

Sex-Steroid Derived Compounds Induce Sex-Specific Effects on Autonomic Nervous System Function in Humans

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The physiological and psychological effects of 2 human sex-steroid derived compounds, 4,16-androstadien-3-one (AND) and 1,3,5(10),16-estratetraen-3-ol (EST) were measured in 24 subjects who participated in a within-subjects, double-blind experiment. A dissociation was evident in the physiological effects of AND, in that it increased physiological arousal in women but decreased it in men. EST did not significantly affect physiological arousal in women or men. Neither compound significantly affected mood. AND is an androgen derivative that is the most prevalent androstene in human male sweat, male axillary hair, and on the male axillary skin surface. The authors argue that AND's opposite effects on physiology in men and women further implicate this compound in chemical communication between humans.

Chemical signaling plays an important role in reproductive states and related behavior in animals. Evidence for such communication exists in animals from insects (Christensen & Hildebrand, 2002; Hansson, 2002) to rodents (Berghard, Buck, & Liman, 1996; Brennan & Keverne, 1997; Dulac & Axel, 1998) to primates (Aujard, 1997; Smith, Tomlinson, Mlotkiewicz, & Abbott, 2001). Yet the nature of chemical signaling in humans is the topic of some debate (Gower & Ruparelia, 1993; McClintock, 1998; Meredith, 2001; Monti-Bloch, Jennings-White, & Berliner, 1998; Preti, Wysocki, Barnhart, Sondheimer, & Leyden, 2003; Sobel & Brown, 2001).

Any odorant may effect human behavior. Common odorants, such as food odors, environmental odors, and manufactured fra-

grances, can influence human mood (Lehrner, Eckersberger, Walla, Potsch, & Deecke, 2000) and autonomic nervous system function (Alaoui-Ismaili, Vernet-Maury, Dittmar, Delhomme, & Chanel, 1997; Bensafi et al., 2002; Brauchli, Ruegg, Etzweiler, & Zeier, 1995; Graham, Janssen, & Sanders, 2000). However, when studying chemical communication between humans, the focus is naturally tuned to odorants not only perceived, but also emitted, by humans.

Of these, research has traditionally focused on steroidal compounds present in axillary secretions (for reviews, see Comfort, 1971; Gower, Nixon, & Mallet, 1988; Gower & Ruparelia, 1993). Recently, the steroidal compound 4,16-androstadien-3-one (AND) has received much attention (Berliner, Monti-Bloch, Jennings-White, & Diaz-Sanchez, 1996; Jacob, Hayreh, & McClintock, 2001; Jacob & McClintock, 2000; Monti-Bloch, Diaz-Sanchez, Jennings-White, & Berliner, 1998; Monti-Bloch & Grosser, 1991; Monti-Bloch, Jennings-White, & Berliner, 1998; Monti-Bloch, Jennings-White, Dolberg, & Berliner, 1994; Savic, Berglund, Gulyas, & Roland, 2001). AND is an androgen derivative and is the most prevalent androstene in human male sweat and other secretions (Nixon, Mallet, & Gower, 1988). A second compound that has also received attention is 1,3,5(10),16-estratetraen-3-ol (EST), an estrogen derivative. In contrast to AND, there is only limited evidence for EST in human secretions (Thyssen, Elliott, & Katzman, 1968).

The reported effects of these compounds on human mood and physiological arousal have not always been consistent. For example, whereas AND was reported to reduce nervousness, tension,

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other negative feelings and sympathetic activity in one study (Grosser, Monti-Bloch, Jennings-White, & Berliner, 2000), in other studies it had no effect on negative feelings but increased sympathetic activity and maintained positive mood in women (Jacob & McClintock, 2000). One interesting effect of these compounds comes from a positron emission tomography (PET) study that reported sex- and compound-specific brain activation of AND and EST (Savic et al., 2001). Compared to smelling plain air, EST preferentially activated the hypothalamic region in men and the olfactory region in women, whereas AND activated the hypothalamic region in women and, to a lesser extent, the olfactory region in men.

External events may influence the effects induced by AND and EST (Jacob, Hayreh, & McClintock, 2001). Thus, environmental differences may underlie the conflicting results in previous studies. In this study, we set out to minimize external events by creating a highly controlled and uneventful environment. Measuring the response to AND and EST within this environment may enable us to establish a baseline response to these compounds, and thus potentially disambiguate some of the previously conflicting findings on AND and EST. In addition, we set out to specifically test for physiological and/or psychological correlates of the dissociation in compound-induced PET brain activation seen by Savic et al. (2001). To these ends, men and women underwent testing with three different compounds: AND, EST and an olfactory control, in a within-subjects repeated-measures design. During each session, subjects were placed in a nonarousing environment where mood and physiology were monitored over time, both before and after compound presentation.

Method

Participants

Twenty-four University of California, Berkeley graduate and undergraduate students (12 women, mean age = 28.00 ± 6.52 , and 12 men, mean age = 24.75 ± 3.81) of mixed ethnic backgrounds participated in this experiment. Exclusion criteria included history of head or nasal passage trauma; history of neurological disease; history of repeated or current sinus infection; chronic use of medication including oral contraceptives; and alcohol, drug, or tobacco abuse. Twenty-two of the subjects described themselves as heterosexual, and 2 described themselves as bisexual women. Women's olfactory acuity may vary across the menstrual cycle (Doty, Snyder, Huggins, & Lowry, 1981; Mair, Bouffard, Engen, & Morton, 1978; Pause, Sojka, Krauel, Fehm-Wolfsdorf, & Ferstl, 1996). We aimed to minimize the variance in this respect by having all female subjects begin testing at approximately the 14th day of their menstrual cycle, counting forward from the 1st day of menstruation as Day 1 to determine the appropriate experimental start date. It is acknowledged that this verbal report by subjects is an inaccurate assessment of the menstrual phase in women, but it was considered helpful toward minimizing experimental variance.

Compounds

Steroidal compounds were obtained from Steraloids (Newport, RI). Both AND and EST were used in this experiment. Fifty milligrams of AND and EST were each deposited in crystal form into identical 60-ml (4.5 cm in diameter at the opening; 9 cm high) opaque jars. Of the various possible methods of delivery and compound concentrations, the above were chosen so as to enable direct comparison with the results of Savic et al. (2001), as these were the methods used in that study. Baking powder served as the

control substance, and 50 milligrams of this was placed into a third identical jar. Baking powder was selected as a control because psychophysical pretesting in the lab suggested it would be a good match on perceived intensity and pleasantness, and it had a similar visual appearance to the experimental compounds, in the event that subjects looked into the jars. Jars were double-containment capped and refrigerated at 4 °C until used. An observer who did not participate further in the experiment numerically coded all jars, thus establishing a double-blind design.

