Running title: Androstadienone and Emotion

Sexually dimorphic effects of human chemosignal on perception of angry but not fearful faces

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Abstract:

Androsta-4,16,-dien-3-one (androstadienone), a steroids implicated as a human social chemosignal, has been reported to impact one's emotional perception along the valence axis. The current study takes a step further to examine whether it modulates the perception of angry and fearful faces, two negative emotions that are similar with respect to valence and arousal, but signal different social values. Systematic comparisons of psychophysical data collected from 40 heterosexual men and 45 heterosexual women revealed that androstadienone subconsciously biased heterosexual men toward perceiving the male faces as less angry, while it biased the heterosexual women toward perceiving the female faces as angrier. Meanwhile, androstadienone did not affect the perception of fearful faces in either men or women. These findings indicate that the modulation of androstadienone on negative emotional perceptions is not uniform, suggesting that it alters the perception of specific rather than general negative emotions. In particular, it impacts one's perception of anger, which signals impending aggression, and hence could further impact an individual's social interaction in a sex-specific manner.

Keywords

androstadienone, emotional perception, angry, fearful, sex-specific

1. Introduction

It is well known that humans, as other odoriferous mammals, communicate chemically. Chemosignals emitted from the human body can exert a range of effects on recipients' behavioral responses, endocrine levels, mood, and cognition (Ye et al., 2021), accompanied by neural activities distinct from those evoked by common odors (Lundstrom et al., 2008; Zhou and Chen, 2008). In the search for specific compounds of human social chemosignals, androstadienone (androsta-4,16,-dien-3-one) has received the most attention among the numerous chemical compounds of human secretions. As a nonandrogenic derivative of gonadal progesterone, androstadienone is the most prominent androstane in male semen, axillary hair and on the axillary skin surface (Gower and Ruparelia, 1993). Accumulating evidence from different labs shows that androtadienone affects recipients' physiology and mood, possibly in a context-dependent as well as sex-specific manner (Bensafi et al., 2004; Bensafi et al., 2003; Jacob and McClintock, 2000; Wyart et al., 2007). Although most works restrict tests in women in their periovulatory phase, there is evidence indicating that the effect of androstadienone varies across the menstrual cycle, which is likely to be related to the changes in sex hormone levels. Specifically, as compared with women in the luteal phase, androstadienone leads women in the fertile phase (periovulatory phase) to perceive more anger from neutral female faces (Wu et al., 2022). Women in the luteal phase show poorer performance in mental arithmetic task with social threat and stronger stress-related hippocampus activation, and exhibit a preference for female faces under the exposure to androstadienone (Chung et al., 2016; Parma et al., 2012), relative to women in the periovulatory phase. Moreover, a series of studies using stringent psychophysical methods have suggested that androstadienone shifts one's perception of social stimuli. For example, androstadienone signals masculinity to heterosexual women and homosexual men as it systematically biases them toward perceiving point-light walkers as more masculine (Zhou et al., 2014). In parallel, it reportedly evokes response in the hypothalamus of heterosexual women and homosexual men (Savic et al., 2001; Savic et al., 2005). However, these sex-specific effects are not without controversies (Chung et al., 2016; Hornung et al., 2018), which may be a results of varying odor qualities and intensities (Burke et al., 2012).

Humans are one of the most intensely social animals. Accurately identifying others' emotional status is essential for successfully navigating human social interactions. Previous research has suggested that androstadienone impacts our processing of emotional stimuli and preferentially allocates mental resources to emotionally significant stimuli (Hummer and McClintock, 2009). For example, it modulates the emotional perception of heterosexual women along the axes of happy-sad and relaxed-nervous, and leads heterosexual women to perceive men as happier and more relaxed and women as sadder (Ye et al., 2019). Also, androstadienone has been shown to bias women in the periovulatory phase toward perceiving neutral female faces as angrier (Wu et al., 2022). Notably, the foregoing effects took place in the absence of individual's awareness, as recipients were oblivious to the nature of the olfactory stimuli and failed to differentiate androstadienone and the carrier control.

Nevertheless, previous studies largely concentrated on the positive-negative axes of emotional perception, leaving the perception of specific negative emotional status unexamined. In particular, anger and fear, two negative emotions that are similar with respect to valence and arousal, have different social-signal value. Specifically, angry faces indicate impending aggression from the poser, while fearful faces indicate potential threat perceived by the poser (Adams et al., 2003). Infants can successfully discriminate angry and fearful faces as young as 5 to 7 months old (Schwartz et al., 1985). In parallel, event-related potential studies have found differential neural activities evoked by these two facial expressions can emerge at 5 months and can be well established at 7 months (Kobiella et al., 2008; Xie et al., 2019). Distinct neural patterns were also evident in adulthood, with angry and fearful faces preferentially activating the insula and amygdala, respectively (Fusar-Poli et al., 2009).

In the present study, we aimed to examine the effects of androstadienone on women's and men's perception of angry and fearful faces from the same sex expressors by comparing their judgements in an emotional identification task under the exposure to androstadienone and the carrier control, so as to expand our knowledge regarding the effect of human chemosignals on negative emotional perception. We opted for facial stimuli as the visual stimuli in the emotion identification task for their richness in social information (Marsh et al., 2005) and effectiveness in conveying these two emotions. To

systematically assess the chemosignal's effect on visual perception, we varied the ambiguity of facial emotion by morphing the facial stimuli between neutral and angry/fearful faces. Sex-specific effects of androstadienone on mood, perception as well as hypothalamic activities have been documented in previous work (Jacob and McClintock, 2000; Savic et al., 2001; Savic et al., 2005; Ye et al., 2019; Zhou et al., 2014), therefore we hypothesized that androstadienone would exert differential effects on men and women.

