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Human chemosignals modulate emotional perception of biological motion in a sex-specific manner

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ABSTRACT

Androsta-4,16,-dien-3-one and estra-1,3,5(10),16-tetraen-3-ol have previously been shown to communicate opposite sex information that is differently effective to the two sex groups. The current study critically examines if the two human steroids could facilitate interactions with potential mates rather than competitors by acting on the recipients' emotional perception in a sex-appropriate manner. Using dynamic point-light displays that portray the gaits of walkers whose emotional states are digitally morphed along the valence and the arousal axes, we show that smelling androstadienone subconsciously biases heterosexual women, but not men, towards perceiving the male, but not female, walkers as happier and more relaxed. By contrast, smelling estratetraenol subconsciously biases heterosexual men, but not women, towards perceiving the female, but not male, walkers as happier and more relaxed. These findings indicate that androstadienone and estratetraenol prime the identification of emotionally receptive states for the potential mates with whom they are associated, in manners contingent upon not only the recipients' own sex but also their sex perception of other individuals that ensure sex-appropriate behavior.

1. Introduction

Pheromones, secreted to the outside by an individual and received by a second individual of the same species, are ubiquitously used in the animal kingdom and mediate a wide range of social interactions (Karlson and Luscher, 1959; Wyatt, 2003). A well-studied class of pheromones is called sex pheromones, which regulate behaviors related to mate choice and reproduction. Their influence on the recipients take many forms, from priming the reproductive system (Keller-Costa et al., 2014), inducing preference, searching behavior and the adoption of a mating stance in the opposite sex (Dorries et al., 1997; Li et al., 2002), to fostering intra-sexual competition among individuals of the same (e.g. male pheromone fostering competition among males) (Hirai et al., 1978), as well as the opposite (e.g. male pheromone fostering competition among females) (Sneddon et al., 2003), sex.

The search for human pheromones, on the other hand, has faced considerable difficulties due to the complexity of human secretions and the multifaceted nature of human behaviors (Wyatt, 2015). Nonetheless, there is accumulating evidence showing that human body odors exert various pheromone-like effects on odor recipients (de Groot et al., 2015; Pause et al., 2004; Stern and McClintock, 1998; Zhou and Chen, 2009). Two endogenous compounds of human secretions have received particular attention in the literature, namely, androsta-4,16,-dien-3-one (Gower and Ruparelia, 1993) and estra-1,3,5(10),16-tetraen-3-ol (Thysen et al., 1968). They have no known androgenic or estrogenic effects. Androstadienone has been found to heighten sympathetic arousal (Bensafi et al., 2003), alter levels of cortisol (Wyart et al., 2007) and promote positive mood states in female as opposed to male recipients (Bensafi et al., 2004; Grosser et al., 2000; Jacob and McClintock, 2000; Lundstrom et al., 2003; Villemure and Bushnell, 2012), probably in a context-dependent manner (Bensafi et al., 2004; Jacob et al., 2001a). Estratetraenol, studied to a lesser extent, has likewise been shown to affect male recipients' autonomic responses and mood under certain context (Bensafi et al., 2004; Olsson et al., 2006). In the brain, androstadienone has been found to activate the hypothalamus in heterosexual females and homosexual males, but not in heterosexual males or homosexual females, while the reverse holds for estratetraenol (Berglund et al., 2006; Savic et al., 2001, 2005).

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Moreover, recent data indicate that androstadienone subconsciously conveys masculinity and estratetraenol femininity to their target recipients (Zhou et al., 2014). These sex-specific effects, while obtained with different paradigms and methods in different labs, are not without controversies (Chung et al., 2016a, b; Ferdenzi et al., 2016; Hornung et al., 2018a, b; Marazziti et al., 2011; Wyatt, 2015).

Ultimately, sex pheromones serve to coordinate reproductive behaviors. As social approach or avoidance is usually consequential to emotional perception (Marsh et al., 2005), we ask whether androstadienone and estratetraenol could differentially impact the recipients' emotional perception of other individuals, thereby facilitating their interactions with potential mates. Based on the animal literature, sex-specific responses to sex pheromones are contingent upon sex discrimination of conspecifics (Stowers et al., 2002). We thus wonder whether humans demonstrate analogous effects to androstadienone and estratetraenol with responses depending on the sex of the recipients as well as the sex of the other individuals they observe — a critical question largely neglected by previous research.

To address the above questions, we adopt point-light walkers (PLWs) — dynamic point-light displays of human gaits — as the visual stimuli in an emotion identification task, considering that affective state is more reliably inferred from human motion than from a static picture (Blake and Shiffrar, 2007). Moreover, biological motion, as captured by PLWs, has been shown to effectively convey sex (Jordan et al., 2006) and emotional state (Dittrich et al., 1996; Heberlein and Saxe, 2005), amongst other social information, yet contains no complex visual details that are present in other types of social stimuli including faces (thus experimentally easy to control). Existing work on the emotional perception of biological motion typically characterizes its mood state along the axes of happy-sad and relaxed-nervous (Troje, 2002, 2008), which roughly correspond to the widely accepted pleasant-unpleasant (valence) and deactivated-activated (arousal) dimensions that underlie our emotional experience (Yik et al., 1999). We therefore set out to assess the influence of androstadienone and estratetraenol on the emotional perception of prototypical male and prototypical female PLWs in heterosexual male and female recipients along the happy-sad as well as the relaxed-nervous axes, with as ultimate aim to help elucidate the nature of human chemosensory cues and their interplays with social cues from other modalities. While we do not pretend that our study will resolve the controversies in the field, we believe a better understanding of the dimorphic reactions to these substances posed by the social context we introduce — i.e. by having both males and females evaluate male and female gaits — may be helpful in teasing apart the contradictory effects. Our hypothesis is that androstadienone would influence the emotional perception of female recipients, particularly when they view male PLWs, whereas estratetraenol would influence the emotional perception of male recipients, particularly when they view female PLWs. Moreover, the target recipients of these chemosignals (i.e. heterosexual females smelling androstadienone and heterosexual males smelling estratetraenol) would be biased towards perceiving opposite-sex PLWs as emotionally more receptive.