Physiological Parameters

The following eight autonomic nervous system parameters were simultaneously and continuously recorded and displayed during the experiment: skin conductance response (SCR), electrocardiogram (ECG), finger pulse (FP), ear pulse (EP), blood pressure (BP), skin temperature (ST), abdominal respiration (AR), and thoracic respiration (TR). In addition to these eight variables, subject body movement or fidgeting was also recorded. All parameters were sampled and recorded at 1 KHz except BP, which was sampled at 250 Hz. Data were converted and amplified with a 16-channel amplifier (PowerLab 16SP; ADInstruments, New South Wales, Australia) and displayed, stored, reduced, and analyzed with the Chart 4.1.1 software package (ADInstruments, 2002).

SCR. SCR was obtained through two bipolar finger Ag/AgCl electrodes (surface: 1 cm²), placed on the second phalanx of the index and the third digit of the nondominant hand, attached with Velcro strap. SCR was measured by applying a 0.5 $\mu\text{A}/\text{cm}^2$ AC current. The SCR amplifier used was fully isolated with low voltage, 75-Hz (~ 40 mV) AC excitation. The variable reduced was the nonspecific skin conductance response (NS-SCR), expressed in number of events per minute. This has been described as the appropriate SCR measure for continuous non-event-dependent SCR (Dawson, Schell, & Filion, 2000). The threshold for an event was a 0.5% deflection from the tracked mean that yielded an average of 3.35 ($SD = 4.18$) NS-SCR events per minute throughout this experiment.

ECG. ECG was obtained through three circular Ag/AgCl conductive adhesive electrodes (0.9 cm diameter). The skin surface was cleaned with alcohol before electrode placement. Electrodes were placed on both the left and the right sides of the abdomen (just under the thoracic cage), and a ground electrode was placed on the left leg. The data were reduced to ECG rate expressed in beats per minute.

FP/EP. FP and EP were recorded with infrared plethysmographs (size: 15.0 mm long \times 15.0 mm wide \times 6.3 mm high) placed on the fifth finger of the nondominant hand (finger pulse) and the ear on the side of the nondominant hand (ear pulse). These devices used an infrared photoelectric sensor to detect changes in tissue blood volume. They were attached with either a Velcro strap (for finger), or a clip (for ear). The data were reduced to pulse rate in beats per minute (BPM).

BP. An arterial tonometer (Colin 7000; National Instruments, San Antonio, TX) enabled continuous measurement of BP waveforms (sampling rate: 250 Hz), as well as determining systolic and diastolic pressures noninvasively and continuously. The data were reduced to mean BP expressed in millimeters of mercury.

ST. A small ceramic-encapsulated metal-oxide semiconductor (9.5 mm in length, 2 mm in diameter), was used to measure ST. The thermistor, designated to operate from 0 °C to 50 °C, was placed directly below the axilla. The data were reduced to temperature change: the difference between ST maximum and ST minimum values.

AR and TR. Two respiratory belt transducers (30 cm rest length, 10 cm maximum elongation, 4.5 cm in width) were used to measure changes in thoracic and abdominal circumference caused by respiration. They contained a piezoelectric device that responded linearly to changes in length (sensitivity: 4.5 ± 1.0 mV/mm). The data were reduced to abdominal and thoracic respiration rates.

Movement. Movement was measured by a high-sensitivity (2,500 mV/g) accelerometer (EGCS, Entran Devices, Fairfield NJ) attached to the

chair armrest. Recordings were used both as an analysis variable (motions reduced to movements per minute) and as a means of detecting movement-related artifact in the physiological recordings.

Psychological Rating

A 17-item test was used to measure compound and sex effects on mood. Subjects rated how strongly they were experiencing each of 17 different emotions on a 9-point scale, with 1 corresponding to *not at all* and 9 corresponding to *very strongly*. This mood test was devised to tap into mood rather than more transient emotional feelings (Ekman, Freisen, & Ancoli, 1980; Levenson, Ekman, & Friesen, 1990). It is well validated and consists of the following variables: afraid, amused, angry, annoyed, anxious, bored, calm, confident, content, contemptuous, disgusted, embarrassed, happy, interested, sad, and stressed. For the purposes of this study, "sexually aroused" was added to this test and used as a descriptor.

Experimental Design

A within-subjects, double-blind, repeated measures design (see Figure 1), was used such that each subject underwent testing every other day for 3 separate days, with each of the three compounds (AND, EST, or control) presented on a different day. Thus, this study contains data from 72 recording sessions, each lasting about 90 min (from subject arrival at the lab to subject departure). Day-by-compound order was counterbalanced across subjects such that AND, EST, and the control occurred with equal frequency on Days 1, 2, and 3 of the experiment. All testing was performed in a temperature- and humidity-controlled, stainless steel-coated, 11 × 8-foot (3.35 × 2.44-m) room equipped with high efficiency particulate air and carbon filtration. This room was designed specifically for olfactory experiments and prevents odor contamination across conditions.

Subjects were left alone in the room during the experiment, and activity in the room was continuously monitored from the adjacent control room via a one-way mirror and video monitor. A same-sex experimenter completed all interactions with subjects except compound presentation, which was performed by an opposite-sex experimenter. This experimenter-to-subject relationship was consistently maintained, in accordance with the suggestion in Jacob, Hayreh, and McClintock (2001) of increased response under these conditions. Presentation of the mood scale, video clips, compound sampling instructions, and recording of physiological data were all

time-locked through one central computer. Participants were tested at the same time of day on each of the 3 days. After completing a demographics questionnaire and providing written informed consent, subjects were taken into the testing room and seated in front of a computer monitor. A keypad was positioned in front of them, and they were instructed to answer the questions that would appear on the monitor after the experimenter had left the room. At this point, the first baseline mood scale was administered via the monitor. After subjects had completed the baseline scale, the experimenter reentered the room and fitted the physiological recording equipment to the subject. When physiological setup was complete, 7 min of habituation and remote BP calibration measurements were performed.

This period enabled subjects to relax and habituate to their new setting. Once physiological measures stabilized, recording was then initiated to obtain a 5-min physiological baseline. During this time, subjects watched a video of the ocean that is commonly used for its nonarousing contents (Piferi, Kline, Younger, & Lawler, 2000). This baseline recording was followed by the second administration of the mood scale. Next, an opposite-sex experimenter entered the room and held the appropriate experimental jar under the participant's nose for each of six sniffs that were timed and cued by computer-generated digitized voice instructions. The digitized voice prompted the subject to sniff at a tone following a count-down (e.g., "three, two, one, sniff"). After each sniff, subjects rated compound intensity, pleasantness, and familiarity on a 1–9-point scale presented on the monitor. There was no verbal interaction between experimenters and subjects during compound presentation. The experimenter then left the room, and subjects watched four consecutive 10-min segments (V1 through V4) of a nature video, answering the mood scale again in between each segment. The nature video was chosen and edited for neutrality. Physiological data were recorded throughout the experiment. The same procedure was carried out with a different compound on each of the 3 experimental days.

