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The influence of androstadienone during psychosocial stress is modulated by gender, trait anxiety and subjective stress: an fMRI study.

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Highlights

- Androstadienone (ANDR) is a synthetic steroid found in male sweat, saliva or semen.
- Males, females during early follicular phase and using contraceptives were tested.
- Neural stress reaction under ANDR was stronger in males than in females.
- ANDR modulated stress reactions in males depending on individual differences.
- Under ANDR, DLPFC activation was negatively associated with trait-anxiety in males.
- ANDR enhanced social threat, potentially facilitating adaptive stress responses.

Abstract

Androstadienone (ANDR), a bodily secreted steroid compound, is a socially relevant chemosignal that modulates subjective and (neuro)physiological responses, predominantly in females. The impact of ANDR on stress responses in males and females has not been explored. Therefore, this fMRI study aimed to examine psychosocial stress reactions induced by mental arithmetic and social evaluation on behavioral and hormonal levels (46 participants: 15 naturally cycling females in their early follicular phase (EF), 15 females on hormonal contraceptives (HC) and 16 males); and on a neural level (40 participants: 13 EF-females, 13 HC-females and 14 males) in an ANDR and placebo treatment repeated-measures design.

While no gender differences emerged in subjective ratings and performance during stress, neural activation patterns differed significantly. Besides, ANDR attenuated the post-stress increase of negative mood in all participants. Region of interest analyses showed that irrespective of treatment, males showed stronger activation of the dorsolateral prefrontal cortex (DLPFC) than females. At the whole brain level, gender differences emerged indicating stronger fronto-parietal activation in males compared to HC-females on both treatments. Males showed stronger visual and fusiform activation than EF-females under ANDR. Both female groups did not show stronger activation than males. Further, error ratio

in the ANDR-stress condition was positively associated with their post-stress cortisol level and increase in subjective stress in males; and male DLPFC activity in the ANDR-stress condition was negatively associated with trait anxiety. Surprisingly, compared to HC-females, EF-female only showed stronger activation of arousal-related areas under placebo treatment.

Taken together, these findings suggest that the male stress reaction under social evaluative threat was stronger than female stress reactions as a function of ANDR. More specifically, this effect on behavioral and neural stress reactions seems to depend on trait anxiety in males only. The study highlights the significance of a chemosignal in enhancing social threat that may facilitate adaptive stress responses.

Key words: androstadienone; social threat; gender; dorsolateral prefrontal cortex; trait-anxiety

1. Introduction

A crucial function of chemosignal communication is to convey messages between conspecifics such as potential threat and modulate adaptive stress behaviors to promote a better survival outcome. In humans, socially relevant chemosensory signals have been shown to enhance processing of socio-emotional information (Haegler et al., 2010; Prehn-Kristensen et al., 2008) and to improve cognitive performance (Bensafi et al., 2004a; Chen et al., 2006). One of many compounds produced in the human body is a steroid found in male semen and axillary sweat, namely androstadienone (4, 16-androstadienon-3-one; ANDR) (Gower and Ruparelia, 1993).

Exposure to ANDR, at an undetectable concentration (250 μM) applied on the skin above the upper lip, has been shown to increase the feeling of attentional focus after viewing a 20-min video clip low in emotional and arousal variables (Lundström et al., 2003a). Hummer and

McClintok (2009) demonstrated that when exposed to ANDR female and male participants directed greater attention to emotional information: reaction time was quicker specifically to emotional information. Also, under ANDR participants spent more time on emotional words in an Emotional Stroop task and findings of higher subjective attentive ratings were replicated. Similarly, processing speed of social threat information was accelerated as indicated by faster reaction times to angry faces in an approach-avoidance task under ANDR (Frey et al., 2012). The improved feeling of being focused was likely due to the sustained attention towards emotional cues (Lundström et al., 2003a). Further support comes from electrophysiological data where ANDR modulated early positive and late positive components (P1 and P3) in electrophysiological cortical responses compared to other odors, suggesting faster sensory and cognitive processing under exposure to ANDR (Lundström et al., 2006b). During human interaction, more attention might be directed to important information such as emotional content as ANDR is likely to be more socially salient than other odors.

Especially the prefrontal cortex (PFC), a region responsible for attentional control, seems to be affected by ANDR. A positron emission tomography (PET) study by Jacob et al. (2001b), including ten females during their mid/late follicular phase in a visual attention task, showed increased activation of PFC, including dorsolateral prefrontal cortex (DLPFC/BA 9, 10), after passively inhaling a low concentration of ANDR. In another PET study, Gulyás et al. (2004) reported stronger activation of DLPFC (BA 8, 10, 25) and other frontal, temporal and occipital regions related to social cognition and attention under exposure to ANDR compared to non-social odors. In other words, ANDR might modulate attention required for the on-going task at hand.

Effects of ANDR were shown to depend on the gender of the receiver. Smelling pure ANDR before viewing emotionally arousing videos (humorous, sad, or erotic videos) led to reduced negative mood, elevated cortisol levels and a range of physiological arousal parameters in

females (Wyart et al., 2007), probably as a function of increased hypothalamic activation (Savic et al., 2001). More recent findings indicated increased hypothalamic activation in both genders (Burke et al., 2012), arguing against a sexually dimorphic neural activation of the hypothalamus. However, these effects of ANDR on peripheral arousal and affective processing were mostly reported in females during their periovulatory phase (7-18 days post-menstruation) (Krajnik et al., 2014). Current evidence of behavioral effects of ANDR in males is limited. Males with higher levels of testosterone exhibited increased pro-social behavior under exposure to pure ANDR (Huoviala and Rantala, 2012)1. In another study, males but not females, showed an increased negative mood to pure ANDR (Bensafi et al., 2004b), while a mixed gender sample showed increased pain perception at a subliminal concentration (250 μM) (Villemure and Bushnell, 2007). Thus, there is tentative evidence that ANDR may modulate behaviors in females and males differently (Baum and Bakker, 2013; Krajnik et al., 2014). Fitting to the purpose of chemosensory signal communication (see Lübke and Pause, 2015), ANDR seems to direct social salience in different experimental contexts (Pause, 2004). By such means, ANDR could enhance social evaluative threat in a negative and stressful context.

Confronted with acute psychosocial stress, males and females showed different physiological responses (Cahill, 2006; Kudielka and Kirschbaum, 2005; Taylor et al., 2000) and divergent neural activation patterns (Kogler et al., 2015a; Wang et al., 2007). Compared to early-follicular females (EF-females, with typically low circulating endogenous ovarian hormone concentrations) and females using hormonal contraceptives (HC-females, stable and suppressed/low circulating ovarian hormone concentrations), males showed stronger stress reactions with significantly higher cortisol responses (Kirschbaum et al., 1999). Previous data also indicated a significant effect of menstrual cycle phase on physiological (Duchesne and Pruessner, 2013; Juster et al., 2015) and neural stress reactions (Andreano and Cahill, 2010; Chung et al., 2016).

The Montreal Imaging Stress Task (MIST, Dedovic et al., 2005), which is commonly used in neuroimaging environments, is a mental arithmetic task incorporated with social evaluative threat elements (Dickerson and Kemeny, 2004) provided by both the program and experimenter (Kogler et al., 2015a; Pruessner et al., 2008). Social evaluative threat is induced in a way similar to the Trier Social Stress Test (TSST) and other mental stress tasks involving mental arithmetics (Kogler et al., 2015a; Wang et al., 2007). The MIST requires trial-by-trial flexibility of attention control as well as regulation of the perceived social evaluative threat. The neural stress network includes fronto-parietal regions (DLPFC and ventromedial prefrontal cortex [VMPFC], middle and inferior frontal gyri and precuneus) associated with executive function, working memory and goal-directed cognitive processes (Cieslik et al., 2013; Dolcos and McCarthy, 2006); and fronto-limbic regions (orbitofrontal cortices, insula, middle and superior temporal sulci) associated with psychosocial stress and regulation of negative emotions (Dedovic et al., 2005; Kogler et al., 2015b; Pruessner et al., 2008). Particularly, the PFC is critical for executive functioning and top-down regulation of attention and has been shown to be influenced by social threat (Bishop et al., 2004, 2007). Attentional resources on the task can be disrupted by potential occurrence or signs of threats, comments, or interpretations of these negative events. Pre-attentive evaluation of threat has been demonstrated to be further modulated by individual differences, in particular trait anxiety, in a response conflict task (Bishop et al., 2007). This function of altering allocation of attentional resources was specific to DLPFC (Bishop, 2009). It has been suggested that amygdala-prefrontal circuitry has a reciprocal relationship in representation and interpretation of social threat, with prefrontal activation exerting inhibitory control over the response to threat-related stimuli. As a result, insufficient attentional resources, as indicated by lower DLPFC activation, would be allocated on the on-going task. Based on these findings we speculated that more anxious participants with higher sensitivity to social threat may have selective attention towards social threat and suffer a lapse of attention and

cognitive control during the task, which can be characterized by the failure to recruit the DLPFC. To test our hypothesis that ANDR enhances social threat perception and evaluation, lower DLPFC activation was expected to be observed in more trait-anxious participants.

