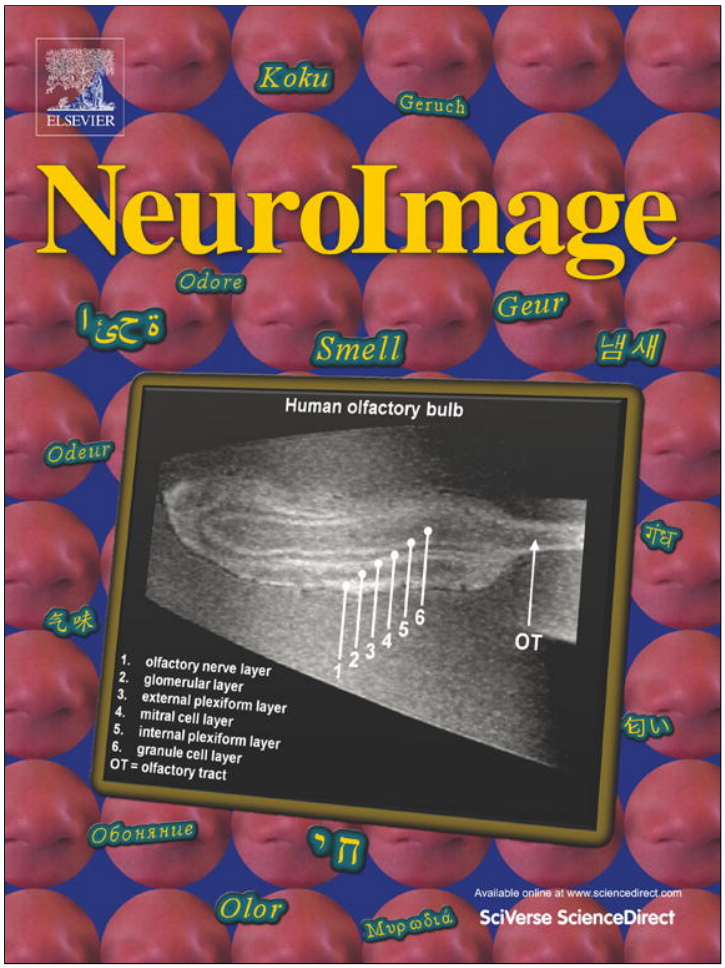

(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

NeuroImage 73 (2013) 1–7

Contents lists available at SciVerse ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Opposite effects of noradrenergic arousal on amygdala processing of fearful faces in men and women

Lars Schwabe [a,*], Oliver Höffken [b], Martin Tegenthoff [b], Oliver T. Wolf [a]

[a] Institute of Cognitive Neuroscience, Department of Cognitive Psychology, Ruhr-University Bochum, Universitaetsstrasse 150, 44780 Bochum, Germany
[b] Department of Neurology, Ruhr-University Bochum, BG-Kliniken Bergmannsheil, Bürkle de la Camp-Platz 1, 44789 Bochum, Germany

ARTICLE INFO

Article history:
Accepted 25 January 2013
Available online 4 February 2013

Keywords:
Stress
Noradrenaline
Glucocorticoids
Amygdala
Fear
Sex differences

ABSTRACT

Fear-related disorders are significantly more prevalent in women than in men. Stress may modulate the neurocircuitry of fear and is a critical factor in the pathogenesis of fear-related disorders. Therefore, we tested in the present experiment the hypothesis that noradrenaline and glucocorticoids, two major stress mediators, have differential effects on fear processing in men and women. In a placebo-controlled, double-blind between-subject design, 80 healthy men and women were administered orally the α2-adrenoceptor antagonist yohimbine and/or the synthetic glucocorticoid hydrocortisone before they rated images of neutral and fearful faces with respect to the degree of fearfulness of the facial expression. During presentation of facial expressions, functional magnetic resonance images were collected. Yohimbine increased subjective ratings of the fearfulness of the faces in women but reduced fearfulness ratings in men. Neuroimaging data showed that yohimbine increased amygdala activity in response to fearful faces in women, whereas it attenuated amygdala responsivity to fearful faces in men. Moreover, yohimbine decreased orbitofrontal activity while viewing fearful faces in women. Hydrocortisone did not affect fear processing, neither in men nor in women. Our findings suggest that noradrenergic arousal may have opposite effects on fear processing in men and women. These sex differences may represent a biological mechanism that contributes to the differential prevalence of fear-related disorders in men and women.

Introduction

Aberrant fear is at the heart of many psychiatric disorders, including panic disorder, post-traumatic stress disorder (PTSD) and phobias. Across cultures and countries, women are at least twice as likely as men to suffer from such fear-related disorders (Kessler et al., 2005; Wittchen et al., 2011). Despite this striking sex difference and great interest in that area, neural mechanisms that contribute to the differential prevalence of fear-related disorders in men and women remain largely elusive.

The key structure in the processing of fear is the amygdala (LeDoux, 2000). Bilateral damage to the amygdala impairs the processing of fearful facial expressions and the acquisition of conditioned fear responses (Adolphs et al., 1994). Neuroimaging data from healthy subjects confirm the critical role of the amygdala in fear processing and learning (Büchel et al., 1998; Morris et al., 1998). In line with the view that altered fear processing is related to psychopathology, exaggerated amygdala activity during the processing of fearful information has been noted in PTSD, depression and social anxiety (Birbaumer et al., 1998; Rauch et al., 2000; Shin et al., 2005; Stein et al., 2002). Moreover, there is considerable evidence for sex differences in amygdala responding during fear-related activities (Cahill, 2006), with some studies reporting stronger amygdala activation in response to fearful information in women than in men (Williams et al., 2005).

Animal and human studies indicate that amygdala activity can be modulated by neurotransmitters and hormones, such as noradrenaline and glucocorticoids (mainly cortisol in humans), that are released in response to stress (Cahill and McGaugh, 1998; Henckens et al., 2010; Rasch et al., 2009; Roozendaal et al., 2009; Strange and Dolan, 2004; van Stegeren et al., 2005). Stressful experiences are also critically involved in the pathogenesis of PTSD and other fear-related disorders (Horowitz, 1997). It is therefore tempting to hypothesize that sex differences in the prevalence of fear-related disorders are (at least partly) related to sex differences in the impact of stress (hormones) on amygdala processing of fear.

The present experiment addressed this hypothesis in a large sample of men and women and examined potential sex differences in the influence of noradrenaline and glucocorticoids on neural processing of fear. We administered healthy men and women orally the α2-adrenoceptor antagonist yohimbine, which leads to increased noradrenergic stimulation, the synthetic glucocorticoid hydrocortisone, or a combination of both drugs in order to assess potential interaction effects of noradrenaline and glucocorticoids on fear processing. After drug intake, participants

* Corresponding author at: Ruhr-University Bochum, Department of Cognitive Psychology, 44780 Bochum, Germany. Fax: +49 234 3214308.
E-mail address: Lars.Schwabe@ruhr-uni-bochum.de (L. Schwabe).