Data Reduction and Data Analysis

Physiological data were first expressed as a change score for each period of interest by subtracting the baseline value from that period (V1, V2, V3, and V4). Results were then expressed as *z*-scores in order to compare between subjects. As a result of technical difficulties in data acquisition, ST, TR, and BP were unavailable for 1 subject each, and movement data were unavailable for 2 subjects.

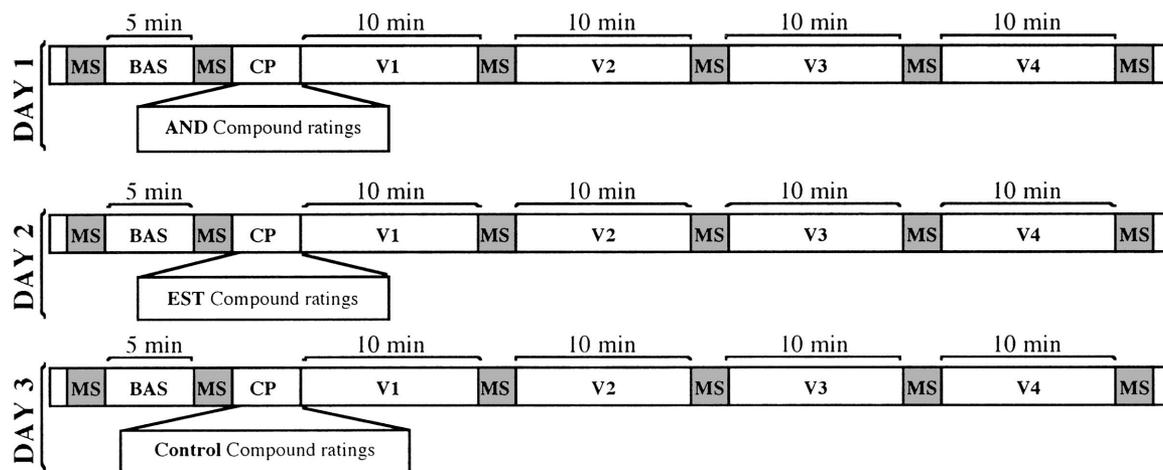


Figure 1. Experimental design. Each subject was tested on 3 separate days with each of the three compounds (4,16-androstadien-3-one [AND], 1,3,5(10),16-estratetraen-3-ol [EST], or control) presented on a different day. MS = administration of the 17-item mood scale; BAS = baseline; CP = compound presentation; V = presentation of the emotionally neutral, 10-min nature video, during which mood-change measures were taken.

Psychological data were also expressed as change scores for each period of interest by subtracting the baseline value from that period (after V1, after V2, after V3, and after V4). In order to reduce the number of comparisons and to provide a clear picture of the psychological effects of AND, EST, and the control compound on men and women, the 16 items of the Ekman's scale were entered into a principal component analysis (with Varimax rotation) and a hierarchical cluster analysis (Pearson distance). The resulting groups were then used in the analysis. Because it was not in the original Ekman inventory, sexual arousal was analyzed separately.

Separate analyses of variance (ANOVAs) for each physiological measure (and each mood category) were performed, including sex (men and women) as a between-subjects variable, and time (V1, V2, V3 and V4) and compounds (AND, EST and control) as within-subject variables. Planned comparisons between AND, EST, and control separately for men and women were performed when significant interactions (Sex \times Compound \times Time, or Sex \times Compound) were observed.

Results

During Compound Presentation

Perception. To ask whether the compounds differed significantly in percept, we entered subjects' estimates of intensity, pleasantness, and familiarity into three separate ANOVAs with sex as the between-subjects variable and compound as the within-subject variable (Figure 2). No significant sex, compound effects, or Sex \times Compound interactions were evident in any of these ANOVAs. In other words, the three compounds did not differ in intensity, pleasantness, or familiarity.

Physiology. To ask whether the compounds differently affected physiological measures during compound presentation, we entered all measures into separate ANOVAs. Despite some trends, no significant sex and compound effects, or Sex \times Compound interactions were observed for any of the physiological measures during the 91 s (\pm 9.4 s) of compound presentation ($p > .05$ in all cases).

After Compound Presentation

Mood. In order to avoid arbitrary inclusion and exclusion of parameters, we first combined psychological descriptors based on principal components analysis. Principal components analysis revealed Primary Components 1 and 2 that together explained around 50% of the variance, and contrasted positive moods with negative moods (see Figure 3a). A hierarchical ascending cluster

analysis (Pearson distance, $r = .428$) performed on the 16 descriptors of Ekman's scale further confirmed the existence of two main clusters, corresponding respectively to the positive moods amused, content, happy, calm, confident and interested, and the negative moods afraid, sad, disgusted, angry, annoyed, anxious, bored, contemptuous, embarrassed, and stressed (Figure 3a).

To ask whether the different compounds uniquely affected these psychological factors in men and women over time, we conducted ANOVAs with sex as a between-subjects variable and time and compounds as within-subject variables. No Sex \times Compound or Sex \times Compound \times Time interactions were revealed in these ANOVAs ($p > .05$ in all cases). In other words, in comparison to an olfactory control, AND and EST did not affect positive or negative moods nor sexual arousal in men and women, regardless of time since stimulation (Figure 3b).

Physiology. In order to avoid arbitrary inclusion and exclusion of parameters, we examined overall physiological effects by constructing a composite physiological arousal index. This was justified in light of Pearson tests that indicated significant positive correlations between the physiological variables measured. Specifically, positive correlations were observed between: (a) NS-SCR and EP ($r = .171$, $p < .01$), ECG ($r = .108$, $p < .05$), and BP ($r = .164$, $p < .01$); (b) EP and ECG ($r = .871$, $p < .01$), FP ($r = .227$, $p < .01$), AR ($r = .221$, $p < .01$), and BP ($r = .206$, $p < .01$); (c) ECG and FP ($r = .185$, $p < .01$), AR ($r = .221$, $p < .01$) and BP ($r = .135$, $p < .02$); (d) TR and FP ($r = .133$, $p < .02$); TR and BP ($r = .223$, $p < .01$). ST was the only variable that showed a significant negative correlation with other physiological variables (ST vs. ECG: $r = -.104$, $p < .05$), a finding consistent with previous results (Jacob, Hayreh, McClintock, 2001). Considering these correlations within the data, all physiological measures were equally weighted in the physiological arousal index such that an increase in physiological arousal was associated with an increase in NS-SCR, ECG, FP, EP, BP, AR, TR and movement, and with a decrease in ST. Figure 4 provides means and standard errors of the overall physiological arousal index data during the four 10-min periods (V1, V2, V3, and V4).

