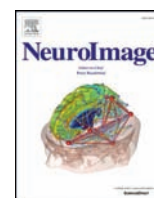


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Sex differences in the functional connectivity of the amygdalae in association with cortisol



Lydia Kogler^{a,h,i,*}, Veronika I. Müller^{b,c}, Eva-Maria Seidel^d, Roland Boubela^{e,f}, Klaudius Kalcher^{e,f}, Ewald Moser^{e,f,g}, Ute Habel^{h,i,j,k}, Ruben C. Gur^g, Simon B. Eickhoff^{b,c}, Birgit Derntl^{a,b,h,i}

^a Department of Psychiatry and Psychotherapy, Medical School, University of Tübingen, Germany

^b Institute of Neuroscience und Medicine, INM-1, Research Centre Jülich, Jülich, Germany

^c Institute of Clinical Neuroscience and Medical Psychology, Medical Faculty, Heinrich Heine University, Düsseldorf, Germany

^d Department of Basic Psychological Research and Research Methods, Faculty of Psychology, University of Vienna, Vienna, Austria

^e MR Centre of Excellence, Medical University of Vienna, Vienna, Austria

^f Centre for Medical Physics and Biomedical Engineering, Medical University, Vienna, Austria

^g Neuropsychiatry Division, Department of Psychiatry, Medical School, University of Pennsylvania, Philadelphia, USA

^h Jülich-Aachen-Research Alliance, Translational Brain Medicine, Germany

ⁱ Department of Psychiatry, Psychotherapy and Psychosomatics, RWTH Aachen University, Aachen, Germany

^j Institute of Neuroscience und Medicine, INM-6, Research Centre Jülich, Research Centre Jülich, Jülich, Germany

^k JARA BRAIN Institute 1: Structure Function Relationship.

ARTICLE INFO

Article history:

Received 27 October 2015

Revised 24 March 2016

Accepted 24 March 2016

Available online 01 April 2016

Keywords:

Task-independent

Resting-state

Cortisol

Striatum

Hippocampus

Frontal cortex

ABSTRACT

Human amygdalae are involved in various behavioral functions such as affective and stress processing. For these behavioral functions, as well as for psychophysiological arousal including cortisol release, sex differences are reported.

Here, we assessed cortisol levels and resting-state functional connectivity (rsFC) of left and right amygdalae in 81 healthy participants (42 women) to investigate potential modulation of amygdala rsFC by sex and cortisol concentration.

Our analyses revealed that rsFC of the left amygdala significantly differed between women and men: Women showed stronger rsFC than men between the left amygdala and left middle temporal gyrus, inferior frontal gyrus, postcentral gyrus and hippocampus, regions involved in face processing, inner-speech, fear and pain processing. No stronger connections were detected for men and no sex difference emerged for right amygdala rsFC. Also, an interaction of sex and cortisol appeared: In women, cortisol was negatively associated with rsFC of the amygdalae with striatal regions, mid-orbital frontal gyrus, anterior cingulate gyrus, middle and superior frontal gyri, supplementary motor area and the parietal–occipital sulcus. Contrarily in men, positive associations of cortisol with rsFC of the left amygdala and these structures were observed. Functional decoding analyses revealed an association of the amygdalae and these regions with emotion, reward and memory processing, as well as action execution.

Our results suggest that functional connectivity of the amygdalae as well as the regulatory effect of cortisol on brain networks differs between women and men. These sex-differences and the mediating and sex-dependent effect of cortisol on brain communication systems should be taken into account in affective and stress-related neuroimaging research. Thus, more studies including both sexes are required.

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Introduction

The amygdalae are involved in a wide range of different functions such as affective (Derntl et al., 2008; Goldin et al., 2009; Ochsner et al., 2004) and stress processing (Veer et al., 2011). The amygdalae contain corticoid receptors that may be activated by cortisol (De Kloet et al.,

1998), a steroid hormone involved in affective and stress-related behavior. It is expressed by the adrenal glands following stressful or arousing situations (Dickerson and Kemeny, 2004) and cortisol patterns are associated with amygdala activity, e.g. while regulating emotions (Urry et al., 2006).

Sex differences have been reported during stress and affective processing on behavioral, physiological as well as neural levels: Women tend to report more frequent and more intense emotions (Grossman and Wood, 1993), report to be more stressed in self-reports

* Corresponding author at: Calwerstr. 14, 72076 Tübingen, Germany.
E-mail address: lydia.kogler@med.uni-tuebingen.de (L. Kogler).

(Kudielka et al., 1998) and show more facial expressions to emotional movies (Kring and Gordon, 1998). Compared to women, men usually show higher cortisol reactions to stress situations, which seem to decrease the recall of unpleasant emotional memory (Buchanan and Tranel, 2008; Kajantie and Phillips, 2006; Kirschbaum et al., 1992). These reports point to divergent patterns in subjective and physiological stress reactions in women and men. BOLD-based amygdala activity also reveals specific patterns in women and in men during affective (Derntl et al., 2009; Domes et al., 2010; McRae et al., 2008; Stevens and Hamann, 2012) and stress (Kogler et al., 2015a) reactions. Women show a stronger amygdala response to negative emotions (Stevens and Hamann, 2012) and stress processing (Kogler et al., 2015a), while in men amygdala activity is increased when processing positive emotions (Stevens and Hamann, 2012). Thus for amygdala activity as well as for cortisol release and their related behavioral domains, sex differences are reported.

However, brain regions, and therefore also the amygdala, do not perform in isolation. Recently, the communication and interaction between them and anatomically separated neural regions came into focus of affective and stress-related neuroimaging research. Temporally dependent activation patterns in spontaneous activity of two different areas might indicate an exchange of information between functionally linked neural regions (Eickhoff and Müller, 2015; Friston et al., 1993). The amygdala interacts with multiple brain regions involved in the regulation of social, affective and stress-related information (Robinson et al., 2011; Roy et al., 2010; Stein et al., 2007), including lateral and medial frontal areas, posterior cingulate cortex, hippocampus, hypothalamus and brainstem regions. These regions also express corticoid receptors (Diorio et al., 1993; Ulrich-Lai and Herman, 2009) and hormones freely fluctuating throughout the brain potentially contribute to connectivity between regions not anatomically connected. Indeed, cortisol has been shown to modify the functional connectivity of the amygdala (Henckens et al., 2010; Vaisvaser et al., 2013; Veer et al., 2012; Vogel et al., 2015). During emotional tasks, exogenous cortisol strengthens the connectivity between the amygdala and the medial prefrontal cortex (Henckens et al., 2010) and a stress-induced increase in connectivity between the amygdala and the striatum is dependent on the availability of corticoid receptors (Vogel et al., 2015). During resting-state, a positive correlation between endogenous cortisol and the coupling of the amygdala with the medial prefrontal cortex as well as with the anterior cingulate cortex (ACC) (Veer et al., 2012) emerged. Moreover, a negative correlation between cortisol levels and the coupling of the amygdala with hippocampus (Vaisvaser et al., 2013) has been reported. Thus, the steroid hormone and its receptor's distribution seem to modify the functional connectivity of the amygdala, and may play a mediating effect in building functional connections between brain regions. However, so far these reports are based on male-only samples (Henckens et al., 2010; Vaisvaser et al., 2013; Veer et al., 2012; Vogel et al., 2015), while specific effects of cortisol in women and possible interactions with sex differences are still lacking.

There is some evidence that the amygdalae show sex specific connectivity patterns, with higher local connectivity in women than in men (Lopez-Larson et al., 2011), and sex differences in resting-state functional connectivity (rsFC) appearing during development in adolescence (Alarcón et al., 2015). One recent study reports sex differences in rsFC of subnuclei of the amygdala in healthy adults, with higher connectivity of the basolateral amygdala to lateral-frontal and striatal regions in women and to medial-frontal regions in men (Engman et al., 2016). Thus, rsFC of amygdala subnuclei with other brain regions involved in stress and emotion processing seems to be sex-specific.

Evidence has accumulated supporting the view that sex differences exist concerning behavioral domains and (stress-related) cortisol release. Therefore, it would be of interest to assess amygdala's rsFC in association with cortisol to determine general and sex-specific neural patterns underlying these phenomena. As shown previously, the connectivity between the amygdala and the ACC may be mediated by

cortisol (Veer et al., 2012). Cortisol is thought to regulate neural network responses to adapt behavior to arousing situations (Henckens et al., 2012; Kloet et al., 2005) and sex differences in cortisol stress response (Kajantie and Phillips, 2006; Kirschbaum et al., 1992) suggest that these regulatory mechanisms may differ in women and men. Thus, the inhibitory effect of the ACC on the amygdala (Stein et al., 2007) may be influenced by cortisol and therefore depend on sex.