2. Materials and methods

2.1. Participants

A total of 192 healthy nonsmokers participated in the study, 96 (mean age ± SEM = 22.34 ± 0.21 yrs; 48 males) in Experiment 1 and 96 in Experiment 2 (22.58 ± 0.23 yrs, 48 males). Sample sizes (n = 24 in each subgroup of each experiment, i.e., male odor recipients watching female PLWs, male odor recipients watching male PLWs, female odor recipients watching female PLWs and female odor recipients watching male PLWs) were determined by G*Power to be adequate to detect a moderate effect of androstadienone and/or estratetraenol ($d \approx 0.6$), at 80% power. The effect size was estimated based on an earlier study that employed identical olfactory stimuli and similar psychophysical testing procedures to those in the current study (Zhou et al., 2014). All participants reported to be Han Chinese, 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. They were generally socially proficient and scored below or equal to 26 (out of 50 total) on the Autism Spectrum Quotient, a self-administered instrument that measures one's autistic-like tendency (Baron-Cohen et al., 2001). All female participants were tested around the periovulatory phase of their menstrual cycles (Experiment 1: mean ± SEM = 14.19 ± 0.91 days from the onset of their last period of a normalized 28 cycle; Experiment 2: 15.52 ± 1.03 days). They were recruited through advertisement, naïve to the purpose of the experiments and gave informed consent to participate in procedures approved by the Institutional Review Board at the Institute of Psychology, Chinese Academy of Sciences. All were paid for participation.

2.2. Olfactory stimuli

The olfactory stimuli consisted of androstadienone (500 μM in 1% v/v clove oil propylene glycol solution), estratetraenol (500 μM in 1% v/v clove oil propylene glycol solution) and the carrier solution alone (control, 1% v/v clove oil in propylene glycol). They were presented in identical 40 ml polypropylene jars, each containing 5 ml clear liquid and connected with two Teflon nosepieces via a Y-structure. The effectiveness of the clove oil carrier solution as a masker for the odors of androstadienone and estratetraenol was verified beforehand in an independent group of 48 healthy nonsmokers (22.3 ± 0.27 yrs, 24 males), who were at chance in discriminating among the olfactory stimuli (6 trials in a triangular procedure described in Section 2.4), mean accuracy ± SEM = 0.37 ± 0.03 vs. chance = 0.33, $p = .24$. In addition, another panel of 24 healthy nonsmokers (23.7 ± 0.80 yrs, 12 males) reported no discernable difference among the olfactory stimuli in perceived intensity, pleasantness or familiarity, ps > .05.

2.3. Visual stimuli

Dynamic parametric point-light walkers (PLWs, Movies 1 and 2) (Troje, 2002, 2008) were generated with MATLAB and presented on a 22-inch LCD monitor using the psychophysics toolbox. Each walker (3.5°×10.0°) was made up of 15 moving dots (0.2°×0.2°), 12 for the major joints, and 3 for the centers of pelvis, thorax and head. The mood state of the PLWs was indexed with a score on an axis reflecting the differences between happy and sad walkers (in Experiment 1, Fig. 1) or between relaxed and nervous walkers (in Experiment 2) in terms of a linear classifier. Specifically, the scores were computed within a 10-dimensional sub-space spanned by the first 10 principle components based on a Fourier-based representation of observers' mood ratings of 80 actual walkers (half male) (Troje, 2008). All the PLWs in Experiment 1 scored 0 on the relaxed-nervous axis, whereas all those in Experiment 2 scored 0 on the happy-sad axis. We varied the sex of the PLWs in a between-subject manner, such that in each experiment half of the participants (24 males and 24 females) were randomly assigned to view prototypical male PLWs (3 SD into the male part of a linear axis reflecting the differences between male and female walkers) and the other half prototypical female PLWs (3 SD into the female part of the linear axis).

2.4. Procedure

Each participant in Experiment 1 was tested at around the same time of the day on three consecutive days. On each day, they firstly completed 4 blocks of an emotion identification task of the PLWs in the absence of olfactory stimuli, which served as the baseline, followed by 6 blocks of the same task where they were continuously exposed to androstadienone, estratetraenol or the carrier solution alone, one on each day in a counterbalanced manner. During the baseline blocks, an empty