To ask whether physiological arousal differed in response to the different compounds over time in men and women, we conducted ANOVAs with sex as a between-subjects variable and time and compounds as within-subject variables. Significant Sex \times Compound, $F(2, 44) = 6.542$, $p < .01$, and Sex \times Compound \times Time,

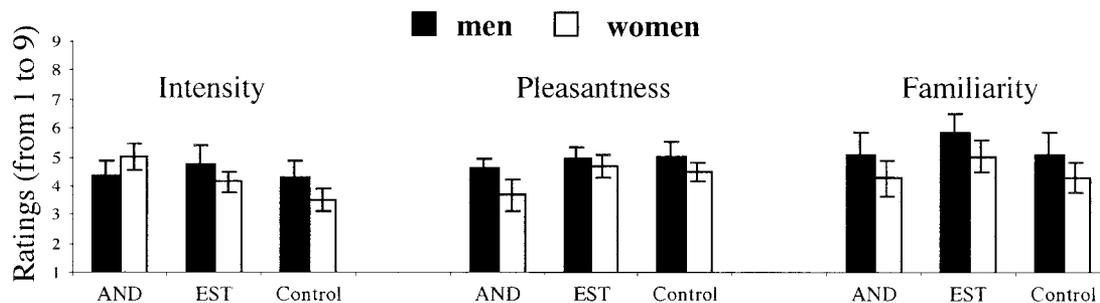


Figure 2. Mean (\pm SEM) ratings of intensity, pleasantness, and familiarity of 4,16-androstadien-3-one (AND), 1,3,5(10),16-estratetraen-3-ol (EST), and the control compound in men and women. The three compounds did not differ across these perceptual measures.

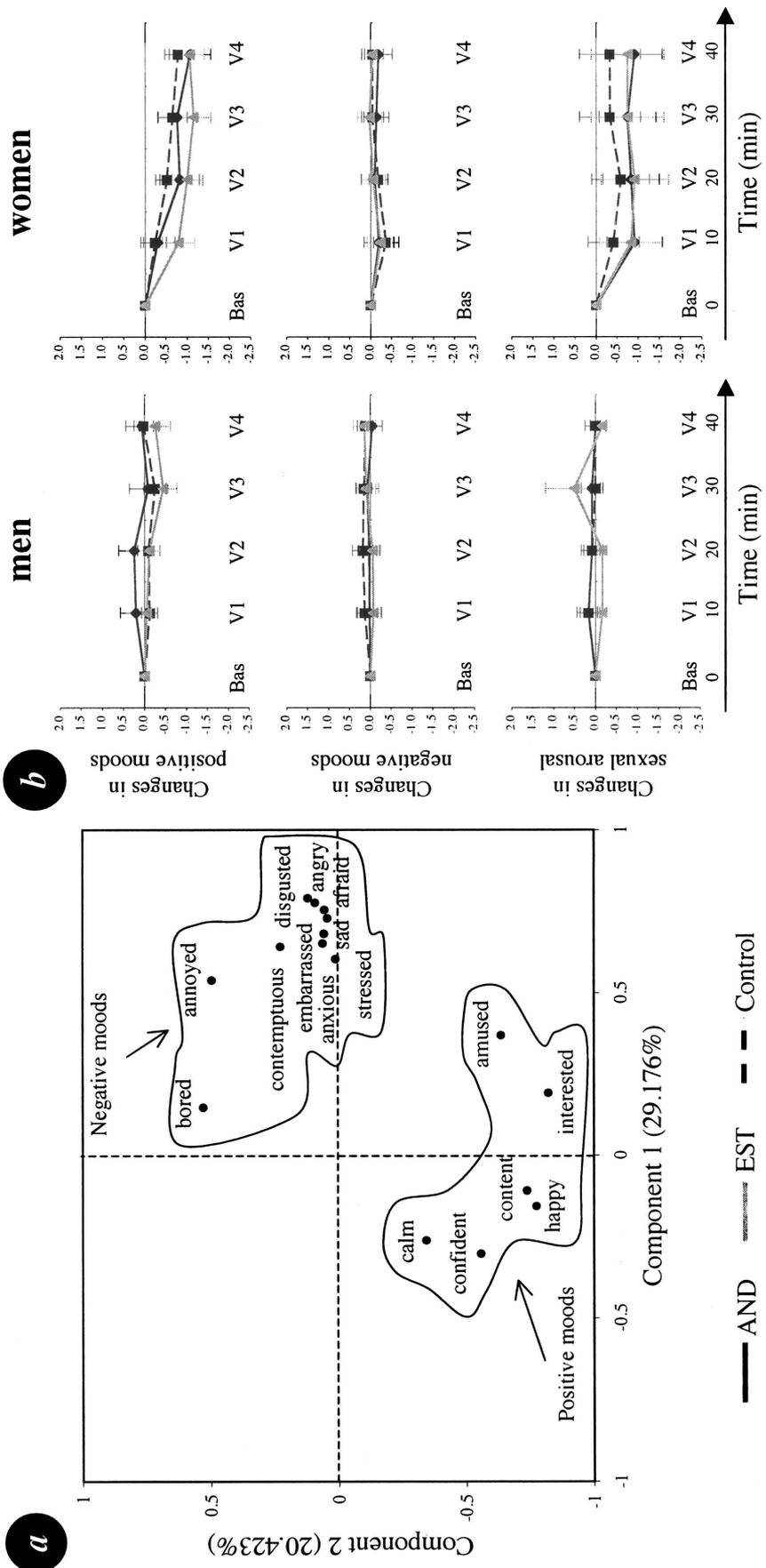


Figure 3. a: Principal component analysis performed on the 16 mood descriptors from Ekman's scale. Components 1 and 2 clearly opposed positive moods to negative moods. The hierarchical ascending clustering (Pearson distance) confirmed the existence of two main clusters corresponding to positive moods and negative moods. b: Mean (\pm SEM) differences in positive moods, negative moods, and sexual arousal as a function of time (from baseline [Bas] to V4) in men and women for 4,16-androstadien-3-one (AND), 1,3,5(10),16-estratetraen-3-ol (EST), and control. No significant differences in the effect of AND, EST, or the control were evident in men or women ($p > .05$ in all cases). V = presentation of the emotionally neutral, 10-min nature video.