1053-8119/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuroimage.2013.01.057

saw, while lying in a 3 Tesla scanner, pictures of fearful (and neutral) facial expressions, which are known to reliably recruit the amygdala and other fear relevant structures such as the medial prefrontal cortex, the insula or the anterior cingulate cortex (Breiter et al., 1996; Stein et al., 2007; Vuilleumier et al., 2001). Given that stress- and fear-related disorders are significantly more prevalent in women than in men and that amygdala activity is often increased during fear processing in these disorders, we expected that yohimbine and glucocorticoids would result in stronger amygdala activation during viewing of fearful faces in women than in men.

Material and methods

Participants

In a placebo-controlled, double-blind between-subject design, 80 healthy, normal weight, right-handed nonsmokers (40 men, 40 women; age: 23.53 ± 0.34 years; body-mass-index (kg/m^2): 23.29 ± 0.33) with normal or corrected-to-normal vision were randomly assigned to one of four experimental groups (n = 20/group): placebo/placebo (PLAC), yohimbine/placebo (YOH), hydrocortisone/placebo (CORT), or yohimbine/hydrocortisone (YOH + CORT). Exclusion criteria for participation were checked by a psychologist (LS) in a standardized interview and included medication intake, hormonal contraceptive use, any current or chronic medical condition, current or lifetime history of any psychiatric or neurological disorder, and any contraindications for MRI. Women were tested only during the late follicular and luteal phase of their menstrual cycle when estrogen and progesterone levels were relatively high in order to reduce variability in sex hormone variations; menstrual cycle phase was determined based on participants' reports. The study protocol was approved by the Review Board of the Medical Faculty of the Ruhr-University Bochum. All participants provided written informed consent.

Drug administration and manipulation check

Participants were administered 20 mg Yohimbine (Desma) and/or 20 mg Hydrocortisone (JenaPharm) orally about 45 min before the MRI session. Drug dosage and timing of drug administration were chosen according to previous studies using these drugs (Buchanan and Lovallo, 2001; Schwabe et al., 2010; van Stegeren et al., 2010). In order to verify the action of the drugs, we collected saliva samples at several time points before and after drug administration. From saliva, we analyzed the biologically active, free fraction of the stress hormone cortisol, the major glucocorticoid in humans, and the enzyme alpha-amylase, an indicator of adrenergic activity (Chatterton et al., 1996). Cortisol concentrations were determined by a luminescence immunoassay (IBL, Hamburg, Germany; Westermann et al., 2004). Mean intra- and inter-assay coefficients of variation are less than 8% and 12%, respectively. Levels of salivary alpha-amylase were determined from the saliva samples using a commercially available kinetic reaction assay (Salimetrics, Penn State, PA; Granger et al., 2007). Mean intra- and interassay coefficients of variation of the salivary alpha-amylase analyses are less than 8% and 6%, respectively.

Stimuli

Stimuli consisted of frontal view images of 36 neutral and 36 fearful faces (18 male and 18 female faces per category) taken from the Radboud Faces Database, a validated set of pictures of models displaying different emotional expressions (Langner et al., 2010). Examples of the face stimuli are shown in Fig. 1.

Procedure

Because of the diurnal rhythm of the stress hormone cortisol, all testing took place in the afternoon between 1.00 and 6.30 pm. After their arrival at the laboratory, all participants completed the State-Trait-Anxiety Inventory (STAI; Spielberger, 2010) to control for potential group differences in state or trait anxiety. Moreover, participants gave a first saliva sample before they received placebo, yohimbine (20 mg) or hydrocortisone (20 mg) pills, depending on the experimental group. After a 30-minute break during which they remained in a quiet room, participants gave another saliva sample and were prepared for the MRI session. About 45 min after pill intake, participants gave another saliva sample immediately before the MRI session started. After a short anatomical scan, participants saw images of 36 fearful and 36 neutral faces that were presented in randomized order at the center of a computer screen (Fig. 1). Each picture was presented for 4 s. Participants were instructed to rate on a scale from 1 ("not at all fearful") to 4 ("very fearful") the degree of fearfulness of the facial expressions by pressing the corresponding button on a four-button response box. Between trials, participants were presented a fixation cross for 5 to 7 s (random jitter: 0–2 s). In total, the face rating task took about 13 min. After scanning, participants gave a final saliva sample out of the scanner.

Image acquisition

Imaging was conducted on a 3.0 Tesla Philips Achieva scanner equipped with a 32-channel head coil. For each participant, one high-resolution T1-weighted anatomical scan was acquired with the following parameters: 220 slices, slice thickness 1 mm, repetition time (TR) = 8.2 ms, echo time (TE) = 3.8 ms. During the face rating task, functional scans (370 volumes) were acquired parallel to the AC–PC plane (30 slices, slice thickness 3 mm, TR = 2.0 s, TE = 30 ms, flip angle = 90°, 64 × 64 matrix, 2 mm × 2 mm pixel size, field of view = 200 × 200 mm). The first 3 images were discarded to allow T1 equilibration.

Data analysis

Cortisol and alpha-amylase data were analyzed by sex × yohimbine (placebo vs. yohimbine) × hydrocortisone (placebo vs. hydrocortisone) × time point of measurement analyses of variance (ANOVAs). The face rating data were subjected to a mixed-design ANOVA with the factors sex, yohimbine, hydrocortisone and facial expression (neutral vs. fearful). Significant main or interaction effects were followed by appropriate post-hoc tests. All reported p-values are two-tailed.

Preprocessing and analysis of the event-related fMRI data were performed using SPM8 (Wellcome Trust Center for Neuroimaging, University College London). Functional imaging data were corrected for slice-timing and head motion. Structural images were segmented into gray matter, white matter, and cerebrospinal fluid. Gray matter images were normalized to the MNI template image. Functional images were co-registered with the structural image and combined with normalization parameters of the gray matter image in the final warp. Finally, data were spatially smoothed using an 8 mm full width half-maximum Gaussian kernel and filtered in the temporal domain using a nonlinear high-pass filter with a 128 s cut-off.

Functional data were analyzed using a general linear model with the regressors neutral face and fearful face. In addition, we included button presses and the six movement regressors counting information about motion correction into our model. Regressors of interest were constructed by a stick function convolved by a hemodynamic response function (HRF). The data were filtered in the temporal domain using a nonlinear high-pass filter with a 128 s cut-off. Contrast estimates were calculated for fearful face–neutral face and neutral face–fearful face.

