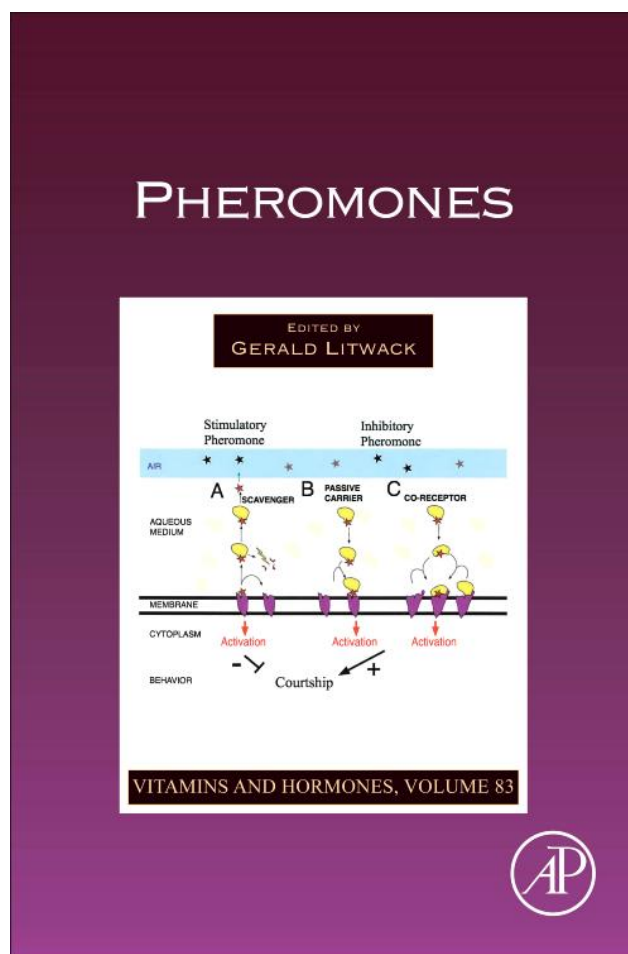


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CURRENT ISSUES IN THE STUDY OF ANDROSTENES IN HUMAN CHEMOSIGNALING

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Abstract

We review research on the 16-androstenes and their special claim, born originally of the finding that androstenes function as boar pheromones, to be human chemosignals. Microbial fauna in human axillae act upon the 16-androstenes to produce odorous volatiles. Both individual variation and sex differences in perception of these odors suggest that they may play a role in mediating social behavior, and there is now much evidence that they modulate changes in interpersonal perception, and individual mood, behavior, and physiology. Many of these changes are sensitive to the context in which the compounds are experienced. However, many key outstanding questions remain. These include identification of the key active compounds, better quantification of naturally occurring concentrations and understanding how experimentally administered concentrations elicit realistic effects, and elucidation of individual differences (e.g., sex differences) in production rates. Until such issues are addressed, the question of whether the androstenes play a special role in human interactions will remain unresolved. © 2010 Elsevier Inc.

I. INTRODUCTION

The cologne of a potential suitor, the smell of freshly baked bread pumped temptingly into a supermarket: the world is full of odors that are designed to alter our mood, perception, and behavior. Odors have tremendous effects on us, and influence us in unexpected ways. For instance, unsurprisingly, people automatically adjust the spread of their fingers to match the size of an object that they reach out to grasp. Yet present someone with a strawberry (a small item) while exposing them to the odor of an orange (a larger item), and people's grasp widens subtly yet perceptibly—and vice versa (Castiello *et al.*, 2006). These cross-modal modulations are not restricted to motor responses: for example, odors perceived as pleasant influence visual ratings of attractiveness (Demattè *et al.*, 2007), while sweet odors influence ratings of different tastes (Stevenson *et al.*, 1999). Yet when it comes to the question of whether odorous chemicals that are of human origin can systematically influence other humans, the answers tend to be more confused.

Human axillary odor derives in part from a range of compounds known as androstenes. Following early findings that some androstenes constitute pheromones produced in boar saliva, giving rise to classic stereotyped behavior in the form of lordosis (Signoret and du Mesnil du Buisson, 1961), research has attempted to establish whether androstenes affect human behavior in similar ways. Yet the question of whether there is any sense in speaking of human pheromones remains open. Some of those who consider the existence of human pheromones to be an unresolved question do so on the basis of what they see as a shortage of empirical data (e.g., Hays, 2003). The concern of

others may be less to do with the specific findings (or lack of them) and more an objection based on definitional semantics, based on a preference to reserve the term “pheromone” for traditional releaser or primer effects (review in [Saxton and Havlicek, 2010](#)). Others (e.g., [Doty, 2010](#)) refute the suggestion that mammals have pheromones at all, preferring to think of them simply as social chemosignals. In this light, social odors influence behavior in the way that a peacock’s train or a human smile might do in the visual domain.

Whether or not they turn out to be pheromones (if these exist in mammals), research continues into the influence of androstenes on human physiology and behavior. Studies have focussed on the production of androstenes in the axillae, the biochemistry and microbiology that influence the origins of human body odor, and the impact of sex differences and puberty on these mechanisms. Others have investigated individual differences in perception, including effects of the menstrual cycle, differences in odor threshold levels, and the effects of sensitization. Finally, some researchers have directed their efforts at understanding whether androstenes impact on human mood, physiology, perception, and behavior. Here we synthesize these different approaches, commenting on problematic areas such as the use of variable methodologies to elucidate relevant effects in humans. We suggest that the lack of a consistent pattern of results may arise through a lack of ecologically valid approaches and an insufficient theoretical framework. We conclude by offering suggestions which may direct future research in this complex and challenging field.

II. BIOCHEMISTRY OF ANDROSTENES

A. Production

The main 16-androstenes occurring in humans are 5 α -androst-16-en-3-one (5 α -androstenone), 5 α -androst-16-en-3 α -ol (5 α -androstenol), and 4,16-androstadien-3-one (androstadienone). Their metabolism has been extensively studied in pigs, in which they are produced in the Leydig cells in the testes from the precursor pregnenolone ([Brooks and Pearson, 1986](#)). In humans, it is thought that these compounds are produced in the adrenal glands and the ovary ([Smals and Weusten, 1991](#)) and that their metabolism follows a common steroidogenic pathway ([Dufort et al., 2001](#)); however, their detailed metabolism is far from understood. Androstenol has been detected in human urine ([Brooksbank and Haslewood, 1961](#)); androstenone ([Claus and Alsing, 1976](#)) and androstadienone ([Brooksbank et al., 1972](#)) occur in plasma and saliva ([Bird and Gower, 1983](#)).

The 16-androstenes are also found in the axillary region, a major source of human body odor (although they represent only a small proportion of the compounds found here ([James et al., 2004](#)) and some have argued they contribute relatively little to the character of armpit odor ([Natsch et al.,](#)

2006)). The axillae are abundant in eccrine and apocrine skin glands. The main function of the eccrine glands, which produce chlorine and magnesium ions and water, is thermoregulation. In contrast, apocrine glands produce a range of chemicals including fatty acids, cholesterol, and 16-androstene steroids. Analysis of fresh apocrine secretion induced by adrenaline injection found that it contained dehydroepiandrosterone, androsterone, and cholesterol (Labows *et al.*, 1979). Other studies detected androstadienone and 5 α -androstenone, but no 3 α -androstenol (Gower *et al.*, 1994).

Differences in androstene production, for example those associated with age and sex, are important for understanding their potential function. Although information is sparse, levels of 5 α -androstenone are on average higher in adult men compared to women (Bird and Gower, 1983), and excretion of androstenol in the urine of prepubertal individuals is negligible compared to postpuberty (Cleveland and Savard, 1964). These findings are indicative of a sexually dimorphic pattern of expression which becomes evident around puberty, a pattern that is characteristic of a trait that is subject to sexual selection (e.g., see Andersson 1986).