Gender differences in neural stress reactions under social evaluative threat have been observed previously, with males relying more strongly on fronto-parietal regions while females seem to recruit emotion-related regions such as amygdala more strongly (Kogler et al., 2015a; Wang et al., 2007). Specifically, DLPFC activity has been shown to differ between genders during social evaluative threat: although no gender difference was reported in a sample of 10 females and 8 males (Bishop et al., 2007), Wang et al. (2007) reported stronger right DLPFC activation during social evaluative threat in 16 males vs. 16 females. Therefore, we investigated whether gender and laterality differences existed in stress-related DLPFC activation and whether DLPFC activation was negatively associated with trait anxiety when social threat could be augmented by ANDR. Notably, direct comparisons of neural stress responses between males and females at different stages of the menstrual cycle or taking hormonal contraceptives are missing.

Millions of females worldwide use hormonal contraceptives, but their effects on the behavioral and neural stress reaction are far from understood (Pletzer and Kerschbaum, 2014). Hormonal contraceptives are likely to modulate behavioral, hormonal and neural stress responses (e.g. Merz et al., 2012; Nielsen et al., 2013). Pletzer et al. (2014) demonstrated similar performance of HC-females and EF-females in a numerical comparison task and a number bisection task, however, fronto-parietal neural activation differed. Thus, despite lacking group differences in performance, neural differences may occur due to hormonal contraceptive intake. To further investigate whether menstrual cycle phase, hormonal contraceptive intake, gender or an interaction of these factors modulates behavioral and neural stress reactions, comparisons between EF-females, HC-females and males are necessary to address further gender and within-female group differences.

Taken together, ANDR not only enhanced socio-emotional information but also altered social and emotional behavior in females and in males (Baum and Bakker, 2013). Thereby, ANDR could also impact psychosocial stress reactions at behavioral, hormonal and neural levels differently in males and females depending on their hormonal status. Therefore, we applied a psychosocial stress task in a repeated-measurement, placebo-treatment design in males, EF-females and HC-females to investigate the interaction of gender and ANDR application on behavioral, hormonal and neural stress reactions.

We expected 1) all participants to have higher post-stress negative mood and stress ratings; 2) similar performance in the stress task between all groups due to the adaptive nature of the task; and 3) a higher post-stress cortisol level in males compared to both female groups (EF- and HC-females) with no between-female group difference (Kirschbaum et al., 1999). 4) If ANDR enhances emotional information on the task at hand (Hummer and McClintok, 2009), here social evaluative threat in the MIST, performance and post-stress cortisol levels should positively correlate with changes in subjective stress. 5) Regarding neural stress reactions, we expected stronger activations of fronto-parietal regions in males and stronger activation in fronto-limbic regions in females (cf. Kogler et al., 2015a; Wang et al., 2007). 6) For the two female groups, we assumed that behavioral performance will be similar but fronto-parietal activation might differ due to hormonal contraceptive intake (cf. Pletzer et al., 2014). 7) Based on previous studies implicating a significant relation between trait anxiety and DLPFC activation (Bishop et al., 2007; Lübke et al., 2014), we expected a negative association with more trait-anxious participants showing weaker DLPFC activation. Here, we further speculated that this effect is particularly present in males, given their stronger DLPFC activation during social evaluative threat (Wang et al., 2007). 8) Under exposure to ANDR, activation of regions related to body odors, such as anterior insula, precuneus, fusiform gyrus, should be stronger.

2. Methods and Material

2.1. Sample

Forty-six healthy, right-handed, heterosexual participants (30 females) aged 19–34 years (M = 25.46, SD = 3.2) were enrolled. The female sample consisted of fifteen females using oral hormonal contraceptives (HC-females), who were tested during their contraceptive intake phase and fifteen early-follicular females (EF-females; day 2–7 post-menstruation). The data of EF-females has also been reported in another study (Chung et al., 2016). Menstrual cycle status was based on self-report. Only EF-females with regular menstrual cycle length (28–30 days) and who were not using hormonal contraceptives in the last six months were included. All HC-females used combined hormonal contraceptives (thirteen HC-females using combined oral progestin/estrogen contraceptives; and two HC-females using an estrogen/antiandrogen combined formulation). At least three previous cycle lengths were surveyed. EF-females were asked to call as soon as their menstrual period started (day 1) to schedule the testing sessions. Additionally, EF-females reported the beginning of their next menstruation to ensure that all testing sessions were conducted in the early-follicular phase.

Please insert Table 1 about here

Exclusion criteria included hormonal and psychotropic medication or illicit drug use in the last six months as well as prior or present neurological and psychological illness confirmed via the German version of the Structured Clinical Interview for DSM (SCID; Wittchen et al., 1997). None of the females were pregnant nor breast feeding within the last year. Due to the nasal delivery method of the steroid, those who frequently or within the previous three days had nosebleeds, chronic nasal disease, rhinitis, upper respiratory tract infections or lung disease were excluded. Subjects smoking up to five cigarettes/day except on the day of

testing were included. The study protocol was approved by the local ethics committee. All volunteers gave written informed consent and received €10/hour for their participation.

2.2. fMRI stress paradigm

We applied the Montreal Imaging Stress Task (MIST, Dedovic et al., 2005; Pruessner et al., 2008), a mental arithmetic task with social evaluative threat and uncontrollability elements (Dickerson and Kemeny, 2004). Three conditions (rest, control and stress) in a block design were presented in two runs (pre-feedback [pre-FB] and post-feedback [post-FB], see Figure 1 for the order of conditions). Through goggles, a black fixation cross was presented in the rest condition. Participants were instructed to solve a series of arithmetic problems. To answer them they were using a three-button touch pad to navigate left or right to the correct digit (between 0 and 9) on a rotary-dial. Via middle button press they submitted their answer. During the control condition, no time pressure was imposed and the participants received trial-by-trial feedback of their performance ("correct", "incorrect") that was shown as "not recorded" on the screen. In the stress condition, social evaluative threat and sense of uncontrollability were increased by the program. To induce pressure to perform, variable time pressure was imposed and performance monitoring on a mock performance bar (in green, yellow; and red as indication of good, medium and low performance) was required. The trial-by-trial feedback of the performance included also "time out" and "recorded". Task difficulty was adaptive with overall 40-50% accuracy with majority of errors committed in the stress condition (see Dedovic et al., 2005). Therefore, the stress condition was of our particular interest for the effect of ANDR on social evaluative threat. To induce additional social stress, scripted negative social feedback was given by the investigator verbally via microphone to discredit participants' poor performance ("Your performance is not good, we will have to do this one more time. We can only use the correct trials for the data analyses. You have to perform better, especially during the "recorded" trials (stress condition).

Concentrate one more time and solve the arithmetic problems as quickly as possible."). Participants were further asked to improve their performance in the post-FB run. This task sequence was the same for both ANDR and placebo (PLAC) treatment testing (see Figure 1).

2.3. Preparation of ANDR and PLAC

A 250µM ANDR solution (with a purity of ≥99%; Steraloids Inc., Newport, RI, USA; diluted in propylene glycol) was masked with 1% musk oil (Sigma-Aldrich, Deisendorf, Germany). This concentration close-to-detection threshold (Lundström et al., 2003b) has been shown to increase autonomic arousal in females (Jacob et al., 2001a). Placebo (PLAC) was solely propylene glycol containing 1% musk oil. ANDR or PLAC was pipetted on a cotton pad with one-direction permeability and attached on the upper lip to avoid transdermal exposure (Albrecht et al., 2010).

2.4. Stress assessment and saliva samples

To assess confounding factors on stress reactivity, body mass index (BMI) was reported (see Table 1); Participants were asked to refrain from exercise and alcohol the day before testing; from black tea, caffeine, highly sugared and carbonated beverages on the testing day; and from eating and drinking, except water, two hours prior to testing. Before entering the MRI scanner, trait anxiety was surveyed using the State-Trait Anxiety Inventory (STAI; Laux et al., 1981) on the first day of testing and state anxiety was surveyed on both days. After the MRI measurement, state anxiety was surveyed again. To assess stress levels, subjective mood (Positive and Negative Affect Scale, [PANAS; Watson et al., 1988]) and subjective stress were verbally reported on a five-point Likert scale. Subjective mood ratings and salivary cortisol samples were drawn at three time points: seven min after exposure to ANDR or PLAC, i.e. immediately before the stress task (T1), 15 min (T2) and 60 min (T3) after the stress experience (see Figure 1). Saliva samples were analyzed by the local laboratory (Uniklinikum

Aachen, Germany) using an ElectroChemiLuminessenzImmunoAssay (ECLIA; functional sensitivity <8.5 nml/l). Data for skin conductance response (SCR) was collected during MRI scanning and analyzed. Please see supplementary materials for information on acquisition parameters, statistical analysis and results.

