

Report

Chemosensory Communication of Gender through Two Human Steroids in a Sexually Dimorphic Manner

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Summary

Recent studies have suggested the existence of human sex pheromones, with particular interest in two human steroids: androstadienone (androsta-4,16,-dien-3-one) and estratetraenol (estra-1,3,5(10),16-tetraen-3-ol). The current study takes a critical step to test the qualification of the two steroids as sex pheromones by examining whether they communicate gender information in a sex-specific manner. By using dynamic point-light displays that portray the gaits of walkers whose gender is digitally morphed from male to female [1, 2], we show that smelling androstadienone systematically biases heterosexual females, but not males, toward perceiving the walkers as more masculine. By contrast, smelling estratetraenol systematically biases heterosexual males, but not females, toward perceiving the walkers as more feminine. Homosexual males exhibit a response pattern akin to that of heterosexual females, whereas bisexual or homosexual females fall in between heterosexual males and females. These effects are obtained despite that the olfactory stimuli are not explicitly discriminable. The results provide the first direct evidence that the two human steroids communicate opposite gender information that is differentially effective to the two sex groups based on their sexual orientation. Moreover, they demonstrate that human visual gender perception draws on subconscious chemosensory biological cues, an effect that has been hitherto unsuspected.

Results

Pheromones are chemical signals that convey information between members of the same species [3, 4]. Chemical communications of sex and reproductive stage are ubiquitous in the animal kingdom, facilitating sexual selection that arises through competition over mates or for matings [5]. Whereas humans are considered the most highly scented ape of all in terms of numbers and sizes of sebaceous and apocrine glands [6], our lack of a functional vomeronasal organ and an accessory olfactory bulb [7]—structures encoding pheromones in most amphibians, reptiles, and nonprimate mammals [8]—has long been considered to negate the possibility of human pheromone communication. This view is challenged by recent findings of human menstrual synchrony [9], socioemotional communications via natural body odor [10] and tears [11],

and, in particular, the gender-specific physiological effects of two human steroids: androstadienone and estratetraenol. Androstadienone is the most prominent androstene present in male semen, in axillary hair, and on axillary skin surface [12]. It heightens sympathetic arousal [13], alters levels of cortisol [14], and promotes positive mood state [15, 16] in female as opposed to male recipients, probably in a context-dependent manner [17, 18]. Estratetraenol, first identified in female urine [19], has been likewise reported to affect men's autonomic responses [18] and mood [20] under certain contexts, albeit with controversies [13, 17]. These effects are further accompanied by distinct hypothalamic response patterns to the two steroids: androstadienone is found to activate the hypothalamus in heterosexual females and homosexual males, but not in heterosexual males or homosexual females, whereas estratetraenol activates the hypothalamus in heterosexual males and homosexual females, but not in heterosexual females or homosexual males [21–23]. Nonetheless, it remains elusive whether any concrete sexual information is relayed by androstadienone or estratetraenol to the proper recipients, an important criterion for these two steroids to qualify as human sex pheromones. Considering that gender corresponds to the biological makeup of an individual's reproductive anatomy and that accurate gender perception is the first key step in constraining subsequent sexual interaction between individuals, we ask whether androstadienone and estratetraenol effectively communicate gender information.

We tackled this issue in a gender identification task (see [Supplemental Experimental Procedures](#) available online) using visually presented point-light walkers (PLWs), a type of stimuli widely employed to represent the essential properties of human biological motion [24]. Each PLW comprised 15 moving dots depicting the trajectories of major body parts during walking: 12 for the major joints and 3 for the centers of the pelvis, thorax, and head. Their genders were quantified [1, 2] and ranged in seven equal steps, from feminine (−0.45 SD) to masculine (0.45 SD), with 0 marking the approximate gender-neutral point that was individually adjusted for each participant in the absence of olfactory stimulus prior to the actual experiment ([Figure 1](#); [Movie S1](#)). Four groups of healthy nonsmokers, including 24 heterosexual males (Kinsey scores = 0), 24 heterosexual females (Kinsey scores = 0), 24 homosexual males (mean Kinsey score ± SEM = 5.25 ± 0.14), and 24 bisexual or homosexual females (Kinsey score = 4.50 ± 0.23) ([Figure S1A](#)), performed the task at around the same time of the day on three consecutive days while being continuously exposed to either androstadienone (500 μM, 4 ml), estratetraenol (500 μM, 4 ml), or their carrier solution alone (control condition, 1% v/v clove oil in propylene glycol, 4 ml total), one on each day, in a counterbalanced manner. In each trial, they viewed a PLW for 500 ms (0.5 walking cycle) and made a forced choice judgment on whether it was a male or a female walker. The three olfactory stimuli all smelled like clove and were perceptually indiscriminable, as first tested in an independent group of 32 people (mean accuracy ± SEM = 0.30 ± 0.03 versus chance = 0.333, $p = 0.21$) and then verified by the participants in the gender identification task (overall accuracy = 0.33 ± 0.03 versus chance = 0.333,