2. Material and methods

2.1 Participants

A total of 85 healthy Han Chinese nonsmokers from a Chinese university participated in the study, including 40 men (mean age ± SD = 21.93 ± 1.99 years) and 45 women (20.49 ± 1.58 years). Sample sizes were determined by G*Power to be adequate to detect a moderate effect of androstadienone (Cohen's $d \approx 0.6$), at 95% power, resulting in a sample size of 39. Nevertheless, we recruited 40 or more participants for each sex group to allow for possible non-compliance or impossibility of model fit. The effect size was estimated based on an earlier study that employed identical olfactory stimuli and similar psychophysical testing procedures (Zhou et al., 2014). All participants reported to be heterosexual, have normal or corrected-to-normal vision, a normal sense of smell, and no respiratory allergy or upper respiratory infection at the time of testing. All female participants were tested around the periovulatory phase of their menstrual cycles (mean ± SD = 14.91 ± 2.77 days and 14.61 ± 3.21 days from the onset of their last period of a normalized 28 cycle, for androstadienone and carrier control condition, respectively), which did not differ between the two olfactory conditions ($t_{44} = 1.10$, $p = 0.28$). The participants were ignorant of the purpose of the experiments and gave informed consent to participate. Data were collected in the summer of 2021. The study was conducted in accordance with the Declaration of Helsinki and was approved by the local research ethics committee.

2.2 Olfactory stimuli

The olfactory stimuli consisted of androstadienone (500μM in 1% v/v clove oil propylene glycol solution) and the carrier solution alone (control, 1% v/v clove oil in propylene glycol). The concentration of androstadienone used in the current study (500μM) was comparable to that in freshly-produced apocrine sweat (mean = 0.44nmol/μl = 0.44×10^{-3} mol/l = 440μM), hence arguably ecologically relevant (Gower et al., 1994). Both stimuli were presented in identical 40 ml polypropylene jars, each containing 5ml of clear liquid and connected with two Teflon nosepieces via a Y-structure (Figure 1B). The effectiveness of the clove oil carrier solution as a mask for the odors of androstadienone has been consistently verified in previous work (Ye et al., 2019; Zhou et al., 2014), and the two olfactory stimuli have been reported to be comparable in perceived intensity, pleasantness and familiarity (Ye et al., 2019). Both stimuli were supra-threshold to all participants.

2.3 Visual stimuli

For both the neutral-angry and neutral-fearful blends, we generated visual stimuli using the procedure as detailed below. We first recruited 62 undergraduate students from the university (age range 18-23, 42 men) as actors. Each actor performed 3 emotional expressions (angry, fearful, neutral), which were photographed indoors by the same experimenter, resulting in 186 images of facial expressions. We kept the lighting and background (white) constant across actors and expressions. To prevent the interference of other confounding factors, we used Photoshop software to process the raw images, e.g., removing other non-facial features such as hair and neck, matching colors (gray-scaled) and cropping to an oval shape.

Next, another 48 participants (age rang: 18-25, 24 men) were recruited to evaluate these facial stimuli (3.57° × 4.32°). They were instructed to identify the emotional expression of the face from the same sex actors (out of three emotions: angry, neutral, fearful) and rate the intensity of the selected emotion (from 1-9, with 9 being the most intense). Each stimulus was randomly drawn and presented to them one at a time, resulting in 60 trials for female participants and 126 trials for male participants. Thirty-six images from 12 actors (6 men) were selected for their high identification accuracies and intensity ratings of the corresponding emotion (for each image, mean identification accuracy > 0.71; mean intensity rating > 4.50; for pooled data from actors and emotions, mean identification accuracies ± SD

= 0.85 ± 0.10 and 0.83 ± 0.16; mean intensity ratings ± SD = 6.07 ± 0.88 and 5.73 ± 0.71, for men and women, respectively). Analyses using identification accuracies as well as rating scores of three emotional expressions from the two sexes as dependent variables showed that the three emotional expressions were equally discriminable, as the identification accuracies were comparable across the three emotions in men ($F(2, 50) = 0.34$, $p = 0.72$) and in women ($F(2, 50) = 1.00$, $p = 0.38$). On the other hand, there were no differences between men and women on the identification accuracies of the three emotions (ps > 0.57), and men and women shared comparable ratings of angry and fearful faces (p > 0.44), but the difference between men and women's ratings of neutral faces reached marginal significance (p = 0.061).

After that, to create ambiguity in the facial emotions, FantaMorph software for Windows (version 5.5.0) was utilized to morph typical images of neutral and emotional faces, generating a continuum of 8 images between each actor's neutral and angry/fearful images, resulting in 48 images (6 actors × 8 images) representing gradual transitions from the typical neutral face (85% neutral and 15% angry/fearful) to the typical emotional face (15% neutral and 85% angry/fearful) per emotion per sex (Figure 1A). All the visual stimuli used in the emotional identification task are available on the project's Open Science Framework (OSF) page: https://osf.io/t2n4b/.

2.4 Emotional identification task

The task contained 3 blocks, each of which had 64 trials, making a total of 192 trials (6 actors × 8 morphs × 2 emotion blends × 2 repetitions). Each trial started with presentation of a central fixation for 500~1000 ms. After that, participants were presented with a facial stimulus (3.57° × 4.32°, same sex as the participant, presented against a black background) for 3s, during which they were instructed to indicate whether they perceived it as a neutral facial expression or an angry/fearful facial expression. Participants were required to press the key F and J (key-response mappings were counterbalanced across participants) as quickly as possible. The trial ended after the response was made or after 3s (a total of 1.11% and 1.16% trials ended without response, for men and women respectively).