2.5. Procedure

All participants were measured twice (with EF-females twice in the early-follicular phase and HC-females twice in their intake phase) in a repeated-measures design, one time with ANDR and another time with PLAC treatment. The treatment sequence was randomized.

Due to diurnal variation of cortisol concentration, all participants were measured in the afternoon between 2pm and 5pm, with both sessions starting at the same time. ANDR or PLAC was applied prior the resting-state scan that was performed to avoid confounding stressors (i.e. MRI scanner environment) and to ensure that ANDR took effect (after approximately six min; Jacob and McClintock, 2000). After seven minutes, the first run of the stress task (pre-FB) began and was followed by negative verbal feedback. Then the second run (post-FB) followed. Both runs lasted for about seven minutes each (see Figure 1).

Please insert Figure 1 about here

Immediately following the MRI session (T2; see Figure 1), participants rated the odor of ANDR and PLAC with regards to pleasantness, intensity and familiarity on 10cm visual analog scales (VAS). Furthermore, threshold tests for ANDR were performed after the last cortisol sample (T3) was collected on the testing day using ANDR (see Figure 1) in order to determine each participant's discrimination ability (ANDR versus PLAC) and their sensitivity to ANDR (see supplementary materials). Additionally, general ability to identify odors using the 40-item Monell Extended Sniffin' Sticks Identification Test (MONEX-40, Freiherr et al.,

2012) was assessed on the testing day using PLAC. After finishing the study, all participants were fully debriefed about the nature of the study.

2.6. Statistical Analysis

Statistical analysis was conducted using IBM SPSS statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY). One-way ANOVAs were applied to test for group differences in sample characteristics. Subjective and psychophysiological variables such as cortisol levels were tested for normal distribution using Kolmogorov-Smirnov tests; logarithmic transformation (y=log10[x+1]; Boucsein et al., 2012) was applied on cortisol and SCR data since assumption of normal distribution was violated.

Ratings for ANDR and PLAC treatment. Pleasantness, intensity and familiarity ratings were analyzed using three separate 2 x 3 repeated measures ANOVAs (rmANOVA) with the factors treatment (ANDR/PLAC) and group (EF-females/HC-females/males).

Subjective mood. PANAS and subjective stress were analyzed using a 2 x 3 x 3 rmANOVA with the factors treatment (ANDR/PLAC), time (T1/T2/T3) and group (EF-females/HC-females/males). For state anxiety, we performed a 2 x 2 x 3 rmANOVA, as only two time points were assessed (before vs. after stress induction, T2).

Task performance. Error ratio was calculated as the sum of number of errors and number of timeout trials divided by the total number of trials processed in the stress condition. It was analyzed by a 2 x 3 rmANOVA with the factors treatment (ANDR/PLAC) and group (EF-females/HC-females/males).

Cortisol. Log-transformed salivary cortisol levels were analyzed using a 2 x 3 x 3 rmANOVA with the factors treatment (ANDR/PLAC), time (T1/T2/T3) and group (EF-females/HC-females/males).

Greenhouse-Geisser correction was applied to the degrees of freedom of the repeated factors when requirements were not met. For significant effects ($p<.05$), estimates of effect size (partial-eta squared) were reported. For significant interactions, all pairwise comparisons were Bonferroni-corrected.

Correlation analyses. Pearson's correlations were applied to change in subjective stress (T2-T1), log-transformed post-stress cortisol (T2), and performance (average error ratio in the stress condition), separately for groups (EF-females/HC-females/males) and treatments (ANDR/PLAC). Significance (two-tailed) was set at $p<.05$. To test for significant group differences of these significant correlations, the Fisher's Z-transformation was applied to the correlation coefficients.

2.7. MRI data acquisition, preprocessing and analyses

Functional and anatomical imaging data were acquired with a 3T TIM Trio scanner (Siemens Medical Solutions, Erlangen, Germany) equipped with the manufacturer's standard twelve-channel head coil. First, a high-resolution anatomical image was acquired using an MPRAGE (3-D Magnetization Prepared Rapid Gradient Echo) sequence consisting of 160 sagittal slices (TR = 1900ms, TE = 2.52ms, TI = 900ms, 1 x 1 x 1mm resolution, field of view (FOV) 250 x 250mm, slice oversampling = 18.2%, flip angle [FA] = 9°). For the task, 34 ascending slices were acquired with a gradient-echo EPI-sequence with distortion correction (TR = 2000ms, TE = 28ms, 3.3 x 3.3 x 3.3mm resolution, FOV = 210 x 210mm, matrix size = 256 x 256, and FA = 77°).

Functional imaging data processing was performed with the Statistical Parametric Mapping software (SPM8; Wellcome Department of Imaging Neuroscience, London, UK) implemented in Matlab (Mathworks Inc., Sherborn, MA, USA) using standard settings unless specified otherwise. This included realignment, coregistration to individual T1-image, spatial normalization to MNI (Montreal Neurological Institute) space (Ashburner and Friston, 2005),

and spatial smoothing (8mm Gaussian kernel). Pre-processed images were then analyzed using a general linear model for each participant.

At the first level, main effects of each condition (rest, control, stress) for each participant were computed. Three translation and three rotation estimates generated by the realignment step served as nuisance regressors for head motion correction. The resulting con-images of all three groups were further fed into a second-level group analysis. Data from six participants (two per group) were discarded due to movement artifacts (>4 mm). The remaining 40 participants (13 EF-females; 13 HC-females; and 14 males) were included in the analyses of the functional data. For all other analyses (subjective ratings, performance, cortisol), the full sample was applied.

A 2 x 2 x 3 whole-brain full-factorial rmANOVA, including treatment (ANDR/PLAC), condition (control/stress) as within-subject factors and group (EF-females/HC- females/males) as between-subjects factor was computed. Whole-brain t-contrasts of stress vs. control across treatment should activate regions within the psychosocial stress network as described previously (Pruessner et al., 2008; Wang et al., 2007). Moreover, to compare stress effects between all groups, a whole-brain full-factorial ANOVA of stress condition across treatment with group as a between-subject factor and post-hoc t-contrasts were applied (see supplementary materials). Here, we were particularly interested in gender differences when applying ANDR. Therefore, neural activations in males and two groups of females during the stress condition were compared for both treatments separately. A total of six t-contrasts (males vs. EF-females, males vs. HC-females and EF-females vs. HC-females for both ANDR and PLAC treatment) were carried out. Results of the whole-brain analyses were thresholded at $p<.05$ family-wise error (FWE) corrected.

The DLPFC was chosen as Region of interest (ROI) due to several reasons. 1) Gender differences in DLPFC activation have previously been reported with males showing stronger right DLPFC activation than females during a psychosocial stress task (Wang et al., 2007).

Moreover, 2) left DLPFC activation during a response conflict task correlated negatively with trait anxiety. This was not observed for the right DLPFC (Bishop, 2009). Therefore, we included laterality as a within-subject factor in the ROI analysis. ROIs were extracted by applying small volume correction using a sphere of 10mm at bilateral DLPFC with SPM8 based on previously reported coordinates ([x, y, z]: ±34, 36, 24; Bishop et al., 2007; Bishop, 2009). The volumes covered parts of the bilateral middle and inferior frontal gyri, BA 10, and borders at BA 46, BA 9 and superior frontal gyri. Activation strength was exported and analyzed in SPSS. A 2 x 2 x 2 x 3 rmANOVA was performed including laterality (left/right), treatment (ANDR/PLAC), condition (control/stress) as within-subject factors and group (EF-females/HC-females/males) as between-subjects factor. Correlation analyses between trait anxiety and DLPFC activation during stress (parameter estimates as derived from ROI analysis) across the whole sample and separately for each group and treatment were also performed. Significance (two-tailed) was set at $p<.05$. To test for significant group differences of these significant correlations, the Fisher's Z-transformation was applied to the correlation coefficients.

3. Results

Groups neither differed significantly in age, BMI or years of education (all $Fs\leq1.67$, all $ps\geq.109$). Moreover, to control for cognitive abilities, direct comparison of processing speed and fluid cognitive abilities (Trial-Making Test, TMT; Reitan, 1956) revealed no significant group effect (all $Fs\leq.24$, all $ps\geq.787$). Groups also did not differ in chronic stress ratings (Trier Inventory for the Assessment of Chronic Stress; Schulz and Schlotz, 1999) as well as self-rated depression scores (Beck's Depression Inventory-II; Hautzinger et al., 2006) and scores of trait anxiety (STAI; Laux et al., 1981) (all $Fs\leq2.35$, all $ps\geq.109$; see Table 1 for means).