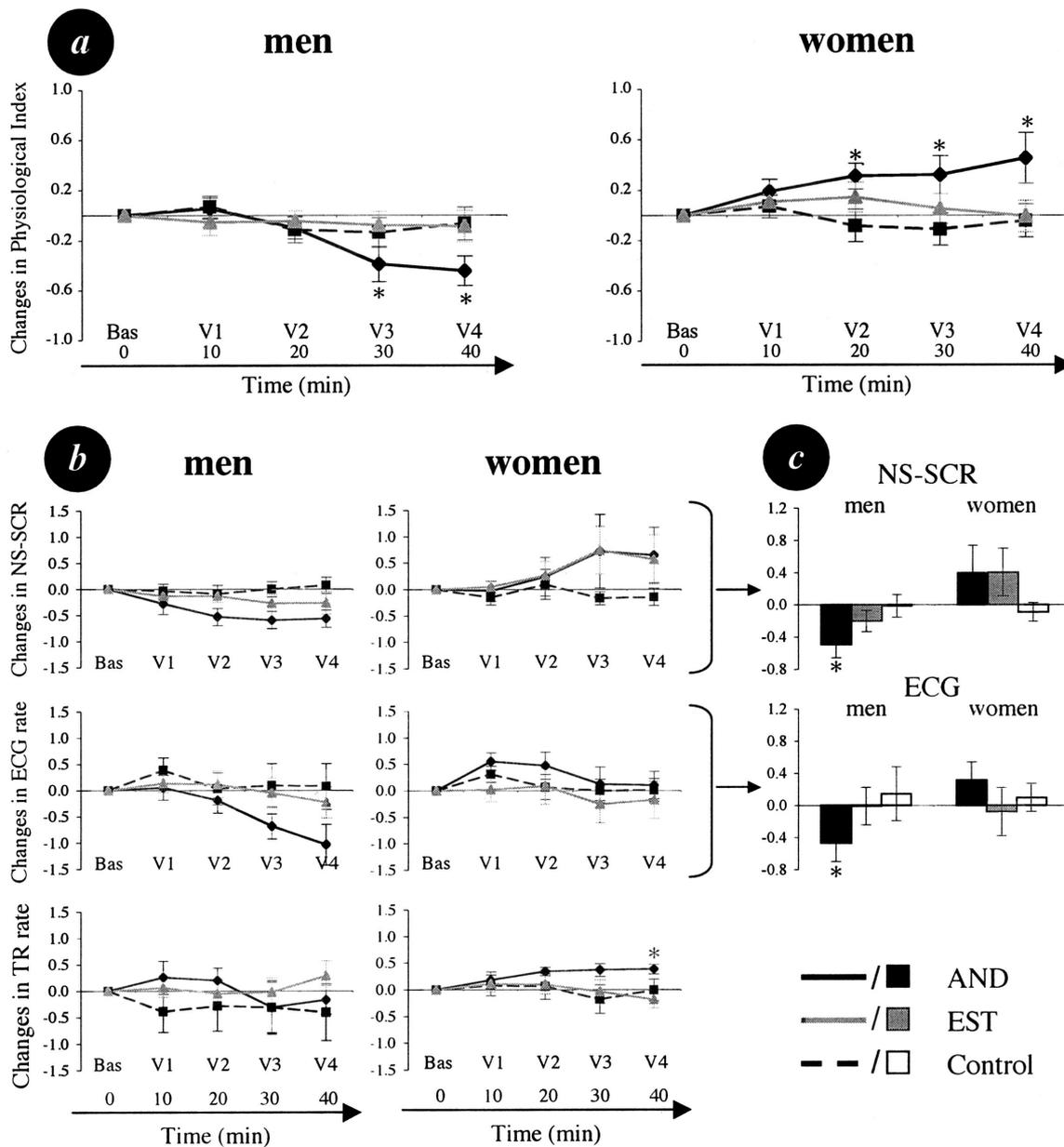


Figure 4. a: Mean (\pm SEM) differences in the physiological arousal index as a function of time (from baseline [Bas] to V4) in men and women for 4,16-androstadien-3-one (AND), 1,3,5(10),16-estratetraen-3-ol (EST), and control. AND induced a significant increase in physiological arousal in women and a significant decrease in men. b: Mean (\pm SEM) changes in nonspecific skin conductance response (NS-SCR), electrocardiogram (ECG) rate, and thoracic respiration (TR) rate in men and women for AND, EST, and control as a function of time (from baseline to V4). Effects were consistent across these three measures but were most pronounced in NS-SCR in women and ECG in men. Normalized data are presented in z scores (c). Mean (\pm SD) raw unnormalized baseline values were NS-SCR (events per minute): men = 3.05 ± 2.86 , women = 2.29 ± 2.37 ; ECG (beats per minute): men = 68.9 ± 10.6 , women = 71.7 ± 13.7 ; TR (breaths per minute): men = 15.8 ± 3.6 , women = 14.6 ± 3.3 . V = presentation of the emotionally neutral, 10-min nature video. Asterisks indicate a significant difference between responses ($p < .05$).

$F(6, 132) = 5.550, p < .01$, interactions were found. These reflected that in women, AND, compared with the control, increased physiological arousal during V2, $t(11) = 2.771, p < .02$; V3, $t(11) = 2.384, p < .04$; and V4, $t(11) = 2.338, p < .04$, and

that AND, compared with EST, increased physiological arousal during V4, $t(11) = 2.815, p < .02$ (Figure 4a). By contrast, in men AND, compared with the control, decreased physiological arousal during V4, $t(11) = -2.569, p < .03$, and AND, compared with

EST, decreased physiological arousal during V3, $t(11) = -2.232$, $p < .05$; and V4, $t(11) = -2.531$, $p < .03$ (Figure 4a). In other words, AND had opposite effects on men and women, increasing arousal in women and decreasing arousal in men.

In order to assess the individual contribution of the different physiological parameters to the physiological arousal index, we examined each measure separately. ANOVAs performed on each physiological parameter, including sex as a between-subjects variable and compounds and time as within-subject variables, revealed significant Sex \times Compound interactions for NS-SCR, $F(2, 44) = 3.777$, $p = .03$, and ECG, $F(2, 44) = 3.805$, $p = .03$, and significant Sex \times Compound \times Time interactions for TR, $F(6, 126) = 2.401$, $p = .03$, and ST, $F(6, 126) = 2.294$, $p = .04$. The Sex \times Compound interaction observed for skin conductance reflected a decrease in NS-SCR in men following AND, $t(11) = -2.383$, $p = .04$, but not EST, $t(11) = -0.769$, $p = .45$, relative to control. In contrast, women displayed a trend toward increased NS-SCR following both compounds: AND versus control, $t(11) = 1.371$, $p = .19$; EST versus control, $t(11) = -1.824$, $p = .09$ (Figure 4c). Mean comparisons following the significant Sex \times Compound interaction on ECG data reflected a decrease in heart rate in men following AND, $t(11) = -2.259$, $p = .04$, but not EST, $t(11) = 0.815$, $p = .43$, relative to control. No equivalent pattern was observed in women (Figure 4c). Regarding the significant Sex \times Compound \times Time interactions, mean comparisons between AND, EST, and Control performed for each 10-min period indicated that, compared with EST, AND increased TR rate in women, specifically during the last period: V4, $t(10) = 3.457$, $p = .006$ (Figure 4b). All other comparisons were not significant. Concerning ST, no significant differences were observed between AND, EST, and control in both men and women. Taken together, the above individual comparisons suggest that NS-SCR, ECG, and TR were the most robust components of the overall physiological index response.