B. The role of the skin microflora

Androstenes and other compounds constitute a substrate for axillary bacteria which produce odorous volatiles (Leyden *et al.*, 1981; Savelev *et al.*, 2008). This is evidenced by experimental treatment with a bactericidal agent (Povidone-iodine) leading to a significant decrease in 5 α -androst-16-en-one (Bird and Gower, 1982). Similarly, other agents (e.g., Farnesol Plus) which inhibit growth of coryneforms result in a decrease in armpit odor intensity (Haustein *et al.*, 1993).

The axillary microflora consist primarily of *Micrococcus*, *Staphylococcus*, *Propionibacteria*, *Corynebacteria*, and eukaryotic *Malassezia* (Leyden *et al.*, 1981; Rennie *et al.*, 1991; Taylor *et al.*, 2003; Wilson, 2005). The *Corynebacteria* appear to be primarily responsible for the intensity of axillary odor (Rennie *et al.*, 1991), and this is supported by *in vitro* studies showing that coryneform bacteria are of special significance in 16-androstene metabolism (Leyden *et al.*, 1981; Rennie *et al.*, 1991), although only a small subset of coryneforms are able to metabolize these steroids (Austin and Ellis, 2003; Decreau *et al.*, 2003). Early *in vitro* studies using both pure and mixed cultures of coryneforms showed that they are able to metabolize testosterone into various breakdown products including dihydrotestosterone and 17-androstenes; however, 16-androstenes were not detected (Nixon *et al.*, 1984, 1986a,b, 1987). Some *Micrococcus luteus* strains, but not *Staphylococcus* or *Propionibacterium*, were also found to metabolize testosterone (Rennie *et al.*, 1989b). Detailed examination of biochemical pathways shows that coryneforms can transform only precursors containing a C16 double bond (Austin and Ellis, 2003). These molecules include androstadienol and androstadienone, which are

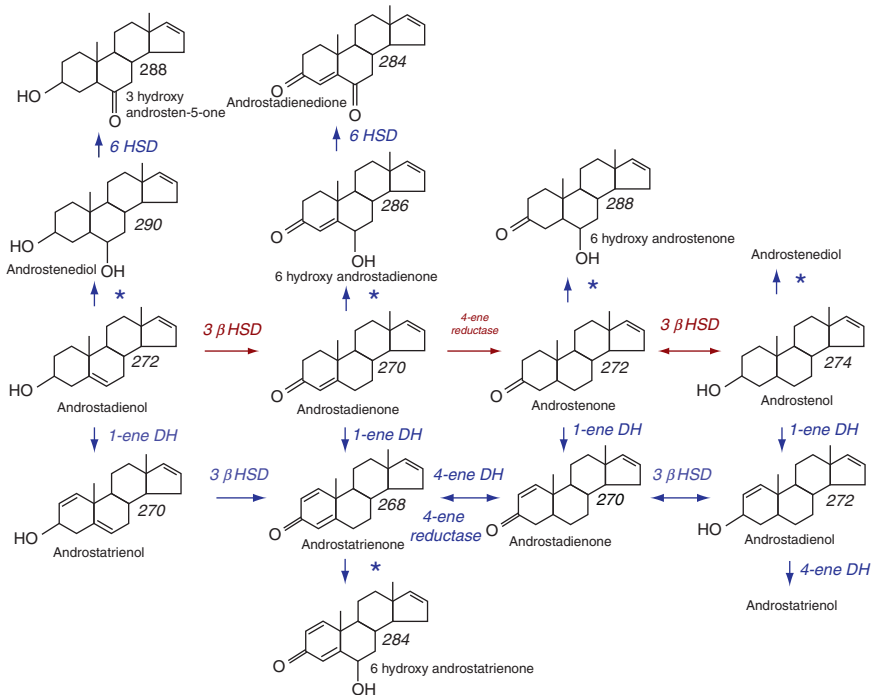


Figure 3.1 Biotransformation of 16-androstenes by corynebacteria (A) axillary isolates. It is important to note that the extent of biotransformation of 16-androstene steroids is likely to be more complex than that presented in this figure, as both α - and β -forms of hydroxylated steroids are probably generated. Key: HSD, hydroxysteroid dehydrogenase; DH, dehydrogenase. *Denotes biotransformations that may involve a number of enzymes (e.g., hydroxylase or dehydroxylase and hydratase activities). Reprinted from *J. Steroid Biochem. Mol. Biol.* 87, Austin and Ellis Microbial pathways leading to steroidal malodour in the axilla. 105–110. (2003) with permission from Elsevier.

subsequently transformed into several different androstenes including 5α -androstenone and 3α -androstenol (Fig. 3.1). There is further evidence for reversible transformations between 5α -androstenone and 3α -androstenol, between 3α -androstenol and androstadienol, and between 5α -androstenone and androstadienone (Rennie *et al.*, 1989a). Another study using androsta-5,16-dien 3β -ol, androsta-4,16-dien 3β -ol, and androsta-5,16-dien 3β -one as substrates for coryneforms found a main reaction at C-3 oxidation which resulted in odorous androsta-4,16-dien-3-one (Decreau *et al.*, 2003).

C. Quantitative assessments of androstene production

Quantitative estimates of axillary extracts find high interindividual variability. Using gas chromatography–mass spectrometry (GC–MS), Nixon *et al.* (1988) found the following concentrations in male axillary hair extracts

(in pmol/total axillary hair weight/24 h): androstenone 0–433, androstadienone 0–4103, androstadienol 0–728, 3 α -androstenol 0–1752, and 3 β -androstenol 0–416. Comparison between the sexes indicates higher levels of androstenone in men (range 5.2–1019 pmol/24 h) than in women (range 1.2–16.6 pmol/24 h, with one outlier of 551 pmol/24 h) (Gower *et al.*, 1985). In another study, male samples contained higher concentrations of dehydroepiandrosterone sulfate, but not androstenol (Preti *et al.*, 1987). Concentrations of androstenol showed cyclic patterns, with a peak in the midfollicular phase of the menstrual cycle in women, and some seasonal fluctuations in men (Preti *et al.*, 1987).

III. PSYCHOPHYSICAL RESEARCH USING ANDROSTENES

A. Prevalence of specific anosmia

Specific anosmia (Amoore, 1967) is a condition in which an individual with normal olfactory sensitivity is incapable of perceiving a particular odor. A classic example, and thus an extensively studied case, is that of androstenone anosmia. Estimates of prevalence range from 7.6% in females and 44.3% in males (Griffiths and Patterson, 1970), to 46% (Amoore *et al.*, 1977) or 50% (Beets and Theimer, 1970) in all subjects (see Table 3.1 for a more comprehensive overview of anosmia studies). Rates of androstenol anosmia may reach 90% in females and 45% in males (Gower *et al.*, 1985). However, it has been claimed that the interpretation of the term “anosmia,” in conjunction with the screening methods employed, may have led to overestimation of rates of nondetection (Bremner *et al.*, 2003). Even after identifying putative nondetectors using standard methods, forced-choice tests showed that these individuals could identify androstenone at rates above chance, despite low confidence in their decision. In light of this, Bremner *et al.* revised downward the rate of androstenone anosmia in a healthy adult population to 1.8–6.0%, considerably lower than previous estimates.