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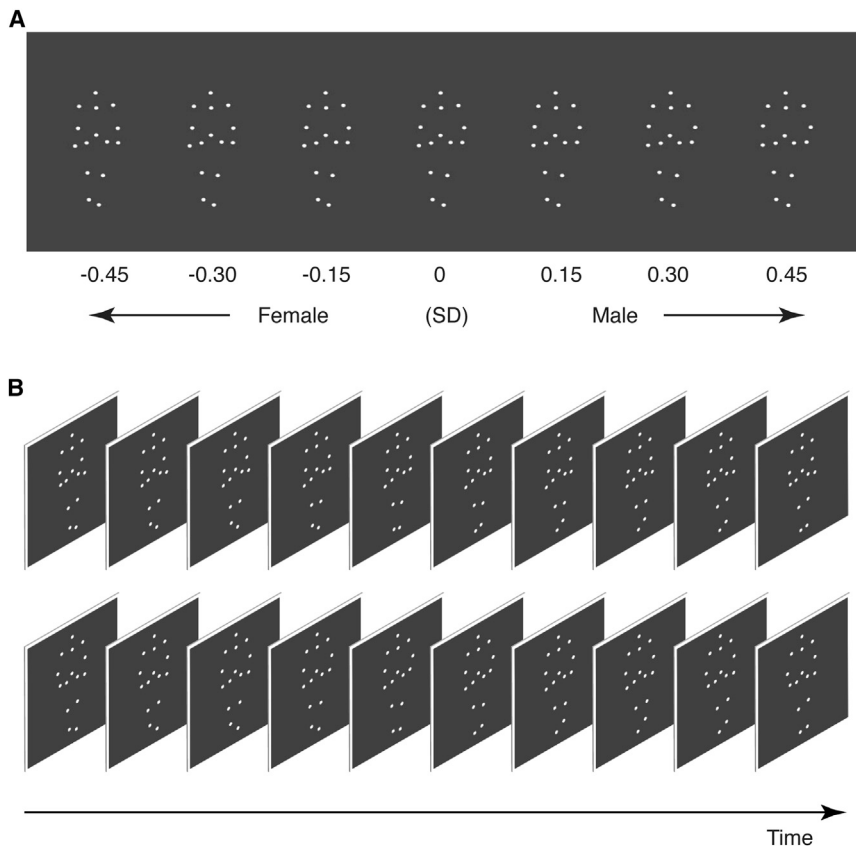


Figure 1. Illustration of PLWs Used in the Gender Identification Task

(A) For each participant, seven PLWs ranging in equal steps, from feminine (-0.45 SD) to masculine (0.45 SD), were employed, with 0 marking the approximate gender-neutral point individually adjusted in the absence of olfactory stimuli.

(B) Moving trajectories of a representative female PLW (top panel) and a representative male PLW (bottom panel) during a walking cycle.

$p = 0.82$, with no difference among the four gender and sexual orientation groups, $F_{3, 92} = 0.73$, $p = 0.54$ (Figure S1B).

Biological motion has been shown to engage a network of distributed neural areas in the form and motion pathways [25] and to naturally convey gender [26] among other social information. Indeed, all participants could decode gender of the PLWs, exhibiting a sigmoidal response pattern in which a more masculine PLW was more frequently judged as a male ($p < 0.0001$; Figure 2, left panels).