Discussion

Our findings suggest that in a highly controlled and uneventful environment, high concentrations of AND induce small but significant effects on physiological arousal in a sex-specific manner, increasing arousal in women but decreasing it in men. Effects of EST were mostly nonsignificant. Furthermore, neither compound significantly affected mood in this setting. The increased physiological arousal was primarily related to changes in skin conductance (NS-SCR), heart rate (ECG), and thoracic respiration (TR), suggesting that increased physiological arousal here refers to increased sympathetic tone (Berntson, Cacioppo, & Quigley, 1993; Bradley, 2000; Critchley, 2002; Dawson et al., 2000; Grosser et al., 2000; Jacob, Hayreh, McClintock, 2001). As noted in the introduction, common odorants may impact mood and physiological arousal (Alaoui-Ismaili et al., 1997; Bensafi et al., 2002; Brauchli et al., 1995; Graham et al., 2000; Lehrner et al., 2000), and these effects may also be sex-specific (Lehrner et al., 2000). The nature of sex specificity seen here, however, appears different from that seen with common odorants. Common odorants may have sex-specific effects in that they affect one sex but not the other, or one sex more than the other. Here, the same compound affected both sexes, but with opposite effects on physiological

arousal. We know of no such response profile to common odorants not emitted from a bodily source.

Jacob, Hayreh, and McClintock (2001) suggested that the effects of AND and EST may be strongly context dependent. Indeed, one may predict that a chemical signal will obtain increased significance in a relevant behavioral context. In order to address this possibility, one must first characterize the baseline response induced by these compounds, that is, the response induced in the absence of any pronounced emotional and/or behavioral context. To this end, we maximized our effort to normalize the environment and rid it of physiologically and psychologically arousing events other than compound presentation. Thus, we consider the responses (or lack thereof) measured here to be a baseline response for comparison to future measurement under varying physiological and psychological conditions.

As noted in the introduction, there have been some conflicting results on the physiological and psychological effects of AND. Regarding psychological effects, Jacob and McClintock (2000) evaluated the effects of AND and EST on mood and found that 6 min after compound exposure, both compounds increased positive mood in women and decreased it in men (Experiment 1 of that report). They then replicated their findings in women and also found long-term effects whereby positive mood was maintained 2 hr after exposure only with AND in these women (Experiment 2 of that report). Similarly, Grosser et al. (2000) also reported a decrease in negative feelings with AND in women 40 min after exposure. In the current study, we did not find significant effects on mood. This difference between the current and previous results may reflect the significant effort to minimize external variance made in the current study.

Regarding physiological effects, Grosser et al. (2000) reported significant decreases in respiratory and cardiac frequency and skin conductance as well as increased body temperature and alpha cortical activity in women 40 min after AND exposure. From these results, it was suggested that AND exerted parasympathetic-like, calming effects on physiology in women. In contrast, Jacob, Hayreh, and McClintock (2001) found that AND and EST both increased temperature in men and decreased it in women, and both compounds increased skin conductance, an effect more prevalent in women than in men. This suggested an arousing effect of AND that is greater in women. Thus, our results on physiology are in general agreement with Jacob, Hayreh, and McClintock (2001) but contradict Grosser et al. (2000).

The differences between our results and those of previous studies may be attributed to methodological variables such as the following:

1. Compound source. Whereas Jacob and McClintock (2000), Savic et al. (2001), and the current study used compounds from Steraloids, Grosser et al. (2000) used compounds from Pherin Pharmaceuticals.
2. Compound dilution. Whereas Grosser et al. and Jacob and McClintock used greatly diluted compounds, Savic et al. and the current study used undiluted compounds.
3. Mode of administration. Whereas Grosser et al. administered AND directly to a pit on the nasal septum (putative vomeronasal organ) via a nasal spray pulse, Jacob

and McClintock swabbed compounds diluted in clove oil on the upper lip of subjects, and Savic et al. and the current study had subjects sniff the compound from a jar.

- Measures for mood estimation. Whereas Jacob and McClintock used the Profile of Mood State, Grosser et al. used the Derogatis Inventory, and we used the 9-point mood ratings scale.
- Study design. Whereas Grosser et al. and Experiment 2 of Jacob and McClintock tested the effects of AND on women only, Experiment 1 of Jacob and McClintock, Savic et al., and the current study used a 3×2 mixed design (within for compounds and between for sex) in which effects of AND, EST, and a control compound were tested on both men and women.

These methodological differences reflect decisions we made regarding this particular study. We chose to use a 3×2 mixed design and an undiluted compound delivered in jars because we set out to probe for physiological and psychological correlates to the patterns of brain activation seen by Savic et al. (2001). Savic et al. used a 3×2 mixed design and undiluted compounds in jars, thus we did the same. We chose compound presentation by opposite-sex experimenter because Jacob, Hayreh, and McClintock (2001) had reported increased effects under these conditions. Finally, we chose the mood rating scale because, in its brevity, it is appropriate under the conditions of concurrent physiological recording (Levenson et al., 1990). Although the above methodological decisions we made were not ideal for the purpose of probing all aspects of these compounds, these methods were deemed appropriate for the current questions. Future studies will address the response to these compounds under different environmental conditions, and following dosage that is biologically realistic. The latter is of utmost significance, as the dosage used here, which was chosen so as to enable comparison to the Savic et al. results, is far from biological,

and may even be considered pharmacological. In fact, one may raise the concern that crystal particles may have been inhaled into the nasal cavity and acted transdermally rather than through transduction of an airborne vapor. Considering that the compounds were deposited at the bottom of a rather deep jar that was sampled by subjects with great hesitation, we find this possibility unlikely, but we cannot rule it out completely.