Similar findings have arisen in relation to levels of 16-androstene anosmia. In a study focussing on the laboratory-synthesized compound 5- α -androst-16-an-3-one (androstanone), it was found that previously labeled anosmics were able to detect androstanone under experimental conditions (Van Toller *et al.*, 1983). The authors likened this to the results of a previous study (Schiffman, 1979) in which subjects were hypnotically induced into a state where they could perceive previously undetectable odors, attributing the newfound detection to a form of altered perceptual state. Anosmic subjects from the same study were found to correctly identify androstanone in a secondary detection task in which they were presented with androstanone and told when to expect it. Here they recognized the odor from the

Table 3.1 Reported androstenone nondetection rates

Study	Method/criterion for nondetection	Concentration	N ^a	Nondetection rate ^a (%)
Beets and Theimer (1970)	One trial; subjective assessment	Unknown (diluted in alcohol)	F (35), M (65)	11
Griffiths and Patterson (1970)	One trial; subjective assessment of smelling strip	Unknown (800 ng residual evaporated from ether as dilutant)	F (145), M (165)	F (7.6), M (44.3)
Amoore <i>et al.</i> (1977)	2/5AFC ^b threshold; lowest conc. with both correct	2.9 ppb solution (water)	764	47
Dorries <i>et al.</i> (1989)	Two AFC runoff series; <5 consecutive correct	1.0 × 10 ⁻¹ (highest conc.); in mineral oil	Not specified	F (24), M (40)
Gilbert and Wysocki (1987) and Wysocki <i>et al.</i> (1991)	Scratch and sniff strip; subjective assessment	Not specified	26, 200	F (24), M (33)
Pause <i>et al.</i> (1999)	2 AFC staircase; <7 reversals	1.25 mg/ml of 1,2-propanediol (highest); 0.04 µg/ml (lowest)	F (132)	F (10.6)
Stevens and O'Connell (1995)	2/5 runoff series, threshold test; <2 consecutive correct trials	5.4 mM binary dilution series, 12 steps	40	75
Sirota <i>et al.</i> (1999)	3AFC runoff series; <4 consecutive correct trials	1.25 mg/ml binary dilution series (mineral oil); 10 steps	M (20)	M (25)
Morofushi <i>et al.</i> (2000)	One/two runoff series, threshold test; <4 consecutive correct trials	5 µM to 5 mM in 1.5 ml mineral oil; 10 steps	F (63)	F (22)
Filsinger <i>et al.</i> (1984)	Passive exposure; subjective assessment of impregnated paper	1 mg crystal residue evaporated from 1% solution in 100% ethanol	F (102), M (98)	F (9), M (13)

(continued)

Table 3.1 (continued)

Study	Method/criterion for nondetection	Concentration	N ^d	Nondetection rate ^a (%)
Bremner <i>et al.</i> (2003)	3AFC screening followed by yes/no forced-choice detection	5 mg crystal androstenone; 30 ml of 7.34×10^{-3} M androstenone in light mineral oil	M (33), F (22)	3AFC: 16.3 (no sex differences) FC: 1.8–6.0
Baydar <i>et al.</i> (1993)	3AFC staircase, <3 consecutive correct trials	19.36 ppb androstanone diluted with humidified air stream	F (41), M (38)	F (15.8), M (26.8)
Boyle <i>et al.</i> (2006) (Expt 1)	3 AFC	Air bubbled through 4 ppm androstenone in propylene glycol, diluted to 80% v/v androstenone	13	0
Knaapila <i>et al.</i> (2008)	Scratch-and-sniff: rate from “no odor” (presumed anosmic) to “extremely strong odor”	0.1% solution in diethyl phthalate	917 (twins; 137 without co-twin)	F (17.6), M (21.9)
Kline <i>et al.</i> (2007)	2AFC; anosmia defined as $\leq 50\%$ accuracy	500 ppm (volume/mass)	F (34), M (34)	F (26.5), M (17.6)
Lübke <i>et al.</i> (2009)	2AFC single staircase	Highest concentration: 1.25 mg/ml in 1,2-propanediol	M (27)	M (25.9)
Wang <i>et al.</i> (2004)	Three-repetition, 2AFC staircase	3.67 mM (0.1% w/v) in silicone oil	58	15.5
Pierce <i>et al.</i> (2004)	Rate odor on 1–9 intensity scale (1 = presumed anosmic)	3.67 mM ($1 \times 10^{-1}\%$ wt/vol; 1 g/L) androstenone in light mineral oil	F (136), M (122)	F (14.7), M (28.7)

Table on page 45 reprinted with permission from Oxford University Press and taken from Bremner *et al.* (2003). The prevalence of androstenone anosmia. *Chem.Senses* **28**, 423–432 and updated on this page to include further studies.

^a F denotes female participants, M denotes males, otherwise sex was not specified.

^b AFC = Alternative forced-choice.

first trial, claiming that they did not respond to the odor initially because they could not give the odor a verbal label. The same subjects exhibited a skin conductance response when exposed to androstanone, despite not consciously detecting its odor (Van Toller *et al.*, 1983).

Worldwide variation in the ability to detect androstenone is apparent (Gilbert and Wysocki, 1987), yet explanations for this have only recently been considered. Boyle *et al.* (2006) attributed this variation to trigeminal interference. The trigeminal system detects chemical irritants in the environment; in doing so, it seems to inhibit olfactory processing of odors (Cain and Murphy, 1980). Most odorants have a trigeminal component (Doty *et al.*, 1978) which is usually concentration-dependent (Cometto-Muniz *et al.*, 1998; Hummel *et al.*, 1992). Accordingly, anosmia variation could be due to trigeminal sensation, which in turn will be dependent upon the concentrations and methodologies of researchers. Indeed, Boyle *et al.* (2006) reported an inverse correlation between androstenone olfactory thresholds and trigeminal sensitivity. Incorporation of trigeminal data might therefore be advantageous in understanding this area of study.

In summary, the number of people who are truly anosmic to androstenone seems to be reliant on many factors. For future studies to control for anosmia occurrence, it seems necessary to establish a standardized method by which it can be reported. Pause *et al.* (1999) showed that altering the conservativeness of methodology resulted in anosmia estimates ranging between 10% and 37%.

B. Thresholds

Olfactory thresholds are usually measured using a geometric concentration scale, consisting of binary dilution steps (see Keller and Vosshall, 2004 for an overview). Early work investigated the idea that sensitivity to androstenone was inherited (Pollack *et al.*, 1982), possibly as a dominant Mendelian trait (Smith, 1974). More recently, twin studies indicate significant heritability (Gross-Isseroff *et al.*, 1992; Knaapila *et al.*, 2008; Wysocki and Beauchamp, 1984) and genetic variation in the odorant receptor OR7D4 has been found to influence both androstenone valence and sensitivity (Keller *et al.*, 2007). However, threshold differences also occur between individuals, between sexes, across the menstrual cycle, and through puberty.

Lundström *et al.* (2003b) identified a bimodal distribution in sensitivity to androstadienone, with a small group of highly sensitive “supersmellers,” but their androstadienone threshold did not correlate with those for phenyl ethyl alcohol (PEA, a rose odor), suggesting that androstadienone sensitivity may be different from general olfactory acuity. A similar pattern of bimodal sensitivity can also be found for androstenone (Amoore, 1991). However, Jacob *et al.* (2006) reported not a bimodal, but a multimodal threshold distribution of androstadienone, arguing that thresholds to related axillary

steroids are likely to be dependent upon individual variation in exposure history, thereby implying that thresholds are plastic and environmentally influenced, at least to some extent (also see “Sensitization”). This could potentially explain the finding that homosexual men have lower thresholds to androstenone than heterosexual men (Lubke *et al.*, 2009).

Lundström *et al.* (2003b) also reported a tendency for women to have lower thresholds for androstadienone than men, confirming previous observations (Koelega and Koster, 1974). However, there appears to be no sex difference in thresholds to androstenone (Gower *et al.*, 1985).

With regard to the menstrual cycle, Lundström *et al.* (2006) reported that women’s sensitivity to androstadienone was higher in the fertile phase than in the luteal phase. They suggested that this was linked to the potential functional role of androstadienone in mate choice (Jacob and McClintock, 2000; Lundström *et al.*, 2003a,b; Savic *et al.*, 2001; Saxton *et al.*, 2008a), and this was also evidenced by the lack of a comparable change in responses to the odor of PEA.