3.1. Subjective ratings and mood

The rmANOVAs revealed a significant time effect in state anxiety ($F(1,43)=17.46$, $p=.002$, $\eta_p^2=.204$) with higher post-stress state anxiety compared to pre-stress state anxiety ($p=.002$). A significant time effect occurred in both subjective stress ($F(2,86)=64.02$, $p<.001$, $\eta_p^2=.598$) and negative mood ratings ($F(2,86)=15.27$, $p<.001$, $\eta_p^2=.262$). Subjective stress and negative mood were significantly higher after the stress induction (T2>T1 and T2>T3, all ps<.001; T1vs.T3, all ps=1.000). A significant treatment-by-time interaction emerged for negative mood ($F(2,86)=3.25$, $p=.043$, $\eta_p^2=.070$), with lower post-stress negative mood under ANDR compared to PLAC (T1: p=.946; T2: p=.017; T3: p=.717; Figure 2A). No group differences (all Fs≤1.61, ps≥.211) or main effect of treatment (all Fs≤.52, ps≥.396) or other interactions (all Fs≤1.26, ps≥.291) emerged.

Please insert Table 1 about here

3.2. Task performance

Participants had to solve mental arithmetic problems under imposed time pressure and performance monitoring. The rmANOVA for error ratio in the stress condition revealed no significant main effect of group ($F(1,43)=.90$, $p=.413$), or treatment ($F(1,43)=1.28$, $p=.265$), nor a significant interaction ($F(1,43)=.21$, $p=.816$). Thus, no differences in performance occurred between groups or between ANDR and PLAC.

Please insert Table 2 and Figure 2 about here

3.3. Cortisol levels

The rmANOVA revealed a significant time effect ($F(2,86)=21.34$, $p<.001$, $\eta_p^2=.332$) with all

participants showing a higher concentration of cortisol at T1>T2 (p=.023), T1>T3 (p<.001); T2>T3 (p=.001). A weak trend for a group effect (F(2,43)=2.77, p=.074, η_p^2=.114) emerged, with the direction of males showing higher cortisol levels than HC-females (p=.070), but not EF-females (p=.690). No group difference between the two female groups (p=.813) occurred. No significant treatment effect (F(1, 43)=.07, p=.788) or interactions (all ps≥.251) emerged.

3.4. Correlation analyses for behavioral parameters

***Subjective stress and error ratio*.** In males, a positive association of increase in subjective stress (T2-T1) and error ratio in the stress condition under ANDR (r=.593, p=.015) occurred, but not under PLAC (r=.014, p=.960) (see Figure 2B). In EF-females, we found a trendwise negative association of change in subjective stress (T2-T1) and error ratio in the stress condition under ANDR (r=-.501, p=.057) but not under PLAC (r=.113, p=.689). This association differed significantly between males and EF-females under exposure to ANDR (Z=3.08, p=.001, two-tailed). Thus under ANDR, males with higher error ratio in their performance reported higher increase in subjective stress, whereas the reverse pattern was observed in EF-females with higher error ratio in their performance reporting lower increase in subjective stress. No significant associations in either treatment occurred in HC-females (all ps≥.291).

***Subjective stress and post-stress cortisol*.** No significant association of change in subjective stress (T2-T1) and post-stress cortisol (T2) in either treatment appeared in EF-females (all ps≥.120), HC-females (all ps≥.258) or in males (all ps≥.188).

***Post-stress cortisol and error ratio*.** In males, a positive association of post-stress cortisol (T2, post-stress) and error ratio in the stress condition under ANDR (r=.568, p=.022) occurred, but not under PLAC (r=.258, p=.334) (see Figure 2C). Hence under ANDR, males with higher error ratio in their performance showed higher post-stress cortisol levels. No significant associations occurred in EF-females (all ps≥.076) or HC-females (all ps≥.599).

3.5. Whole-brain analyses

The whole-brain rmANOVA did not reveal a significant treatment effect, treatment-by-group, treatment-by-condition or treatment-by-condition-by-group interactions. However, there was a significant main effect of condition. In the stress>control contrast across all participants and treatments, robust activation of psychosocial stress regions including clusters at right middle frontal gyrus (MFG) extending to the right superior temporal gyrus (STG), precentral gyrus, precuneus and visual association cortices as well as left inferior frontal gyrus (IFG) were observable (see Table 3). To address our pre-defined interest in gender differences in neural stress reactions under ANDR, gender specific neural activations in males and two groups of females during the stress condition were compared for both treatments separately.

Insert Table 3 about here

Gender-specific neural activations during PLAC. Comparing males with EF-females in the PLAC stress condition, we observed stronger tertiary visual cortex activation in males. EF-females did not demonstrate significantly stronger activation than males. Compared to HC-females, males had significantly stronger activations in bilateral DLPFC, bilateral IFG, left MTG (extending to the angular gyrus), mid-line superior parietal region (including right precuneus) and visual association regions (see Table 3 and Figure 3). Again, HC-females did not show stronger activation than males.

Between-female group neural activations during PLAC. Finally, EF-females showed stronger activation of left somatosensory association cortex and right pre-motor and supplementary motor area under stress during PLAC treatment compared to HC-females, while HC-females showed no significant stronger activation than EF-females (for details see Table 3).

***Gender-specific neural activations during ANDR*.** Here, males exhibited significantly stronger activation of the left fusiform gyrus and the right visual association cortex than EF-females in the stress condition (see Figure 3). The direct comparison of males with HC-females under ANDR showed a stronger activation of left insula, right IFG (pars triangularis) and the left anterior DLPFC among others (see Table 3 and Figure 3). The reverse contrasts (EF-females or HC-females > males in the ANDR stress condition) did not reveal any significant clusters.

Between-female group neural activations during ANDR. No significant group difference was observed.

Full details on coordinates, cluster size, and statistics are listed in Table 3.

Insert Figure 3 about here

3.6. Region-of-interest analyses

DLPFC. A main effect of group ($F(2,37)=8.09$, $p=.001$, $\eta_p^2=.304$) emerged, with males showing stronger activation than EF-females ($p=.010$) and HC-females ($p=.002$). Female groups did not differ ($p=1.000$). A main effect of condition ($F(1,37)=49.73$, $p<.001$, $\eta_p^2=.573$), with stronger activation in the stress compared to control condition occurred. Additionally, a significant condition-by-group interaction ($F(2,37)=3.44$, $p=.043$, $\eta_p^2=.157$) emerged, with stronger activations in the stress condition compared to the control condition in EF-females ($p=.004$), HC-females ($p=.006$) and males ($p<.001$). Males also showed stronger activations in the stress condition than EF-females ($p=.002$), and HC-females ($p<.001$) did (see Figure 4B). No such differences appeared in the control condition (all ps≥.104). No between-female-group differences appeared in the control or stress condition (all ps=1.000). No significant laterality, treatment effect or interactions emerged (all ps≥.105).

Insert Figure 4 about here

3.7. Individual differences and DLPFC activation

***Trait anxiety*.** No group differences for trait anxiety scores (see Table 1) or laterality effect of the DLPFC activation emerged. Therefore, a negative correlation of the DLPFC activation (mean activation of stress condition averaged across left and right and both runs) and trait anxiety across the whole sample under exposure to ANDR (r=-.356, p=.024) and PLAC (r=-.138, p=.396) were conducted. Further, group-specific analyses indicated no significant relationship after collapsing both female groups (r=-.181, p=.377). In males, DLPFC activation during the stress condition under ANDR was negatively correlated with trait anxiety (r=-.571, p=.033) (see Figure 4C), but not in the PLAC condition (r=-.080, p=.787). Similarly, the same negative association appeared in HC-females at trend level (r=-.544, p=.054), with no such association in the PLAC condition (r=.160, p=.602). This association, however, did not differ between males and HC-females under exposure to ANDR (Z=.09, p=.466, two-tailed). The association of DLPFC activation and trait anxiety differed significantly between ANDR and PLAC treatment for males (Z=-1.73, p=.042, one-tailed) and HC-females (Z=-1.76, p=.039, one-tailed). No correlation appeared significant in either treatment for EF-females (all ps>.290).

3.8. Odor quality of ANDR vs. PLAC

For the ratings of stimulus intensity, a significant group effect (F(2,43)=8.84, p=.001, η_p^2=.291) emerged: HC-females perceived the stimuli more intense than the males (p<.001), and trendwise than the EF-females (p=.059), while males and EF-females did not differ (p=.275). No significant effect of stimulus (ANDR/PLAC) or interaction occurred (all Fs≤.74, ps≥.394). No significant main effect of group, treatment or interactions for ratings of odor pleasantness (all Fs≤2.17, ps≥.126) or familiarity (all Fs≤1.97, ps≥.168) emerged (see Table

1). Judging from the mean pleasantness ratings of each group given immediately after the scanning sessions (shown in Table 1), participants perceived application of both, ANDR and PLAC, indifferently as neutral-pleasant odors inside the MRI scanner. It is, however, worth mentioning that in the subsequent discrimination tests, four EF-females, four HC-females and two males could discriminate between ANDR and PLAC (see supplementary materials). Moreover, threshold tests revealed that two EF-females, two HC-females and one male were anosmic for ANDR. Based on the current understanding of the communication properties of chemosignals, it is not necessary for ANDR to elicit a physiological reaction in our olfactory system (Baum and Bakker, 2013). Furthermore, it is often presumed that all participants would perceive or respond similarly to the chemosensory signals regardless of conscious or unconscious exposure (high vs. low concentration), albeit the large variance of sensitivity to ANDR (see Baum and Bakker, 2013). For exploratory reasons, we excluded the anosmic subjects of our sample to address this uncertainty; however, this did not change the direction and significance of results. Visual inspection of the data in the decrease in post-stress negative mood under ANDR compared to PLAC also occurred in four of five anosmic subjects. Therefore, we included the anosmic participants in the final analyses.