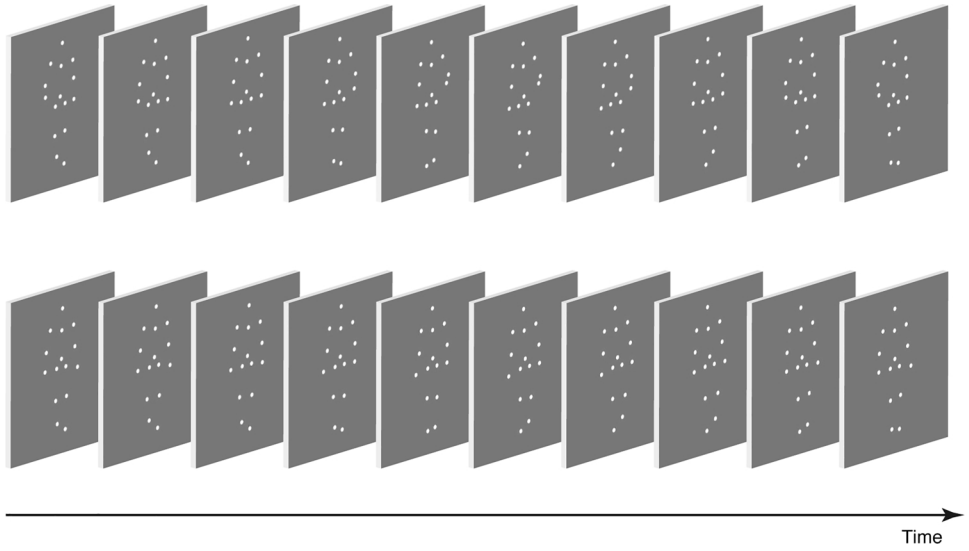

Fig. 1. Moving trajectories of a representative happy male PLW (top panel) and a representative sad male PLW (bottom panel) during a walking cycle.

jar was used in place of those containing the olfactory stimuli. Participants were instructed to hold the jar, empty or containing an odorant, with their non-dominant hand, position the nosepieces inside their nostrils and continuously inhale through the nose and exhale through their mouth while performing the emotion identification task. There was a break of at least 1 min in between every two blocks, during which the participants were not exposed to any odor. The experimenter (female) was blind to the identity of the olfactory stimuli used in the experimental blocks and was not in the test room when the participants performed the task.

Each trial of the emotion identification task started with a 500 ms fixation cross ($0.5^{\circ} \times 0.5^{\circ}$), followed by a PLW presented for 1000 ms (1 walking cycle) at a random location 0 - 1° away from fixation. Participants then pressed one of two buttons to indicate whether the walker was happy or sad. The next trial began 500 ms after a response was made. Each participant viewed seven PLWs ranging in equal steps designated from -3 to 3 along the happy-sad axis (Movie 1), with 0 marking approximate perceived emotional neutrality (50% sad responses) individually adjusted and set prior to the actual experiment on the first day of testing. The initial frame of each motion sequence was randomized. There were 70 trials in each block (7 PLWs × 10 repetitions in random order) and 6 experimental blocks per olfactory condition, resulting in 60 trials per PLW per olfactory condition per participant.

On the third day of testing, each participant also completed three trials of a triangle odor discrimination task. In each trial, they were presented with three smells, two identical (control) and the other one different (target), and reported which one was the odd one out. Each of the three olfactory stimuli served as the target once and as the control once. The probability of arriving at a correct response by chance was 1/3. There was at least a 30 s break in between two trials.

The procedure of Experiment 2 was largely identical to that of Experiment 1 except that the mood state of the PLWs was varied along the relaxed-nervous axis. Each participant viewed seven PLWs ranging in equal steps designated from -3 to 3 along the axis, with 0 marking approximate perceived emotional neutrality (50% nervous responses) individually adjusted and set prior to the actual experiment on the first day of testing. In each trial, participants pressed one of two buttons to indicate whether the presented PLW was relaxed or nervous.

86 participants (89.6% of the total number, 45 males) in Experiment 1 and all 96 participants in Experiment 2 also completed the Profile of Mood States (POMS) (McNair et al., 1971), a 65-item self-reported rating scale assessing transient, distinct mood states, right after the completion of the emotion identification task on each day of testing. POMS consists of six subscales, i.e., tension, depression, fatigue, confusion, anger and vigor, based on which a total mood disturbance score is calculated.

2.5. Experimental design and statistical analyses

Participants' responses from the emotion identification task were baseline normalized (mean shifting) per olfactory condition per participant prior to any statistical analysis to account for olfactory irrelevant day-to-day variations in emotion judgment criterion. For each of the seven PLWs, the baseline adjusted proportion of 'sad' (in Experiment 1) or 'nervous' (in Experiment 2) responses $p'$was calculated as $p' = p_{\exp} - p_{base} + \bar{P}_{base}$, where $p_{\exp}$ is the averaged proportion of 'sad' or 'nervous' responses in the experimental blocks, $p_{base}$is the averaged proportion of 'sad' or 'nervous' responses in the preceding baseline blocks on the same day and $\bar{P}_{base}$ is the mean proportion of 'sad' or 'nervous' responses in the baseline blocks across the three days of testing.

For Experiment 1, the data from male and female participants were then analyzed separately in two repeated measures ANOVAs, using olfactory condition (androstadienone, estratetraenol, carrier control) and PLW's mood state along the happy-sad axis (each axis with 7 levels) as the within-subject factors and PLW's sex (male vs. female) as the between-subject factor. We subsequently zoomed in on the most ambiguous emotion-neutral point of the PLWs (point 0), where responses should be most susceptible to chemosensory impact according to the rule of inverse effectiveness (Stein and Stanford, 2008), and performed pairwise t tests between androstadienone and carrier control as well as between estratetraenol and carrier control for the participants watching male PLWs and those watching female PLWs, respectively, using the proportion of 'sad' responses at point 0 as the dependent variable.