Savic *et al.* (2001) reported a sexually dimorphic effect whereby women, but not men, exhibited anterior hypothalamic activation following exposure to androstadienone (androstenol has similar effects: Savic and Berglund, 2010). Since the anterior hypothalamus plays a significant role during puberty (causing the release of gonadotropins from the pituitary gland), it might be expected that androstadienone thresholds would also change during puberty. Consistent with this prediction, a relatively high proportion of very young children may be able to detect androstenone (Schmidt and Beauchamp, 1988), suggesting a decrease in sensitivity between early childhood and adulthood. Furthermore, a peripubertal decrease in androstenone sensitivity has been reported in men but not in women (Dorries *et al.*, 1989), possibly due to the sexual dimorphism in production discussed above. A similar trend occurs for androstadienone thresholds (Hummel *et al.*, 2005). Furthermore, Chopra *et al.* (2008) reported the same effect not only for androstadienone, but also for 2-methyl, 3-mercapto-butanol (2M3M; a malodorous component of human sweat), using both chemosensory event-related potentials (CSERPs) and psychophysically measured thresholds. The results of the CSERPs were consistent with psychophysical findings, whereby increases in latencies in male pubescents highlighted their higher thresholds to androstadienone.

C. Sensitization

Sensitization is the process of becoming more sensitive to a stimulus. Repeated exposure to an odor normally leads to adaptation and decreased sensitivity; but unusually, the reverse seems to occur for androstenone and androstadienone, a characteristic that contributes to their unique properties. Wysocki *et al.* (1989) noticed that heightened sensitivity could be induced in some individuals by repetitive exposure. They reported that the ability to

smell androstenone was induced in 10 of 20 initially insensitive subjects after they had been systematically exposed to androstenone (for 3 min, 3 times a day, for 6 weeks). Their explanation likened the olfactory system to an immune system response, with clonal expression of olfactory receptors occurring by a yet unknown mechanism. Subsequent studies have suggested that other sites mediate these changes, including the olfactory epithelium (Wang *et al.*, 2004; Yee and Wysocki, 2001) and central components of the olfactory system (Mainland *et al.*, 2002).

Similarly, Jacob *et al.* (2006) reported a reduction in thresholds for androstadienone, in both men and women, of more than 4 orders of magnitude (from 3.5×10^{-3} M to 0.3×10^{-6} M) after repetitive exposure (following the same exposure schedule as Wysocki *et al.*, but for 2 weeks only). Using this same methodology, with the addition of CSERP and olfactory-evoked potential (OEP) readings, Boulkroune *et al.* (2007) shed light on gender dimorphisms during this sensitization process. Although they found a decrease in detection thresholds for androstadienone similar to those found by Jacob *et al.*, changes in the later components of the evoked potentials were specific to women and apparently linked to cognitive and perceptual functioning, indicative of a female-specific “learning” component in androstadienone perception. Again, these results are consistent with androstenes bearing characteristics fitting of sexually selected chemosignals.

D. Hedonic perception

A range of studies have investigated how the hedonic perception of androstene compounds differs across time and between individuals. Verbal labels range from being reminiscent of “strong urine” (Ohloff *et al.*, 1983) to “musky” (Jacob *et al.*, 2002) and “unpleasant” (Lundström *et al.*, 2003b), and compounds vary in intensity: androstadienone has been described as a “low-odor androstene” in comparison with androstenone (Pause, 2004). While investigating the effects of repetitive exposure to androstadienone, Jacob *et al.* (2006) reported an additional change in perceived odor quality with increased sensitivity, a result confirmed by Boulkroune *et al.* (2007). Low-sensitivity subjects initially rated androstadienone with a wide range of negative and positive descriptors. Following exposure-induced sensitization, negative adjectives such as “putrid,” “vegetable,” and “woody” came to predominate. This process was explained in terms of two putative odor channels, one responding to broadly pleasant odors and the other to putrid odors (Jacob *et al.*, 2006), via low- and high-affinity receptors, respectively (Polak, 1973; Stevens and O’Connell, 1995). Thus, exposure to androstadienone in sensitized individuals might activate the high-affinity receptors responsive to putrid odors while the low-affinity receptors remain inactive.

Most studies of hedonics have focussed on changes in odor perception across the menstrual cycle. Hummel *et al.* (1991) investigated the hedonic

estimates of several odorants, including androstenone. Only with regard to androstenone did trend analysis reveal a significant change across cycle phase, with more pleasant perceptions at midcycle. Similarly, Grammer (1993) reported that, while females rated androstenone as unattractive, their response became less negative around ovulation.

IV. PSYCHOLOGICAL EFFECTS

A. Changes in interpersonal perception

A summary of studies describing effects of androstenes on perception, mood, and behavior is provided in Table 3.2. In an early experiment, Cowley *et al.* (1977) asked participants to wear surgical masks impregnated with androstenol, volatile fatty acids, or a control (nontreated) mask. Participants then assessed suitability of three job applicants of each sex who were pictured in photographs accompanied by verbal descriptions. Women exposed to androstenol rated applicants more positively than those wearing masks impregnated with fatty acids; men exposed to androstenol rated applicants more negatively than the control group. A similar technique (i.e., impregnated masks and rating of images) was also used by Kirk-Smith *et al.* (1978), this time with participants rating photographs of people, animals, and buildings. Androstenol exposure caused both women and men to judge female images as more attractive (images of both sexes were also rated as emotionally warmer), but there was no effect on ratings of images depicting animals or buildings (although they found the opposite in a follow-up study: Kirk-Smith and Booth, 1990). More recently, the impregnated mask technique was used to explore the effect of androstenol in a marketing context (Ebster and Kirk-Smith, 2005). Raters judged three magazines regarded by an independent panel as masculine, neutral, and feminine in terms of their philosophy, target audience, and purchasing intention. When exposed to androstenol, men but not women increased their ratings of the masculinity and positivity of the “masculine” journal. Together, these results imply a degree of context specificity, discussed further below.

The impregnated mask technique has been criticized because it exposes experimental participants to far higher androstene concentrations than those found in axillary odor. To avoid this, Black and Biron (1982) aimed to use a more natural setting. In their design, men and women watched a 15-min slide-show, together with a confederate of the opposite sex who had applied androstenol, exaltolide (a synthetic musk), or a nonsmelling substance. Participants afterward judged the confederate’s attractiveness. There was no significant effect of androstenol or exaltolide. However, as noted by Filsinger *et al.* (1985), there was a statistical tendency ($p = 0.07$) for confederates to be rated as less attractive by those in the androstenol condition.

Table 3.2 A summary of key studies investigating effects of androstenone, androstenol, and androstadienone on perception, mood, and behavior

Study	Compound ^a	Conc./ Quantity	Target sex ^b (N)	Mask ^c	Exposure method	Measure	Main effect ^b
Perception Cowley <i>et al.</i> (1977)	AL	1 mg/1 ml	F, M (183)	×	Surgical mask	Perception of others	F attributed more positive traits to others, M attributed more negative traits to others.
Kirk-Smith <i>et al.</i> (1978)	AL	0.3 mg	F (12), M (12)	×	Surgical mask	Perception of others	F rated more attractive by both sexes.
Black and Biron (1982)	AL	1% in 95% ethanol	F (39), M (39)	×	Confederate's odor	Attractiveness perception	None
Filsinger <i>et al.</i> (1984)	AN	1 mg	F (102), M (98)	×	Envelopes	Self and other perception	M rated M more passive. F rated themselves as less sexy
Filsinger <i>et al.</i> (1985)	AL, AN	1 mg, 1 mg	F (132), M (122)	×	Envelopes	Self and other perception	AL: M rated M more attractive. AN: F rated M and F less attractive
Kirk-Smith and Booth (1990)	AN	0.25 mg	F (8), M (8)	×	Surgical mask	Perception of others	Rated others as less sexy
Ebster and Kirk-Smith (2005)	AL	1 mg/ml	F (60), M (60)	×	Surgical masks	Product evaluation	M rated men's magazines more masculine and had more positive buying intentions
Saxton <i>et al.</i> (2008a)	AND	250 μ M	F (22,19,12)	✓	Upper lip	Attractiveness perception	F rated M more attractive
Hummer and McClintock (2009)	AND	250 μ M	F (30), M (20)	✓	Upper lip	Perception of emotion	Raters more engaged to emotionally significant stimuli