Each block contained 2 sections, which either utilized the neutral-angry blend (32 trials) or the neutral-fearful blend (32 trials) as visual stimuli, with a 30s interval between them to prevent fatigue. During each section, morphs from 4 actors (8 morphs each actor) were used as visual stimuli and randomly presented to the participants. The actors were kept the same for the two sections in each block. The order of the section was randomized across participants, with 22 men and 21 women beginning with the identification of the neutral-angry blend. Each block lasted approximately 2.5 minutes. There was a break of at least 1 minute in between every second block to eliminate olfactory adaptation.

2.5 Experimental procedure

Each participant completed 2 testing sessions: one session per day with an interval of approximately 48 hours. On each day of testing, participants performed 3 blocks of emotion identification task under the continuous exposure to androstadienone or the carrier control solution alone at around the same time of day. The order of the olfactory conditions was randomized across participants (24 men and 19 women exposed to androstadienone in the first session). Specifically, while performing the task, participants were instructed to hold the jar with their non-dominant hand while positioning the nosepieces inside their nostrils, continuously inhale through the nose and exhale through their mouth. To ensure the participants followed the instructions, their activities were continuously observed via a video monitor placed in an adjacent room. The experimenter (woman) was not in the testing room when the participants performed the task.

Previous work have linked the traits of social anxiety and aggression to the perception of emotional faces (Brennan and Baskin-Sommers, 2020; McTeague et al., 2018), hence we further measured these two traits of the participants to test whether the effects of olfactory cues differed in participants with different traits. Specifically, on the first day of testing, each participant had to complete the Liebowitz Social Anxiety Scale (a 24-item self-report measure of social anxiety) (Liebowitz, 1987) and the Buss-Perry Aggression Questionnaire (BPAQ, a 29-item self-report measure of aggression) (Buss and Perry, 1992) before the formal experiment.

2.6 Data analyses

Participants were excluded from analyses if (1) they only completed one of the two sessions, and (2) their task data failed to fit with the model.

Firstly, to verify the comparability of the perceptions of visual stimuli between the two sexes as well as between the two emotion blends, we conducted an omnibus ANOVA with emotion blend and facial morph as the within-subjects factors, and sex of participants as the between-subjects factor, using judgements of the emotional identification task under the exposure to the carrier control as the dependent variable. Additionally, we also performed an omnibus ANOVA on response time with emotion blend, olfactory condition and facial morph as the within-subjects factor, and sex of participants as the between-subjects factor.

Then, we performed an omnibus ANOVA on the judgements with emotion blend, olfactory condition and facial morph as the within-subjects factors, and sex of participants as the between-subjects factor. Then we dissected the effects by conducting the analyses in male and female participants separately. To verify the effectiveness of the visual stimuli, we analyzed the judgements at 8 morph levels from each blend under the exposure of the carrier control with repeated measures ANOVA. Afterwards, data was analyzed in a 2 (olfactory condition: androstadienone, carrier control) × 8 (morph level: 1 to 8 in each blend) repeated measures ANOVA. In addition, to investigate the main effect of olfactory condition on response time, a 2 (olfactory condition) × 8 (morph level) repeated measures ANOVA was conducted on the response time in each blend separately.

We subsequently zoomed in on the morphs with the highest ambiguity, where responses were assumed to be most susceptible to chemosensory effects according to the rule of inverse effectiveness (Stein and Stanford, 2008). We opted for the fourth morph (55% neutral and 45% angry) and the fifth morph (45% neutral and 55% angry), where judgements under the exposure of the carrier control were closest to 50%, suggesting that they were nearly perceived as equally neutral and angry/fearful). We first ran a repeated measures ANOVA with olfactory condition, morph level of the facial stimuli (fourth or fifth morph) and the emotion blend as within-subjects factors. After that, to clarify the specific direction of the effect of androstadienone, a series of pairwise-t tests between

androstadienone and the carrier control were conducted, using the mean proportion of 'angry/fearful' responses at the fourth and fifth morphs as the dependent variable.

Furthermore, to better characterize the participants' response criteria and sensitivities, we conducted a sigmoidal-curve fit using the function $P = c + d/(1 + e^{-\frac{x-a}{b}})$ (Moradi et al., 2005; Zhou and Chen, 2009), which is frequently used to obtain psychometric curves that depict the probability of emotion judgments as a function of facial morphs (Moradi et al., 2005; Zhou and Chen, 2009). Specifically, *P* denotes the proportion of the face being judged as angry/fearful, x is the morphed levels (8 levels), and *c, d, a,* and *b* correspond to coefficients for the y-offset, height, center, and width of the curve, respectively. Based on the function, we can calculate the x (point of subjective equality, PSE) where *P* = 0.5, indicating that the face was judged as equally neutral and angry/fearful, and d/b indicates the slope, as an index of an individual's sensitivity (Figure 1C). For each sex, we further conducted a series of paired-t tests to compare the emotion judgment criteria and sensitivity, under the exposure to the different olfactory conditions.

To explore the contribution of social anxiety trait and aggression trait, we also conducted the aforementioned analyses with PSEs as dependent variables and each participant's scores of the Liebowitz Social Anxiety scale and the Buss-Perry Aggression Questionnaire as covariates.