4. Discussion

The primary aim of the study was to test how gender (males vs. EF-females vs. HC-females) and ANDR application influence behavioral, hormonal and neural stress reactions. Additionally, we examined how individual differences in trait anxiety modulate the effect of ANDR on DLPFC activation in a stressful context in males, and in two female subgroups measured at a time when ovarian hormone levels are typically low.

Current findings revealed that 1) a treatment effect of ANDR on mood appeared with post-stress negative mood being lower under ANDR compared to PLAC. 2) HC-females showed overall lower cortisol levels than males (weak trend), however no treatment effect of ANDR

occurred for cortisol levels. Further, male post-stress cortisol levels were positively correlated with error ratio in the MIST.

Regarding neural activation, a significant condition effect occurred. A wide-spread neural network was activated when contrasting stress vs. control condition across both ANDR and PLAC treatments in all participants. Here, gender differences appeared in neural activations: During PLAC treatment, 3a) males showed stronger activation of visual association cortex compared to EF-females; and stronger activation of fronto-parietal regions, including DLPFC (BA9, 10), IFG, precuneus, pre-SMA, angular gyrus and visual association areas compared to HC-females; 3b) Compared to HC-females, EF-females showed stronger activation in somatosensory association cortex and pre-SMA under PLAC.

4) An overall treatment effect of ANDR or other treatment-related interactions were missing, however, neural stress responses under exposure to ANDR seemed to differ between groups. Besides typically activated regions in psychosocial stress processing, we observed a striking difference in processing of ANDR between males and both female groups: 5a) Males exhibited stronger activation of the fusiform gyrus compared to EF-females; 5b) Males also showed stronger activation of insula, IFG and angular gyrus compared to HC-females; 5c) Application of ANDR did not induce differences in behavioral and neural stress reactions between EF-females and HC-females; As hypothesized, 6) DLPFC activity was negatively associated with trait anxiety under ANDR only in males. Finally, the stress task was perceived as stressful as all participants showed a post-stress increase in subjective stress and negative mood. No group differences in task performance occurred.

In the following sections, the observed gender differences in neural activation, the effect of ANDR and its association with trait characteristics will be discussed in detail.

4.1. Gender differences in psychosocial stress reactions

At the whole-brain level, our male participants showed stronger activation of regions frequently observed during mental stress tasks (Lederbogen et al., 2011; Pruessner et al., 2008; Wang et al., 2007), particularly compared to HC-females during PLAC treatment. The right IFG plays a prominent role in psychosocial stress responses as it was recently highlighted as one of two regions that are particularly associated with stress reactions in a meta-analysis (Kogler et al., 2015b). It is involved in inhibition processes of motor responses (Aron et al., 2014), cognitive (Cole and Schneider, 2007) and attentional control (Nelson et al., 2010), provided that the task does require mental flexibility in order to obtain the quickest answer (e.g. the shortest possible click to the correct digit by going left or right on the rotatory response dial) during the stress condition. Additionally, right IFG and MFG activation have been reported during conscious negative memory suppression (Depue et al., 2007). These studies support the assumption that males tend to cope with controlling over psychosocial stressors while activating prefrontal regions (Wang et al., 2007). Our current findings revealed that compared to both EF-females and HC-females, males exhibited a consistently stronger activation at the visual association cortex. Increased activation in occipital areas was previously shown for stronger negative valence visual input encoding in males after stress induction via movie clips of aggressive behaviors. It was suggested that during stress, participants became hypervigilant and sensory encoding increased (Henckens et al., 2009). This probably reflects stronger selective assessment of social threat in males in this cognitive challenge.

A possible cause of the observed gender differences in neural stress reactivity is the influence of ovarian hormone concentration. Previous studies using numerical tasks indicated that EF-females showed stronger neural activation of SFG, MFG, MTG, SMA and precentral gyrus than males and HC-females, while there were no differences between EF-females and HC-females in performance (Pletzer et al., 2014). The authors concluded that

decreased endogenous ovarian hormone concentration due to hormonal contraceptive intake may change fronto-parietal activation, hence, activation patterns in HC-females were similar to males in that study. Similarly, no neural activation differences between HC-females and males in a verbal generation task occurred in another study (Rumberg et al., 2010). Contrarily, here we observed a pattern of stronger neural stress reaction in males compared to HC-females in bilateral MFG and IFG, suggesting that males might be more engaged in down-regulating stress than HC-females. Future studies should further investigate the impact of hormonal contraceptive intake on psychosocial stress processing.

Our ROI analyses yielded a consistent pattern of stronger DLPFC activation in males than both female groups. DLPFC is a region known for its executive function as well as for response inhibition (Ridderinkhof et al., 2004) and modulating emotional responses. Higher cognitive control was linked to usage of cognitive strategies in controlling emotions (Ochsner et al., 2012). During induced anger control, stronger male DLPFC activation was associated positively with testosterone levels when cortisol levels were low; the same is true for the PFC-amygdala connectivity among these males (Denson et al., 2012). Of three other prefrontal areas (left orbitofrontal cortex, insula and dACC), the right PFC was shown to be the most important in clustering sex differences of neural stress reactions (Wang et al., 2007). It is possible that circulating testosterone in males facilitates HPA-activity and prefrontal activation in regulating stress. This may support the assumption that prefrontal regions can be sexually dimorphic with gender differences in executive function, albeit no such gender differences in behavioral performance were present in our study. Whether psychosocial stress induces changes in ovarian/gonadal hormones and how it mediates prefrontal activation remains to be tested.

4.2. The influence of contraceptives on stress reactions

Our findings showed that although performance and mood in HC-females were similar to males or EF-females, lower cortisol levels in HC-females (at a weak trend level) compared to males and no difference compared to EF-females were observed. The same blunted cortisol pattern in HC-females has been reported previously after the TSST (Kirschbaum et al., 1999). Regarding neural stress responses in our data, stronger activation of pre-SMA and somatosensory sensory cortex in EF-females compared to HC-females emerged. Previous studies addressing the impact of hormonal contraceptives on neural activation reported heterogeneous results: Gingnell et al. (2013) observed lower reactivity in the left MFG and bilateral IFG in an emotion matching task in four week contraceptive user compared to non-users. The authors assumed that mood changes and involvement of MFG and IFG in social interaction processes were strongly affected by hormonal status (Craig et al., 2007).

In our findings, both female groups appeared to share common neural stress activations. Interestingly, previous studies investigating impact of hormonal contraceptive intake on neural activation underlying social cognitive processes (i.e. emotion processing, reward processing) reported significant differences between female groups, with HC-females demonstrating stronger activation of the fusiform face area during facial emotion processing (Mareckova et al., 2014) or monetary reward processing (Bonenberger et al., 2013). In the same sample, watching erotic scenes did not elicit group differences, however, females taking hormonal contraceptives exhibited significantly reduced anterior insula activation during expectance of erotic stimuli (Abler et al., 2013). In all mentioned studies including ours sample sizes were relatively small (Mareckova: n=10; Bonenberger/Abler: n=12). Additionally, type of hormonal contraceptives as well as adherence to hormonal contraceptive intake was not assessed. More stringent control of hormonal contraceptives (i.e. formulation, switching on-/off-pill period, adherence, etc.) in inclusion criteria and comparison with different menstrual cycle sub-phases in the future is recommended.

4.3. ANDR modulates psychosocial stress

ANDR was shown to modulate attention, mood and psychophysiological responses, particularly in females (for review see Krajnik et al., 2014). In our study, negative mood after stress was lower during ANDR treatment compared to PLAC, but this effect was not modified by gender. Hence, ANDR, at an imperceptible threshold, attenuated an increase in negative mood in males as well as in females. This novel finding reveals that ANDR also prevented an increase of negative mood after psychosocial stress in EF-females and HC-females. However, this should be treated with caution, as the effect size was small. Additional analyses for potential order effects (i.e. perceiving ANDR on the first testing day might have been more stressful than on the second testing day) revealed no significant results in negative mood, subjective stress, cortisol levels or task performance (all ps>.104). Nevertheless, this extended to the positive mood maintenance in females during their periovulatory phase reported previously (e.g. Jacob and McClintock, 2000). With these, we indirectly address the interaction of hormone levels and ANDR on negative mood. ANDR was shown to increase negative mood in males while viewing sad movie clips (Bensafi et al., 2004b). Here, ANDR seems to have beneficial effects on mood during stress. Previously, improved feeling of attentional focus by ANDR was reported (Hummer and McClintok et al., 2009; Lundström et al., 2003a). ANDR seemed to influence more complex social behaviors (Huoviala and Rantala, 2012) in the context of enhanced social evaluative threat, and possibly increased its significance in self-related processes during a task that requires motivation. Evidence for stronger frontal activation in males indicates increased neural processes such as top-down control of attention, which is required for a task related to problem-solving. The stress-induced allocation of attentional focus may in turn decrease negative feelings in males. However, in females the underlying neural processes that mediate such mood changes could not be determined.

