b> ≤ .025
Parahippocampal Gyrus	-.003	.494	≤ .05	<b>.510</b>	<b>.001*</b> ≤ .0125
Amygdala	-.079	.336	≤ .04375	<b>.516</b>	<b>.001*</b> ≤ .0125
Cingulate Cortex	-.196	.145	≤ .01875	.311	.032* ≤ .03125
Thalamus	-.395	.014*	≤ .0125	-.020	.456 ≤ .05
Ncl. Caudatus	<b>-.449</b>	<b>.006*</b>	≤ .00625	.290	.043* ≤ .0375
Ncl. Accumbens	-.193	.149	≤ .025	<b>.621</b>	<b>.001*</b> ≤ .00625
Putamen	-.116	.266	≤ .03125	.234	.085 ≤ .04375

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