Considering that the effects of sex pheromones are typically sex specific [5], we first examined in heterosexual participants whether their own gender interacted with the olfactory stimulus they were being exposed to in their gender judgments of the PLWs. Repeated-measures ANOVA with olfactory condition (androstadienone, estratetraenol, or carrier control) and PLW's gender (seven levels, with Z scores from -0.45 SD to 0.45 SD) as the within-subject factors and odor recipient's gender (male versus female) as the between-subject factor indeed showed a significant three-way interaction of olfactory condition, recipient's gender, and PLW's gender ($F_{12, 552} = 2.00$, $p = 0.023$). Zooming in on the most ambiguous gender-neutral point of the PLWs ($z = 0$), where the rule of inverse effectiveness [27] dictates that chemosignals would exert the largest impact on visual gender perception, we found that smelling estratetraenol relative to the carrier solution alone decreased "male" responses in heterosexual males ($t_{23} = -3.35$, $p = 0.003$; Figure 2A, middle panel) but did not significantly affect gender judgments in heterosexual females ($t_{23} = -0.013$, $p = 0.99$; Figure 2B, middle panel). Conversely, smelling androstadienone relative to the carrier control increased "male" responses in heterosexual females ($t_{23} = 2.34$, $p = 0.028$; Figure 2B, middle panel) but did not

significantly affect gender judgments in heterosexual males ($t_{23} = -0.66$, $p = 0.52$; Figure 2A, middle panel).

To further characterize the interplays between the human steroids and the recipients' gender, we fitted the gender judgments of each participant per olfactory condition with a Boltzmann sigmoid function containing two parameters: point of subjective equality (PSE), the point at which the observer perceived a PLW as equally masculine and feminine, and difference limen, an index of discrimination sensitivity (essentially the slope of the fitted psychometric function near the PSE). With the carrier control condition serving as the reference, we found that smelling estratetraenol systematically biased heterosexual males toward perceiving the

PLWs as more feminine, resulting in a PSE shifted to the masculine PLW side ($t_{23} = 2.84$, $p = 0.009$), whereas androstadienone had no obvious effect ($t_{23} = 0.53$, $p = 0.60$) (Figure 2A, right panel). By contrast, in heterosexual females, smelling androstadienone significantly shifted PSE to the feminine PLW side ($t_{23} = -2.84$, $p = 0.009$), reflecting a bias to perceive the PLWs as more masculine, whereas estratetraenol showed no apparent effect ($t_{23} = -0.33$, $p = 0.75$) (Figure 2B, right panel).

The above results from heterosexual participants revealed clear sexually dimorphic effects of androstadienone and estratetraenol in communicating masculine and feminine information, respectively. We next turned to homosexual/bisexual participants to assess whether such effects also depend on recipients' sexual orientation.

At the most ambiguous gender-neutral point of the PLWs ($z = 0$), we found that smelling androstadienone relative to the carrier solution alone increased "male" responses in homosexual males ($t_{23} = 2.18$, $p = 0.04$; Figure 2C, middle panel) but did not significantly affect gender judgments in bisexual/homosexual females ($t_{23} = -0.16$, $p = 0.87$; Figure 2D, middle panel). On the other hand, smelling estratetraenol as compared with the carrier control failed to show an effect in both homosexual males ($t_{23} = 0.68$, $p = 0.51$; Figure 2C, middle panel) and bisexual/homosexual females ($t_{23} = -0.76$, $p = 0.46$; Figure 2D, middle panel). Analyses of PSEs yielded parallel results. Homosexual males were not influenced by estratetraenol ($t_{23} = -0.60$, $p = 0.55$) but exhibited a significant PSE shift to the feminine PLW side under the exposure of androstadienone ($t_{23} = -2.86$, $p = 0.009$) (Figure 2C, right panel). This response pattern was similar to that of heterosexual females and opposite to that of heterosexual males. For bisexual/homosexual females, no significant effect of androstadienone