It is so far unknown, whether the same association appears in women and men or whether specific mediating effects on amygdala connectivity contribute to sex differences during negative emotional or stress related processing. Thus, differences in rsFC of the amygdala in association with cortisol may underlie reported sex differences in behavioral domains (Kogler et al., 2015a; Stevens and Hamann, 2012).

Notably, no direct comparison of the functional connectivity of the amygdala and its relationship to the steroid hormone in women and men has been published. However, the amygdala's strong involvement in modulating affective and stress-related functions, its association with cortisol and previous reports of sex differences in rsFC of the amygdala warrant further investigation. Therefore, the aims of the current study are 1) to assess and analyze rsFC of the amygdala in women and men; and 2) to investigate whether women and men express different patterns of amygdala rsFC in association with cortisol. Based on previous findings, we hypothesize that 1) women and men differ in rsFC of the amygdala: Given increased amygdala activity during negative affective and stress related processing in females (Stevens and Hamann, 2012), we assume that women compared to men show higher connectivity of the amygdala with other limbic regions associated with affective and stress processing. 2) Furthermore, we hypothesize that higher cortisol levels are associated with increased connectivity of the amygdala with medial and ventrolateral prefrontal regions in men (Henckens et al., 2010; Veer et al., 2012). Due to the lack of studies on the impact of cortisol on the functional connectivity of the amygdala in women, and based on reports of sex differences in behavior, psychophysiology and amygdala activity, however, we can only assume sex differences in the association of cortisol and amygdala rsFC.

Material and methods

Sample

Resting-state data and anatomical scans were obtained in ninety-four right-handed non-smoking university students. All participants were screened for the following exclusion criteria: history of neurological and psychiatric disorders, chronic illnesses, drug or hormone intake, night shift working, competitive sports, recent or current pregnancy, premenstrual dysphoric disorder, allergic asthma and the common factors of MR-incompatibility. Only naturally cycling women without intake of oral contraception were included.

Participants were asked to refrain from exercise or alcohol consumption for 24 h prior to the session, medication, caffeine and drug intake on the test day, and food or drinks other than water for two hours before the session. The measurement sessions took place at the MR Center of Excellence at the Medical University of Vienna, Vienna, Austria, and were scheduled between 2:30 pm and 5:30 pm. Data assessment in all subjects was scheduled in the afternoon to control for circadian cortisol rhythm. Upon arrival, participants received detailed instructions, gave subjective mood ratings (positive and negative affect scale, PANAS) (Watson et al., 1988) and provided saliva samples for hormone analyses (approx. 15 min after arrival).

Written informed consent was obtained from all subjects. The study was approved by the local Institutional Review Board (Ethik Kommission, Medizinische Universität Wien).

Due to missing hormone data ($n = 1$), outliers in hormone data (mean \pm 2 standard deviations; $n = 8$), sickness (1 subject vomited in the scanner) and as a result of scanner movement matching between the groups ($n = 3$; groups were matched for the following scanner

movement parameters: FD, DVARS, RMS (Power et al., 2012; Satterthwaite et al., 2013)), in total thirteen subjects had to be excluded. Women and men did not differ regarding the three movement parameters (all p s > 0.689). Furthermore, no significant correlation between cortisol and any of the movement parameter emerged (all p s > 0.504).

The final sample consisted of eighty-one participants (42 women; see Table 1 for sample description).

Saliva samples

To obtain hormone concentration, saliva samples were obtained and stored at $-20\text{ }^{\circ}\text{C}$ at least until shipping to the analysis laboratory (SwissHealthMed, Aying, Germany), where they were frozen at $-20\text{ }^{\circ}\text{C}$ over-night, then thawed and centrifuged. Competitive luminescence immunoassay kits (LUMI) were used to obtain cortisol concentrations. These kits have minimal cross reactivity to other steroid-hormones and achieved reliable measurements (cortisol: intra-assay CV < 4% and inter-assay CV < 5%). Cortisol data were normalized using a log transformation ($y = \log_{10}(x + 1)$) prior to statistical analyses.

Data and statistical analysis of behavioral and hormone data

Statistical analyses of sex differences in demographic and cortisol data were conducted using IBM SPSS statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). In cases where assumptions for parametric testing did not apply, non-parametric tests were performed (see Table 1, “negative affect”). The level of significance was set at $p < .05$ for all tests.

Resting-state functional connectivity analysis

Acquisition and preprocessing

Images were acquired on a 3T TIM Trio scanner (Siemens Medical Systems, Erlangen, Germany) using BOLD contrast (gradient-echo EPI-sequence with distortion correction; 23 interleaved slices, TE/TR = 38/1800 ms, voxel size $1.5 \times 1.5 \times 3$ mm, 90° flip angle; bandwidth = 1446 Hz/pixel, 1.8 mm slice gap) in an axial plane with 167 images per subject. Using a high spatial resolution at 3T, in particular a lower slice thickness, avoids signal dephasing especially in the ventral brain including the amygdala (Robinson et al., 2009, 2004, 2008). As a consequence, brain coverage is limited and a larger gap is needed. This should not be a critical issue as smoothing is applied in preprocessing (see below). Hence, this approach provides high sensitivity and specificity particularly around the amygdala. Data was processed using SPM8 (Wellcome Trust Centre for Neuroimaging, London, <http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>) implemented in Matlab (Version R20112b; Mathworks Inc., Sherborn, MA, USA). The first four volumes were discarded from each subject prior to further analyses. EPI images were corrected for head movement by affine registration using a two-pass procedure. In a first step, images were aligned to the initial volumes and subsequently to the mean of all volumes. Next, the mean EPI image

was spatially normalized to the non-linear MNI152 template for each subject (Holmes et al., 1998) by using the “unified segmentation” approach (Ashburner and Friston, 2005). Ensuing deformation was applied to the individual EPI volumes. Images were smoothed by a 5 mm full-width-at-half-maximum Gaussian kernel to improve signal-to-noise ratio and to compensate for residual anatomical variations. Time-series of each voxel were processed as follows (Weissenbacher et al., 2009): Variance was excluded to reduce spurious correlations by the following nuisance variables: (1) the six motion parameters derived from image realignment; (2) their first derivatives. All nuisance variables entered the model as first and also as second order nuisance terms (Satterthwaite et al., 2013). These corrections have been shown to increase specificity and sensitivity of the analyses and to detect valid correlation and anti-correlations during rest (Satterthwaite et al., 2013). In turn, given recent reports of spurious effects specifically in between group comparisons due to global signal removal (Murphy et al., 2009; Saad et al., 2012; Weissenbacher et al., 2009), we did not employ global signal regression. Finally, data was band-pass filtered (cut-off frequencies of 0.01 and 0.08 Hz). Furthermore and prior to the 2nd level analyses, rsFC of each subject was adjusted for the effect of age via a regression analysis.

Functional connectivity analyses

Left and right amygdala regions of interest as seed regions were provided from AnatomyToolbox v2.0 (Eickhoff et al., 2005) implemented in SPM8. For each subject, time-courses of all voxels within the seeds were extracted and expressed as the first eigenvariate. Linear (Pearson) correlation coefficients were computed between the ensuing characteristic time series of the seed regions and the time series of all other gray matter voxels of the brain to quantify rsFC. The voxel-wise correlation coefficients of each subject and seed were transformed into Fisher's Z-scores. Then, these scores for the left and right amygdala were fed into a second-level GLM, including an appropriate non-sphericity correction as implemented in SPM8. The GLM included the factor sex as well as cortisol as a covariate, to test for group differences and effects of the steroid hormone. Firstly, we tested for sex differences in the rsFC of the left and right amygdala on a whole-brain level. Secondly, we tested for correlations between cortisol and rsFC of the left and right amygdala for the whole group (women and men). Then, we tested for differences between women and men in the association of cortisol and rsFC of the left and right amygdala on a whole-brain level. For all analyses testing for associations with cortisol and group differences in these associations we conducted conjunctions with the main effect of the amygdala's (positive or negative) rsFC [cortisol \cap amygdala]. This conjunction approach restricts the analyses in association with cortisol to regions having significant functional connectivity with the amygdala. Furthermore and in addition to the conjunction with the main effect of the amygdala, sex differences in the pattern of the covariate in association with rsFC of the amygdala [cortisol \times sex \cap amygdala] were masked with the effects of cortisol for each sex: positive or negative correlations in women as well as positive or negative correlations in men. This approach a) restricts the analyses to regions that are significantly functionally connected with the amygdala and b) shows whether rsFC is positively or negatively associated with the cortisol within women or men.