(continued)

Table 3.2 (continued)

Study	Compound ^a	Conc./ Quantity	Target sex ^b (N)	Mask ^c	Exposure method	Measure	Main effect ^b
Mood							
Cowley <i>et al.</i> (1980)	AL	1 mg/1 ml	F (153)	×	Surgical masks	Mood during the menstrual cycle	Increased irritability during menses
McCullough <i>et al.</i> (1981)	AL	2 second spray of Boar Mate	F (161), M (59)	×	Surgical masks	Emotional responsiveness	No effect
Benton (1982)	AL	150 μ g	F (18)	×	Upper lip	Mood during the menstrual cycle	F more submissive during middle of menstrual cycle
Jacob and McClintock (2000); Expt 1	AND	250 μ M	F (10), M (10)	×	Upper lip	Mood and alertness	Increased positive mood state in F
Jacob and McClintock (2000); Expt 2	AND	250 μ M	F (31)	✓	Upper lip	Mood and alertness	Prevention of mood deterioration. Modulatory effect
Jacob <i>et al.</i> (2001a)	AND	250 μ M	F (44), M (21)	✓	Upper lip	Skin temp, skin conductance, and mood	M increase in positive mood with a F experimenter
Jacob <i>et al.</i> (2002)	AND, AL	250 μ M	F (18), M (19)	✓	Smelling swab/ upper lip	Mood	Reduced negative mood and increased positive mood
Lundström <i>et al.</i> (2003a)	AND	250 μ M	F (38,40)	✓	Upper lip	Mood and concentration	Increased feelings of focus

Bensafi <i>et al.</i> (2003)	AND	50 mg	F (12), M (12)	×	Jars	Mood	No effect on mood
Bensafi <i>et al.</i> (2004a)	AND	50 mg	F (36), M (36)	×	jars	Mood, memory and autonomic function	Maintained positive mood and decreased memory of events in F
Bensafi <i>et al.</i> (2004b)	AND	6250 μ M, 250 μ M	F (30), M (30)	×	Jars	Mood and autonomic function	High concentration increased positive mood and decreased negative mood in F only
Lundström and Olsson (2005)	AND	250 μ M	F (37)	✓	Upper lip	Mood and arousal	Increase in psychophysical arousal and mood when experimenter was M
Villemure and Bushnell (2007)	AND	250 μ M	F (48)	×	Jars	Pain thresholds and mood	Increased positive mood in F in the absence of pain
Wyart <i>et al.</i> (2007)	AND	30 mg	F (21)	×	Jars	Cortisol levels, mood, and physiological arousal	Maintained positive mood and increased sexual arousal
Behavior							
Kirk-Smith and Booth (1980)	AN	3.2 μ g, 16 μ g, 32 μ g	540	×	Sprayed chairs	Preference of seats	F preferred to sit on 3.2 μ g or 32 μ g sprayed seats. M avoided 32 μ g sprayed seats
Gustavson <i>et al.</i> (1987)	AL	2.5 mg	480	×	Sprayed Perspex	Choice of toilet cubicle	M avoided sprayed stalls, F had no preference
Cowley and Brooksbank (1991)	AL	1 mg	F (38), M (38)	×	Impregnated necklace	Opposite sex exchanges	F reported more exchanges with males

^a Compound abbreviations: AN refers to androstenone, AND to androstadienone; AL to androstenol.

^b Target sex refers to the individuals exposed to the compound (F = female, M = male).

^c Masking odor: used/not used (in all cases where used, masking odor was eugenol/clove oil).

It is not known whether the short duration of exposure to the compound, and length of time between exposure and rating, could have minimized a possibly transient effect.

Another method for testing the psychological effects of the androstenes was introduced by [Filsinger *et al.* \(1984, 1985\)](#). A male photograph, impregnated with different compounds, was rated by participants on several personality and appearance scales. In their first study, they compared androstenone with three controls: no odor, a negative odor (scatol, a fecal odor), and a positive odor (methyl anthranilate, a fruit odor). This procedure aimed to control for effects based on a possible hedonic quality of androstenone. Men judged the man in the photograph as more passive if they were exposed to androstenone compared with the positive odor; there was no difference in women's ratings. However, women in the androstenone condition rated themselves as less sexy than controls. A follow-up study compared effects of androstenone, androstenol, and exaltolide ([Filsinger *et al.*, 1985](#)). Here, photos of both sexes were rated. Men judged male images less positively if exposed to androstenone compared with androstenol and exaltolide. They also rated images of men treated with androstenol as sexier than those assessing unscented photos. Men exposed to all the three compounds rated themselves less sexy than the men in the no-odor condition. Women exposed to androstenone and androstenol, compared with the no-odor condition, rated men as more weak and less sexy; and women in the androstenone condition rated women's photos as less sexy. No effect on self-perception was detected.

Most recently, [Hummer and McClintock \(2009\)](#) tested whether the effects of androstadienone on attention and perception are restricted to social and emotional contexts. They found that inhalation of androstadienone caused men and women to react faster to affective facial expressions but not to neutral faces or nonsocial stimuli (shapes). Similarly, in Stroop tests, androstadienone affected attention to both positive and negative emotional words, but not attention to neutral stimuli.

All of these studies took place under laboratory conditions. To discover whether androstenes might have effects in more naturalistic settings, [Saxton *et al.* \(2008a\)](#) carried out three speed-dating experiments (speed-dating involves a series of time-limited interactions as a means to meet potential partners). In two out of three experiments, women exposed to androstadienone masked in clove oil (to avoid possible detection of androstadienone) rated the men they interacted with as more attractive than did women exposed to clove oil alone (all women met the same men).

B. Changes in mood

As described in the introduction, odors are powerful modulators of affective states, so it is not surprising that researchers have explored the effects of androstenes on mood (see [Table 3.2](#)). In an early study, [Cowley *et al.*,](#)

(1980) used masks impregnated with androstenol to test changes in mood across the menstrual cycle. Women exposed to androstenol (as compared to no-odor or naphthalene conditions) reported higher levels of irritability and depression during their menstrual bleeding. A similar study found that androstenone elicited higher ratings of alertness and excitability compared to a nonodor control (Kirk-Smith and Booth, 1990). Benton (1982), in contrast, applied androstenol or a control compound every morning to the woman's filtrum, finding that androstenol-exposure induced higher submissiveness self-ratings at midcycle. In a similar vein, Jacob and McClintock (2000) daubed participants' necks and filtrums with androstadienone, estratetraenol, or a control (propylene glycol, in which the steroids were dissolved). Estratetraenol is a steroid substance detected in pregnant women (Thyssen *et al.*, 1968), with several physiological effects (Monti-Bloch and Grosser, 1991). At intervals of 6 min, 2 h, 4 h, and 9 h postapplication, participants completed questionnaires on their emotional state. After 6 min, androstadienone and estratetraenol were associated with increased positive mood in women, and decreased mood in men. Androstadienone-exposed women scored higher on some subscales (i.e., "Stimulant," "High") even after 2 h, and experienced lower postexperiment decrease in positive mood.

Another paper compared the effects of androstadienone, androstenol, and muscone (a synthetic steroid) (Jacob *et al.*, 2002), which share similar chemical structure and perceptual qualities. Compounds were first inhaled passively, then applied to the participant's filtrum 25 min later. Androstadienone again prevented a decrease in positive mood (factor "Elation-vigor") compared to both androstenol and muscone, and also prevented an increase in negative mood compared to muscone.