Next, to better characterize the participants' response criteria and sensitivities, the baseline normalized emotion judgments were fitted with a Boltzmann sigmoid function $f(x) = 1/(1 + exp((x - x_0)/\omega))$, where x_0 corresponds to the point of subjective equality (PSE), at which the observer perceived a PLW as equally happy and sad; and half the interquartile range of the fitted function corresponds to difference limen, an index of discrimination sensitivity. For each of the four subgroups of participants (male and female participants watching male PLWs and female PLWs), paired sample t tests were then conducted on the PSEs to compare the emotion judgment criteria under the exposures to androstadienone and estratetraenol, respectively, with that under the

exposure of the carrier solution alone.

The data from Experiment 2, where participants made relaxed/nervous judgements of the PLWs, were analyzed in the same manner except that the dependent variables changed to the proportion of 'nervous' judgements and the PSE in making nervous/relaxed judgments of the PLWs.

Repeated measures ANOVAs were also performed on difference limens and POMS ratings to compare the participants' discrimination sensitivities in making emotional judgments as well as their mood states across the three olfactory conditions (within-subject factor) when presented with male and female PLWs (between-subject factor).

3. Results

3.1. Sexually dimorphic influences of androstadienone and estratetraenol on the emotional perception of males and females along the valence dimension

Experiment 1 examined in men and women the influences of androstadienone and estratetraenol, as compared with their carrier clove oil solution, on the emotional judgments of male and female PLWs along the happy-sad axis. In the male participants, an omnibus repeated measures ANOVA with olfactory condition (androstadienone, estratetraenol, carrier control) and PLW's mood state along the happy-sad axis (7 levels) as the within-subject factors and PLW's sex (male vs. female) as the between-subject factor showed a significant three-way interaction among the factors ($F(4.87, 224.08) = 3.47$, $p = .005$, $\eta_p^2 = .07$, Fig. 2a-b left panels). We firstly examined the most ambiguous emotion-neutral point (point 0), where according to the rule of inverse effectiveness (Stein and Stanford, 2008) the steroids should exert the largest impact on visual emotional perception. Paired-sample t tests showed that, in the men who viewed female PLWs, smelling estratetraenol relative to the carrier control decreased the proportion of 'sad' responses at point 0 by 10.9% ($t(23) = -3.05$, $p = .006$, $d = 0.65$, 95% CI = [-0.18, -0.03]), whereas smelling androstadienone had no obvious effect ($t(23) = 0.51$, $p = .616$) (Fig. 2a inset). For those who viewed male PLWs, neither androstadienone nor estratetraenol showed a significant effect as compared with the carrier control ($t(23) = -0.28$ and 0.87, $p = .781$ and .392, respectively; Fig. 2b inset).

This pattern was also reflected by shifts in the participants' judgment criteria, namely PSEs. Smelling estratetraenol as opposed to the carrier control systematically biased the male participants towards perceiving the female PLWs as happier, resulting in a PSE significantly shifted towards the sad PLW side ($t(23) = 2.60$, $p = .016$, $d = 0.57$, 95% CI = [0.04, 0.34]; Fig. 2a right panel), but did not alter the emotional perception of the male PLWs ($t(23) = -0.43$, $p = .675$; Fig. 2b right panel). On the other hand, smelling androstadienone relative to the carrier control did not affect the male participants' judgments regardless of the sex of the PLWs ($t(23) = -0.48$ and 0.61, $p = .638$ and .551, for female and male PLWs, respectively; Fig. 2a-b right panels).

Turning to the female participants, we found that their happy/sad judgments also exhibited a significant three-way interaction among olfactory condition, PLW's mood state and PLW's sex ($F(5.47, 251.51) = 2.30$, $p = .040$, $\eta_p^2 = .05$; Fig. 2c-d left panels). At the most ambiguous emotion-neutral point (point 0), smelling androstadienone relative to the carrier control increased the proportion of 'sad' responses by 10.5% in the women who viewed female PLWs ($t(23) = 3.31$, $p = .003$, $d = 0.69$, 95% CI = [0.04, 0.17]), whereas smelling estratetraenol had no obvious effect ($t(23) = 0.80$, $p = .433$) (Fig. 2c inset). In those who viewed male PLWs, neither androstadienone nor estratetraenol showed a statistically significant effect ($t(23) = -1.17$ and 0.14, $p = .252$ and .890, respectively), although there was a trend that smelling androstadienone decreased the proportion of 'sad' responses