Results were thresholded at a cluster-level FWE corrected threshold of $p < .05$ ($k = 80$; cluster-forming threshold at voxel-level $p < .001$) (e.g., Müller et al., 2013). Cytoarchitectonic assignment was based on AnatomyToolbox v2.0 (Eickhoff et al., 2005). In order to visualize and investigate the direction of sex differences in correlations of cortisol and rsFC, the rsFC of the amygdala with regions that appeared to be significant on a whole-brain level were extracted and further investigated.

Table 1
Sample description.

	Women ($n = 42$)		Men ($n = 39$)		p Value
	Mean	STD	Mean	STD	
Age	24.31	3.9	24.00	3.1	0.695
Positive affect ^a	26.80 ^b	7.3	26.56	6.2	0.878
Negative affect ^a	12.63 ^c	3.5	11.95	2.2	0.878 ^e
Cortisol ^d	0.99	0.1	0.92	0.2	0.082

STD = standard deviation.

^a Raw scores (PANAS) (Watson et al., 1988).

^b $n = 40$.

^c $n = 41$.

^d pg/ml (log-transformed).

^e U-test (negative affect was not normally distributed).

Functional characterization

BrainMap (<http://brainmap.org/>) is a large database of published functional neuroimaging experiments with coordinate-based results, also including information of meta-data across these experiments. The meta-data are useful to characterize functions of a specific brain region (e.g., being involved in emotion, cognition or perception, amongst others). For the current study we used the database to identify behavioral phenomena underlying the simultaneous activation of the derived brain region with the amygdala. Although these regions may not mandatorily be related to the same functions during task-dependent and task-independent processing, an understanding of task-related functions objectively supports the interpretation underlying a specific cluster of a brain region (which is potentially included in various functions). We performed a functional characterization for a) the regions derived from the current rsFC analyses combined with b) either left or right amygdala seed. Therefore, we used meta-data categories that classify each single experimental contrast from the BrainMap database according to the assessed “behavioral domain” (BD) (such as emotion, cognition or perception) and the “paradigm class” (PC) (such as flanker task or reward task) (Turner and Laird, 2012) (complete list for BD and PC: <http://brainmap.org/subscribe/>). The forward and reverse inference approach were calculated for the analyses as described in previous papers (Kogler et al., 2015b; Müller et al., 2013) and only results of BD and PC that appear to be significant in both approaches (forward and reverse) will be reported in this manuscript. The forward inference approach determines the probability of observing activity in a brain region when a mental process (such as BD emotion, cognition or perception or PC flanker task, mental rotation, reward task) is present. Thus, it tests whether the conditional probability of activation given a particular task [P(Activation|Task)] is higher than the baseline probability of activation [P(Activation)]. The baseline denotes the probability of finding a (random) activation from BrainMap in the region of interest. Significance was tested using a binominal test ($p < .05$, corrected for multiple comparisons [FDR]). Additionally, the reverse inference approach tests the probability of the presence of a mental process given knowledge of activation in a particular region of interest. This likelihood [P(Task|Activation)] can be derived from P(Activation|Task) as well as P(Task) and P(Activation) using Bayes' rule. Significance was assessed by means of a chi-square test ($p < .05$, corrected for multiple comparisons [FDR]).

Correlational analyses

We performed correlation analyses across the whole group as well as separately for women and men between positive and negative affect and the significant rsFC of the amygdala to investigate associations between subjective mood and amygdala's rsFC. Correlations were corrected for multiple comparisons where appropriate.

Voxel-based-morphometry (VBM) analysis

To account for potential sex differences in amygdala volume, a high-resolution anatomical image using an MPRAGE sequence (3-D Magnetization Prepared Rapid Gradient Echo: 160 sagittal slices, TR = 2300 ms, TE = 4.21 ms, $1 \times 1 \times 1.1$ mm resolution, flip angle 9° , inversion time 900 ms) was acquired from each participant. The anatomical scans were preprocessed with VBM8 toolbox (dbm.neuro.uni-jena.de/vbm) in SPM8 using standard settings (DARTEL normalization, spatially adaptive non-linear means denoising) (see also Müller et al., 2015). Within a unified segmentation model (Ashburner and Friston, 2005), images were corrected for biasfield inhomogeneity. The brain tissue was classified into gray matter, white matter and cerebrospinal fluid, adjusted for partial volume effects and spatially normalized to the Montreal Neurological Institute (MNI) template. The segmented images were non-linearly modulated to adjust them to the amount of expansion and contraction, which was applied during normalization. We computed the

volume of the left and right amygdala by integrating the (non-linearly) modulated voxel-wise gray matter probabilities for each subject. Age was included as nuisance variable. As we did not multiply the segmented images by linear components but rather modulated the images by the non-linear components only, the calculated gray matter volume represents the amount of gray matter corrected for individual brain size.

We tested for sex differences in gray matter volume of left and right amygdala, first. Then, we investigated the associations between cortisol and left or right amygdala volume and further, we tested for sex differences in the correlations of cortisol with left or right amygdala volume. Statistical significance was evaluated at $p < 0.05$, and Bonferroni-corrected for multiple comparisons.

Results

Mood and hormones

Female and male groups did not differ in age ($p = .695$), positive and negative affect (PANAS) (both $ps = .878$), nor in cortisol level ($p = .082$). Cortisol levels were not correlated with positive or negative affect, neither for the whole group nor in sex-specific analyses (all $ps > .119$). See Table 1 for sample description.

Sex differences in the resting-state functional connectivity of the amygdalae

Analysis of sex differences in amygdala connectivity revealed significantly higher rsFC of the left amygdala with the left middle temporal gyrus (MTG), left inferior frontal gyrus (IFG) (triangularis), left hippocampus, and left postcentral gyrus in women compared to men (Fig. 1 and Table 2). Given our threshold, no sex difference for right amygdala connectivity emerged, and also no stronger connectivity of either left or right amygdala appeared in men as compared to women.

The functional decoding analyses (BrainMap, <http://brainmap.org/>), to characterize behavioral phenomena (BD and PC) associated with the derived clusters, revealed the following results: *Left MTG* and left amygdala co-activation were found to be associated with the PC [face monitoring and discrimination]. No BD emerged to be significant. *Left IFG* and left amygdala co-activation were significantly linked to the PC [face monitoring and discrimination as well as semantic monitoring and discrimination]. No BD emerged to be significant. Co-activation of the *left hippocampus* and the left amygdala was significantly associated with the BD [fear, happiness, emotion, memory, explicit memory] and the PC [affective words and pictures, face monitoring and discrimination, emotion induction, encoding, passive viewing]. *Left postcentral gyrus* and left amygdala co-activation was found to be related to BD [pain perception] and PC [classical conditioning].

Functional connectivity of the left amygdala: women > men

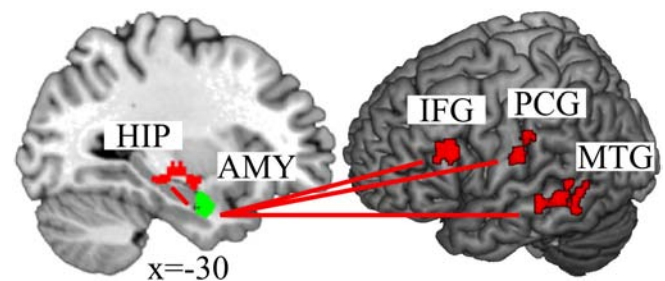


Fig. 1. Women had a stronger functional connectivity of the left amygdala (AMY, green) to the left hippocampus (HIP), the left inferior frontal gyrus (IFG), the left postcentral gyrus (PCG) and the left middle temporal gyrus (MTG), all in red, than men.

Table 2
Sex differences in the resting-state functional connectivity (rsFC) of the left amygdala.