Changes in women's mood due to androstadienone were subsequently confirmed in two experiments by another research group (Lundström *et al.*, 2003a). Here, women reported feeling more focussed following application of androstadienone in eugenol (a synthetic clove odor); results were identical when those women who reliably discriminated androstadienone were excluded. Similarly, Villemure and Bushnell (2007) reported mood changes and increased tolerance of pain following androstadienone exposure (in women but not men).

Other studies suggest that the mood effects of androstadienone could be modulated by various contexts. McCollough *et al.* (1981) induced mood by asking participants to read a passage of erotic fiction, but found no differences in ratings of 11 emotional scales between those exposed (via masks) to androstenol, rose, or a no-odor control. Subsequently, Bensafi *et al.* (2004a) induced emotional reactions by presenting excerpts from happy, sad, arousing, and neutral movies. Exposure to androstadienone while watching a sad movie was associated with positive mood in women and sad mood in men. Androstadienone and estratetraenol were linked with increased arousal in

both sexes while watching an erotic movie, but there was no change when participants viewed neutral and funny movies, consistent with previous studies of mood effects in neutral contexts (Bensafi *et al.*, 2003; Hummer and McClintock, 2009).

Finally, another study of emotional contexts found that androstadienone increased positive mood and sexual arousal in women (Wyart *et al.*, 2007). A further development arose when Jacob *et al.* (2001a) noted that mood changes depend on the administrator's sex: women's positive mood in the presence of androstadienone increased in the presence of a male but not a female administrator (see also Lundström and Olsson, 2005).

As some critiques have acknowledged (e.g., Black and Biron, 1982), the key variable in these studies appears to be the concentration of the compound under investigation. The only study on this issue has showed that a high concentration of androstadienone (625 μM) increased positive and decreased "high arousal negative mood" in women in contrast to men, whereas no mood effect at a low concentration (250 μM) was observed (Bensafi *et al.*, 2004b).

C. Behavioral effects

Evidence about context-dependent effects of androstenes on mood change and perception is certainly important, but leaves open the question of whether these effects translate into behavioral changes. To answer this, we should examine evidence on behavioral effects, and in particular those investigated in naturalistic settings. In the first study of this kind, researchers impregnated seats in a dentist's waiting room with androstenone (Kirk-Smith and Booth, 1980). Female patients approached the treated seats, while male patients avoided them. In a second elegant study, researchers impregnated doors of student restroom stalls with ethanol, androstenol, or androsterone (a compound that smells similar to androstenol, but is not a constituent of human body odor), and monitored men's and women's usage (Gustavson *et al.*, 1987). Men but not women avoided cubicles impregnated with androstenol, but not the two controls.

A third group of researchers used a "necklace technique" to test the effect of androstenol and copulines in natural settings (Cowley and Brooksbank, 1991). Participants wore a plastic tube with open ends that had been impregnated with the target substance (0.25 ml chloroform with 1 mg/ml androstenol) as a necklace from the afternoon until the next morning, when they were asked to record details of all their morning social interactions, including sex of their interlocutor, length and depth of the conversation, and details of who initiated the interaction. Women who wore androstenol reported more interactions with men but not with other women, and longer and deeper conversations.

D. Effects on physiology

The research group led by Luis Monti-Bloch in the late 1980s focussed on the question of human vomeronasal organ (VNO) function. One of the substances they used in their experiments was androstadienone. Its application directly into the VNO led to neural activity (measured by an electrovomeronasogram) in men but not in women, whereas application to the main olfactory mucosa did not reveal any such activity (Monti-Bloch and Grosser, 1991; for a detailed overview of key physiological studies, see Table 3.3). Another study of women by the same team found electrovomeronasal activity immediately after the application of the substance, together with an increase in skin conductance, decrease in respiration rate and pulse 35 min after the application, and a decrease in negative mood (Grosser *et al.*, 2000). Other studies, reviewed below, did not set out to differentiate whether androstadienone was perceived by the main olfactory organ or the VNO.

Several of the studies reviewed above also assessed physiological changes, and most revealed sex-specific changes. For instance, Jacob *et al.* (2001a) found an increase in skin conductance in women (but not men) following exposure to androstadienone, and that women's skin temperature decreased while it increased in men. These effects were particularly evident in women when the experimenter was male; there was no effect of the experimenter's gender on male participants.

Sex-specific changes were also observed in a study by Bensafi *et al.* (2003), who constructed an overall physiological index to measure arousal. Androstadienone increased arousal in women and decreased it in men. This was mainly due to changes in skin conductance, pulse, and respiration. A follow-up study by the same team, to test the effect of context (Bensafi *et al.*, 2004a), found increases in skin temperature, and a decrease in respiration rates in men but not women, following exposure to androstadienone in the context of an erotic video. In other contexts (induced by viewing a neutral, happy, or sad movie), androstadienone did not induce physiological changes. A second study (Bensafi *et al.*, 2004b) reported that only a high dose (625 μM) and not a low dose (250 μM) of androstadienone was associated with affective and physiological changes. Finally, Wyart *et al.* (2007) suggest that androstadienone raises levels of cortisol in women (men were not tested). The authors also replicated findings of the effects of androstadienone on composite measures of physiological arousal.

E. Brain imaging

If androstenes work as human chemosignals, they should give rise to specific brain responses. This issue has been explored by several brain imaging studies (see Table 3.3). Smelling androstadienone in clove oil activates the right

Table 3.3 A summary of the key studies investigating the effects of androstadienone and androstenone on human physiology and brain function

Study	Compound ^a	Conc.	Target sex ^b (N)	Mask ^c	Exposure method	Measure	Main effect ^b
Electrophysiology							
Van Toller <i>et al.</i> (1983)	AN	0.6 mg, 6 mg, and 10 mg	M (11) F (16)	×	Smelling strip	Skin conductance	Increased skin conductance in reported anosmics
Grosser <i>et al.</i> (2000)	AND	100 pg	F (40)	×	VNO	Tension and autonomic function	Reduction in nervousness, tension, and other negative feelings. Altered autonomic function
Jacob <i>et al.</i> (2001a)	AND	250 μ M	F (44), M (21)	✓	Upper lip	Skin temperature and skin conductance	Skin temp increases in M and lowered in F Increased skin conductance in both
Bensafi <i>et al.</i> (2003)	AND	50 mg	F (12), M (12)	×	Jars	Physiological state	Increased physiological arousal in F but not M
Wyart <i>et al.</i> (2007)	AND	30 mg	F (21)	×	Jars	Cortisol levels, mood and physiological arousal.	Increased cortisol levels in saliva and physiological arousal
Brain imaging							
Jacob <i>et al.</i> (2001b)	AND	250 μ M	F (10)	×	Passive inhalation	FDG—PET scan	Activated the right prefrontal cortex, amygdala, and hypothalamus in F
Savic <i>et al.</i> (2001)	AND	200 mg	F (12), M (12)	×	Passive inhalation	PET	Activated anterior-ventral hypothalamus in F

<i>Gulyas et al.</i> (2004)	AND	5% solution	F (5)	×	Jars	PET	Activated orbitofrontal cortex, inferior prefrontal cortex and fusiform gyrus
<i>Savic et al.</i> (2005)	AND	200 mg	F (12), M (24)	×	Bottles	PET	Activated areas associated with sexual behavior in homosexual M and heterosexual F
<i>Berglund et al.</i> (2006)	AND	200 mg	F (24), M (12)	×	Bottles	PET	Lesbian F processed AND more similarly to heterosexual M than heterosexual F.

^a Compound abbreviations: AN refers to androstenone; AND to androstadienone.

^b Target sex refers to the individuals exposed to the compound (F = female, M = male).