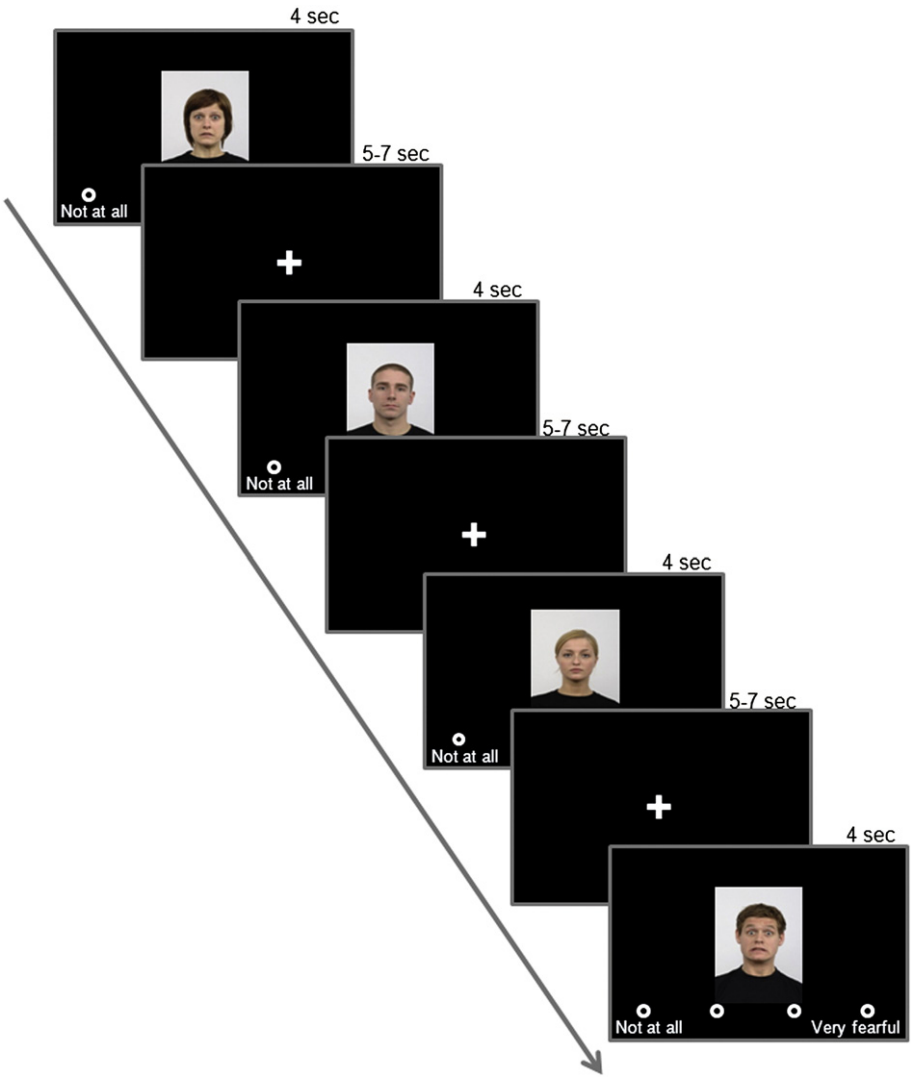

Fig. 1. Task procedure. Participants saw front view images of neutral and fearful facial expressions and were asked to rate the fearfulness of the facial expressions. Images of faces were shown for 4 s; between images there was a fixation period of 5 to 7 s.

Linear contrasts were used to obtain subject-specific estimates for each effect of interest, which were then entered into a second-level (group) analysis, treating subject as a random effect and using a full factorial model with the factors yohimbine (placebo vs. yohimbine), hydrocortisone (placebo vs. hydrocortisone), and participants' sex. We used explorative whole brain analyses as well as region of interest (ROI) analyses. For the explorative whole brain analyses, the significance threshold was set to $p<0.05$ on voxel-level, corrected for multiple testing (family-wise error (FWE) correction). ROI analyses were performed using the small volume correction options of SPM8 ($p<0.05$). A priori ROIs included the amygdala, the insular cortex, the medial prefrontal and orbitofrontal cortex, the anterior cingulate cortex, and the fusiform cortex which were identified in previous neuroimaging studies on emotion and face processing (Stein et al., 2007; Vuilleumier et al., 2001). The respective ROI masks were taken from the Harvard–Oxford cortical and subcortical atlases.

Results

Changes in cortisol and alpha-amylase after hydrocortisone and yohimbine intake

Significant changes in salivary cortisol and alpha-amylase confirmed the action of the drugs. Salivary alpha-amylase, an indicator of noradrenergic activity (Chatterton et al., 1996), increased after yohimbine intake (time × yohimbine: $F(3,210)=3.89$, $p=0.01$, $\eta^2=0.05$) but not after hydrocortisone intake (time × hydrocortisone: $F(3,210)=0.38$, $p=0.76$; Fig. 2A). Conversely, salivary cortisol increased significantly after hydrocortisone intake (time × hydrocortisone: $F(3,210)=33.77$, $p<0.0001$, $\eta^2=0.33$) but not after yohimbine intake (time × yohimbine: $F(3,210)=0.53$, $p=0.66$; Fig. 2B). There were no interaction effects between yohimbine and hydrocortisone, neither for alpha-amylase nor for cortisol (all $F<1$, all $p>0.42$). Furthermore, men and women did not differ in their alpha-amylase or cortisol levels (all main and interaction effects: $F<2.10$, $p>0.11$).

Moreover, experimental groups did not differ in their state and trait anxiety scores, nor did men and women (all main and interaction effects: $F<1.61$, $p>0.20$; Supplementary Table S1).

Yohimbine increases fearfulness ratings in women but decreases fearfulness ratings in men

As expected, participants rated fearful facial expressions as significantly more fearful than neutral facial expressions ($F(1,72)=1852.93$, $p<0.0001$, $\eta^2=0.96$). Interestingly, however, fearfulness ratings were modulated by yohimbine intake and participants' sex. In a sex × yohimbine × hydrocortisone × facial expression ANOVA, we obtained a main effect of sex ($F(1,72)=5.77$, $p=0.019$, $\eta^2=0.07$),