All the data and analysis scripts are available on the project's Open Science Framework (OSF) page: https://osf.io/t2n4b/

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3 Results

We first conducted an omnibus ANOVA on the judgements of the emotional identification task under the exposure to the carrier control in order to prevent the interference of olfactory condition. Analysis revealed neither a significant main effect of sex of participants ($F(1, 83) = 0.13$, $p = 0.72$), emotion blend ($F(1, 83) = 1.68$, $p = 0.20$), nor a significant interaction between sex of participants and emotion

blend ($F(1, 83) = 0.91$, $p = 0.34$). These results validated that the perceptions of visual stimuli were comparable between the two sexes and the two emotion blends. On the other hand, male and female participants did not differ in response time, as ANOVA with emotion blend, olfactory condition, facial morph, and sex of participants revealed no main effect of sex of participants ($F(1, 83) = 1.68$, $p = 0.20$).

Another omnibus ANOVA of the judgements from both male and female participants (emotion blend × olfactory condition × morph level × sex of participants) identified a significant three-way interaction between emotion blend, olfactory condition and sex of participants ($F(1, 83) = 14.02$, $p < 0.001$, partial $\eta^2 = 0.14$), suggesting that the effects of androstadienone on emotional judgments were dependent on the emotion blend, olfactory condition and the sex of the participants. To dissect the effect of androstadienone in each emotion blend in each group of participants, we conducted analyses on male and female participants separately.

3.1 Exposure to androstadienone led heterosexual men to perceive male faces as less angry, while it had no effect on the perception of fearful faces

Repeated measures ANOVAs on participants' responses from the emotional identification task under the exposure to the carrier control revealed a pronounced main effect of morph level of the facial stimuli (8 levels, from 15% angry/fearful to 85% angry/fearful) in both emotion blends (neutral-angry blend: $F_{5.05, 196.90} = 356.64$, $p < 0.001$, partial $\eta^2 = 0.90$; neutral-fearful blend: $F_{3.56, 138.86} = 367.96$, $p < 0.001$, partial $\eta^2 = 0.90$). The datapoints displayed sigmoidal response patterns where facial morphs with a higher proportion of angry/fearful were more frequently judged as angry/fearful (Figure 2A-B), corroborating the effectiveness of the morphed facial stimuli. Then we conducted another repeated measures ANOVA with olfactory condition, morph level of the facial stimuli and the emotion blend as within-subjects factors. There was a significant interaction between olfactory condition and emotion blend ($F(1, 39) = 6.29$, $p = 0.016$, partial $\eta^2 = 0.14$), suggesting that androstadienone had differential impacts on the perception of angry and fearful faces. Separate analyses on responses from each emotion blend revealed that participants had differential responses under the two olfactory

conditions while judging along the neutral-angry blend (F(1, 39) = 5.47, p = 0.025, partial η^2 = 0.12, Figure 2A) rather than the neutral-fearful blend (F(1, 39) = 1.38, p = 0.25, Figure 2B). On the other hand, analyses of the response time with olfactory condition and morph level as within-subjects factors failed to find any significant effect of androstadienone (F(1, 39) = 0.24 and 0.20, ps = 0.63 and 0.66, for the neutral-angry and neutral-fearful blends, respectively).

To clarify the specific direction of the abovementioned effect of androstadienone, we then zoomed in on the most ambiguous morphs, where judgements would be most susceptible to the influence of chemosensory cues based on the rule of inverse effectiveness (Stein and Stanford, 2008). Repeated ANOVA on judgments of the fourth and fifth morphs (45% angry and 55% angry, respectively, where the average judgements under the exposure to the carrier control were closest to 0.5, suggesting that they were nearly perceived as equally neutral and angry/fearful) found a marginal significant interaction between olfactory condition and emotion blend (F(1, 39) = 3.91, p = 0.055, partial η^2 = 0.091), but no significant three-way interaction between olfactory condition, morph level, and emotion blend (F(1, 39) = 0.46, p = 0.50). These results suggested that the interaction between olfactory condition and emotion blend were comparable between the fourth morph and the fifth morph. To determine the directions of androstadienone's effect, we calculated the average of data of the fourth and fifth morphs and ran a series of pairwise-t tests to compare responses under different olfactory condition in different emotion blend. The results showed that compared with the carrier control, exposure to androstadienone decreased "angry" responses when the visual emotional information was ambiguous (mean ± SD = 0.44 ± 0.15 and 0.51 ± 0.14 for androstadienone and carrier control condition, respectively; t(39) = -2.72, p = 0.010, Cohen's d = 0.43, Figure 2A, inset). On the other hand, average judgements of ambiguous faces in the neutral-fearful blend were unaffected by the olfactory conditions (mean ± SD = 0.54 ± 0.17 and 0.54 ± 0.16 for androstadienone and carrier control condition, respectively; t(39) = -0.006, p > 0.99, Figure 2B, inset).

To quantify the judgement, emotion identification performances under the exposure to androstadienone and the carrier control were separately fitted with a sigmoid function to obtain psychometric curves. This allowed us to determine the points of subjective equality (PSEs) which

yield neutral and angry/fearful judgement with equal probability, as well as the slope of the curve. Comparisons between the PSEs, which indicated one's judgment criteria, dovetailed with the results for the ambiguous morphs. Specifically, androstadienone biased male participants toward perceiving the faces as less angry, since the face had to be angrier (PSEs moving rightward, Figure 1C) to be perceived as equally neutral and angry (mean ± SD = 4.97 ± 0.93 and 4.41 ± 0.67 for androstadienone and carrier control condition, respectively; $t(39) = 3.40$, $p = 0.002$, Cohen's $d = 0.54$, Figure 2C). In contrast, the perception of fearful faces was not influenced by the exposure to androstadienone (mean ± SD = 4.36 ± 0.72 and 4.53 ± 0.88 for androstadienone and carrier control condition, respectively; $t(39) = -1.09$, $p = 0.28$, Figure 2C). Moreover, the divergent effects of androstadienone on different blends cannot be attributed to the intrinsic difference between the two blends, indicated by comparable judgements on the ambiguous morphs as well as PSEs of the two blends under the exposure to the carrier control (t(39s) = 0.89 and 0.74, $ps = 0.38$ and 0.46, for judgement on the ambiguous morphs and PSEs, respectively). Furthermore, androstadienone did not exert influence on sensitivity of emotion identification, which was indexed by the quotient of the height and width of the fitted curve (t(39s) = -0.97 and 0.89, $ps = 0.34$ and 0.38, for the neutral-angry and neutral-fearful blends, respectively).