	<i>t</i> Value	<i>p</i> Value	X	Y	Z	Macroanatomical location	Cytoarchitectonic location
<i>Women > men</i>							
Cluster 1 (<i>k</i> = 163)	3.98	0.001	-58	-40	-6	L middle temporal gyrus	
Cluster 2 (<i>k</i> = 118)	4.77	0.008	-46	20	24	L IFG (<i>p. triangularis</i>)	
Cluster 3 (<i>k</i> = 105)	4.98	0.014	-32	-16	-8	L hippocampus	
Cluster 4 (<i>k</i> = 104)	4.67	0.015	-56	-16	22	L postcentral gyrus/ rolandic operculum/ supramarginal gyrus	Area OP 1/ Pft (IPL)/OP 4

Note. No stronger rsFC appeared for men nor for the right amygdala. Only the maximum peak of each cluster is reported. Degrees of freedom (1, 154). Macroanatomical and cytoarchitectonical locations indicate the extensions of each cluster. L = left. Coordinates *x, y, z* of local maxima refer to Montreal Neurological Institute space (MNI). *k* = number of voxels in cluster. References for histological assignments: Pft (Caspers et al., 2006); OP1–4 (Eickhoff et al., 2006a, 2006b).

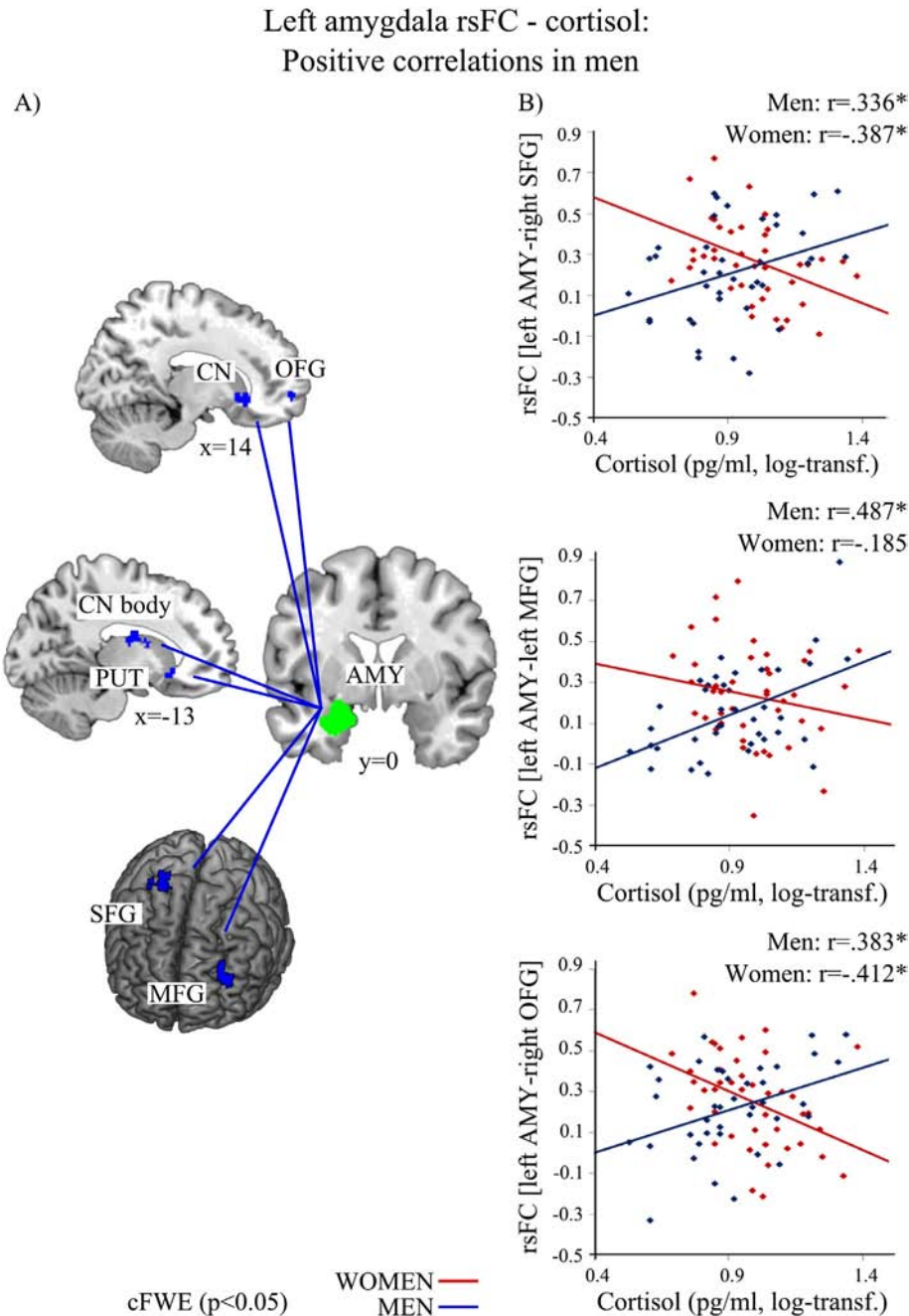


Fig. 2. In men (blue) higher cortisol levels were associated with stronger resting-state functional connectivity (rsFC) of the left amygdala (AMY, green) with bilateral caudate nucleus (CN), left putamen (PUT), right mid-orbital frontal gyrus (OFG), left middle frontal gyrus (MFG), right superior frontal gyrus (SFG). A) depicts the regions; B) shows the association between cortisol level and rsFC of the left amygdala and SFG, MFG as well as OFG in men (blue, positive) and women (red, negative). (log-transf. = log-transformed). Significant correlations of the whole-brain analysis ($p < 0.05$) are marked with an asterisk.