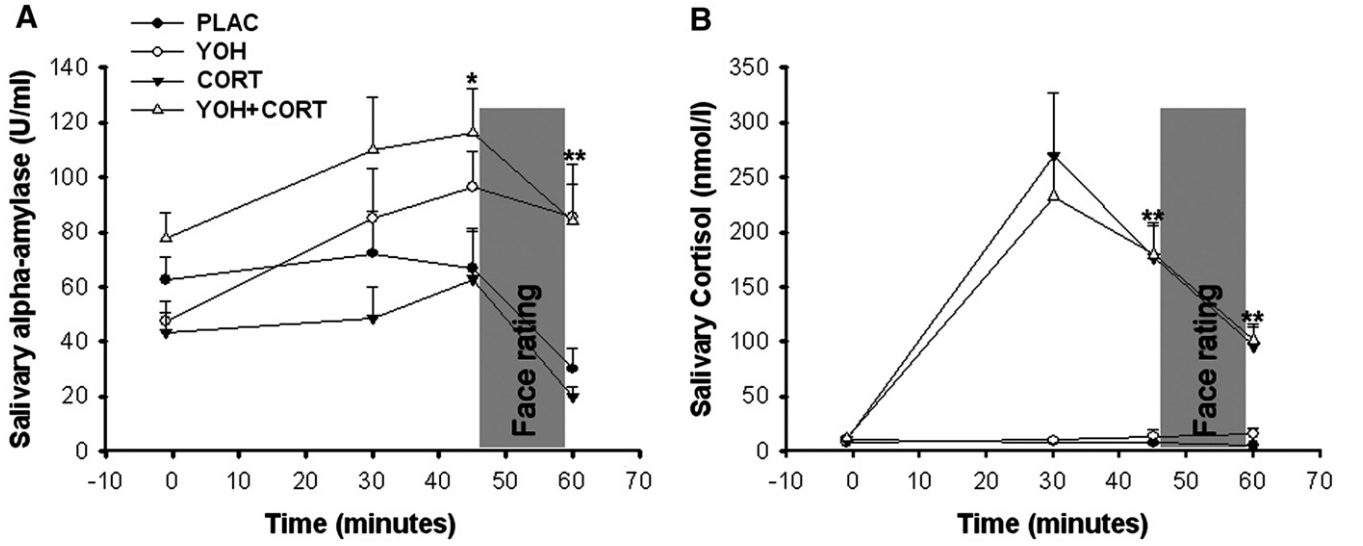

Fig. 2. Alpha-amylase and cortisol levels across the experiment. (A) Salivary alpha amylase increased in the groups that had received yohimbine (YOH and YOH + CORT, respectively) but not in the groups that had received a placebo (PLAC) or hydrocortisone only (CORT). (B) Conversely, salivary cortisol levels were elevated in participants that were administered hydrocortisone but not in those that were administered a placebo or yohimbine only. ** $p<.01$; * YOH + CORT vs. PLAC and CORT, respectively: both $p<.05$. Data represent means and standard error of the mean.

a sex × yohimbine interaction ($F(1,72)=6.19$, $p=0.015$, $\eta^2=0.08$), a sex × facial expression interaction ($F(1,72)=5.34$, $p=0.024$, $\eta^2=0.07$), and, most importantly, a three-way interaction between sex, yohimbine, and facial expression ($F(1,72)=11.03$, $p=0.001$, $\eta^2=0.13$). Whereas sex and yohimbine did not affect participants' ratings of the neutral facial expressions (all main and interaction effects: $F<1.40$, $p>0.40$), yohimbine had opposite effects on the ratings of fearful faces in men and women (sex × yohimbine interaction: $F(1,72)=10.93$, $p=0.009$, $\eta^2=0.13$). As shown in Fig. 3, yohimbine increased fearfulness ratings in women ($t(38)=2.45$, $p=0.019$) but reduced the ratings of fearfulness in men ($t(38)=2.22$, $p=0.032$). Hydrocortisone did not influence the ratings of the facial expressions (all main and interaction effects: $F<2.52$, $p>0.15$).

Although the absence of any interaction effects with hydrocortisone suggests that hydrocortisone did not modulate the yohimbine effects, we ran an additional sex × group (PLAC, CORT, YOH, and YOH + CORT) × facial expression ANOVA to rule out the possibility that the effects of yohimbine were affected by hydrocortisone (as the observed main effect of yohimbine collapsed across the YOH and YOH + CORT groups). This analysis confirmed the opposite effects of yohimbine in men and women and showed that these effects were independent of hydrocortisone. We obtained a significant sex × group × facial expression effect ($F(3,72)=6.25$, $p=.001$, $\eta^2=.21$) showing a differential effect of the pharmacological treatment in men and women for negative (sex × group: $F(3, 72)=6.92$, $p<.001$, $\eta^2=.22$) but not for neutral faces ($p>.50$). Follow-up analyses revealed that women in the YOH and YOH + CORT groups rated the fearful faces as more fearful than women in the PLAC and CORT groups (group effect: $F(3, 36)=4.13$, $p=.01$, $\eta^2=.26$; LSD post-hoc tests: all $p<.07$). In men, the fearfulness ratings of the YOH and YOH + CORT groups were reduced compared to the PLAC and CORT groups (group effect: $F(3, 36)=3.00$, $p=.04$, $\eta^2=.26$; LSD post-hoc tests: all $p<.08$). Importantly, there were no differences between the YOH and the YOH + CORT groups (neither in men nor in women; both $p>.38$), nor were there any differences between the PLAC and CORT groups (both $p>.63$).

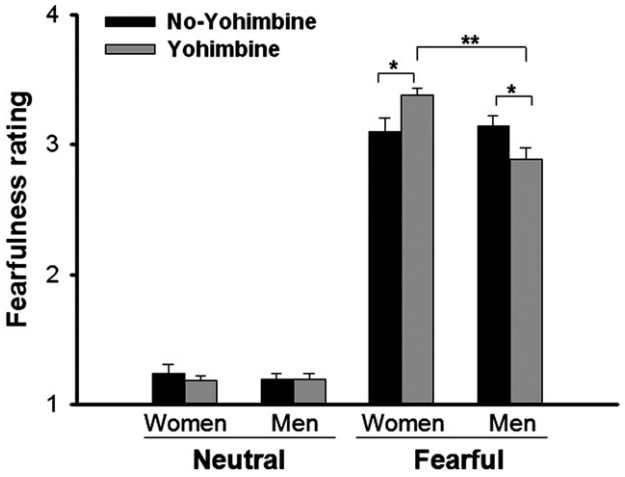

Fig. 3. Opposite effect of yohimbine on fearfulness ratings in women and men. Fearful facial expressions were rated as significantly more fearful than neutral facial expressions. Administration of the α2-adrenoceptor antagonist yohimbine increased fearfulness ratings in women whereas yohimbine administration decreased fearfulness ratings in men. Facial expressions were rated on a scale from 1 ("not at all fearful") to 4 ("very fearful"). * $p<.05$, ** $p<.01$. Data represent means and standard error of the mean.