For both blends, repeated measures ANOVAs on PSEs found that scores of the Liebowitz Social Anxiety Scale and the Buss-Perry Aggression Questionnaire did not interact with the effect of odor ($ps > 0.37$).

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3.2 Exposure to androstadienone led heterosexual women to perceive female faces as angrier, but had no effect on the perception of fearful faces

We applied similar analyses to judgments of female participants. Repeated measures ANOVA on data from different emotion blends under the exposure to the carrier control suggested that the morphs

were highly distinguishable in both blends (neutral-angry blend: $F(4.39, 193.33) = 389.50$, $p < 0.001$, partial $\eta^2 = 0.90$; neutral-fearful blend: $F(4.14, 182.06) = 614.16$, $p < 0.001$, partial $\eta^2 = 0.93$). Similar to the results for male participants, there was a significant interaction between emotion blend and olfactory condition ($F(1,308) = 7.86$, $p = 0.008$, partial $\eta^2 = 0.15$). Subsequent analyses conducted on each blend showed that while odor evoked different response patterns in the judgment of the neutral-angry blend ($F(1, 44) = 4.63$, $p = 0.037$, partial $\eta^2 = 0.095$, Figure 2D), it did not bias the judgment of the neutral-fearful blend ($F(1, 44) = 1.34$, $p = 0.25$, Figure 2E). Meanwhile, analyses of response time found no effect of olfactory condition ($F(1, 44s) = 0.013$ and 0.042, $ps = 0.91$ and 0.84, for the neutral-angry and neutral-fearful blends, respectively).

We further narrowed down our analyses on the most ambiguous morphs and found a significant interaction between olfactory condition and emotion blend ($F(1, 44) = 5.70$, $p = 0.021$, partial $\eta^2 = 0.12$), but no significant interaction between olfactory condition, morph level and emotion blend ($F(1, 44) = 1.29$, $p = 0.26$). Further analyses with averaged data from the fourth and fifth morphs showed that in contrast to men, smelling androstadienone resulted in judging the ambiguous morphs as angrier (mean ± SD = 0.55 ± 0.20 and 0.50 ± 0.19 for androstadienone and carrier control condition, respectively; $t(44) = 1.99$, $p = 0.053$, Cohen's $d = 0.30$, Figure 2D, inset). Nevertheless, androstadienone still had a null effect on the judgment of neutral-fearful faces (mean ± SD = 0.51 ± 0.18 and 0.54 ± 0.17 for androstadienone and carrier control condition, respectively; $t(44) = -0.89$, $p = 0.38$, Figure 2E, inset).

Likewise, the analyses of PSEs suggested that compared with the carrier control, exposure to androstadienone systematically biased the female participants toward perceiving the female faces as angrier, as indexed by the leftward shift of PSE (mean ± SD = 4.26 ± 0.87 and 4.55 ± 0.91 for androstadienone and carrier control condition, respectively; $t(44) = -2.34$, $p = 0.024$, Cohen's $d = 0.35$, Figure 2F). A repeated measures ANOVA of the PSEs from both male and female participants revealed a significant two-way interaction between olfactory condition and sex of participants ($F(1, 83) = 17.42$, $p < 0.001$, partial $\eta^2 = 0.17$), further corroborating the dissociable effects of androstadienone on anger perception in men and women. Androstadienone did not shift the perception

of the neutral-fearful blend (mean ± SD = 4.50 ± 0.65 and 4.50 ± 0.76 for androstadienone and carrier control condition, respectively; $t(44) = -0.008$, $p = 0.99$, Figure 2F). These distinct patterns in the two blends cannot have been induced by inherent differences between the two emotion blends, as under the exposure to the carrier control, neither judgements at the most ambiguous morphs nor PSEs differed between the two blends (t(44s) = 0.92 and -0.36, ps = 0.37 and 0.72, for judgement on the ambiguous morphs and PSE, respectively). Moreover, androstadienone did not impact the sensitivity of female participants' judgments (t(44s) = 0.36 and 0.29, ps = 0.72 and 0.77, for the neutral-angry and neutral-fearful blends, respectively).

For both blends, as indicated by analyses of PSEs, scores of the Liebowitz Social Anxiety Scale and the Buss-Perry Aggression Questionnaire did not interact with the effect of odor ($ps > 0.39$).

4 Discussion

In the present study, we have demonstrated the sex-specific effect of chemosensory cues on human emotional perception. Specifically, with respect to the neutral-angry blend, androstadienone subconsciously biased heterosexual men toward perceiving male faces as less angry, while it biased heterosexual women toward perceiving female faces as angrier. In the meantime, androstadienone did not influence the perception of fearful faces. Collectively, these results suggest that androstadienone exerts differential impacts on the perception of the two negative emotions that are similar with respect to valence and arousal, indicating that androstadienone elicits change to perception of specific rather than general negative emotions.

