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Bayesian informed evidence against modulation of androstadienone-effects by genotypic receptor variants and participant sex: A study assessing Stroop interference control, mood and olfaction

Jonas Hornung[a,*], Hannes Noack[a,c], Mara Thomas[a], Gisbert Farger[a], Vanessa Nieratschker[a,b], Jessica Freiherr[d,e], Birgit Derntl[a,b,c]

[a] Department of Psychiatry and Psychotherapy, University of Tübingen, Tübingen, Germany
[b] Werner Reichardt Centre for Integrative Neuroscience, University of Tübingen, Tübingen, Germany
[c] Lead Graduate School, University of Tübingen, Tübingen, Germany
[d] Diagnostic and Interventional Neuroradiology, Universitätsklinikum RWTH Aachen, Aachen, Germany
[e] Fraunhofer Institute for Process Engineering and Packaging IVV, Freising, Germany

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ABSTRACT

The androgen derivative androstadienone (AND) is present in human sweat and may act as human chemosignal. Though effects of AND have been reported with respect to emotional and cognitive processes, results have been highly inconsistent. For this reason, it is likely that AND-action is dependent on modulatory factors. Here we wanted to specifically investigate the impact of genotypic variations of the AND-receptor OR7D4, as well as the influence of participant sex and concomitant hormonal fluctuations on AND-action during emotional interference processing, olfactory performance and mood assessments.

To this end 80 healthy individuals (women taking oral contraceptives; naturally cycling women measured during the luteal phase and men) were tested twice on two consecutive days (AND vs. placebo exposure) with an emotional Stroop task. Also, olfactory performance and mood was assessed. Participants provided saliva samples to measure testosterone, progesterone and estradiol and a blood sample to assess genotypic variations of the AND-receptor OR7D4.

We found a small task-dependent reduction of overall error rates under AND but no modulation of effects by genetic variation or group (female OC, female NC, male) with respect to olfactory performance and mood. Additional analyses with help of Bayesian statistics gave strong evidence in favor of specific null hypotheses suggesting that the action of AND was not modulated by either genotypic variations or sex of participants with respect to interference control (bias indices), olfactory self-reports and mood parameters.

Additional effects of AND in connection with hormonal fluctuations are reported.

1. Introduction

Human sweat contains androgens like androstenone, androstenol and androstadienone (4,16-androstadien-3-one, AND; Wyatt, 2015). Especially AND was repeatedly detected in male and female axillary hair (Nixon et al., 1988; Gower et al., 1994) and was found to affect mood states by reducing nervousness and tension (Grosser et al., 2000) and increasing positive mood in women (Grosser et al., 2000; Jacob and McClintock, 2000; Villemure and Bushnell, 2007).

Given its occurrence in human sweat, it has been surmised that AND may especially influence attention in social contexts. Support for this claim comes from studies reporting higher pain intensity under AND exposure (Villemure and Bushnell, 2007) and higher self-reports of being more focused in women (Grosser et al., 2000). In addition, heterosexual women spent more time looking at female faces and both men and women responded more slowly to social negative and quicker to social positive images only under AND but not placebo (PLAC) exposure (Hummer et al., 2016). Finally, an emotion-specific reduction of reaction times to angry human faces under AND exposure has recently been reported (Frey et al., 2012; Hornung et al., 2017). However, studies often report inconsistent findings regarding AND-action. There are e.g. debates whether AND increases attractiveness ratings of opposite sex-faces (Saxton et al., 2008; Ferdenzi et al., 2016) or not (Hare et al., 2017) and whether AND increases positive mood (Grosser et al., 2000;

* Corresponding author at: Department of Psychiatry & Psychotherapy, University of Tübingen, Calwerstrasse 14, 72076 Tübingen, Germany.
E-mail address: jonas.hornung@med.uni-tuebingen.de (J. Hornung).

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Jacob and McClintock, 2000; Villemure and Bushnell, 2007) or has no effect on mood (Hummer and McClintock, 2009; Ferdenzi et al., 2016). For this reason, it is likely that AND-action is dependent on modulatory factors. Identification of such factors may help to explain why only some individuals are affected by AND. Here we want to investigate (i) the impact of genotypic variation of the AND-receptor OR7D4, as well as (ii) the influence of participant sex and concomitant fluctuations in sex hormone levels on AND-action.

First, there are repeated claims that genetic traits may explain why some people are able to smell steroids like androstenone and AND and differ with respect to intensity and pleasantness ratings for these steroids. In this respect, one study found that identical compared to fraternal twins only had higher similarity for sensitivity of the steroid androstenone but not for a control odor (pyridine) suggesting a heritable cause for differences in this steroid's perception (Wysocki and Beauchamp, 1984). Further studies have shown that some people rate androstenone as pleasant (sweet, flower-like) while others rate it as unpleasant (sweat, urine-like) (Beets and Theimer, 1970; Van Toller et al., 1983). However, the potential molecular mechanisms of these effects have only recently been addressed. In an in vitro experiment, Keller et al. (2007) identified the olfactory receptor OR7D4 to be selectively activated by androstenone and AND but not by a panel of another 64 odors. The authors also identified different polymorphisms of the OR7D4 gene: human carriers of the receptor polymorphism RT/RT rated AND as more intense and were more sensitive in detecting AND in a threshold test than carriers of the RT/WM or WM/WM genotypes. These findings are in accordance with Lunde et al. (2012) who found increased androstenone-sensitivity of RT/RT carriers and additionally showed that androstenone-rich pork was rated as more unpleasant by RT/RT compared to carriers of the RT/WM or WM/WM genotypes.