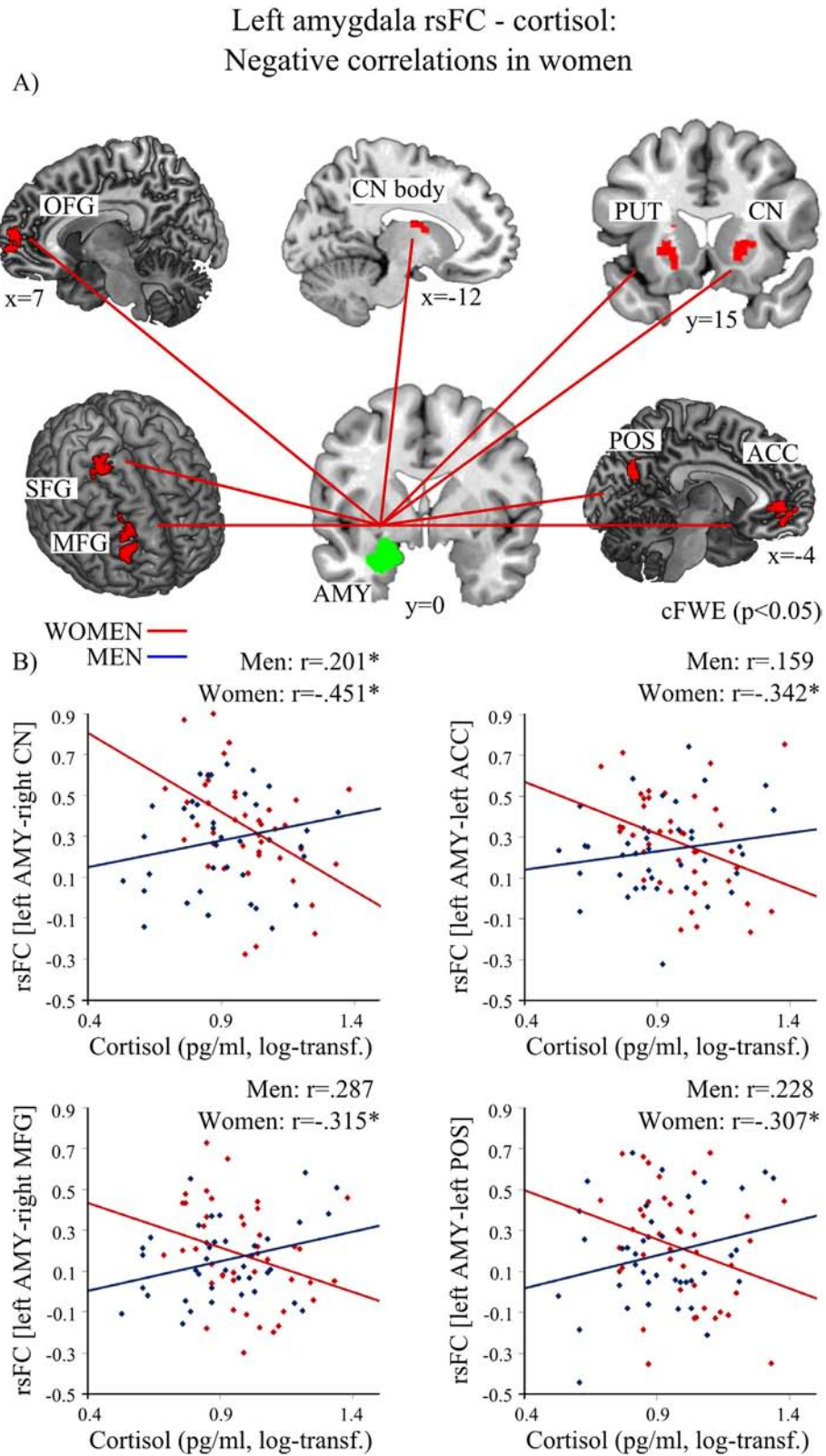


Fig. 3. In women (red) higher cortisol levels were associated with decreased resting-state functional connectivity (rsFC) of the left amygdala (AMY, green) with bilateral caudate nucleus (CN), left putamen (PUT), left anterior cingulate gyrus (ACC), right mid-orbital frontal gyrus (OFG), right middle frontal gyrus (MFG), right superior frontal gyrus (SFG) and left parietal-occipital sulcus (POS) with adjacent cortical regions precuneus and cuneus. A) depicts the regions; B) shows the association between cortisol level and rsFC of the left amygdala and CN, ACC, MFG, and POS in women (red, negative) and men (blue). (log-transf. = log-transformed). Significant correlations of the whole-brain analysis ($p < 0.05$) are marked with an asterisk.

Association of amygdala's functional connectivity with cortisol

Whole group

For the whole group no significant association emerged in association with cortisol for rsFC of the left and right amygdala.

Interaction with sex

Sex differences appeared for the association of cortisol with rsFC of both amygdala with striatal, frontal and parietal regions (Figs. 2–4 and Tables 3 & 4). While in men higher cortisol levels were associated with a stronger rsFC of those regions with amygdala, women showed the opposite pattern, i.e., less connectivity went along with higher cortisol concentrations.

Positive associations in men vs. women. Investigation of these interaction effects for the left amygdala indicated significantly positive correlations of cortisol with bilateral striatal regions (caudate nucleus, putamen), right mid-orbitofrontal gyrus (OFG), left middle frontal gyrus (MFG) and right superior frontal gyrus (SFG) in men in contrast to women (see Fig. 2 and Table 3). For men, neither a negative association was found for cortisol with rsFC of the left amygdala, nor any significant positive or negative association for rsFC of the right amygdala.

Functional decoding analyses revealed the following results for these clusters: Co-activation of *right caudate nucleus* and left amygdala was associated with the BD [emotion, cognition] and the PC [reward, face monitoring and discrimination]. Concurrent activation of *left caudate nucleus* and left amygdala was particularly related to the BD [emotion, sadness, cognition] and the PC [reward, film viewing]. Neither BD nor PC emerged to be significant for co-activation of *left putamen* and left amygdala applying our threshold. Lowering the threshold ($p < .05$, uncorr.), the BD [emotion, cognition, speech execution] and the PC [reward, face monitoring and discrimination] emerged. Concurrent

activation of *right OFG* and left amygdala was associated with the BD [emotion] and the PC [passive viewing, face monitoring and discrimination]. Co-activation of *left MFG* and left amygdala was associated with the PC [passive viewing, face monitoring and discrimination]. No BD emerged to be significant. Co-activation of the *right SFG* and left amygdala was associated with the BD [explicit memory]. No PC emerged to be significant.

Negative associations in women vs. men. For women, cortisol was negatively associated with rsFC of the left amygdala and bilateral striatal regions (caudate nucleus, putamen), left ACC, right OFG, right MFG, right SFG as well as adjacent medial cortical areas of the parietal–occipital sulcus (POS) (precuneus, cuneus) (see Fig. 3 and Table 4). Furthermore, cortisol correlated negatively with rsFC of the right amygdala with left supplementary motor area (SMA) as well as with right SFG (see Fig. 4 and Table 4). No positive association between cortisol and left or right amygdala emerged in women.

Functional decoding analyses for the derived clusters revealed the following results: Co-activation of *right caudate nucleus* and left amygdala was associated with the BD [emotion, cognition] and the PC [reward, face monitoring and discrimination]. Concurrent activation of *left caudate nucleus* and left amygdala was particularly related to the BD [emotion]. No PC emerged to be significant. Co-activation of *left putamen* and left amygdala was linked to the BD [explicit memory]. No PC emerged to be significant. Concurrent activation of *right OFG* and left amygdala was associated with the BD [emotion, sadness, interoception sexuality, perception olfaction, cognition] and the PC [reward, olfactory monitoring and discrimination, film viewing]. No BD or PC emerged to be significant applying our threshold for *right MFG*. Lowering the threshold ($p < .05$, uncorr.), right MFG and left amygdala co-activation was associated with the BD [cognition, explicit memory] and the PC [reward]. Concurrent activation of *left ACC* and

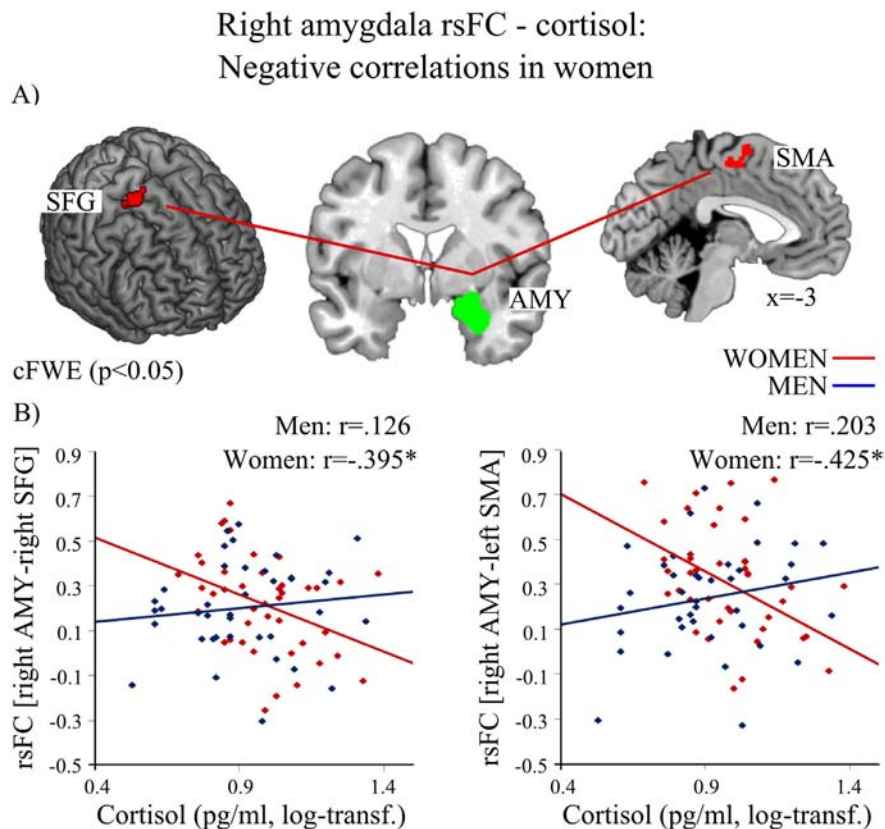


Fig. 4. In women (red) higher cortisol levels were associated with decreased resting-state functional connectivity (rsFC) of the right amygdala (AMY, green) with left supplementary motor area (SMA) and right superior frontal gyrus (SFG). A) depicts the regions; B) shows the association between cortisol level and rsFC of the right amygdala and SFG as well as SMA in women (red, negative) and men (blue). (log-transf. = log-transformed). Significant correlations of the whole-brain analysis ($p < 0.05$) are marked with an asterisk.