Consistent with the existing literature, our study revealed sex-specific effects of androstadienone. On the one hand, its effects on women have been repeatedly reported. Physiologically, it heightens women's sympathetic arousal and maintains their elevated levels of cortisol (Bensafi et al., 2003; Wyart et al., 2007). Psychologically, it exerts a positive influence on women's mood (Bensafi et al., 2004; Jacob and McClintock, 2000; Lundstrom et al., 2003a; Lundstrom and Olsson, 2005), and affects women's perception of sex and emotion (Ye et al., 2019; Zhou et al., 2014). On the other hand, although studied to a lesser extent, there is evidence suggesting that androstadienone also acts on men (Banner et al., 2019; Banner and Shamay-Tsoory, 2018). In our study, we found that androstadienone

affected both sexes. Importantly, the effects differed between men and women, further corroborating it as a putative sex pheromone, which should have differential rather than indiscriminate impacts on male and female individuals.

The majority of work investigating the effects of human chemosignals on emotion processing concentrates on emotion along the valence axis, such as happy-fearful and happy-angry (de Groot et al., 2015; Wu et al., 2022; Zhou and Chen, 2009). However, instead of a uniform pattern, perception of negative emotions has exhibited a distinctive profile of neutral and autonomic nervous system activity (Blair et al., 1999; Levenson, 1992; Murphy et al., 2003; Williams et al., 2005). Specifically, anger and fear, two negative emotions evaluated as comparably unpleasant, stressful, threatening, and anxiety provoking (Strauss et al., 2005), evoke differentiated neural responses. For example, the perception of anger elicits a rapid onset and slow recovery arousal response as well as activation of anterior cingulate activity, whereas perception of fearful faces leads to large arousal responses together with an enhanced amygdala response (Williams et al., 2005). A recent study found that relative to neutral sweat, exposure to fearful sweat altered processing of fearful faces but not angry or disgusted faces (Kamiloglu et al., 2018), which demonstrated that perception of discrete rather than general negative emotions is biased by human chemosignals. Another study utilizing androstadienone as the olfactory stimulus found that the effects of androstadienone are emotion-specific, as it reduces interference in the processing of angry faces by non-relevant emotional words, but not fearful faces (Hornung et al., 2017). In line with these findings, our results show that androstadienone did not shift the perception of negative emotions uniformly, refuting that human chemosignals induce a general state. Specifically, instead of a fearful face that signals an indirect threat, androstadienone affects the perception of angry faces, which are rated as more likely to directly inflict harm and less likely to produce positive emotional outcomes, hence signaling impending aggression (Davis et al., 2011; Strauss et al., 2005). Our results add to the growing literature indicating androstadienone as a chemosignal of dominance, impacting one's perception of dominance and reducing interference with threatening facial expressions (Banner and Shamay-Tsoory, 2018; Hornung et al., 2017).

Several studies point out that androstadienone increases collaboration and decreases aggression-related behavior between men. At the perceptual level, androstadienone has been reported to increase men's perceived dominance of other men (Banner and Shamay-Tsoory, 2018). Behaviorally, it promotes cooperative behavior in decision making tasks (Huoviala and Rantala, 2013), increases gaze avoidance of dominant poses (Banner et al., 2019), and reduces men's reactive and proactive aggressive behaviors toward other men (Wu et al., in revision). Notably, emotional expressions are prominent social cues that impact interpersonal interactions (Lerner et al., 2015), and anger perception of others can result in less cooperative behaviors while excessive aggression has been associated with atypical processing of angry faces (Crago et al., 2019). In agreement with that, our findings suggest that androstadienone biases men toward perceiving faces as less angry, which is likely to lead to more collaborative and less aggressive behaviors in men.

A separate line of research demonstrates that androstadienone promotes intrasexual competition in heterosexual women, resulting in spending more time viewing female faces (Parma et al., 2012), biasing them toward perceiving other women's emotions more negatively (Wu et al., 2022; Ye et al., 2019). Moreover, results from our recent study showed that women responded to androstadienone by increasing their reactive aggression when confronted with provocation (Wu et al., in revision). These responses to male signals have adaptive value, since male aggression typically targets physical harm, in particular towards women (Pause et al., 2020). Furthermore, androstadienone has been reported to convey masculinity to heterosexual women, biasing them towards perceiving neutral point light walkers as more masculine (Zhou et al., 2014). Since masculinity is associated with the perception of anger, as angry expressions bias sex perception toward men (Hess et al., 2009), the communication of masculinity induced by androstadienone could also underline the effects observed in our study.

Some issues warrant further investigation. First, the present study adopts only faces from same-sex expressors as visual stimuli (i.e., male participants viewed male faces, and female participants viewed female faces), to avoid the influence of confounding factors regarding perception toward opposite-sex faces. Note that, in addition to the sex of the recipients, the effect of androstadienone is also contingent upon their sex perception of the expressors (Ye et al., 2019). Therefore, the effect of