Second, sex differences and fluctuations of sex hormone levels like testosterone, estradiol and progesterone are likely predictors of olfactory differences. In general, women outperform men on olfactory assessments like threshold and identification tests although findings are mixed and do not seem to pose a strong effect (see Doty and Cameron, 2009 for a review). Furthermore, also within women differences are apparent when the menstrual cycle, hormonal contraceptive (OC) use and concomitant hormonal fluctuations are taken into account: while OC-use leads to a relatively stable and low hormonal profile of natural estradiol and progesterone, naturally cycling women show stronger fluctuations of gonadal hormones (Sundström-Poromaa and Gingnell, 2014). In general, OC-users seem to have lower sensitivity for a range of odors compared to women in their follicular and periovulatory phase (Caruso et al., 2001). This is in line with another study showing that during their periovulatory phase, naturally-cycling women were more sensitive to AND, androstenone and musk than OC-users (Renfro and Hoffmann, 2013). However, olfactory performance may not be generally different between OC-users and naturally cycling women but rather be odor-specific as suggested by Lundstrom et al. (2006) who showed that around ovulation women were more sensitive to AND but less sensitive to phenylethyl alcohol than OC-users suggesting better detection of a putative chemosignal when conception risk is high in naturally cycling women. Thus, different levels of natural female sex hormones (OC-use: low levels; luteal cycle phase: high levels) may also differently impact the response to the chemosignal AND.

Based on above findings we propose the following hypotheses:

1. **Genotypic influence**: we expect carriers of the RT/RT-OR7D4-receptor genotype to show higher AND-sensitivity and higher intensity ratings for AND than carriers of the RT/WM and WM/WM genotypes. This should also be visible in stronger AND-effects in a task tapping interference as AND is believed to draw attention more strongly to socio-affective stimuli like human faces, thus reducing interference processes. Similarly, the change of positive/negative affect under AND exposure should be pronounced for carriers of the RT/RT genotype.
2. **Sex influence**: we expect that women show higher AND-sensitivity and higher intensity ratings for AND than men. Furthermore, we expect naturally cycling women during their luteal phase to show stronger AND-effects than OC-users. This higher sensitivity and intensity ratings for AND should also be visible in stronger AND-effects during interference control and in a stronger change of positive/negative affect.
3. **Hormonal influence**: given that hormonal fluctuations were linked to olfactory performance and the liking of masculinity in women (Garver-Apgar et al., 2008), we suspect that these fluctuations may also influence AND-action. Based on one previous study (Lübke and Pause, 2015), we expect that in men higher endogenous testosterone levels are linked to decreased pleasantness ratings of AND. In women, we expect higher estradiol levels to be linked to decreased liking of AND. Given these associations it seems reasonable to assume that fluctuations of sex hormones also influence olfactory performance, in specific regarding the chemosignal AND. In the present study we therefore explored the differential impact of testosterone, estradiol and progesterone under AND compared to PLAC with respect to interference processing and olfactory performance.

This is the first study to combine task-dependent measures of AND-action with molecular, hormonal and self-report measures. We hope that this helps to identify factors that explain interindividual differences with respect to the action of the putative human chemosignal AND.

2. Materials and methods

2.1. Participants

2.1.1. Group information

A total of 80 male and female students of the University of Tübingen were recruited and measured twice (once under AND, once under PLAC) on two consecutive days at the same time of day. Female participants were either taking oral contraceptives (OC-users: n = 31, combination of ethinyl-estradiol and progestin) and were measured during their active pill-intake phase or were naturally cycling and measured during their luteal cycle phase (luteal women; n = 21). The menstrual cycle of naturally cycling women was recorded at least three times before an experimental appointment was scheduled. Experimental dates were scheduled for luteal women between days 18–24 within the standardized 28 day cycle when natural female sex hormones are high in contrast to the low natural hormone profile that is normally observed during OC-use (Sundström-Poromaa and Gingnell, 2014). To account for varying cycle lengths, for each luteal woman a standardization to a 28-day cycle was performed. Based on previous reports (Garver-Apgar et al., 2008), we kept the luteal phase constant at a length of 14 days and calculated a time window backwards from expected onset of the next menses. Women also reported onset of the first menses after the end of the experiment. This information confirmed that on average luteal women were measured on the 21st and 22nd day within the standardized 28 day cycle (SD = 2.99 days; range = 17th–27th day).

2.1.2. Genotypic information

Blood samples from finally included participants were drawn (n = 73), 48 of whom were carriers of the RT/RT genotype (thereof 18 OC-users, 20 men, 10 luteal women), 22 were carriers of the RT/WM genotype (thereof 8 OC-users, 7 men, 7 luteal women) and 3 were carriers of the WM/WM genotype (3 OC-users). As the loss of one RT-allele (RT/WM) was expected to have as severe consequences on the ability to detect AND (Keller et al., 2007; Lunde et al., 2012), we collapsed carriers of RT/WM with WM/WM thus yielding a group of 25 subjects (= any WM).