Table 3

Positive correlations between resting-state functional connectivity (rsFC) of the left amygdala and cortisol in men.

	t Value	p Value	X	Y	Z	Macroanatomical location	Cytoarchitectonic location
Cluster 1 (k = 161)	4.78	0.001	14	22	−8	R caudate nucleus/mid orbital gyrus	
Cluster 2 (k = 131)	4.49	0.004	−18	12	−8	L putamen/caudate nucleus	
Cluster 3 (k = 108)	4.47	0.013	−8	−2	6	L caudate nucleus/pallidum	Thal: Temporal
Cluster 4 (k = 98)	4.59	0.020	14	58	−6	R mid orbital gyrus/superior medial gyrus	Area Fp1/FP2
Cluster 5 (k = 85)	3.99	0.038	−30	46	32	L middle frontal gyrus	
Cluster 6 (k = 80)	4.03	0.050	24	−8	62	R superior frontal gyrus/precentral gyrus	

Note. Sex differences in the association of cortisol with rsFC of the left amygdala: Positive correlations in men. Degrees of freedom (1, 154). R = right, L = left. Coordinates x, y, z of local maxima refer to Montreal Neurological Institute space (MNI). k = number of voxels in cluster. Only the maximum peak of each cluster is reported. Macro- and cytoarchitectonical locations indicate the clusters' extensions. References for histological assignments: Thalamus (Behrens et al., 2003); Fp1, Fp2 (Bludau et al., 2014).

left amygdala was associated with the BD [emotion, fear] and the PC [face monitoring and discrimination, passive viewing]. Right SFG and left amygdala co-activation was linked to the BD [emotion, fear] and PC [face monitoring and discrimination, passive viewing]. Co-activation of the left POS and left amygdala was particularly related to the BD [emotion] and the PC [face monitoring and discrimination, cued explicit recognition]. Co-activation of SMA and amygdala was found to be associated with the BD [action execution]. No PC emerged to be significant.

In summary, cortisol and rsFC of amygdala with striatal regions, right OFG, and right SFG were significantly positively associated in men and significantly negatively in women, whereas the association of cortisol and rsFC of the amygdala with left MFG was significant only in men and rsFC of ACC, right MFG, SMA and CAL was significant only in women (Figs. 2–4 and Tables 3 & 4).

Correlational analyses with subjective mood

In women, negative affect was negatively correlated with the rsFC of the amygdala and the IFG (Spearman $r_s = -0.377$; $p = 0.015$). No other significant association appeared for negative mood nor any with positive mood, neither in women nor in men nor across the whole group.

Gray matter volume of the amygdala in association with cortisol

For VBM analyses three subjects had to be excluded due to outlying data in amygdala gray matter volume (mean \pm 2STD, 2 women, 1 men). No sex differences emerged for gray matter volume of left or right amygdala (both $p > .105$, see supplemental material and Fig. S1). Performing correlation analyses revealed a positive association of gray matter volume of the right amygdala with cortisol levels across the whole group (Fig. 5), while no such correlation occurred for the left amygdala. No significant sex differences emerged for correlation analyses of amygdala-volume with cortisol.

Table 4

Negative correlations between resting-state functional connectivity (rsFC) of left and right amygdala and cortisol in women.

	t Value	p Value	X	Y	Z	Macroanatomical location	Cytoarchitectonic location
<i>Left amygdala: Negative correlations in women</i>							
Cluster 1 (k = 215)	4.78	< 0.001	14	22	−8	R caudate nucleus/mid orbital gyrus/pallidum	
Cluster 2 (k = 210)	4.49	< 0.001	−18	12	−8	L putamen/caudate nucleus	
Cluster 3 (k = 169)	4.46	0.001	−4	40	−2	L ACC/mid orbital gyrus	Area Fp2
Cluster 4 (k = 132)	4.59	0.004	14	58	−6	R mid orbital gyrus/superior medial gyrus	Area Fp1/FP2
Cluster 5 (k = 102)	4.47	0.017	−8	−2	6	L caudate nucleus/pallidum	Thal: Temporal
Cluster 6 (k = 91)	4.03	0.029	24	−8	62	R superior frontal gyrus/precentral gyrus	
Cluster 7 (k = 91)	4.01	0.029	−4	−68	22	L calcarine gyrus/cuneus/precuneus	
Cluster 8 (k = 86)	4.08	0.037	34	46	26	R middle frontal gyrus	
Cluster 9 (k = 85)	4.06	0.038	24	28	30	R superior frontal gyrus/middle frontal gyrus	
<i>Right amygdala: Negative correlations in women</i>							
Cluster 1 (k = 96)	4.47	0.022	−2	−4	64	L supplementary motor area/MCC	
Cluster 2 (k = 87)	4.72	0.035	24	−8	62	R superior frontal gyrus/posterior-medial frontal	

Note. Negative correlations of cortisol with rsFC of the left and right amygdala in women. Degrees of freedom (1, 154). R = right, L = left. Coordinates x, y, z of local maxima refer to Montreal Neurological Institute space (MNI). k = number of voxels in cluster. Only the maximum peak of each cluster is reported. Macro- and cytoarchitectonical locations indicate the clusters' extensions. References for histological assignments: Thalamus (Behrens et al., 2003); Fp1, Fp2 (Bludau et al., 2014).

Discussion

The aim of the current study was to assess sex differences in the functional connectivity of the amygdala, a brain region associated with emotional and stress-related behavior, and its association with cortisol. Sex differences are reported for certain functions (such as stress and affective processing) as well as in mental health issues including diverging prevalence rates for psychiatric disorders (e.g., attention deficit hyperactivity disorder, depression, anxiety disorder) (Cahill, 2006; McCarthy et al., 2012). Also, for cortisol release sex differences are reported. Unfortunately, studies investigating associations with hormones often focus either on female- or male-only samples. However, conclusions that are based on results of only one sex have limited value in understanding the same phenomena in the other sex (McCarthy et al., 2012). The current results therefore crucially contribute to the knowledge of sex specific effects of cortisol and its underlying neural networks.

In accordance with previous literature (Quaedflieg et al., 2015; Veer et al., 2011), our results show that steroid hormone levels are associated with the amygdala's functional connectivity. Furthermore, these associations differ between women and men particularly regarding the functional connectivity of the amygdala with frontal and striatal regions. The coupling between the amygdala and the frontal cortex as well as the basal ganglia in interaction with hormone levels may drive sex specific differences in a variety of behaviors, which are discussed in the following.

Stronger functional connectivity of the amygdala in women than in men

Our results revealed stronger rsFC of the amygdala in women compared to men, while no stronger rsFC in men vs. women was observed. One of the regions showing increased functional connectivity with the amygdala in women compared to men was the posterior MTG. Based on our functional decoding analysis co-activation of both regions is associated with face monitoring and discrimination. More

Gray matter volume of the right amygdala in association with cortisol: whole group

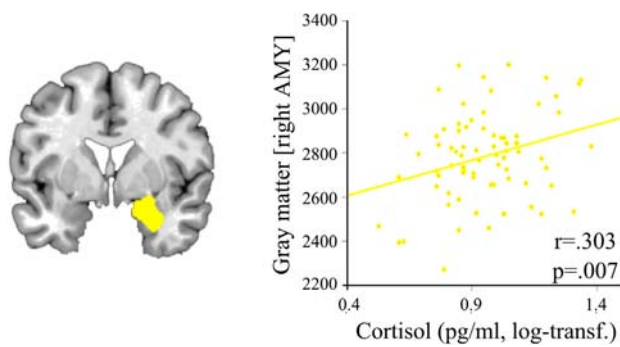


Fig. 5. Higher cortisol levels are associated with higher gray matter volume of the right amygdala (AMY, yellow) in both, women and men (log-transf. = log transformed).

specifically, studies investigating processing faces under different circumstances (e.g., emotion induction or imitation, rating of attractiveness; infant facial cues (Habel et al., 2005; Lee et al., 2006; Strathearn et al., 2008; Winston et al., 2007)) report activation of both brain regions. There are various reports on sex differences in emotional face processing (for a review see e.g., Forni-Santos and Osório, 2015) with women outperforming men in accuracy and identification (Megreya et al., 2011). Increased connectivity between amygdala and a multimodal area like the MTG may contribute to a superior performance in women compared to men during face processing, independent of the specific task requirements. Taken together, stronger coupling between amygdala and MTG in women may contribute to the reported sex differences in face processing.