ANDR in combination with social evaluative threat only seemed salient to males at this stage. Our interpretation is limited by the lack of an overall treatment effect of ANDR or a significant treatment-by-group interaction. Nevertheless, our male participants demonstrated significantly stronger activations under ANDR: compared to the HC-females, males showed significantly stronger activations of left anterior insula and right precuneus, in regions known for regulation of social emotions (Prehn-Kristensen et al., 2009). Compared to EF-females, males showed stronger activation of the left fusiform gyrus in our study. Fusiform gyrus activation was often related to enhanced perceptual processes during presence of body odors in close proximity (Lübke et al., 2014). Notably, all of these regions (anterior insula, precuneus, fusiform gyrus) were associated with processing of body odors (Lundström et al., 2008) and anxiety sweat collected before academic exams (Prehn-Kristensen et al., 2009). Corresponding to the mental stress context, our findings support the notion that ANDR could modulate cognitive evaluation during psychosocial stress particularly in males.

In our study, activation of the left insular during exposure to ANDR in males was stronger than in HC-females. Anterior insula activation has been associated with error awareness and performance monitoring (see Klein et al., 2007 for a review). Considering the significant associations between error ratio, change in subjective stress ratings and cortisol levels were only observed in males under exposure to ANDR. We speculated that self-relevant processes in males seem to be particularly affected by social evaluative threat when it was enhanced by the social saliency of ANDR. Functional connectivity studies showed that the anterior insula receives regulatory signals from DLPFC, VMPFC and anterior cingulate cortex in the executive control network that is important during motivational, social and cognitive challenges (Seeley et al., 2007). Therefore, it is possible that males were more reactive to the psychosocial stressors in the MIST and had stronger error awareness during

performance monitoring. The correlational nature of the current findings should be treated with caution.

Under exposure of ANDR, the triangular part of the left IFG bordering the insula in males was activated. Its role in motor execution was enhanced by the maintenance and manipulation of working memory resources (Binkofski and Buccino, 2004). We suspect that ANDR facilitated motor responses particularly in males as the social threat assessment was enhanced. Indeed, it has been proposed that stress reactions can be amplified by the sensory driven attention to social threat (Krusemark and Li, 2012). At the same time, more socially stressed participants might have engaged in competitive processes in sensory encoding of socially relevant chemosignals during the MIST.

The effect of ANDR on social threat in a stress task and the associated DLPFC activity might depend on individual differences. Implicit exposure to ANDR was not linked to a specific psychological process, but we suspect that self-aware and trait-anxious participants were likely to be influenced by non-specific appraisals of a social odor during negative social evaluation. The negative association between trait anxiety and DLPFC activation, and a concomitant positive association of post-stress cortisol level and error ratio, together point to a classical “fight or flight” response as in an adaptive stress response in males. It was argued previously that males were more drawn to “fight” responses in social competition to secure their social status (Eisenegger et al., 2011). The robustness of the effect of social evaluative threat during stress was further strengthened by the positive association between increase in subjective stress and the error ratio observed in males under exposure to ANDR.

In previous studies, more trait-anxious individuals, as characterized by a deficit in DLPFC activation, were shown to have selective attention to social threat (Bishop et al., 2007; Bishop, 2009). In agreement, our data also support that trait anxiety could modulate an executive control region in males as a function of ANDR. DLPFC was also modulated by

stress-related reward processes such as social openness (Lübke et al., 2014). The associations with DLPFC activation in our findings are highly relevant in predicting selective attention to social threat in stressed males. It is worth noting that similar to males, HC-females showed a negative association between trait anxiety and DLPFC activation, however, without parallel associations between subjective stress, post-stress cortisol and performance. It is plausible that hormonal contraceptives affect neural activation and behaviors differentially (cf. Pletzer and Kerschbaum, 2014). Therefore, more research elucidating the impact of synthetic steroids and trait anxiety on behavioral and neural responses is needed.

Previous chemosensory studies have predominantly examined social threat processing in females with airborne chemosignals of males (e.g. Zhou and Chen, 2009). ANDR was found to be more available in males and thus might have a stronger socially relevant salience to males in modulating a wide range of behaviors (Gower et al., 1994). Our data not only extend to situations, in which males showed enhanced social threat processing evoked by a male steroid beyond sensory dynamics, but also support the assumption that individual differences in sensitivity to social stressors may alter the attentional control over social threat processing.

4.4. Potential limitations

Whole brain analyses revealed no significant treatment effect nor a treatment-by-group interaction. The concentration of ANDR used in the current study (250µM/0.25mM) was close to naturalistic occurrences during human interaction (Gower et al., 1994), with a concentration far lower than those in previous studies reporting functional differences of the hypothalamus (e.g. Savic et al., 2001; Burke et al., 2012). However, hypothalamus activation appeared not to be exclusive to exposure to ANDR (Frasnelli et al., 2011). Hence, future studies need to clarify the potentially modulating effects of activation in regions

associated with social cognition and emotion processing by applying different concentrations of ANDR, testing females during ovaluation and using different tasks.

We acknowledge that the stress effect observed here might be constrained by the fixed order of the condition presented, however, we argue that stress should also be built up over time. Moreover, due to using only ANDR and PLAC we cannot infer that the reported findings are substance specific (see Sorge et al., 2014). Thus, future studies might want to compare ANDR to other chemosignals in order to highlight its specificity for social cognition in general and stress responsivity in detail. Although subsequent discrimination and threshold tests indicated a high sensitivity to ANDR in all our participants, the exposure in the scanner remained implicit, as the ratings in both treatments (ANDR vs. PLAC) were similar and rather neutral. This highlights that the experimental manipulation worked even at a subliminal threshold. However, at this threshold we did not observe an increase in cortisol, possibly because a real social interaction in the stress task was lacking. This nevertheless fits to the consensus that non-fertile females (EF-females, HC-females) are not as reactive to socially relevant ANDR as fertile females during periovulation (Lundström et al., 2006a).

Given the fMRI environment could be an additional stressor for some participants, baseline changes in cortisol might be a confound for our results. It has previously been shown that using the MIST, half of the participants were cortisol non-responders and some responders had a long delay until cortisol rose (Dedovic et al., 2009). Our study raised a concern that cortisol levels were not necessarily reflecting perceived stress in females during on- or off-pill period (Hellhammer et al., 2009). Ovarian hormones were not measured, thereby limiting possible hormones-brain-behavior explanations for the effect of ANDR. Moreover, we reported a weak trending group effect for cortisol levels with higher cortisol levels in males than in HC-females. To better explain this group difference, measurement of ovarian hormone concentration in the naturally cycling females and control for contraceptive use in

HC-females should be overcome in future studies. Our study was limited by a small sample size, therefore, the current findings should be considered preliminary.

Future studies will be necessary to elucidate how social odors prime attention to social threat and what other individual differences modulate social threat processes with cognitive load. More specifically, social anxiety in addition to general trait anxiety should be assessed. Moreover, direct evidence (a treatment effect) on whether subgroups of males and females (e.g. with different hormonal levels) process ANDR similarly in a complex social setting is still missing. Limited by our repeated-measures design, we were not able to test individual ANDR threshold concentration. Moreover, we have employed different subgroups of females than the previous studies. In order to test for possible interactions of ANDR and protective mood effects of estradiol, the influence of ANDR on stress reactions in females during their ovulatory period should be tested.

4.5. Conclusion

The present study is unique in that males and two groups of females typically showing similar sex hormonal levels were investigated. We demonstrated significant gender differences in stress reactivity on behavioral and neural levels, with a consistent pattern in males showing stronger DLPFC activation than females. Subjective mood and stress ratings did not differ between females and males. ANDR modulated psychosocial stress: while it yielded less increase in post-stress negative mood, post-stress subjective stress remained unaffected in both genders; males showed significantly stronger neural activation in regions associated with social odors perception and stress-related self-evaluation processing. Inasmuch the effect of ANDR on males, individual differences in trait anxiety further influenced DLPFC activation during psychosocial stress.

Taken together, psychosocial stress reactions and the effects of ANDR differed between females and males and thus our results highlight gender-specific and individual differences

in stress reactions and extend existing knowledge of chemosensory influences on stress processing.

The possible implications of these findings should be discussed with respect to psychobiological processes linking salience of chemosignals and selective attention to social evaluative threat. Accordingly, we gain an understanding that a single chemosignal could enhance social information in a stressful context and how this relates to the biological stress response.

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Conflict of interests

We declare that no conflict of interests exist.