Yohimbine has opposite effects on amygdala responses to fearful faces in men and women

In line with earlier studies on fearful face processing (Stein et al., 2007; Vuilleumier et al., 2001), our imaging data showed that, compared to viewing of neutral faces, viewing of fearful faces was associated with significant activation of the amygdala, the anterior cingulate cortex, the insular cortex, the orbitofrontal cortex and the fusiform cortex. In addition to these ROI, exploratory whole brain analyses revealed activations in a number of other frontal, parietal, temporal, and occipital regions (Supplementary Table S2). In order to assess whether yohimbine, hydrocortisone and participants' sex modulated the neural structures involved in processing of fearful faces, we analyzed brain activations associated with viewing fearful versus neutral faces with a full factorial model. This analysis revealed a significant (bidirectional) interaction of yohimbine administration and participants' sex in the amygdala ([−30, 0, −16]; $Z=3.72$, $p=0.011$; FWE corrected; Figs. 4A and B). To pursue this analysis, we assessed the influence of yohimbine on brain activations for the contrast fearful minus neutral faces in men and women, separately. We found that yohimbine reduced amygdala activity in men (no-yohimbine–yohimbine:

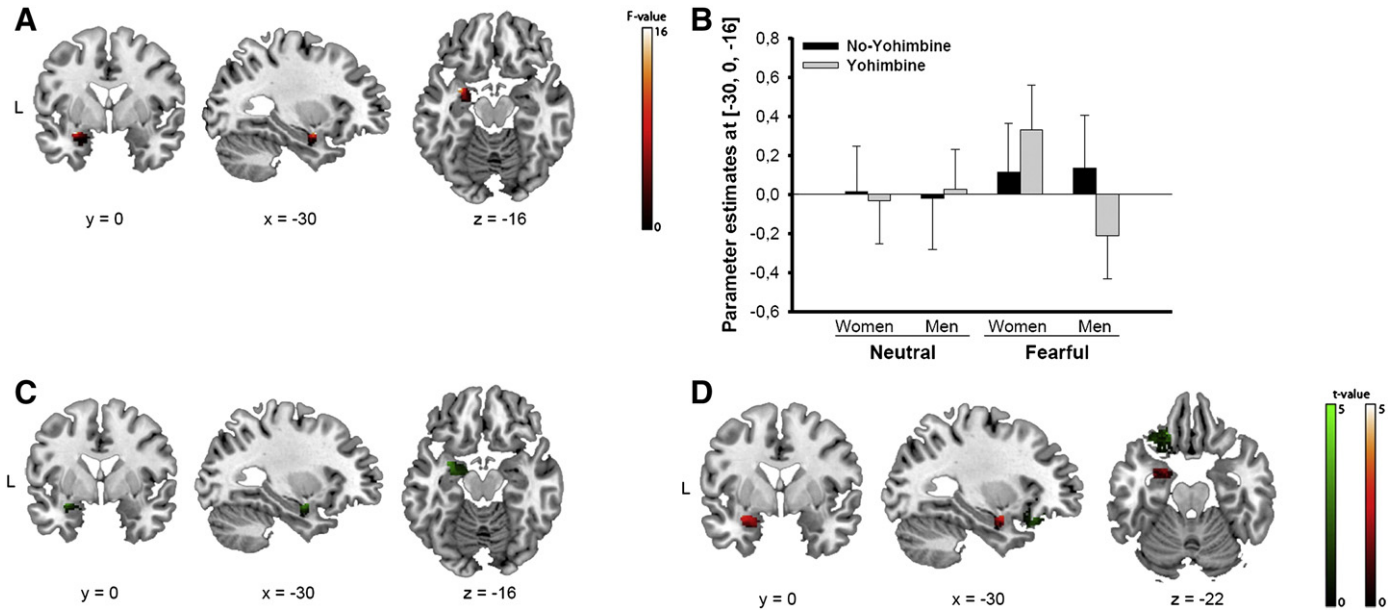

Fig. 4. Modulation of brain activity during viewing of fearful (versus neutral) facial expressions by yohimbine and sex. (A) Interactive effect of yohimbine and participants' sex on brain activity associated with viewing of fearful faces. (B) Parameter estimates are shown for the peak voxel in the amygdala. Values are mean ± SEM. (C) In men, yohimbine intake reduced amygdala activity during viewing of fearful faces. (D) In women, however, yohimbine increased amygdala activity but decreased activity in the orbitofrontal cortex during viewing of fearful facial expressions. Coronal, sagittal, and transverse sections are shown, superimposed on a T1 template image. Increased activations after yohimbine intake are shown in red/yellow; deactivations after yohimbine intake are shown in green.

[−30, 0, −16], $Z = 3.35$, $p = 0.034$, FWE corrected; Fig. 4C; yohimbine–no-yohimbine: no suprathreshold activations). In women, however, yohimbine increased amygdala activity during viewing of fearful faces (yohimbine–no-yohimbine: [−30, 0, −16], $Z = 3.10$, $p = 0.057$, FWE corrected; Fig. 4D). At the same time, yohimbine reduced activity in the orbitofrontal cortex in women (no-yohimbine–yohimbine: [−20, 30, −22], $Z = 3.62$, $p = 0.05$, FWE corrected). In contrast to yohimbine, hydrocortisone did not affect brain activity during viewing of fearful faces. There were no significant main effects of hydrocortisone, nor any interaction effects with yohimbine or participants' sex. Moreover, we did not obtain any effects of yohimbine, hydrocortisone or sex for the reverse contrast neutral minus fearful faces. The exploratory whole brain analyses revealed a significant sex × yohimbine interaction in the superior orbital lobe ([24, 30, −12], $Z = 3.61$, $p < 0.001$, cluster size: 384 voxel) when the threshold was set to $p < .001$ in 10 contiguous voxels. Follow-up tests showed that this effect was owing to reduced superior orbital activation after yohimbine intake in men ([24, 32, −10], $Z = 3.33$, $p < 0.001$, cluster size: 169 voxel) whereas yohimbine did not affect superior orbital activation in women.

Because the reported main effects of yohimbine in men and women include both the YOH and the YOH + CORT groups, we contrasted these two groups (both in men and women) in an additional analysis directly to rule out the possibility that there were any modulatory influences of hydrocortisone. We did not find any differences in brain activation between these groups, neither in men nor in women. Even when the statistical threshold was set to the much more lenient level of $p = .005$ (uncorrected), no differences occurred. Similarly, we obtained no differences in brain activation between the PLAC and CORT groups. Thus, the observed effects of yohimbine were not modulated by hydrocortisone. Moreover, we compared brain activation during viewing of fearful (vs. neutral) faces in men and women separately in the YOH and YOH + CORT groups. These analyses showed that women had reduced amygdala activity compared to men, both in the YOH ([−30, 0, −16]; $Z = 3.44$, $p = 0.034$; FWE corrected) and YOH + CORT group ([−16, −4, −14]; $Z = 3.19$, $p = 0.056$; FWE corrected). In addition to these differences in amygdala activation, we obtained further differences between men and women, mainly in frontal areas, when the statistical threshold was set to the more lenient level of $p = .005$ (uncorrected; see supplementary Table S3).