A secondary aim of this study was to probe for physiological and psychological correlates to the pattern of compound-induced PET brain activation reported by Savic et al. (2001). The overall pattern of results in the present study resembled compound-specific hypothalamic activation primarily for AND (see Figure 5). Specifically, in men, AND induced less hypothalamic activation compared with the control compound (in Savic et al.), which was reflected in the present study in reduced heart rate and skin conductance responses. In contrast, in women, AND induced more hypothalamic activation compared with control (in Savic et al., 2001), which was reflected in the present study in increased skin conductance and respiration rate. That increased hypothalamic response as measured with PET would be reflected in increased physiological arousal as measured in our study is consistent with the role of the hypothalamus in driving skin conductance (Koss & Davison, 1976) and respiratory (von Euler, Wexler, & Herrero, 1970) responses. In comparison to AND, the effects of EST on mood and physiology were far less convincing in this study, and we found no physiological correlate to the dissociation in PET response between men and women following EST.

Taken together, these and previous findings support a sex-specific hypothalamic response to AND. Such a response would be consistent with the consideration of AND as a putative human pheromone. Unfortunately, the question of human pheromones is currently largely semantic; that is, it is strongly dependent on the definition of pheromone one chooses to use. The term *pheromone* is an adaptation coined by Karlson and Lüscher (1959) relating to the term *ectohormones* used by Bethe (1932). Karlson and Lüscher

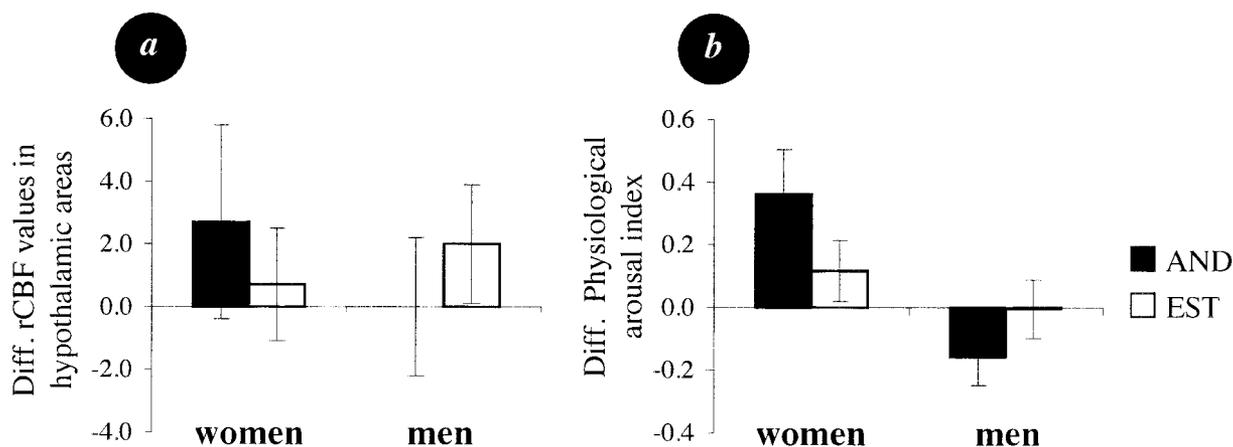


Figure 5. Physiological measures recorded here compared with the hypothalamic PET response. Comparison of (a) differences in cerebral activation from Savic et al. 2001 (extrapolated from data in Figure 2b of that paper), and (b) physiological effects seen within each sex in the present study, based on the difference between the tested compound and control. Whereas the physiological response to 4,16-androstadien-3-one (AND) mirrored the PET response, the physiological response to 1,3,5(10),16-estratetraen-3-ol (EST) did not. Diff. = difference in; rCBF = regional cerebral blood flow.

defined pheromones as “substances which are secreted to the outside by an individual of the same species, in which they release a specific reaction, for example, a definite behavior or a developmental process” (p. 55). Regarding AND, it would be tempting to define it as a putative human pheromone because it is released by one member of the species (primarily in men through sweat), and as the combination of previous studies and ours here suggests, it modulates a set of reactions. Indeed, we find it impressive that a brief exposure to a compound significantly affected physiology 40 min later, in opposing directions in men and women. That said, one may question use of the term *pheromone* altogether in relation to mammalian chemosignaling (Beauchamp, Doty, Moulton, & Mugford, 1976; Doty, 2003). Indeed, the combination of multiple definitions that are also incredibly broad does not help in the application of this term in a scientific context. We prefer to circumvent this semantic debate and conclude in saying that our findings suggest that AND, a steroidal compound present in human sweat, affects physiological arousal in a sex-specific manner, and in an extended time-frame that suggests a cascade of responses different from those induced by common odorants not derived from bodily sources (e.g., baking powder in this study). Furthermore, our present findings, combined with numerous findings on the behavioral effects of human-derived chemical signals (Ackerl, Atzmueller, & Grammer, 2002; McCoy & Pitino, 2002; Nicholson, 1984; Preti et al., 2003; Rothardt & Beier, 2001; Shinohara, Morofushi, Funabashi, & Kimura, 2001), which induce brain activation even when not consciously detected (Jacob, Kinnunen, Metz, Cooper, & McClintock, 2001; Sobel et al., 1999) together suggest that chemical signaling among humans may play a much larger role in human behavior than is usually acknowledged.