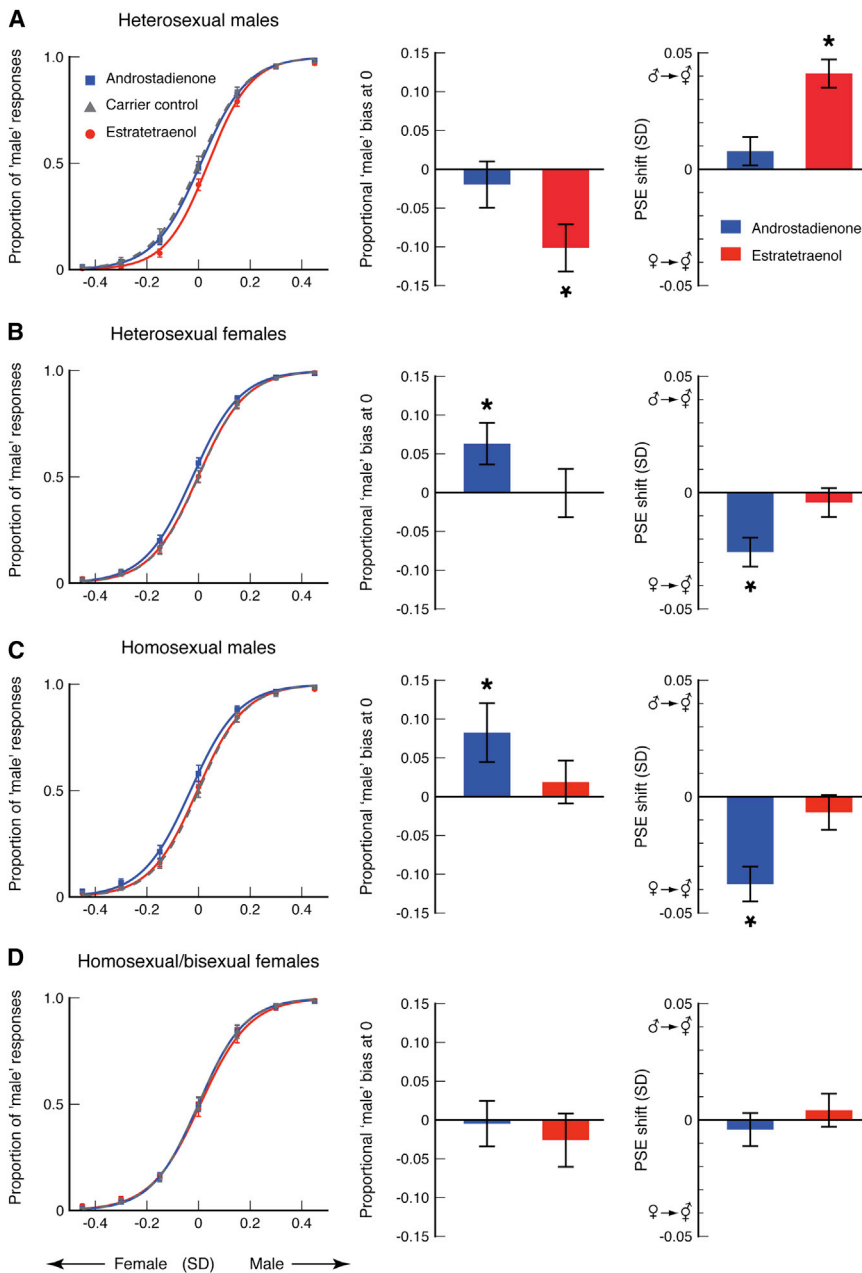


Figure 2. Androstadienone- and Estratetraenol-Induced Visual Gender Judgment Biases (A–D) Androstadienone- and estratetraenol-induced visual gender judgment biases in heterosexual males (A), heterosexual females (B), homosexual males (C), and homosexual/bisexual females (D). Left panels: gender identification performances of the four gender/sexual orientation groups under the exposures of androstadienone, estratetraenol, and the carrier control, respectively, fitted with sigmoidal curves. Dashed curves are the sigmoidal curve fits for the gender identification performances of the four gender/sexual orientation groups under the exposure of the carrier control. Middle and right panels: androstadienone- and estratetraenol-induced proportional “male” biases at the gender-neutral point $z = 0$ (middle panels) and overall PSE shifts (right panels) with respect to the carrier control in the four gender/sexual orientation groups. Error bars show SEM adjusted for individual differences; * $p < 0.05$.

homosexual/bisexual groups are higher compared to the heterosexual groups [29]. Meanwhile, the difference limens of the four participant groups did not differ from one another ($F_{3, 92} = 0.73$, $p = 0.54$) and remained unchanged across the three olfactory conditions (main effect of olfactory condition: $F_{2, 184} = 0.13$, $p = 0.88$; interaction: $F_{6, 184} = 0.30$, $p = 0.94$). Thus, it was the criterion (reflected in the PSEs) rather than the sensitivity (reflected in the difference limens) of gender judgment that was altered by the chemosensory cues, in manners contingent on the recipient’s gender and sexual orientation.

It could be argued that the above effects are not pheromonal in nature but rather result from learned associations between walking gaits and chemical cues for the gender one is attracted to. To examine this alternative, we conducted a supplemental experiment using isovaleric acid, an odoriferous

fatty acid present in axillary apocrine sweat that partly causes body odor [30]. Although men have more apocrine glands than women in all axillary regions [6], isovaleric acid did not significantly bias gender judgments of the PLWs in either heterosexual males or heterosexual females, as compared with the clove oil carrier solution alone (Supplemental Experimental Procedures; Figure S2). This led us to conclude that associative learning is unlikely the basis for the observed gender communication through androstadienone and estratetraenol.