Furthermore, in the current study the left IFG was more strongly coupled with the left amygdala in women than in men. Our functional characterization revealed that the IFG and the amygdala are associated with tasks involved in semantic processing such as judging semantics in different languages (Longe et al., 2007; Luke et al., 2002). The IFG is also involved in self-referenced inner speech (Morin and Hamper, 2012) as well as in rumination, which is a perseverative focus on perceived threats, negative events and emotions, thus kind of a negative afflicted inner speech (Kühn et al., 2014). Interestingly, the connectivity between the amygdala and the IFG is reported to be higher in healthy, trauma-exposed controls compared to trauma-exposed subjects with posttraumatic-stress disorder (Brown et al., 2014). This may indicate that successful coping and the use of positive self-referenced inner speech goes along with higher amygdala–IFG coupling. Women tend to use self-referenced positive speech as well as rumination more frequently than men (Nolen-Hoeksema, 2012; Tamres et al., 2002; Trapnell and Campbell, 1999). The amygdala–IFG coupling may underlie coping mechanisms related to self-referenced speech and may result in a health-preserving mechanism such as a positive, self-referenced speech to cope with stressful situations, but also in negatively biased rumination. In concert with this we observed a negative correlation between negative affect and amygdala–IFG rsFC: Higher rsFC was associated with reduced negative affect. Thus, higher coupling between the amygdala and the IFG may indicate a stronger positive self-related talk thereby reducing negative affect probably serving as a coping mechanism. Therewith, the amygdala–IFG coupling may subserve coping mechanisms, related to inner-speech.

Furthermore, our results show a stronger coupling between the left hippocampus and the left amygdala in women than in men. The hippocampus is often associated with fear conditioning, extinction and memory (Hartley and Phelps, 2010; Maren and Holmes, 2015; Myers and Davis, 2007; Pape and Pare, 2010), and also the current functional decoding analysis of the hippocampus in association with the

amygdala shows its involvement in fear and memory processing. An increased coupling between two regions processing fear and memory may lead to a disproportionate consolidation of fearful experiences in women. Indeed, women give higher subjective fear ratings in fear learning and retrieval than men (Lonsdorf et al., 2015) and women have a higher prevalence rate of anxiety and panic disorder (McCarthy et al., 2012; World Health Organization, 2004). Although the current results are based on healthy subjects, a higher coupling of hippocampus and amygdala in women may act as a neural contributing factor to sex-specific prevalence rates in anxiety and panic disorders.

Furthermore, women show stronger functional connectivity between the amygdala and a part of the inferior postcentral gyrus. This region has previously been shown to be part of a network of higher order somatosensory processing (Eickhoff et al., 2010) and found to be activated during somatosensory and pain perception (Bingel et al., 2006). Our functional decoding analyses also revealed the association of postcentral gyrus and amygdala co-activation with painful stimulation. In female pain patients, an increased functional connectivity is reported for inferior parietal regions also to other brain regions than the amygdala (Napadow et al., 2010). Furthermore, there is some evidence for higher chronic pain perception and increased reports of pain intensity in women compared to men (Mogil, 2012). The coupling between a subcortical region driving emotional and stress reactions, such as the amygdala, and a cortical area associated with somatosensory pain processing, may be associated with the increased reports in pain sensitivity and chronic pain in women (Mogil, 2012).

Sex specific association of cortisol with functional connectivity of the amygdalae

The analyses of the amygdala's functional connectivity in association with cortisol revealed a significant interaction with sex. In men, cortisol is positively associated with the functional connectivity of the amygdala with striatal and frontal regions, whereas in women this association is negative. Our results support previous findings showing a stress-induced increase in connectivity between the amygdala and the striatum (Vogel et al., 2015), as well as with frontal regions (Veer et al., 2011) in male-only samples. Also, they support previous reports on functional connectivity of the amygdala in association with cortisol: Exogenous cortisol administration increases the functional coupling of amygdala and the prefrontal cortex in men (Henckens et al., 2010), and a positive association between circadian cortisol decrease and a decrease in amygdala–ACC connectivity was shown in previous studies for healthy men (Veer et al., 2012). In addition, the current results crucially extend those previous studies by demonstrating that cortisol-connectivity associations are sex dependent.

Our functional decoding analyses demonstrate a tight coupling of cortisol with regions that are associated with emotion, reward and face processing. Indeed, cortisol has been shown to be associated with reward processing (Montoya et al., 2014) as well as emotion regulation (Urry et al., 2006). In particular, in a male sample, exogenous cortisol decreased reward preference and activity in the striatum (Montoya et al., 2014), and circadian cortisol decrease was associated with activity in the amygdala during emotion regulation (Urry et al., 2006). In the current study, increased cortisol levels in men were associated with increased connectivity between the left amygdala and regions of the reward and limbic system, such as caudate nucleus, putamen, OFG and MFG. Aversive stimuli (Domes et al., 2010), monetary loss (Beck et al., 2009), decision-making in learners in a card betting game (Schönberg et al., 2007), as well as processing pleasant or infant faces (Jabbi et al., 2007; Strathearn et al., 2008) increases activity in the amygdala as well as caudate nucleus, putamen and MFG. Contrarily when retrieving stressful events, the OFG as well as the amygdala seems to be deactivated in healthy subjects (Britton et al., 2005). Taken together with the current results on rsFC, it seems that cortisol may increase connectivity of amygdala and these specific regions in men, while in

women the connectivity may decrease with increasing cortisol. Cortisol is thought to regulate neural network responses that underlie behavioral adaptation to arousing situations (Henckens et al., 2012; Kloet et al., 2005). However, the current results indicate sex-dependent regulatory effects of cortisol on regions associated with emotion and reward processing.

Particularly, the pattern of cortisol in association with amygdala–ACC coupling seems to be sex-dependent. In men, this association was reported to be positive (Veer et al., 2012). This previous study, however, did only test men and could thus not provide any information how cortisol may affect amygdala–ACC connectivity in women. Our study now demonstrates an interaction between sex and cortisol in amygdala–ACC connectivity, with a negative association in women, while men showed an opposite (though not significant) relationship. The ACC is thought to down-regulate amygdala activity (Stein et al., 2007; Veer et al., 2012), playing an important role in emotion regulation and adapted stress response (Diorio et al., 1993; Ulrich-Lai and Herman, 2009; Veer et al., 2012). Our results in addition with the literature indicate that the mediating effect of cortisol on ACC activity, and consequently its influence on amygdala activity, differs between women and men.

Another sex-specific effect was shown for the connectivity between amygdala and SFG in association with cortisol. Our functional decoding analysis revealed that concurrent activation of the SFG and the amygdala is associated with explicit memory such as recognition of objects (e.g., Achim and Lepage, 2005). Sex differences in association with stressful situations are already reported for memory retrieval: Stress impairs memory more strongly in men than in women, and memory performance is also correlated negatively with cortisol in men (Hidalgo et al., 2015; Wolf et al., 2001). This together with our current results indicates that men feature an altered ability to retrieve information during stressful situations which is possibly mediated by a stronger coupling of the SFG and the amygdala with increased cortisol levels. In contrast, in women, increased cortisol levels going along with decreased SFG–amygdala connectivity may indicate less emotional interference during explicit memory processing, shown by the decoupling of an area associated with emotion processing such as the amygdala and an area associated with memory such as the SFG with higher cortisol concentrations in women. Thus, sex differences in association of cortisol and functional connectivity of amygdala and SFG as found in our study may underlie sex specific effects that cortisol exerts on memory performance in women and men.

In addition, the connectivity of the left amygdala and the left POS, including adjacent precuneus and cuneus, demonstrated sex-specific associations with cortisol. Increased connectivity between precuneus and the amygdala following stress in a male-only sample has been reported previously (Veer et al., 2011), and additionally in the current study a negative association was found in women in association with cortisol. Interestingly, in women with post-traumatic stress disorder compared to healthy women the connectivity between the medial parietal area and the amygdala is decreased (Bluhm et al., 2009). Besides being functionally associated with emotion and face processing (Schilbach et al., 2012), as also revealed by the current decoding analysis, the medial parietal area is often associated with “default mode”, mind-wandering and self-generated thoughts (Cavanna and Trimble, 2006; Fox et al., 2015) that appear during resting-states (Mason et al., 2007; Tusche et al., 2014). Furthermore, cortisol release is associated with self-generated thoughts: While future oriented social thoughts are associated with decreased cortisol levels, an increase in cortisol is positively correlated with negative thoughts (Engert et al., 2014). Thus, increased cortisol concentrations in association with decreased connectivity of the amygdala and the POS in women may indicate a higher engagement of women in future oriented thoughts, whereas an increased connectivity of POS and amygdala with higher cortisol levels in men suggests a focus on negative thoughts during resting-state. Hence, one could speculate that in the scanner environment, aroused women distract themselves with thinking about the future, while

aroused men tend to concentrate on negative thoughts. Whether women and men deal with different contents during mind-wandering, and whether this is directly related to cortisol release, remains to be tested in future research.