In order to assess whether the interactive influence of participants' sex and yohimbine on amygdala activation was mediated by subjective ratings of fear (Dyck et al., 2011; Williams et al., 2006), we performed first a regression analysis in which we regressed fear ratings on brain activity, including participants' sex as an additional factor. This analysis, however, revealed no significant associations between fear ratings and brain activation. Nevertheless, we entered in a next step subjective fear ratings as a covariate into our sex × yohimbine × hydrocortisone full factorial model. This ANCOVA showed that the sex × yohimbine interaction effect in the amygdala ([−30, 0, −16]; $Z = 3.66$, $p = 0.014$; FWE corrected) remained when we controlled for differences in subjective fear ratings. Even when controlling for subjective fear ratings, yohimbine increased amygdala activation in men ([−30, 0, −16]; $Z = 3.53$, $p = 0.020$; FWE corrected) and tended to decrease amygdala activation in women ([−30, −2, −16]; $Z = 2.72$, $p = 0.145$; FWE corrected). However, the decrease in orbitofrontal activation after yohimbine intake in women disappeared when we controlled for the influence of fear ratings ($p > .001$, uncorrected), suggesting that activation in the orbitofrontal cortex was associated with explicit fear in women.

Discussion

Stress modulates fear processing networks in the brain (Roozendaal et al., 2009; van Stegeren et al., 2005) and is a key factor in the pathogenesis of fear-related disorders (Horowitz, 1997). Most of these fear-related disorders are significantly more prevalent in women than in men (Kessler et al., 2005; Wittchen et al., 2011). We therefore hypothesized that stress may, mediated by noradrenergic arousal and/or glucocorticoid stress hormones, have a differential effect on fear processing in men and women. To test this hypothesis, healthy subjects received the α2-adrenoceptor antagonist yohimbine, which leads to increased noradrenergic stimulation, or the synthetic glucocorticoid hydrocortisone before they viewed pictures of fearful facial expressions in the scanner. Our results indicate that noradrenergic arousal, but not glucocorticoids, had indeed a differential impact on fear processing in men and women. Yohimbine increased subjective fearfulness ratings in

women, whereas it decreased fearfulness ratings in men. At the neural level, this sex-dependent influence of yohimbine was reflected in opposite changes in amygdala activity during viewing of fearful faces, with increased amygdala activity after yohimbine intake in women and reduced amygdala activity after yohimbine intake in men.

Sex differences have been reported in a variety of emotion-related behaviors and neuroimaging studies suggest that these differences are closely linked to differences in amygdala responsivity (Cahill, 2006; Hamann, 2005). These functional differences may be related to structural and developmental sex differences that have been found for the amygdala (Newmann, 1999). Most interestingly for the present study, first evidence points to possible sex differences in amygdala responsivity to fear-related stimuli which are associated with autonomic arousal (Williams et al., 2001, 2005). Previous pharmacological studies that addressed the role of noradrenergic arousal on amygdala activity in response to emotional stimuli directly found that beta-adrenergic blockade reduced amygdala responsivity to emotional material (Hurlemann et al., 2010; van Stegeren et al., 2005), whereas the noradrenaline reuptake inhibitor reboxetin increased amygdala activity (Onur et al., 2009). These studies, however, tested only relatively moderate sample sizes that did not allow focusing on possible sex differences. To the best of our knowledge, the present study is the first that examined noradrenaline (and glucocorticoid) effects on fear processing in a large sample of men and women. We found increased fear ratings and amygdala responsivity after yohimbine intake in women but decreased fear ratings and amygdala responsivity after yohimbine intake in men. How can these opposite effects of noradrenergic arousal in men and women be explained? Some authors argued that women have developed a more sensitive fear detection system during evolution that can be activated more easily (Campbell, 1999). According to this view, one might speculate that women have a higher density or sensitivity of adrenergic receptors in the amygdala than men. However, as far as we know, direct evidence to support this claim is missing.

Another, more likely, explanation takes the role of sex hormones into account. Major female sex hormones (estrogen and progesterone) have a strong impact on amygdala functioning. For example, estrogen increases synaptogenesis and spontaneous activity in the amygdala (Matsumoto, 1991; Scheiss et al., 1988) and interacts with catecholaminergic systems to increase amygdala activity (McEwen and Alves, 1999). Male sex hormones (in particular, testosterone), however, may have the opposite effect than estrogen, they may counterbalance the effects of arousal on the amygdala. For instance, it has been shown that stress increases fear responses and α2-adrenergic binding sites in the amygdala and that the injection of testosterone decreased emotional responses and α2-adrenoceptor expression in the medial amygdala (Flügge et al., 2001). These data suggest a critical influence of sex hormones in the impact of noradrenergic arousal on amygdala activity. In the present study, women were tested only in the late follicular and luteal phase when both estrogen and progesterone were relatively high. Future studies, however, should also directly measure or manipulate experimentally estrogen, progesterone and testosterone levels to elucidate the role of sex hormones in the effect of arousal on fear processing.

In addition to the alterations in amygdala activity, yohimbine decreased in women also activity in the orbitofrontal cortex while viewing images of fearful faces. This finding is in line with other studies showing reduced orbitofrontal cortex functioning after noradrenergic stimulation (Schwabe et al., 2012; Sun et al., 2010). The orbitofrontal cortex is another key region for the processing of emotional information. However, its role in emotion processing appears to differ from the role of the amygdala. Whereas the amygdala is implicated in rather automatic, 'reflexive' responding to emotional stimuli (Whalen et al., 2004), the orbitofrontal cortex is related to a more thorough analysis of emotional information (Schwabe et al., 2011). Thus, the finding that yohimbine increases amygdala activity but decreases orbitofrontal activity suggests that noradrenergic arousal alters fear processing in women in two ways: by enhancing the 'reflexive' fear system and by impairing the system involved in the more elaborative evaluation of emotional stimuli. Interestingly, the same pattern of results, i.e., increased amygdala activity and decreased orbitofrontal activity, has been observed in several fear-related disorders (Shin et al., 2004, 2006).