androstadienone on angry/fearful perception of opposite-sex faces (male participants view female faces, and female participants view male faces) awaits future investigation. Second, a recent study found that smelling hexadecanal, a human body volatile whose molecular structure is different from androstadienone, blocks aggression in men but increases aggression in women (Mishor et al., 2021). It would be interesting to examine whether hexadecanal also exhibits sex-specific effects on the perception of angry but not fearful faces. As neither androstadienone nor hexadecanal alone is smelled in nature, it calls for future research to test the effects when they are presented together with other chemosignals as body odor. Third, since we only tested young male participants and young female participants in their periovulatory phases, this conclusion may not be generalized to all. Albeit most studies in this field focused on participants in their periovulatory phases, there is evidence indicating that the sensitivity to androstadienone and the effect of androstadienone may be subject to the influence of the phase of the menstrual cycle as well as oral contraception use (Lundstrom et al., 2006; Wu et al., 2022). It would be interesting to examine the modulatory effect of these two factors in future work. Fourth, individual differences in sensitivity to the odor of androstadienone have been documented, indicating a bimodal distribution with a smaller group of individuals with a high sensitivity to androstadienone (supersmellers) (Lundstrom et al., 2003b). Unfortunately, we did not assess the perception to the two olfactory stimuli in our sample. Whether the effects of androstadienone differed between supersmellers and others needs further research. Fifth, since androstadienone's positive influence on women's mood has been widely reported (Bensafi et al., 2004; Bensafi et al., 2003; Jacob and McClintock, 2000; Lundstrom et al., 2003a; Lundstrom and Olsson, 2005; Ye et al., 2019), we did not examine whether the mood states altered after exposure to androstadienone. Whether the observed changes in the participants' emotional perception were manifestations of "emotional contagion" — their own mood changes under the interactive influences of the chemosignals and the faces in turn influenced their judgements of the face's emotion remain to be tested.

Conclusions

We tested the modulation of the perception of two negative facial emotions by a human chemosignal, androstadienone. The findings demonstrate that androstadienone subconsciously biased heterosexual men and women toward perceiving the faces as less angry and angrier, respectively, while leaving the perception of fearful faces unaffected. These data indicate that androstadienone does not uniformly affects negative emotional perception, but specifically impacts one's perception of angry faces, which signals impending aggression, and could further impact one's social interaction.

Conflict of interests declaration

We declare we have no competing interests.

Role of the funding source

This work was supported by The Hong Kong Polytechnic University Start-up Fund for New Recruits (P0039779), Departmental General Research Fund (P0041484) and Research Institute for Sports Science and Technology (P0043556) to YW, and National Natural Science Foundation of China grant (32000789) to YY.

Author contributions

YY and YW designed the study. YN and RW collected and analysed the data. YY and RW wrote the first version of the paper, YN and YW provided critical revisions. All authors approved the final version for submission.

Acknowledgement

We thank Dr. Wen Zhou for her help with the study.

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Figure Captions

Figure 1. (A) Examples of the morphed faces of a female actor, with upper panel and lower panel demonstrating neutral-angry and neutral-fearful blends, respectively. (B) Illustration of the device for odor presentation. (C) Each participant's emotion judgments for each emotion blend and each olfactory condition were fitted with a sigmoid function that contained two parameters: point of subjective equality (PSE, where proportion of 'angry/fearful' responses is equal to proportion of neutral responses ($p = 0.5$), indicating that the face was judged as equally neutral and angry/fearful) and slope. A leftward shift of PSE (PSE shift < 0) indicates an angry/fearful bias, as the face has to be less angry/fearful to be perceived as equally neutral and angry/fearful. A rightward shift of PSE (PSE shift > 0) indicates a neutral bias, as the face has to be angrier/more fearful to be perceived as equally neutral and angry/fearful.

Figure 2. (A) Exposure to androstadienone led heterosexual men to perceive male faces as less angry, (B) while it had no effect on the perception of fearful faces. Judgements of emotional identification under the exposure to androstadienone (blue) or the carrier control (gray) were respectively fitted with sigmoid curves, with insets showing the androstadienone-induced proportional 'angry/fearful' biases at the most ambiguous morphs (the average proportion of the fourth and the fifth morphs). (C) Androstadienone-induced positive PSE shifts with respect to the carrier control in neutral-angry blend, but not in neutral-fearful blend. (D) Exposure to androstadienone led heterosexual women to perceive female faces as angrier, (E) but had no effect on the perception of fearful faces. (F) Androstadienone-induced negative PSE shifts with respect to the carrier control in neutral-angry blend, but not neutral-fearful blend. Error bars stand for standard errors of the mean adjusted for individual differences; *, $p \leq 0.05$, **, $p \leq 0.01$.