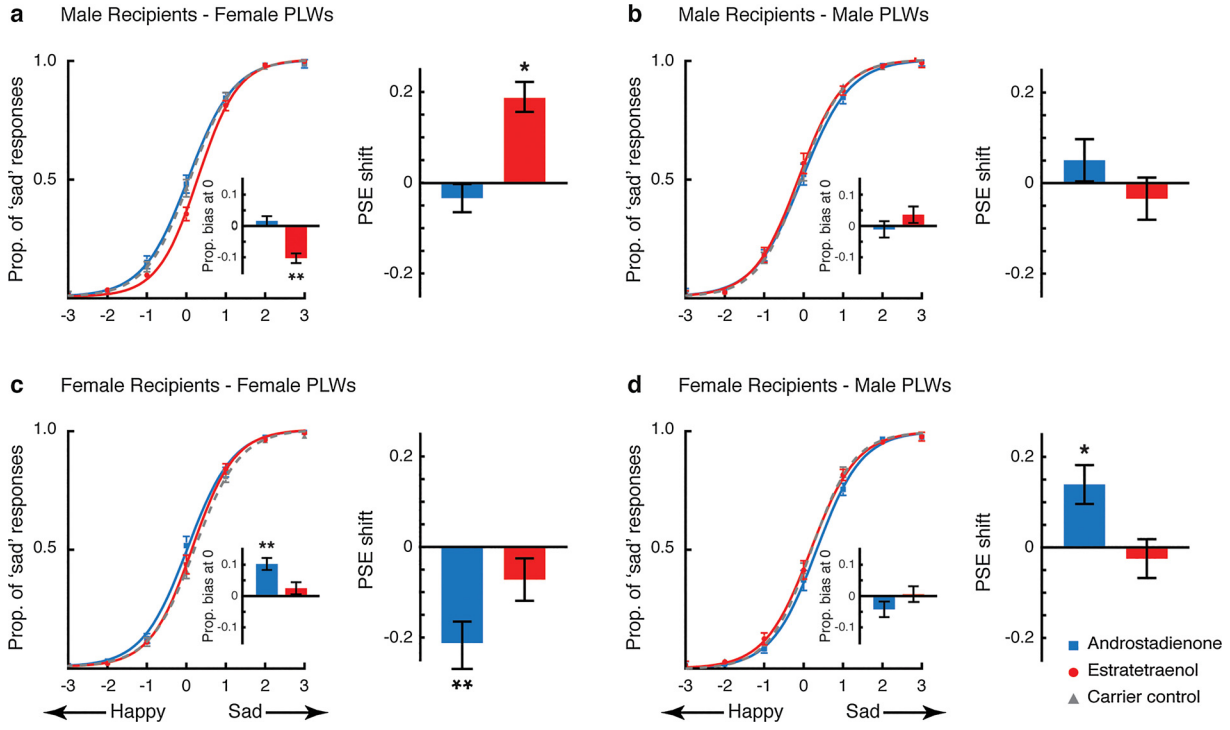

Fig. 2. Androstadienone- and estratetraenol- induced emotional judgment biases along the valence dimension. (a–d) Androstadienone- and estratetraenol- induced changes in the happy/sad judgments of male recipients viewing female PLWs (a), male recipients viewing male PLWs (b), female recipients viewing female PLWs (c) and female recipients viewing male PLWs (d). Left panels: emotion identification performances of the four groups of participants under the exposures of androstadienone (blue squares), estratetraenol (red dots) and the carrier control (gray triangles), respectively, fitted with sigmoidal curves. Insets illustrate the corresponding androstadienone- and estratetraenol- induced proportional 'sad' biases at the emotion-neutral point 0, relative to the carrier control. Right panels: androstadienone- and estratetraenol-induced overall PSE shifts with respect to the carrier control when male and female recipients viewed female and male PLWs. Error bars represent standard errors of the mean adjusted for individual differences; *: $p \leq .05$; **: $p \leq .01$.

(Fig. 2d inset).

Analyses of the PSEs, which characterized the entire psychometric curves rather than performances at only the emotion-neutral point, yielded a clearer pattern. As compared with the carrier control, smelling androstadienone not only systematically biased the female participants towards perceiving the female PLWs as sadder but also led them to view the male PLWs as happier, resulting in a PSE significantly shifted towards the happy PLW side in the former case ($t(23) = -2.87$, $p = .009$, $d = 0.59$, 95% CI = [-0.35, -0.06]; Fig. 2c right panel) and towards the sad PLW side in the latter case ($t(23) = 2.27$, $p = .033$, $d = 0.47$, 95% CI = [0.01, 0.27]; Fig. 2d right panel). Conversely, smelling estratetraenol failed to affect the female participant' judgments regardless of the sex of the PLWs ($t(23) = -0.83$ and -0.30, $p = .416$ and .768 for female and male PLWs, respectively; Fig. 2c-d right panels).

In both the male participants, whose happy/sad judgments of the PLWs were modulated by estratetraenol but not androstadienone, and the female participants, whose judgments were modulated by androstadienone but not estratetraenol, we identified a significant interaction between olfactory condition and sex of the PLWs in their judgment criteria along the valence dimension (PSEs, $F(2, 92) = 4.16$ and 5.47, $p = .019$ and .006, $\eta_p^2 = .08$ and .11, respectively). This interaction highlighted that the steroids' effects on the perception of emotional valence varied by the sex of the PLWs presented, in addition to the recipients' own sex. In the meantime, the participants' difference limens, which indexed their sensitivities in discriminating between happy and sad PLWs (see *Experimental design and statistical analyses*), were comparable across the olfactory conditions ($F(2, 184) = 1.06$, $p = .348$) and for the male and female PLWs ($F(1, 92) = 0.38$, $p = .539$), with no difference between men and women ($F(1, 92) = 0.38$, $p = .541$; all interactions, $ps > .50$).

3.2. Sexually dimorphic influences of androstadienone and estratetraenol on the emotional perception of males and females along the arousal dimension

Experiment 2 focused on the relaxed-nervous axis (Movie 2), orthogonal to the happy-sad axis examined in Experiment 1. We tested whether androstadienone and estratetraenol could also impact the recipients' perception of emotional arousal, and if so, whether the effects would similarly be contingent upon the sex of the recipients as well as the sex of the PLWs presented.