Discussion
Our results provide strong behavioral evidence that the human steroids androstadienone and estratetraenol effectively communicate masculine and feminine information, respectively, in a gender- and sexual orientation-dependent manner.

($t_{23} = -0.32$, $p = 0.75$) or estratetraenol ($t_{23} = 0.29$, $p = 0.77$) was evident (Figure 2D, right panel), probably because their sexual orientations were more ambiguous than those of homosexual males (comparison of Kinsey scores, $t_{38,12} = -2.84$, $p = 0.007$; Figure S1A).

To facilitate comparison, the central tendencies of the androstadienone- and estratetraenol-induced PSE shifts for the four participant groups are respectively highlighted in Figure 3, which was generated by using a standard bootstrapping procedure [28]. They form three distinct clusters: the bootstrapped sample means of heterosexual males (cyan dots) fall around the vertical axis on the positive side; those of heterosexual females (yellow dots) and homosexual males (lime dots) fall around the horizontal axis on the negative side; in between lie bisexual/homosexual females (orange dots) centered around the origin. The variances in the

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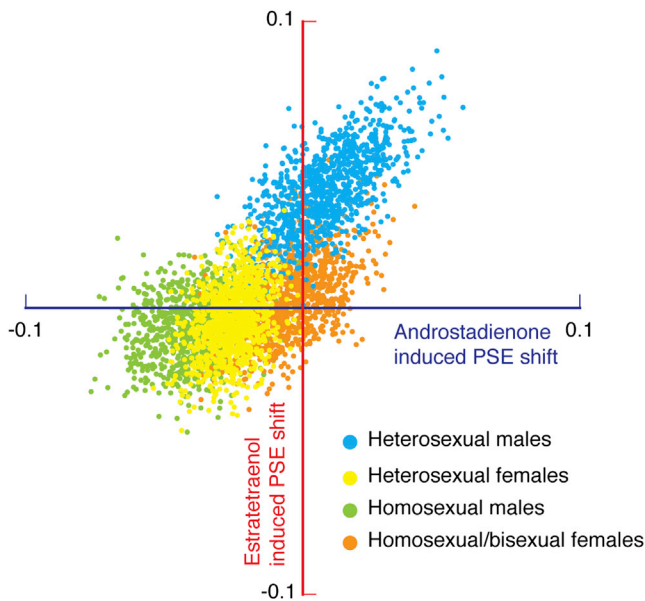


Figure 3. Central Tendencies of Androstadienone- and Estratetraenol-Induced PSE Shifts

Bivariate distributions of 1,000 bootstrapped sample means for each group plotted against the horizontal and vertical axes, respectively representing androstadienone- and estratetraenol-induced PSE shifts.

On average, the two substances induced ~8% change in the gender judgments of heterosexuals (Figures 2A and 2B) and homosexual males (Figure 2C) at the most ambiguous gender-neutral point of the PLWs. The size of the effects is actually comparable to that of gender adaptation using visually presented faces or bodies [31], which is quite noteworthy in view of the dominance of vision in daily gender perception.

Pheromones generally exercise their influence in a dose-dependent manner [32]. Androstadienone and estratetraenol are likely no exception [33]. To maximize experimental power, we followed standard practice in the field and used concentrations significantly higher than those naturally occurring in human secretions [12, 19]. It is thus expected that the effects of androstadienone and estratetraenol in daily social encounters would be smaller. The dose-response relationships remain to be tested. Nevertheless, we were able to demonstrate qualitatively that androstadienone signals masculinity to heterosexual females and homosexual males, whereas estratetraenol signals femininity to heterosexual males, without the recipients being aware of the odors. Importantly, the specific sexual information conveyed by androstadienone and estratetraenol strongly supports them as human sex pheromones.

It has been shown in the mouse that the main olfactory bulb recognizes social signals [34] and projects to sexually dimorphic hypothalamic nuclei controlling reproduction and fertility [35]. We suspect a similar pathway underlies the observed gender- and sexual orientation-specific processing of the chemosensory sexual cues in humans, where sex differences in the hypothalamus and adjacent structures have been related to heterosexuality and homosexuality [21–23, 36]. Remarkably, such chemosensory processing operates below awareness yet significantly modulates visual gender perception, indicating itself as part of the human gender code in the brain.

Supplemental Information

Supplemental Information includes Supplemental Results, Supplemental Experimental Procedures, two figures, and one movie and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2014.03.035>.

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