Whereas the α2-adrenoceptor antagonist yohimbine had a significant impact on fear processing, glucocorticoids did not affect brain activity while viewing fearful faces nor did they modulate the effect of noradrenergic stimulation, neither in men nor in women. It is well-known that glucocorticoids interact with noradrenergic arousal to enhance memory consolidation (Roozendaal et al., 2009). These effects, however, are presumably mediated by genomic glucocorticoid actions that develop over hours. In addition to genomic actions, glucocorticoids may also exert rapid non-genomic actions via membrane-bound receptors (de Kloet et al., 2008). A recent study contrasted the rapid, non-genomic vs. slow, genomic effects of glucocorticoids on the processing of fearful faces in men (Henckens et al., 2010). The results showed that slow glucocorticoid actions reduced amygdala responsivity to fearful faces, whereas rapid glucocorticoid action had no emotion-specific effect on amygdala processing. Another neuroimaging study showed that slow glucocorticoid actions may also interact with noradrenaline to modulate amygdala reactivity to fearful stimuli (Kukolja et al., 2008). Here, we administered glucocorticoids about 45 min before stimulus presentation, when genomic glucocorticoid actions had most likely not yet developed. Although glucocorticoids may rapidly modulate amygdala activity in general (Lovallo et al., 2010), our results suggest that these rapid glucocorticoid effects are not specific to the processing of fearful information. At this point, it is to be noted however, that the hydrocortisone dose that was used in the present study was relatively moderate. It cannot be ruled out that, at a higher hydrocortisone dose, even non-genomic glucocorticoid actions might alter fear processing.

In summary, our findings show that noradrenergic arousal has opposite effects on fear processing in men and women, with increased fear processing in women and reduced fear processing in men. Such sex differences in fear processing in times of high arousal may represent a biological mechanism that contributes to the differential prevalence of fear-related disorders in men and women.

Acknowledgment

This study was supported by a grant of the German Research Foundation (DFG; SCHW 1357/2-2). We gratefully acknowledge the technical assistance of Tobias Otto and we thank Carsten Siebert and Florian Watzlawik for their help during data collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2013.01.057.

References

Adolphs, R., Tranel, D., Damasio, H., Damasio, A., 1994. Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. Nature 372, 669–672.
Birbaumer, N., Grodd, W., Diedrich, O., Klose, U., Erb, M., Lotze, M., Schneider, F., Weiss, U., Flor, H., 1998. fMRI reveals amygdala activation to human faces in social phobics. Neuroreport 9, 1223–1226.
Breiter, H.C., Etcoff, N.L., Whalen, P.J., Kennedy, W.A., Rauch, S.L., Buckner, R.L., Strauss, M.M., Hyman, S.E., Rosen, B.R., 1996. Response and habituation of the human amygdala during visual processing of facial expression. Neuron 17, 875–887.
Buchanan, T.W., Lovallo, W.R., 2001. Enhanced memory for emotional material following stress-level cortisol treatment in humans. Psychoneuroendocrinology 26, 307–317.
Büchel, C., Morris, J., Dolan, R.J., Friston, K.J., 1998. Brain systems mediating aversive conditioning: an event-related fMRI study. Neuron 20, 947–957.
Cahill, L., 2006. Why sex matters for neuroscience. Nat. Rev. Neurosci. 7, 477–484.
Cahill, L., McGaugh, J.L., 1998. Mechanisms of emotional arousal and lasting declarative memory. Trends Neurosci. 21, 294–299.