As shown in Fig. 3a-b, in the male participants, we found that the exposure to estratetraenol as opposed to the carrier control led to a systematic bias to perceive the female PLWs as more relaxed, resulting in a reduction of 'nervous' responses by about 8.4% at the most ambiguous emotion-neural point (point 0, $t(23) = -2.06$, $p = .051$, $d = 0.42$, 95% CI = [-0.17, 0.00]) and a PSE significantly shifted towards the nervous PLW side ($t(23) = 2.61$, $p = .016$, $d = 0.53$, 95% CI = [0.04, 0.31]). Meanwhile, their judgements of the male PLWs were unaffected ($t(23) = 0.69$ and -0.48, $p = .499$ and .633 for the proportional bias at point 0, overall PSE shift, respectively). Smelling androstadienone, by contrast, did not influence the male participants' relaxed/nervous judgments for the female ($t(23) = -0.93$ and 1.78, $p = .363$ and .088, respectively) or the male PLWs ($t(23) = 1.28$ and -1.36, $p = .214$ and .186, respectively).

In the female participants, we observed the opposite pattern (Fig. 3c-d). As compared with the carrier control, estratetraenol had no influence on the female participants' nervous/relaxed judgments, regardless of the sex of the PLWs (female PLWs: $t(23) = -0.32$ and 0.02, $p = .749$ and .982, respectively; male PLWs: $t(23) = 0.73$ and 0.15, $p = .475$ and .885, respectively). However, the exposure to androstadienone significantly biased them towards perceiving the male PLWs as more relaxed, causing a 6.5% reduction of 'nervous' responses at the emotion-neutral point (point 0, $t(23) = -2.32$, $p = .030$, $d = 0.48$, 95% CI = [-0.12, -0.01]) and a PSE significantly shifted towards the nervous PLW side ($t(23) = 2.95$, $p = .007$, $d = 0.60$, 95% CI = [0.04, 0.24]). In the meantime, their judgments of the female PLWs remained unaltered ($t(23) = 0.85$ and -0.78, $p = .405$ and .442, respectively).

Repeated measures ANOVA on the pooled PSEs from the male and female participants revealed a significant three-way interaction among olfactory condition, sex of the recipients and sex of the PLWs presented ($F(2, 184) = 4.21$, $p = .016$, $\eta_p^2 = .04$), which confirmed that the effects of androstadienone and estratetraenol on the recipients' perceptual criteria of emotional arousal depended on both the recipients' own sex and the sex of the PLWs they viewed. None of these factors significantly influenced the participants' difference limens — like in Experiment 1, their sensitivities in discriminating between relaxed and nervous PLWs were comparable across the olfactory conditions ($F(2, 184) = 2.27$, $p = .106$) and for the male and female PLWs ($F(1, 92) = 0.07$, $p = .792$), with no difference between the male and female participants ($F(1, 92) = 0.43$, $p = .513$; all interactions, $ps > .10$).

3.3. Assessments of recipients' mood states and olfactory awareness

It could be argued that the observed changes in the participants' emotional perception were manifestations of "emotional contagion" — their own mood changes under the interactive influences of the steroids and the PLWs in turn influenced their interpretations of the PLWs' emotions (Bouhuys et al., 1995; Hatfield et al., 1993; Pause, 2012; Semin and Groot, 2013). To verify this possibility, we analyzed the participants' self-reported mood states (POMS ratings) on each day of testing, right after the completion of the emotion identification tasks in Experiments 1 and 2. For the female participants, the only significant effect emerged was that of olfactory condition on their feelings of depression ($F(2, 174) = 4.87$, $p = .009$, $\eta_p^2 = .05$). Specifically, they reported feeling less depressed after the exposure to androstadienone ($t(88) = -2.32$, $p = .023$, $d = 0.25$, 95% CI = [-2.13, -0.17]), but not estratetraenol ($t(88) = 0.92$, $p = .363$), relative to the carrier control, in line with previous findings of androstadienone's positive influence on women's mood (Bensafi et al., 2004, 2003; Jacob and McClintock, 2000; Lundstrom et al., 2003; Lundstrom and Olsson, 2005). Regarding the male participants, their mood states were not significantly affected by olfactory condition ($ps > .20$ for all POMS subscales and total mood disturbance). Instead, they reported experiencing less tension, depression, confusion and overall less mood disturbance after viewing female as opposed to male PLWs ($F(1, 91) = 8.34$, 8.30, 8.70, and 5.18, $p = .005$, .005, .004, and .025, $\eta_p^2 = .08$, .08, .09, and .05, respectively). Critically, there was no interaction between olfactory condition and PLW's sex in the mood states of the female or the male participants ($ps > .20$). It was therefore unlikely that changes of the recipients' own mood states could give rise to the observed effects of androstadienone and estratetraenol on their emotional perception of the PLWs, which were contingent upon both their own sex and the PLWs' sex.

Throughout Experiments 1 and 2, the participants were naïve to the nature of the olfactory stimuli and could not differentiate androstadienone, estratetraenol and the carrier clove oil solution by smell (mean accuracy = 0.35 vs. chance = 0.33, $t(190) = 1.26$, $p = .211$), with no difference between men and women ($t(189) = -0.12$, $p = .904$). Thus, the sex-specific influences of androstadienone and estratetraenol on emotional perception took place in the absence of olfactory awareness.