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Figure legends

Figure 1. Timeline of the paradigm of each testing day: Patch application order of androstadienone (ANDR) and placebo (PLAC) was randomized for all groups. To avoid pre-exposure to additional stressors (e.g. scanning environment) and to ensure ANDR to take effect, a 7min resting state scan was performed before the stress task. The order of conditions in each of the two runs of the stress task, and experimenters gave negative feedback between the two runs. Between each condition in each run, a 6-8 second jittered fixation cross was presented.

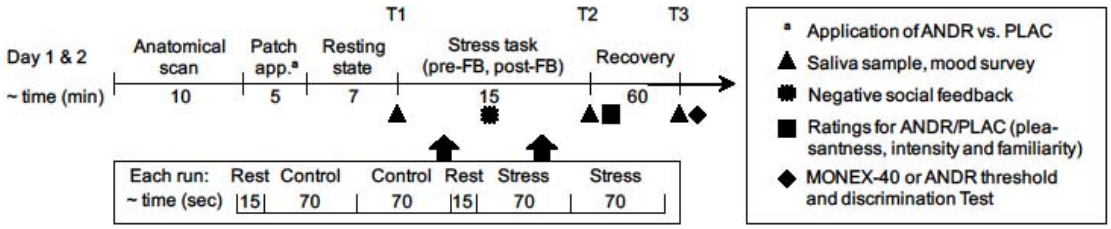

Figure 2. Mean scores and standard errors of (A) negative mood of all participants (n=46) illustrating the significant treatment-by-time interaction with lower negative mood under ANDR compared to PLAC at T2; (B) Correlations of error ratio and change in subjective stress (T2-T1) in males under ANDR (p=.015) and PLAC (p=.960); (C) Correlations of error ratio and log transformed post-stress cortisol level at T2 (µg/dl) in males under ANDR (p=.022) and PLAC (p=.334). * Significant difference at p<.05.

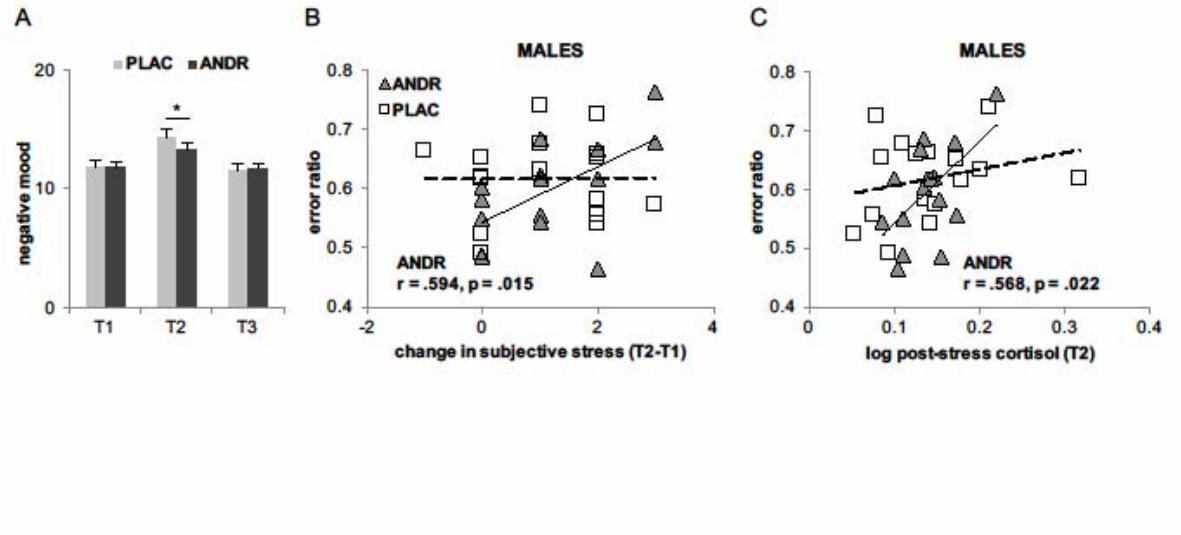

Figure 3. Whole-brain contrasts (13 EF-females vs. 13 HC-females vs. 14 males) for both treatments: (A) Stress condition: males > EF-females contrast for androstadienone (ANDR) and placebo (PLAC) treatment; (B) stress condition: males > HC-females contrast for ANDR and PLAC treatment. FWE corrected at p=.05 (t>4.42). For details of coordinates and statistics, see Table 3.

Inferior occipital gyrus (IOG), dorsolateral prefrontal cortex (DLPFC), angular gyrus (AG),

Note: Inferior frontal gyrus (IFG), pre-motor and supplementary motor area (pre-SMA),

tertiary visual association cortex (V3).

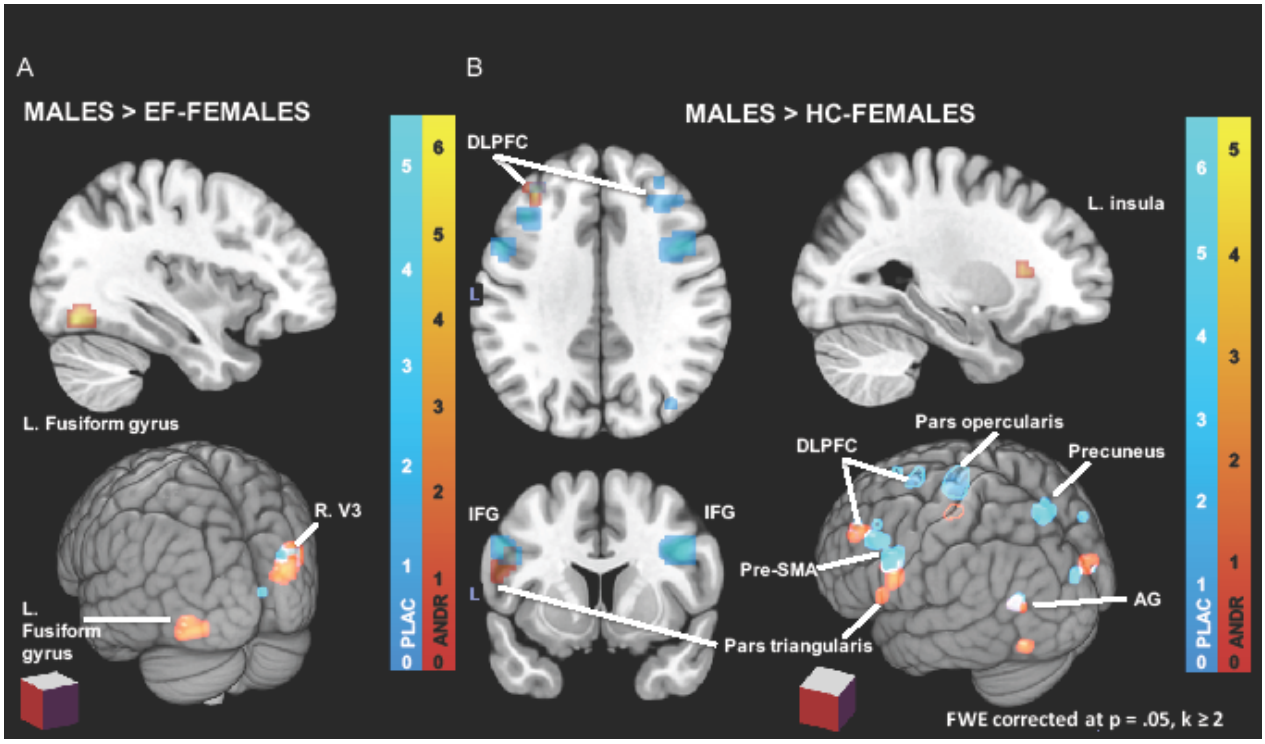

Figure 4. (A) Results of the DLPFC ROI analysis with peak activation at left (-35, 40, 32, k=16) and right (37, 36, 32, k=43) DLPFC; (B) Mean parameter estimates extracted from the peak voxel depicting the significant condition-by-group (13 EF-females vs. 13 HC-females vs. 14 males) interaction; (C) Significant positive correlation between trait anxiety and activation of the DLPFC in the stress condition in males under ANDR. Data represents mean and standard error. ** Significant difference at p<.01; *** Significant difference at p<.001.

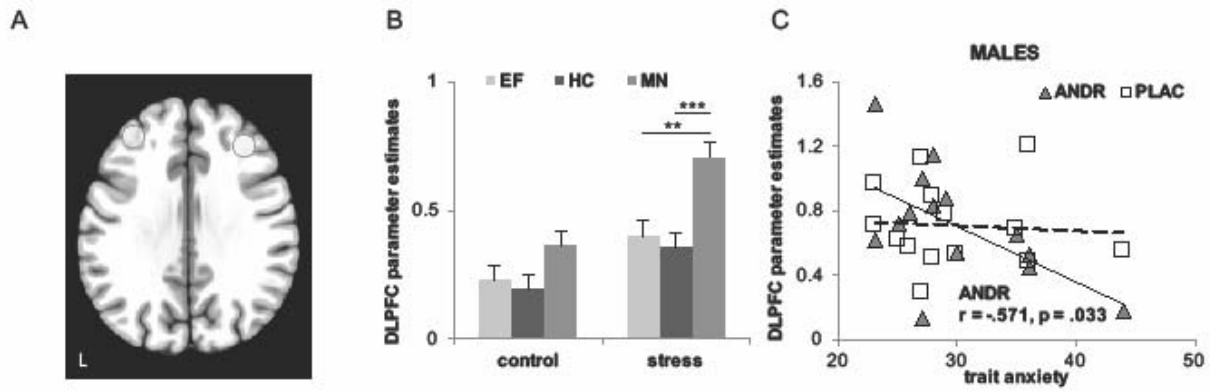

Table 1. Sample description and perception scores of androstadienone (ANDR) and placebo (PLAC) for females in early-follicular (EF, n=15) phase, females taking hormonal contraceptives (HC, n=15) and males (MN, n=16) (mean (standard deviation)).