Campbell, A., 1999. Staying alive: evolution, culture, and women's intrasexual aggression. Behav. Brain Sci. 22, 203–214.
Chatterton, R.T.J., Vogelsong, K.M., Lu, Y.C., Ellman, A.B., Hudgens, G.A., 1996. Salivary alpha-amylase as a measure of endogenous adrenergic activity. Clin. Physiol. 16, 433–448.
de Kloet, E.R., Karst, H., Joels, M., 2008. Corticosteroid hormones in the central stress response: quick-and-slow. Front. Neuroendocrinol. 29, 268–272.
Dyck, M., Loughead, J., Kellermann, T., Boers, F., Gur, R.C., Mathiak, K., 2011. Cognitive versus automatic mechanisms of mood induction differentially activate left and right amygdala. Neuroimage 54, 2503–2513.
Flügge, G., Kramer, M., Fuchs, E., 2001. Chronic subordination stress in male tree shrews: replacement of testosterone affects behavior and central alpha2-adrenoceptors. Physiol. Behav. 73, 293–300.
Granger, D.A., Kivlighan, K.T., El-Sheikh, M., Gordis, E., Stroud, L.R., 2007. Salivary alpha-amylase in biobehavioral research: recent developments and applications. Ann. N. Y. Acad. Sci. 1098, 122–144.
Hamann, S., 2005. Sex differences in the responses of the human amygdala. Neuroscientist 11, 288–293.
Henckens, M.J.A.G., van Wingen, G.A., Joels, M., Fernandez, G., 2010. Time-dependent effects of corticosteroids on human amygdala processing. J. Neurosci. 30, 12725–12732.
Horowitz, M.J., 1997. Stress Response Syndromes: PTSD, Grief, and Adjustment Disorders, 3rd ed. Jason Aronson, Lanham.
Hurlemann, R., Walter, H., Rehme, A.K., Kukolja, J., Santoro, S.C., Schmidt, C., Schnell, K., Musshoff, F., Keysers, C., Maier, W., Kendrick, K.M., Onur, O.A., 2010. Human amygdala reactivity is diminished by the β-noradrenergic antagonist propranolol. Psychol. Med. 40, 1839–1848.
Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R., Walters, E.E., 2005. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. Arch. Gen. Psychiatry 62, 593–602.
Kukolja, J., Schläpfer, T.E., Keysers, C., Klingmüller, D., Maier, W., Fink, G.R., Hurlemann, R., 2008. Modelling a negative response bias in the human amygdala by noradrenergic–glucocorticoid interactions. J. Neurosci. 28, 12868–12876.
Langner, O., Dotsch, R., Bijlstra, G., Wigboldus, D.H.J., Hawk, S.T., van Knippenberg, A., 2010. Presentation and validation of the Radboud Faces Database. Cogn. Emot. 24, 1377–1388.
LeDoux, J.E., 2000. Emotion circuits in the brain. Annu. Rev. Neurosci. 23, 155–184.
Lovallo, W.R., Robinson, J.L., Glahn, D.C., Fox, P.T., 2010. Acute effects of hydrocortisone on the human brain: an fMRI study. Psychoneuroendocrinology 35, 15–20.
Matsumoto, A., 1991. Synaptogenic action of sex steroids in developing and adult neuroendocrine brain. Psychoneuroendocrinology 16, 25–40.
McEwen, B.S., Alves, S.E., 1999. Estrogen actions in the central nervous system. Endocr. Rev. 20, 279–307.
Morris, J., Friston, K.J., Büchel, C., Frith, C.D., Young, A.W., Calder, A.J., Dolan, R.J., 1998. A neuromodulatory role for the human amygdala in processing emotional facial expressions. Brain 121, 47–57.
Newmann, S.W., 1999. The medial extended amygdala in male reproductive behavior: a node in the mammalian social behavior network. Ann. N. Y. Acad. Sci. 877, 242–257.
Onur, O.A., Walter, H., Schlaepfer, T.E., Rehme, A.K., Schmidt, C., Keysers, C., Maier, W., Hurlemann, R., 2009. Noradrenergic enhancement of amygdala responses to fear. Soc. Cogn. Affect. Neurosci. 4, 119–126.
Rasch, B., Spalek, K., Buholzer, S., Luechinger, R., Boesiger, P., Papassotiropoulos, A., de Quervain, D.J.F., 2009. A genetic variation of the noradrenergic system is related to differential amygdala activation during encoding of emotional memories. Proc. Natl. Acad. Sci. U. S. A. 106, 19191–19196.
Rauch, S.L., Whalen, P.J., Shin, L.M., McInerney, S.C., Macklin, M.L., Lasko, N.B., Orr, S.P., Pitman, R.K., 2000. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. Biol. Psychiatry 47, 769–776.
Roozendaal, B., McEwen, B.S., Chattarji, S., 2009. Stress, memory and the amygdala. Nat. Rev. Neurosci. 10, 423–433.
Scheiss, M.C., Joels, M., Shinnick-Gallagher, P., 1988. Estrogen priming effects active membrane properties of medial amygdala neurons. Brain Res. 440, 380–385.
Schwabe, L., Tegenthoff, M., Höffken, O., Wolf, O.T., 2010. Concurrent glucocorticoid and noradrenergic activity shifts instrumental behavior from goal-directed to habitual control. J. Neurosci. 30, 8190–8196.
Schwabe, L., Merz, C.J., Walter, B., Vaitl, D., Wolf, O.T., Stark, R., 2011. Emotional modulation of the attentional blink: the neural structures involved in capturing and holding attention. Neuropsychologia 49, 416–425.
Schwabe, L., Tegenthoff, M., Höffken, O., Wolf, O.T., 2012. Simultaneous glucocorticoid and noradrenergic activity disrupts the neural basis of goal-directed action in the human brain. J. Neurosci. 32, 10146–10155.
Shin, L.M., Orr, S.P., Carson, M.A., Rauch, S.L., Macklin, M.L., Lasko, N.B., Peters, P.M., Metzger, L.J., Dougherty, D.D., Cannistraro, P.A., Alpert, N.M., Fischmann, A.J., Pitman, R.K., 2004. Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female Vietnam veterans with PTSD. Arch. Gen. Psychiatry 61, 168–176.
Shin, L.M., Wright, C.I., Cannistraro, P.A., Wedig, M.M., McCullin, K., Martis, B., Macklin, M.L., Lasko, N.B., Cavanagh, S.R., Krangel, T.S., Orr, S.P., Pitman, R.K., Whalen, P.J., Rauch, S.L., 2005. A functional magnetic resonance imaging study of amygdala and medial prefrontal cortex responses to overtly presented fearful faces in posttraumatic stress disorder. Arch. Gen. Psychiatry 62, 273–281.
Shin, L.M., Rauch, S.L., Pitman, R.K., 2006. Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. Ann. N. Y. Acad. Sci. 1071, 67–79.
Spielberger, C.D., 2010. State-Trait Anxiety Inventory.
Stein, M.B., Goldin, P.R., Sareen, J., Zorilla, L.T., Brown, G.G., 2002. Increased amygdala activation to angry and contemptuous faces in generalized social phobia. Arch. Gen. Psychiatry 59, 1027–1034.
Stein, M.B., Simmons, A.N., Feinstein, J.S., Paulus, M.P., 2007. Increased amygdala and insula activation during emotion processing in anxiety prone subjects. Am. J. Psychiatry 164, 318–327.
Strange, B.A., Dolan, R.J., 2004. β-adrenergic modulation of emotional memory-evoked human amygdala and hippocampus responses. Proc. Natl. Acad. Sci. U. S. A. 101, 11454–11458.
Sun, H.S., Green, T.A., Theobald, D.E.H., Birnbaum, S.G., Graham, D.L., Zeeb, F.D., Nestler, E.J., Winstanley, C.A., 2010. Yohimbine increases impulsivity through activation of cAMP response element binding in the orbitofrontal cortex. Biol. Psychiatry 67, 649–656.
van Stegeren, A.H., Goekoop, R., Everaerd, W., Scheltens, P., Barkhof, F., Kuijer, J.P.A., Rombouts, S.A.R.B., 2005. Noradrenaline mediates amygdala activation in men and women during encoding of emotional material. Neuroimage 24, 898–909.
van Stegeren, A.H., Roozendaal, B., Kindt, M., Wolf, O.T., Joels, M., 2010. Interacting noradrenergic and corticosteroid systems shift human brain activation patterns during encoding. Neurobiol. Learn. Mem. 93, 56–65.
Vuilleumier, P., Armony, J., Driver, J., Dolan, R.J., 2001. Effects of attention and emotion on face processing in the human brain: an event-related fMRI study. Neuron 30, 829–841.
Westermann, J., Demir, A., Herbst, V., 2004. Determination of cortisol in saliva and serum by a luminescence-enhanced enzyme immunoassay. Clin. Lab. 50, 11–24.
Whalen, P.J., Kagan, J., Cook, R.G., Davis, F.C., Kim, H., Polis, S., McLaren, D.G., Somerville, L.H., McLean, A.A., Maxwell, J.S., Johnstone, T., 2004. Human amygdala responsivity to masked fearful eye whites. Science 306, 2061.
Williams, L.M., Phillips, M.L., Brammer, M.J., Skerret, D., Lagopoulos, J., Rennie, C., Bahramali, H., Olivieri, G., David, A.S., Peduto, A., Gordon, E., 2001. Arousal dissociates amygdala and hippocampal fear responses: evidence from simultaneous fMRI and skin conductance recording. Neuroimage 14, 1070–1079.
Williams, L.M., Barton, M.J., Kemp, A.H., Liddell, B.J., Peduto, A., Gordon, E., Bryant, R.A., 2005. Distinct amygdala-autonomic arousal profiles in response to fear signals in healthy males and females. Neuroimage 28, 618–626.
Williams, L.M., Liddell, B.J., Kemp, A.H., Bryant, R.A., Meares, R.A., Peduto, A.S., Gordon, E., 2006. Amygdala-prefrontal dissociation of subliminal and supraliminal fear. Hum. Brain Mapp. 27, 652–661.
Wittchen, H.U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jönsson, B., Olesen, J., Allgulander, C., Alonso, J., Faravelli, C., Fratiglioni, L., Jennum, P., Lieb, R., Maercker, A., van Os, J., Preisig, M., Salvador-Carulla, L., Simon, R., Steinhausen, H.C., 2011. The size and burden of mental disorders and other disorders of the brain in Europe 2010. Eur. Neuropsychopharmacol. 21, 655–679.