In women, contrarily to men, cortisol was negatively associated with the coupling of the amygdala and the SMA, two regions which co-activation has been associated with action execution. Interestingly, theories on stress reactions claim that neuroendocrine mechanisms regulate stress responses in women and men differently (Taylor et al., 2000): In women a predominant “tend-and-befriend” reaction to arousing situations is assumed (Taylor et al., 2000) suggesting that in arousing states the need for action in women may be decreased. This pattern was not seen in men. The results thus further support the notion that cortisol exerts different functions in women and men.

Positive association of gray matter volume and cortisol levels

Analyses of gray matter volume revealed a significant association between right amygdala and cortisol levels, with greater amygdala volumes going along with higher cortisol concentrations across women and men. Notably, no sex differences in amygdala volume emerged. Previously, larger volumes in men than in women have been reported for bilateral subcortical clusters, including the amygdala, for an age range of 7–80 years (Ruigrok et al., 2014). Differences to the current results might be explained by the relatively homogenous age range of the sample used in the current study. Furthermore, sex hormones are known to have an influence on amygdala volume (Giedd et al., 2012; Neufang et al., 2009; Pletzer et al., 2010). Given that we only included naturally cycling women in the current study, sex hormone release throughout the menstrual cycle may also contribute to the missing sex differences here. Furthermore, the observed positive association between amygdala volume and cortisol levels is in line with the assumption that the amygdala has an excitatory effect on the hypothalamus–pituitary–adrenal axis, leading to increased corticoid secretion (Ulrich-Lai and Herman, 2009), in a healthy sample.

Interestingly, we observed a positive association between amygdala volume and baseline cortisol levels. The amygdala, as a key brain structure in response to emotionally salient situations such as stress, rapidly responds to environmental demands by triggering physiological stress responses (Evans et al., 2015; McEwen and Gianaros, 2010) and exerts excitatory effects on the hypothalamus–pituitary–adrenal gland (HPA) axis (McEwen and Gianaros, 2010; Ulrich-Lai and Herman, 2009). Increased life stress during childhood leads to larger amygdala volumes in children and adults (Evans et al., 2015; Lupien et al., 2011; Tottenham et al., 2010) emphasizing the association of amygdala volume and stress reactivity. The positive correlation between amygdala volume and baseline cortisol levels in our data underlines the excitatory effect of the amygdala on HPA-axis leading to increased corticoid secretion. Furthermore, our data show that this is already apparent in healthy subjects under baseline conditions.

Limitations

There are some suggestions for future research that may be seen as limitations of the current study. Interestingly, previous reports partly support the current findings on sex differences in amygdala rsFC, however they appeared to be right lateralized: Higher coupling of right basolateral amygdala with right IFG and right postcentral gyrus were reported in women than in men (Engman et al., 2016). Differences in lateralization between studies may be explained by the area used as seed region (subnuclei vs. whole amygdala). Due to the current scanning parameters, which were not optimized to assess small amygdala subnuclei, and the ubiquitous smoothing factor, we decided to use the whole amygdala as seed region for the current analyses (e.g., Robinson et al., 2004). Furthermore, differences in preprocessing approaches may influence the data (Weissenbacher et al., 2009). Global signal regression is

suspected to rather create than reveal relationships between brain regions (Saad et al., 2012), which further may suggest non-existent group differences. To avoid false positive results, we did not include global signal as nuisance variable. However, replication studies are needed to confirm the results.

Importantly, it has to be considered that amygdala connectivity measures might be confounded by signal from nearby veins, especially when using spatial smoothing. While direct assessment of venous influences has been investigated in short-TR (temporal resolution) datasets (Boubela et al., 2015) and corrections might be implemented with these types of data, it is not yet clear how to appropriately account for vascular effects in a dataset with long TR, where multiple high-frequency fluctuations present in the amygdala region are not critically sampled. The cleansing of long TR data with respect to venous effect is a topic for future investigation, but at present the possibility of potential distortion of results by venous effects should be considered when interpreting results.

Hormone levels vary across the female menstrual cycle and cortisol levels are reported to differ between follicular and luteal cycle phase in response to arousing or stressful situations (Andreano et al., 2008; Duchesne and Pruessner, 2013; Kirschbaum et al., 1999). For exploratory reasons we directly compared cortisol levels between our naturally cycling women with independent *t*-tests (15 follicular vs. 5 ovulation vs. 22 luteal) and did not find significant group differences (ovulation vs. follicular $p = .716$; ovulation vs. luteal: $p = .980$; follicular vs. luteal: $p = .514$) (menstrual cycle phase was assessed via self-reports surveying the last three cycles and the onset of the following menses). In the current study we did not induce a stressful, arousing situation and the missing difference between sexes and also between menstrual cycle phases in baseline cortisol are consistent with previous reports (Chung et al., 2016; Duchesne and Pruessner, 2013; Kirschbaum et al., 1999; Maki et al., 2015). Furthermore, sex hormones seem to critically modulate cortisol response in emotion and stress regulation during different stages of the menstrual cycle (Albert et al., 2015; Andreano and Cahill, 2010; Duchesne and Pruessner, 2013). Thus, we cannot exclude that diverging levels of sex steroids may interact with the reported findings regarding cortisol, specifically as we included females from different menstrual cycle phases with different sex hormone concentrations. Hence, future studies might want to specifically analyze the interaction between sex hormones and cortisol across the menstrual cycle and further investigate the combined impact on functional connectivity. Moreover, oral contraception seems to have an impact on brain networks of rsFC (Petersen et al., 2014). Here we only included naturally cycling women, but the impact of oral contraceptives on functional connectivity of the amygdala has to be elucidated in upcoming studies.

The current study is based on correlational analyses, thus we cannot deduce causal effects between cortisol and functional connectivity. Furthermore, we can only speculate about the direction of the described connections, and did this based on previous reports on effective or anatomical neural connections. Additionally, the current study mainly focused on associations between physiological arousal and rsFC and many important variables, e.g. personality traits, stress-related behavior, etc. were not included in the analyses but would be important to further strengthen our assumptions.

Also, we wish to note that the current results reflect states of sex differences in healthy young adults in association with cortisol. We do not assume that the reported sex and cortisol differences are determined by biological sex only (Miller and Halpern, 2014). They may also be a result of socialization and gender roles. A longitudinal study of the functional connectivity of the amygdala in association with steroid hormones and gender roles remains to be tested.

Summary and conclusion

Taken together, resting-state functional connectivity of the amygdala differs between women and men. Women showed stronger connections

of the amygdala with the MTG, IFG, hippocampus and the postcentral gyrus. This indicates a stronger coupling in women compared to men of regions associated with face processing, inner-speech, fear and pain processing with a region of emotion processing. These sex-specific connectivities may underlie sex differences in those behavioral phenomena.

Furthermore, an interaction of sex and cortisol levels was apparent for rsFC of the amygdala: Higher levels of cortisol were positively correlated with functional connectivity in men and negatively in women. Cortisol seems to enhance the crosstalk of regions included in emotion, reward, memory and action processing in men, while the coupling of these regions is reduced with increasing cortisol levels in women. Our results thus indicate that the regulatory mechanism of cortisol on neural networks to adapt behavior in arousing situations differs between women and men. Given the amount of literature based on male-only samples, the current results significantly extend our knowledge and should lead to a more sensitive way of dealing with biological sex and hormonal influence on functional connectivity and stress research. Therefore, sex differences in functional connectivity and interactions with hormones should be taken into account much stronger in future research.

Acknowledgments

This study was supported by the Deutsche Forschungsgemeinschaft (DFG; IRTG-1328 [LK, RCG, UH, BD]; EI 816/4-1 [SBE]; EI 816/6-1 [SBE]; LA 3071/3-1 [SBE]), Jülich-Aachen-Research-Alliance, Translational Brain Medicine (LK, UH, BD), the National Institute of Mental Health (R01-MH074457) (SBE), the European EFT program (Human Brain Project) (SBE) and Austrian Science Fund (FWF Project P-23533 [RB, KK, EMS, EM, RCG, UH, BD]). The funding sources had no involvement in study design, collection, analysis or interpretation of the data, in writing the article and in decision for publication. Preliminary results of parts of this study were presented at the annual Meeting of the Organization of Human Brain Mapping (OHBM) in Honolulu 2015.

All authors declare no conflict of interest in relation to the manuscript.

BD, UH, RCG, EMS, EM designed research; EMS, RB, KK performed research; VIM, SBE contributed analytic tools; LK, VIM, SBE, BD analyzed data; LK, VIM, SBE, BD wrote and EMS, RB, KK, UH, RCG, EM revised and approved the paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.neuroimage.2016.03.064>.

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