4. Discussion

The combined results from Experiments 1 and 2 (summarized in Table 1) show that, with respect to the emotional valence axis, estratetraenol biased heterosexual men towards perceiving the mood state of female, but not male, PLWs as more positive. Androstadienone, on the other hand, shifted heterosexual women's perception in opposite ways depending on the PLW's sex, with male PLWs being perceived as more positive but females PLWs as more negative. With respect to the arousal axis, estratetraenol biased men towards perceiving female, but not

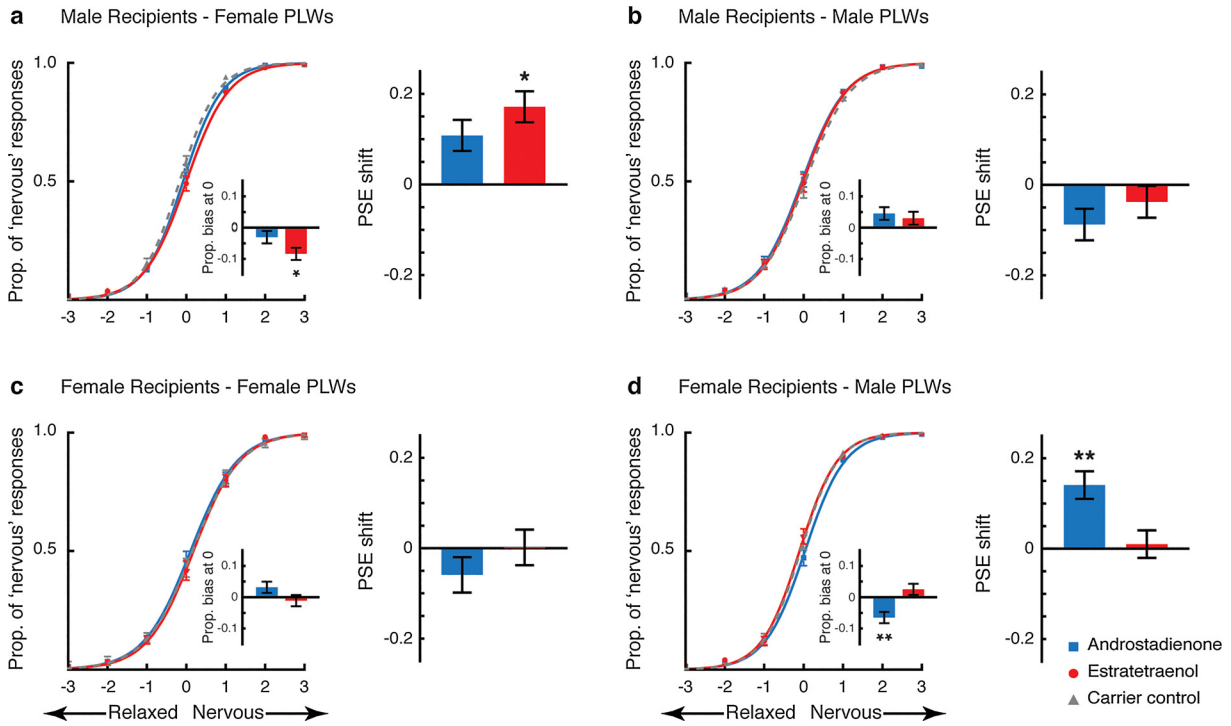

Fig. 3. Androstadienone- and estratetraenol- induced emotional judgment biases along the arousal dimension. (a–d) Androstadienone- and estratetraenol- induced changes in the relaxed/nervous judgments of male recipients viewing female PLWs (a), male recipients viewing male PLWs (b), female recipients viewing female PLWs (c) and female recipients viewing male PLWs (d). Left panels: emotion identification performances of the four groups of participants under the exposures of androstadienone (blue squares), estratetraenol (red dots) and the carrier control (gray triangles), respectively, fitted with sigmoidal curves. Insets illustrate the corresponding androstadienone- and estratetraenol- induced proportional 'nervous' biases at the emotion-neutral point 0, relative to the carrier control. Right panels: androstadienone- and estratetraenol-induced overall PSE shifts with respect to the carrier control when male and female recipients viewed female and male PLWs. Error bars represent standard errors of the mean adjusted for individual differences; *: $p \leq .05$; **: $p \leq .01$.

Table 1
Effects of androstadienone (A) and estratetraenol (E) on male and female recipients' emotional judgment criteria (PSEs) of male and female point-light walkers (PLWs) along the happy-sad (Experiment 1) and relaxed-nervous (Experiment 2) axes. Each cell represents results from 24 participants. −: no significant effect, ↑: a bias towards perceiving the PLWs as happier (in Experiment 1) or more relaxed (in Experiment 2), ↓: a bias towards perceiving the PLWs as sadder (in Experiment 1).

	Experiment 1 (happy-sad axis)		Experiment 2 (relaxed-nervous axis)	
	Male PLWs	Female PLWs	Male PLWs	Female PLWs
Male recipients	A − E −	A − E ↑	A − E −	A − E ↑
Female recipients	A ↑ E −	A ↓ E −	A ↑ E −	A − E −

male, PLWs as more relaxed. Androstadienone, by contrast, biased women towards perceiving male, but not female, PLWs as more relaxed. Consistent with our earlier findings (Zhou et al., 2014), estratetraenol influenced heterosexual men but not women; androstadienone, on the contrary, affected heterosexual women but not men. That is, not only do the human steroids androstadienone and estratetraenol respectively signal masculinity and femininity (Zhou et al., 2014), they also elicit changes in emotional perception along the valence and arousal axes in the targeted recipients, namely heterosexual women for androstadienone, and heterosexual men for estratetraenol. Moreover, these chemosensory cues are subconsciously processed by the targeted recipients and differently act on their emotional perception of male and female individuals, independent of the recipients' own mood states.