Figure 1

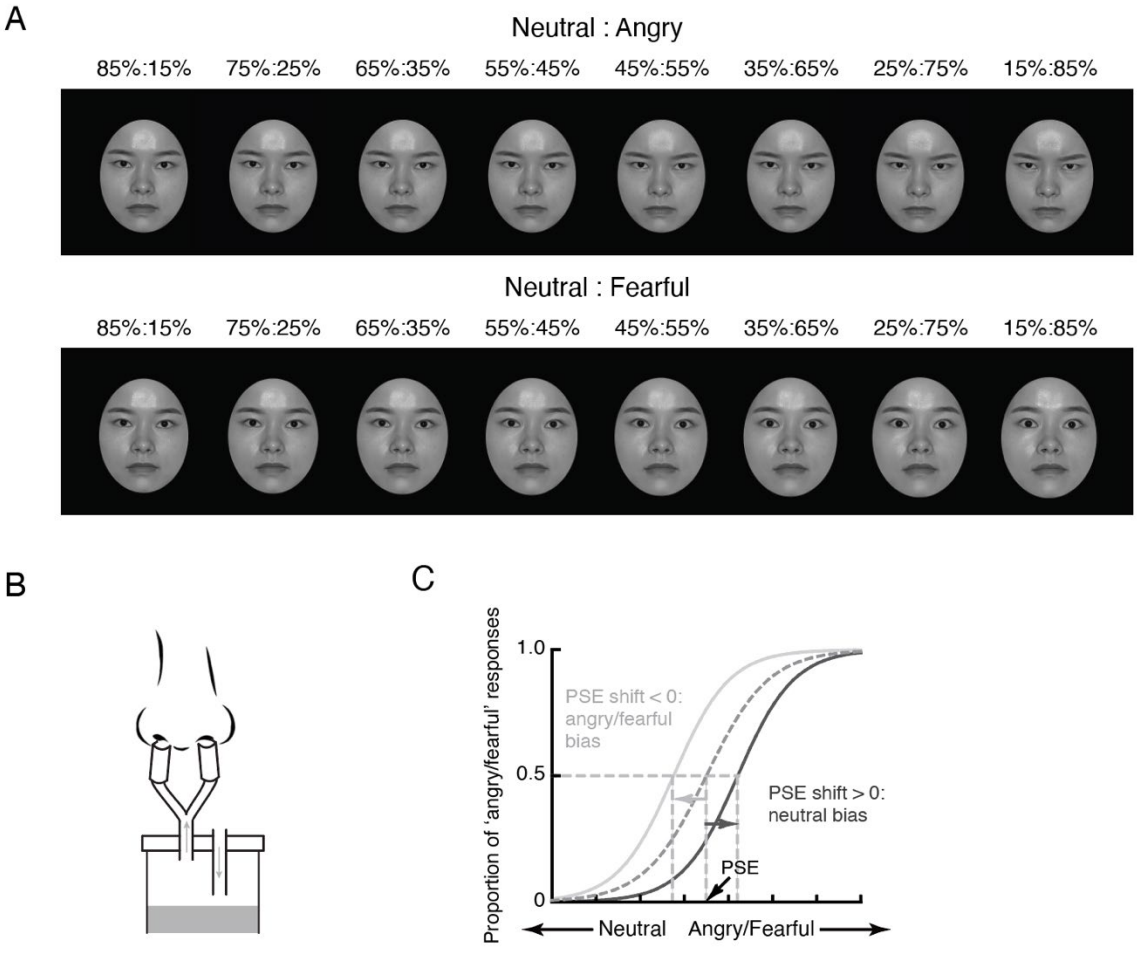
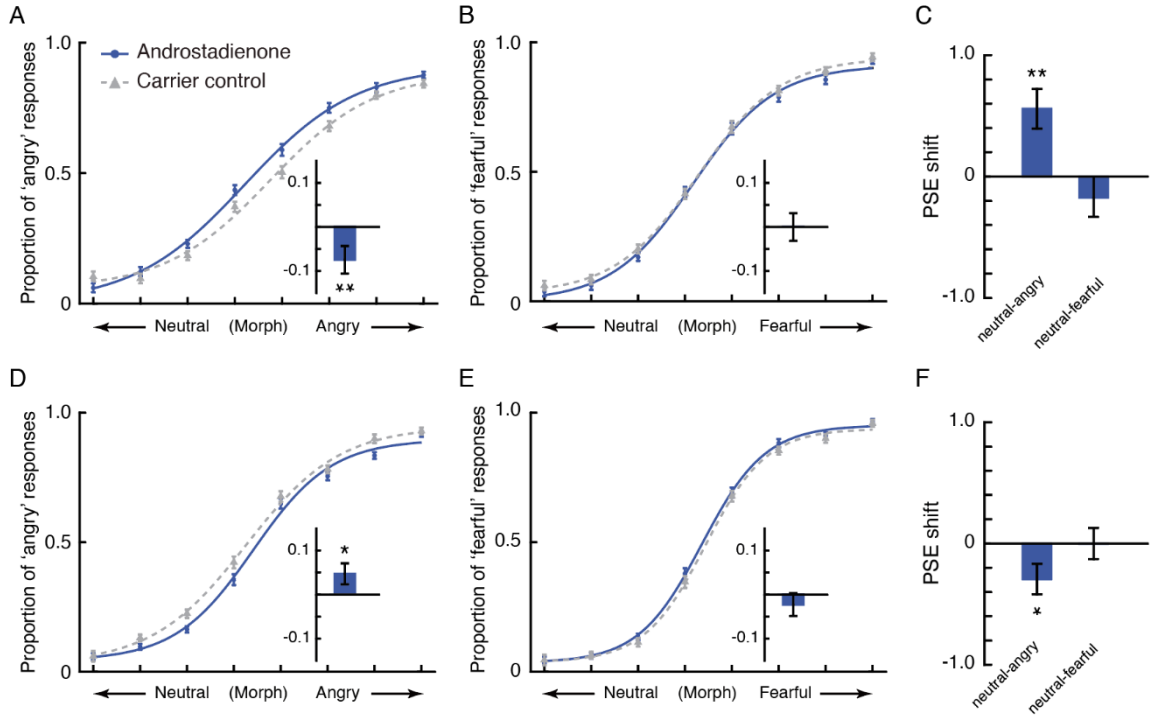

A
Neutral : Angry
85%:15%
75%:25%
65%:35%
55%:45%
45%:55%
35%:65%
25%:75%
15%:85%
Neutral : Fearful
85%:15%
75%:25%
65%:35%
55%:45%
45%:55%
35%:65%
25%:75%
15%:85%
B
C
Proportion of 'angry/fearful' responses
1.0
0.5
0
PSE shift < 0:
angry/fearful
bias
PSE shift > 0:
neutral bias
PSE
Neutral
Angry/Fearful

Figure 2


A
B
C
D
E
F
Androstadienone
Carrier control
Proportion of 'angry' responses
Proportion of 'fearful' responses
PSE shift
Neutral
(Morph)
Angry
Fearful
neutral-angry
neutral-fearful
1.0
0.5
0
-1.0
0.1
-0.1
**
*