^c Masking odor: used/not used (in all cases where used, masking odor was clove oil).

prefrontal cortex, amygdala, and hypothalamus in women (Jacob *et al.*, 2001b). Specific comparisons of the sexes have revealed sex-specific activations: androstadienone (200 mg in crystalline form) activated the anterior-ventral hypothalamus in women, while estratetraenol activated the same brain area in men (Savic *et al.*, 2001). These areas are sexually dimorphic and are presumably involved in sexual behavior, including sexual orientation. A subsequent study showed that homosexual men exhibited activation patterns similar to heterosexual women (Savic *et al.*, 2005). Moreover, heterosexual (but not homosexual) women showed an activation of the hypothalamus after inhalation of androstadienone (Berglund *et al.*, 2006). Interestingly, both compounds activate areas known to be involved in processing odor perception (e.g., amygdala, piriform, orbitofrontal, and insular cortex).

Gulyas *et al.* (2004) compared brain activations in four odor conditions: androstadienone (5% solution in dipropylene glycol), pleasant (gamma-methyl-ionon), unpleasant (methyl-thio-butanoate), and a control (dipropylene glycol). Androstadienone showed activation in the orbitofrontal cortex, inferior prefrontal cortex, and fusiform gyrus, compared to the control, and activation in the inferior prefrontal cortex and superior temporal cortex compared to the pleasant and unpleasant odors. The superior temporal cortex area is known to be involved in face recognition and in mental states connected with social interactions. The inferior prefrontal cortex is activated in social cognitive and emotional processes. Thus, these activational patterns emphasize the potential role of androstenes in social interactions.

V. DISCUSSION

The literature we have reviewed leads us to several overarching questions that we believe remain largely unanswered, but which necessitate satisfactory answers before we can even approach a full understanding of the role of androstenes in human social interactions.

A. What compound(s) are responsible for social function?

Most of the research to date has investigated effects of androstenone, androstenol, or androstadienone. While there is plenty of mixed evidence and conflicting results from these studies, the fact that the majority of studies find some form of effect, and most that investigate a specific effect find somewhat complementary findings, suggests that there is reasonable evidence that androstenes do influence physiology, psychology, and behavior to some degree. However, given that very few studies have compared the effects of two or more of these compounds within the same study,

and because most studies use widely varying methodologies, it remains a very difficult task to form a coherent picture.

The selection of the compound of interest (from the three previously mentioned) for different experiments appears, at least to us, somewhat arbitrary. We perceive there to have been a gradual shift in focus of interest from androstenone and androstenol in earlier work to androstadienone more recently. The earlier work was inspired by findings in pigs that androstenone functioned as a releaser pheromone in sexual activity. However, the reason for the shift from androstenone to androstadienone is unclear. In our view, the choice of compound appears to be more akin to the vagaries of fashion than a logical and rational process of falsification. We should hasten to admit that we have not been immune to this ourselves (e.g., in the selection of androstadienone for our speed-dating study [Saxton et al., 2008a](#)). However, what is clearly needed in future work is a concerted effort to actively compare the effects of the three compounds within the same experiments. This will enable us to determine the extent to which reported effects are compound-specific or compound-general. This is a question that is critical to the discussion of possible pheromonal effects, since any definition of a pheromone (strict or not) will require us to determine the compound responsible.

What if it emerges that such studies demonstrate that androstene compounds exert comparable effects? Would this mean that they are all pheromones, or none? The answer to this question may depend critically on a greater understanding of the biochemical metabolic pathways involved in the formation of each compound, and in the compound-specific contributions to the chemosignal (discussed below). That is, which of the compounds are actually perceived and contribute to appropriate responses by the signal receivers, and which are simply either precursors or byproducts of the active compound(s)?

We also need to be aware of the possibility that the true functional signal carried in axillary odor may be a mixture of compounds, rather than a single one acting in isolation. At least in vertebrates, almost all chemical signals are composed of compound mixtures, with the precise communicative message being determined by precise ratios of a subsample of key compounds. For example, colony-specific signatures of ants are formed by ratios of different cuticular hydrocarbons ([Guerrieri et al., 2009](#)). Similarly in mice, individual variation in urinary odor associated with genes in the major histocompatibility complex, which influences MHC-disassortative mate choice independently of other cues ([Roberts and Gosling, 2003](#)), is coded by characteristic ratios of volatile carboxylic acids ([Singer et al., 1997](#)). In this light, it is possible that each of the three compounds, at given ratios, contributes to an overall signal. It is also possible that other, yet unknown, compounds are involved. If either of these possibilities turns out to be true, we will have barely begun to understand how the androstenes influence behavior. Also, each of the

compounds may have a slightly different effect; for instance, it was reported recently that distinct cuticular hydrocarbons affect species and sex recognition and sex attractiveness in fruit flies (Billeter *et al.*, 2009).

B. What is the relevant concentration to enable social function?

A glance at Tables 3.2 and 3.3 shows the diversity of concentrations which have been used to assess effects of androstenes. To some extent, these are due to the differing intensities of the three most commonly studied compounds—androstadienone is the least intense and thus researchers have tended to use this at a comparably higher concentration than in the case of the other two. Further variation across studies using any given compound is largely due to individual preferences of different researchers, and subsequent studies often tend to copy the chosen concentration. An example is the choice of a concentration of 250 μM , at which Jacob and McClintock (2000) tested the effects of androstadienone and estratetraenol on mood. No justification was given for the use of this concentration, which is far higher than any measured concentration (see section on Quantitative Assessment), but this has since been used by a substantial number of studies (Tables 3.2 and 3.3).

Does the use of substantially higher compound concentrations in experiments invalidate the results? This approach was criticized almost three decades ago by Black and Biron (1982). However, the use of higher concentrations might not alter the nature or direction of an effect, though this awaits testing—indeed, studies that have compared effects at different concentrations within the same study are fewer than those that have compared the effects of different compounds in the same study. Results of a study by Bensafi *et al.* (2004b) support the notion that concentration matters. It will certainly be interesting to see results of studies that test supranormal and substantially reduced concentrations. However, what seems indisputable is that a higher concentration must enhance the likelihood of signal detection, and thus the likelihood of any response. In this regard, it will be interesting to discover what the typical concentrations of relevant compounds are in the headspace surrounding the axillae—the medium in which any natural chemoreception will take place. To date, quantitative measures of concentrations stem from measurements on axillary skin or hair, or from artificially elicited apocrine secretion. At best, this markedly overestimates the concentrations at which potential functionally relevant detection takes place.

Furthermore, supranormal concentrations are likely to introduce potentially anomalous results if they elicit responses in individuals who would not detect or react to more ecologically valid concentrations. As we have outlined, substantial variation in detection thresholds and rates of anosmia are

hallmarks of androstene psychophysical research. Detection thresholds vary not just between individuals, but also at different times (e.g., across the menstrual cycle) and depending on the degree of prior exposure. The latter may be relevant, for example, when comparing women who experience male axillary odor on a regular basis, such as via the odor of their male partners, with those who do not (e.g., those without current partners). Indeed, recent studies investigating women's perception of male odors find that partnered women perceive odors in different ways compared with currently single women, whether the effect is concerned with the relationship between odor pleasantness and either male dominance (Havlicek *et al.*, 2005) or MHC-similarity (Roberts *et al.*, 2008).

To fully understand responses to androstenes, we need to take into account these kinds of effects, whether it is by collecting relevant background information (e.g., relationship status) and/or by undertaking some form of psychophysical screening before behavioral/psychological measurement. One example of the benefits of such an approach is the study by Morofushi *et al.* (2000), which found that individual women with higher sensitivity to androstenol were more likely to exhibit synchronization in their menstrual cycles, compared to those who were less sensitive. Without this information, the possible implication of androstenol in cycle synchronization would have been missed.

C. Is individual variation in production, detection, and sensitivity to behavioral change consistent with a signaling function?

As we have described, there is huge diversity in the kinds of effects that have been attributed to the androstenes, and in the consistency of findings within specific experimental paradigms. However, some themes have emerged that bear upon the question that underpins most of the research, namely whether androstenes carry communicative significance. Two of the most important appear to be that there are sex differences in both production and response to androstene compounds, and that the context in which testing occurs is critical to obtaining apparent responses.