At the most ambiguous emotion-neutral point of the PLWs, the two steroids on average induced ~8.4% change in the targeted recipients' emotional judgments along the happy-sad axis, and ~7.4% change along the relaxed-nervous axis — similar to that observed with sex perception (Zhou et al., 2014) and quite noteworthy given the dominance of vision in everyday emotional perception. Nonetheless, the values should not be interpreted as the extent that sex-related chemosensory cues influence our emotional perception in daily life, where visual emotional information is typically salient. Besides, like animal pheromones, the effects of androstadienone and estratetraenol are probably dose-dependent (Bensafi et al., 2004; Nakagawa et al., 2005). Although the concentration of androstadienone used in the current study (500 μM) was comparable to that in freshly-produced apocrine sweat (mean = 0.44 nmol/μl = 0.44×10^{-3} mol/l = 440 μM), hence arguably ecologically relevant (Gower et al., 1994), the dose-response relationships remain to be tested. Besides, estratetraenol has been studied to a lesser degree than androstadienone and its concentration in sweat is unknown. Indeed, it was not the goal of the current study to quantify the impacts of sex-related chemosignals on real-life social encounters. Rather, we set out to qualify and characterize their impacts, which humans are nonconscious of.

By systematically assessing the interplays among the two steroids, recipients' sex and PLWs' sex in the recipients' emotional perception of the PLWs, our findings demonstrate for the first time that androstadienone and estratetraenol modulate emotional perception along both the valence and arousal axes in manners contingent upon not only the recipients' own sex but also their sex perception of other individuals. Such contingency critically ensures sex-appropriate behavior and represents a hallmark of sex pheromones' effects in facilitating sexual reproduction (Wyatt, 2003). The evolutionary significance of these findings is open to multiple interpretations, but they may suggest that the chemosignals androstadienone and estratetraenol prime the identification of emotionally receptive states for the potential mates with whom they are associated (i.e. androstadienone with males, and estratetraenol with females). In other words, for heterosexual men

estratetraenol may facilitate the identification of positive relaxed state in potential female mates, and thereby social interactions with them; and for heterosexual women, androstadienone likely serves a similar role in the interactions with potential male mates. On the other hand, androstadiene's negative impact on women's emotional perception of other females could mediate female intra-sexual competition (Fisher, 2004; Parma et al., 2012). Future studies including homosexual participants and/or measures of approach tendencies would help further clarify the potential roles of androstadienone and estratetraenol as human sex chemosignals.

It has been shown in the mouse that the main olfactory bulb recognizes social signals (Lin et al., 2005) and projects to sexually dimorphic hypothalamic nuclei controlling reproduction and fertility (Yoon et al., 2005), as well as the amygdala, which plays an important role in emotional processing (Kang et al., 2009). Human brain imaging studies also indicate that, aside from the hypothalamus (Berglund et al., 2006; Burke et al., 2014; Savic et al., 2001, 2005), androstadienone elicits significant changes in regional cerebral glucose metabolism in a broad range of regions in females including the prefrontal cortex, cingulate cortex and the amygdala independent of whether they are aware of its odor (Jacob et al., 2001b) and modifies amygdala connectivity (Hummer et al., 2017). These could underlie the observed sex-specific influences of androstadienone and estratetraenol on the recipients' emotional perception. The exact mechanism awaits further research.

In a recent publication, Wyatt cautioned that positive results in studies involving androstadienone and estratetraenol need to be treated with scepticism and that there is no robust evidence for the claim that the two steroids are human pheromones (Wyatt, 2015). He proposed that establishing a pheromone relies on demonstration of an odor-mediated behavioral or physiological response, identification and synthesis of the bioactive molecule(s), followed by bioassay confirmation of activity. While we agree with Wyatt to this approach, along with other researchers in the field (Endevelt-Shapira et al., 2018), we do not agree with the statement that existing (positive) results regarding androstadienone and estratetraenol are likely false-positives. We also note that the proposed approach does not preclude androstadienone and estratetraenol as candidates of human sex pheromones. Although the effects of androstadienone and estratetraenol have been controversial in the literature (Chung et al., 2016a, b; Ferdenzi et al., 2016; Hornung et al., 2018a, b; Marazziti et al., 2011; Wyatt, 2015), the current study, through rigorous psychophysical testing of a large sample of 192 participants, replicates earlier findings that the effects of androstadienone and estratetraenol are sexually dimorphic — different between male and female recipients (Bensafi et al., 2004; Jacob and McClintock, 2000; Lundstrom et al., 2003; Savic et al., 2001; Zhou et al., 2014) and critically demonstrates that their effects are also contingent upon sex perception of other individuals, thus fostering sex-appropriate interactions with potential mates and/or competitors. We believe these results contribute to a systematic quest across various laboratories around the world for human pheromones and add to the accumulating evidence suggesting that certain compounds, in this case androstadiene and estratetraenol, exert sex pheromone-like effects on the recipients.

Author contributions

M. S. and W. Z. designed research; Y. Y. and Y. Z. performed research; Y. Y. and W. Z. analyzed data; and Y. Y., M. S. and W. Z. wrote the manuscript.

Conflicts of interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.10.014.

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