	EF (n=15)	HC (n=15)	MN (n=16)	t/F	Sig.
Cycle Day (post-menstruation)	4.87 (1.0)				
Cycle length (days)	28.07 (1.9)	28.47 (0.5)		1.63	0.121
Contraceptive use (years)		5.80 (3.8)			
Age	26.40 (3.9)	25.67 (2.6)	24.38 (2.8)	1.67	0.201
Years of education	16.13 (2.4)	15.57 (2.1)	16.13 (2.1)	0.29	0.750
BMI	23.11 (3.4)	21.31 (3.0)	23.12 (1.1)	2.34	0.109
BDI	5.00 (6.9)	2.77 (4.0)	2.06 (1.8)	1.62	0.209
TMT-A (sec)	16.08 (5.1)	16.71 (2.8)	17.28 (3.8)	0.13	0.880
TMT-B (sec)	28.97 (10.4)	28.06 (7.2)	30.03 (7.1)	0.24	0.787
Chronic stress screening	17.00 (9.0)	15.00 (9.0)	11.00 (6.5)	2.35	0.114
Trait anxiety (raw score)	31.00 (11.8)	33.67 (9.5)	29.69 (5.8)	0.73	0.489
MONEX-40	32.73 (3.4)	31.40 (8.8)	31.90 (5.5)	1.75	0.186
Pleasantness					
ANDR	6.03 (3.0)	7.26 (1.8)	6.43 (1.4)	1.27	0.292
PLAC	5.83 (2.0)	7.35 (2.3)	6.69 (1.6)	2.20	0.124
Intensity[a]					
ANDR	5.20 (3.0)	6.42 (0.9)	4.30 (0.5)	5.59	0.007
PLAC	4.65 (2.3)	6.60 (0.9)	3.57 (0.5)	9.66	<0.001
Familiarity					
ANDR	4.31 (2.9)	4.40 (2.9)	3.94 (2.8)	0.94	0.911
PLAC	4.75 (3.2)	4.80 (2.6)	4.81 (2.9)	0.02	0.977

Note: Body-mass-index (BMI), depression scale (BDI-II; Hautzinger et al., 2006), visual attention and task switching (TMT; Reitan, 1956), screening scale for chronic stress (TICS; Schulz and Schlotz, 1999), trait anxiety (STAI; Laux et al., 1981), Monell Extended Sniffin' Sticks Identification Test (MONEX40; Freiherr et al., 2012), androstadienone (ANDR), placebo (PLAC).

[a] HC-females perceived PLAC treatment stronger than EF-females ($p < .025$) and males ($p < .001$), but no group difference appeared between EF-females and males ($p = .388$). HC-females also perceived ANDR treatment stronger than males ($p = .018$) but not EF-females ($p = .451$), while no such difference appeared between EF-females and males ($p = .551$).

Table 2. Presentation of subjective stress, negative mood ratings, error ratio in the stress condition and cortisol levels (µg/dl) of EF-females (n=15), HC-females (n=15) and males (n=16) on both, placebo (PLAC) and androstadienone (ANDR) (mean (standard error)).

	EF (n=15)				**HC (n=15)**				**MN (n=16)**			
	ANDR		**PLAC**		**ANDR**		**PLAC**		**ANDR**		**PLAC**	
Stress (subjective rating)												
T1	1.74	(0.24)	1.59	(0.19)	1.67	(0.24)	1.57	(0.19)	1.48	(0.23)	1.49	(0.19)
T2	2.78	(0.32)	3.05	(0.32)	2.53	(0.32)	2.78	(0.32)	2.65	(0.31)	2.52	(0.32)
T3	1.55	(0.21)	1.59	(0.23)	1.54	(0.21)	1.46	(0.23)	1.60	(0.20)	1.90	(0.22)
Negative mood (PANAS)												
T1	11.56	(0.77)	11.80	(0.92)	12.67	(0.77)	12.87	(0.92)	11.40	(0.75)	10.88	(0.89)
T2	14.33	(0.96)	15.07	(1.28)	13.00	(0.96)	14.13	(1.28)	12.69	(0.93)	13.88	(1.24)
T3	12.20	(0.71)	11.53	(0.82)	11.93	(0.71)	11.40	(0.82)	11.13	(0.69)	11.81	(0.80)
Error ratio	0.63	(0.02)	0.64	(0.02)	0.62	(0.02)	0.62	(0.02)	0.60	(0.02)	0.62	(0.01)
Cortisol (µg/dl)												
T1	0.13	(0.01)	0.14	(0.01)	0.13	(0.01)	0.11	(0.01)	0.14	(0.01)	0.15	(0.01)
T2	0.11	(0.01)	0.13	(0.01)	0.12	(0.01)	0.11	(0.01)	0.14	(0.01)	0.14	(0.01)
T3	0.11	(0.01)	0.12	(0.01)	0.10	(0.01)	0.09	(0.01)	0.13	(0.01)	0.13	(0.01)

Table 3. Whole brain contrasts showing stronger activation in the stress compared to control condition across treatment for all participants; stronger activations in males (n=14) compared to EF-females (n=13) and HC-females (n=13) in both treatments; and stronger activations in EF-females compared to HC-females in both treatments.

	MNI			k	T	Sig.	Region
Contrasts	**X**	**Y**	**Z**				
All participants across treatment							
Stress > control	51	-66	3	10348	13.2	<0.001	R. MTG
	-19	30	-1	134	6.35	<0.001	L. IFG
	-32	36	29	42	5.91	<0.001	L. MFG
	47	-20	-7	12	4.98	<0.001	R. MTG
	47	-3	-17	4	4.73	0.014	R. MTG
Control > stress							No sig. activation
MN vs. EF							
ANDR MN > EF	31	-86	26	40	6.43	<0.001	R. V3
	-35	-72	-14	35	6.17	<0.001	L. fusiform gyrus
PLAC MN > EF	31	-86	26	15	5.57	<0.001	R. V3
ANDR EF > MN						n.s.	No sig. activation
PLAC EF > MN						n.s.	No sig. activation
MN vs. HC							
ANDR MN > HC	-48	-72	19	4	5.38	0.001	L. angular gyrus
	-42	-76	-14	4	4.95	0.006	L. fusiform gyrus
	-35	40	32	9	4.83	0.009	L. DLPFC (BA9, 10)
	-52	3	22	12	4.8	0.010	L. IFG
	51	17	3	5	4.78	0.011	R. IFG
	-29	20	9	4	4.73	0.014	L. insula
	-45	17	3	4	4.63	0.020	L. IFG
	18	-89	16	6	4.58	0.025	R. V2
PLAC MN > HC	44	10	29	46	6.67	<0.001	R. IFG
	4	-66	52	17	5.68	<0.001	R. precuneus
	-52	10	32	24	5.34	0.001	L. pre-SMA
	-48	-72	19	6	5.31	0.001	L. angular gyrus
	-38	23	32	12	4.93	0.006	L. DLPFC (BA9, 8)
	34	33	32	7	4.92	0.006	R. DLPFC (BA9)
	11	-82	9	3	4.77	0.012	R. V1
	-32	40	32	3	4.58	0.025	L. DLPFC (BA9, 10)
	41	-79	-4	2	4.45	0.041	R. V3
ANDR HC > MN						n.s.	No sig. activation
PLAC HC > MN						n.s.	No sig. activation
EF vs. HC							
ANDR EF > HC						n.s.	No sig. activation

PLAC EF > HC	-19	-63	36	5	4.92	0.006	L. SSC
	28	3	45	2	4.42	0.044	R. pre-SMA
ANDR HC > EF						n.s.	No sig. activation
PLAC HC > EF						n.s.	No sig. activation

Note: Androstadienone (ANDR), placebo (PLAC), MN (males), early follicular females (EF), females on hormonal contraceptive (HC), middle temporal gyrus (MTG), middle frontal gyrus (MFG), inferior frontal gyrus (IFG), primary visual cortex (V1), secondary visual cortex (V2), tertiary visual cortex (V3), dorsolateral prefrontal cortex (DLPFC), somatosensory cortex (SSC), pre-motor and supplementary motor area (pre-SMA).
Results of the whole-brain analyses were thresholded at $p < .05$ family-wise error (FWE) corrected.