Whether or not androstenes are produced at different levels in men and women is clearly a question of fundamental importance. A sex difference would allow us to infer which sex is the signaler, and it appears that this is males, as might be expected from the starting point of the field (and of this chapter): the boar pheromone. Very few studies have produced quantitative estimates of androstene expression in individuals, but the existing evidence (albeit based on a very small sample) suggests that levels are higher in men than women (Gower *et al.*, 1985). This, coupled with the finding that androstene production becomes upregulated around puberty (Cleveland and Savard, 1964), indicates that the signal is likely to be involved in sexual selection. However, what it does not conclusively help us with is the

identity of the signal receiver. The nature of chemical communication, in contrast with most visual and acoustic signals, means that this is usually open to question, since the same signal may be used by conspecifics of both sexes in different contexts (see e.g., Gosling and Roberts, 2001).

It is possible, for example, that androstenes are produced by males as part of an intrasexual signaling system linked to competition over access to resources or mates. In light of links between mammalian scent-marking and dominance (Gosling and Roberts, 2001), this explanation resonates particularly well with findings that androstene exposure leads to physiological arousal (Jacob *et al.*, 2001a; but see Bensafi *et al.*, 2003), higher masculinity ratings of male magazines (Ebster and Kirk-Smith, 2005), and avoidance by men but not women of impregnated restroom stalls (Gustavson *et al.*, 1987). However, it seems that females also respond to androstene exposure in a manner consistent with the notion that androstene expression is an intersexual signal. Women exposed to androstenes rate males more positively than controls (androstadienone: Saxton *et al.*, 2008a; but see opposite effect of androstenone: Filsinger *et al.*, 1985), experience more exchanges with men (Cowley and Brooksbank, 1991), and experience heightened physiological arousal (Bensafi *et al.*, 2003). Furthermore, brain imaging studies suggest that smelling androstadienone affects brain activity according to sexual orientation (Savic *et al.*, 2005). Teasing apart the differential responses and sensitivity to androstenes is of utmost importance in understanding their communicative function, and more work is needed to attain a clearer picture of which sex is the principal receiver (although it is possible that a signal selected for communication with one sex might have been co-opted, during evolutionary history, as a signal to the other).

Sensitivity to context is one way to address this, and it is becoming clear that the context in which experiments take place has a large bearing on the nature of the result. Ecological validity in the study of androstenes has been discussed in Saxton *et al.* (2008a,b). Two examples will suffice here. First, in two experiments, Jacob *et al.* (2001a) and Lundström and Olsson (2005) showed that exposure to androstadienone influenced women's mood, but only in a subsample of female participants who were tested by a male experimenter; those tested by a female experimenter experienced no alteration in mood. This again suggests that androstenes function as a signal to women. Second, in contrast with previous experiments in the laboratory that found no effect on women's attributions of male attractiveness (e.g., Black and Biron, 1982), or even reduced them (Filsinger *et al.*, 1985), when these effects were tested in a face-to-face scenario (a speed-dating context), attributions increased as predicted (Saxton *et al.*, 2008a).

If androstenes are produced by men, perhaps mainly to signal to women, what is the information that is being signaled? It is conceivable that androstenes are simply sex-markers, indicating that the signaler is male. However, it seems likely that, in common with other sexually selected signals, the message is more subtle and complex than this, signaling individual variation in male quality and

thus suitability as a mate and father. Studies of female perceptions of male axillary odors show that women who express a relatively high preference for androstadienone also have relatively high preferences for masculine male faces; this suggests cross-modal concordance in preference strength for masculine traits (Cornwell *et al.*, 2005). Furthermore, axillary odor pleasantness correlates with psychometric dominance in men (Havlicek *et al.*, 2005) and physical indicators of attractiveness in other sensory modalities (e.g., Rikowski and Grammer, 1999), results that link odor quality with other physical traits that indicate putative male quality. Although the chemical basis for the latter studies is unknown, it seems reasonable in light of Cornwell *et al.*'s results that androstenes could be at least partly responsible for mediating these important behavioral effects. Finally in this regard, differential sensitivity (Lundström *et al.*, 2006) and higher hedonic ratings (Grammer, 1993; Hummel *et al.*, 1991) to androstenes at midcycle, when conception is most likely, also point to an evolutionary adaptation to optimal discrimination of good-quality mates, who would be predicted to have characteristic androstene profiles.

D. To what extent are androstenes special?

What we have discussed thus far assumes that the three androstenes that have been most studied (or others, or a mixture composed of these) are responsible for a set of physiological and/or psychological responses, and that these responses are specific and unique to these compounds. This seems a reasonable conclusion, though it is by no means certain.

One way that this conclusion can be made more plausible is the use of odor controls in experimental designs. It is possible that some of the effects we have reviewed are at least partly due to nonspecific sensory stimulation inherent in the presentation of the androstene odors. Perhaps this is most likely in the measurement of physiological responses such as arousal, but in principle it applies equally to any outcome variable. To circumvent this flaw in design, a number of studies have included odor controls where the control is a nonandrostene. Several studies (e.g., Saxton *et al.*, 2008a) have used clove oil; in this approach, the clove odor is presented together with the androstene, and without it (Saxton *et al.* additionally used a water control). Although this has the additional advantage of acting as a masking odor to prevent experimental participants from detecting the androstene, it means that the androstene is presented within an odor mixture, and in a way that is in no way ecologically valid. Another alternative is to include another condition altogether, ideally another nonandrostene odor that occurs naturally within axillary odor (to enhance ecological validity) or is perceptually similar. For example, Jacob *et al.* (2002) used muscone, a synthetic musky odor, finding that androstadienone, but not muscone (or androstenol), was responsible for maintaining positive mood. Such examples of odor specificity support the idea that androstenes have specific effects and that it is thus a reasonable

exercise to focus on these particular compounds, which after all represent only a small proportion of the cocktail of compounds present in axillary odor.

Another way to investigate the specificity of androstene effects could be to use a comparative approach; in other words, to examine the extent to which responses to androstenes are conserved across closely related species (e.g., nonhuman primates), relative to other odor compounds. [Laska *et al.* \(2005\)](#) measured thresholds for androstenone and androstenol in spider monkeys, squirrel monkeys, and pig-tailed macaques. They found that androstenone thresholds are similar to those of humans, yet androstenol thresholds are considerably higher. Although there are no details of sexual dimorphisms in thresholds, nor a comparison involving other, nonandrostene compounds, this kind of research is an appropriate start to what we think could potentially be a fruitful approach.

E. Conclusions

We have outlined a considerable body of research that collectively describes a set of compounds, expressed in natural axillary odor at higher levels in males, which have specific physiological and behavioral effects in other individuals, particularly in females. Put like this, androstenes sound very much like pheromones, according to almost any definition. Despite this, we remain cautious about this conclusion and prefer to use the term chemosignal (or even semi-chemical). Reasons for this caution include the facts that it remains unclear which compound or mixture of compounds is responsible for the most potent effects, that we think it far from conclusively demonstrated that any one of these compounds is responsible for the effects as opposed to a generic androstene or odor effect (although there is some limited support for this notion), and that effects remain to be shown at ecologically appropriate concentrations (which are as yet unknown). At another level, we wish to distance ourselves from what we see as the naive search for a human sex pheromone that encapsulates much of the media's coverage of the research described here, and that perhaps represents the motivation of some researchers. The simplistic view of such pheromones, in which involuntary, stereotypical responses are triggered by the faintest whiff of a compound, arises from an uncritical extrapolation from the kind of pheromonal response observed in insects. In contrast to this, our primary concern is that an ecologically appropriate framework, informed by evolutionary approaches where appropriate, should be used to explore and elucidate the action of this group of compounds. Although a good start has been made, we think that this work has some way yet to go.

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