References

- Ackerl, K., Atzmueller, M., & Grammer, K. (2002). The scent of fear. *Neuroendocrinology Letters*, 23(2), 79–84.
- ADIstruments. (2002). Chart (Version 4.1.1) [Computer software]. Colorado Springs, CO: Author.
- Alaoui-Ismaili, O., Vernet-Maury, E., Dittmar, A., Delhomme, G., & Chanel, J. (1997). Odor hedonics: Connection with emotional response estimated by autonomic parameters. *Chemical Senses*, 22, 237–248.
- Aujard, F. (1997). Effect of vomeronasal organ removal on male socio-sexual responses to female in a prosimian primate (*Microcebus murinus*). *Physiology & Behavior*, 62, 1003–1008.
- Beauchamp, G. K., Doty, R. L., Moulton, D. G., & Mugford, R. A. (1976). The pheromone concept in mammals: A critique. In R. L. Doty (Ed.), *Mammalian olfaction, reproductive processes, and behavior* (pp. 143–160). New York: Academic Press.
- Bensafi, M., Rouby, C., Farget, V., Bertrand, B., Vigouroux, M., & Holley, A. (2002). Autonomic nervous system responses to odours: The role of pleasantness and arousal. *Chemical Senses*, 27, 703–709.
- Berghard, A., Buck, L. B., & Liman, E. R. (1996). Evidence for distinct signaling mechanisms in two mammalian olfactory sense organs. *Proceedings of the National Academy of Sciences, USA*, 93, 2365–2369.
- Berliner, D. L., Monti-Bloch, L., Jennings-White, C., & Diaz-Sanchez, V. (1996). The functionality of the human vomeronasal organ (VNO): Evidence for steroid receptors. *Journal of Steroid Biochemistry and Molecular Biology*, 58(3), 259–265.
- Berntson, G. G., Cacioppo, J. T., & Quigley, K. S. (1993). Cardiac psychophysiology and autonomic space in humans: Empirical perspectives and conceptual implications. *Psychological Bulletin*, 114(2), 296–322.
- Bethe, A. (1932). Vernachlassigte Hormone [The neglected hormone]. *Naturwissenschaften*, 11, 177–181.
- Bradley, M. M. (2000). Emotion and motivation. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), *Handbook of psychophysiology* (pp. 602–642). New York: Cambridge University Press.
- Brauchli, P., Ruegg, P. B., Etzweiler, F., & Zeier, H. (1995). Electrodermal and autonomic alteration by administration of a pleasant and an unpleasant odor. *Chemical Senses*, 20, 505–515.
- Brennan, P. A., & Keverne, E. B. (1997). Neural mechanisms of mammalian olfactory learning. *Progress in Neurobiology*, 51, 457–481.
- Christensen, T. A., & Hildebrand, J. G. (2002). Pheromonal and host-odor processing in the insect antennal lobe: How different? *Current Opinion in Neurobiology*, 12(4), 393–399.
- Comfort, A. (1971, April 16). Likelihood of human pheromones. *Nature*, 230, 432–433.
- Critchley, H. D. (2002). Electrodermal responses: What happens in the brain. *Neuroscientist*, 8(2), 132–142.
- Dawson, M. E., Schell, A. M., & Filion, D. L. (2000). The electrodermal system. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), *Handbook of psychophysiology* (pp. 200–223). New York: Cambridge University Press.
- Doty, R. L. (2003). Mammalian pheromones: Fact or fantasy? In R. L. Doty (Ed.), *Handbook of olfaction and gustation* (2nd ed., pp. 345–383). New York: Marcel Dekker.
- Doty, R. L., Snyder, P. J., Huggins, G. R., & Lowry, L. D. (1981). Endocrine, cardiovascular, and psychological correlated of olfactory sensitivity changes during the human menstrual cycle. *Journal of Comparative and Physiological Psychology*, 95, 45–60.
- Dulac, C., & Axel, R. (1998). Expression of candidate pheromone receptor genes in vomeronasal neurons. *Chemical Senses*, 23, 467–475.
- Ekman, P., Freisen, W. V., & Ancoli, S. (1980). Facial signs of emotional experience. *Journal of Personality and Social Psychology*, 39(Supp. 6), 1125–1134.
- Gower, D. B., Nixon, A., & Mallet, A. I. (1988). The significance of odorous steroids in axillary odour. In S. Van Toller & G. H. Dodd (Eds.), *Perfumery: The psychology and biology of fragrance* (pp. 45–76). London: Chapman and Hall.
- Gower, D. B., & Ruparelia, B. A. (1993). Olfaction in humans with special reference to odorous 16-androstenes: Their occurrence, perception and possible social, psychological and sexual impact. *Journal of Endocrinology*, 137(2), 167–187.
- Graham, C. A., Janssen, E., & Sanders, S. A. (2000). Effects of fragrance on female sexual arousal and mood across the menstrual cycle. *Psychophysiology*, 37(1), 76–84.
- Grosser, B. I., Monti-Bloch, L., Jennings-White, C., & Berliner, D. L. (2000). Behavioral and electrophysiological effects of androstadienone, a human pheromone. *Psychoneuroendocrinology*, 25(3), 289–299.
- Hansson, B. S. (2002). A bug’s smell—research into insect olfaction. *Trends in Neuroscience*, 25, 270–274.
- Jacob, S., Hayreh, D. J., & McClintock, M. K. (2001). Context-dependent effects of steroid chemosignals on human physiology and mood. *Physiology & Behavior*, 74, 15–27.
- Jacob, S., Kinnunen, L. H., Metz, J., Cooper, M., & McClintock, M. K. (2001). Sustained human chemosignal unconsciously alters brain function. *NeuroReport*, 12, 2391–2394.
- Jacob, S., & McClintock, M. K. (2000). Psychological state and mood effects of steroidal chemosignals in women and men. *Hormones and Behavior*, 37, 57–78.
- Karlson, P., & Lüscher, M. (1959). “Pheromones”: A new term for a class of biologically active substances. *Nature*, 183, 55–56.
- Koss, M. C., & Davison, M. A. (1976). The electrodermal response as a model for central sympathetic reactivity: The action of clonidine. *European Journal of Pharmacology*, 37, 71–78.
- Lehrner, J., Eckersberger, C., Walla, P., Potsch, G., & Deecke, L. (2000).

- Ambient odor of orange in a dental office reduces anxiety and improves mood in female patients. *Physiology & Behavior*, 71, 83–86.
- Levenson, R. W., Ekman, P., & Friesen, W. V. (1990). Voluntary facial action generates emotion-specific autonomic nervous system activity. *Psychophysiology*, 27(4), 363–384.
- Mair, R. G., Bouffard, J. A., Engen, T., & Morton, T. H. (1978). Olfactory sensitivity during the menstrual cycle. *Sensory Processes*, 2(2), 90–98.
- McClintock, M. K. (1998). On the nature of mammalian and human pheromones. In C. Murphy (Ed.), *Annals of the New York Academy of Sciences: Vol. 855. Olfaction and Taste XII: An International Symposium* (pp. 390–392). New York: New York Academy of Sciences.
- McCoy, N. L., & Pitino, L. (2002). Pheromonal influences on sociosexual behavior in young women. *Physiology & Behavior*, 75, 367–375.
- Meredith, M. (2001). Human vomeronasal organ function: A critical review of best and worst cases. *Chemical Senses*, 26, 433–445.
- Monti-Bloch, L., Diaz-Sanchez, V., Jennings-White, C., & Berliner, D. L. (1998). Modulation of serum testosterone and autonomic function through stimulation of the male human vomeronasal organ (VNO) with pregna-4,20-diene-3,6-dione. *Journal of Steroid Biochemistry and Molecular Biology*, 65(1–6), 237–242.
- Monti-Bloch, L., & Grosser, B. I. (1991). Effect of putative pheromones on the electrical activity of the human vomeronasal organ and olfactory epithelium. *Journal of Steroid Biochemistry and Molecular Biology*, 39(4B), 573–582.
- Monti-Bloch, L., Jennings-White, C., & Berliner, D. L. (1998). The human vomeronasal system. A review. In C. Murphy (Ed.), *Annals of the New York Academy of Sciences: Vol. 855. Olfaction and Taste XII: An International Symposium* (pp. 373–389). New York: New York Academy of Sciences.
- Monti-Bloch, L., Jennings-White, C., Dolberg, D. S., & Berliner, D. L. (1994). The human vomeronasal system. *Psychoneuroendocrinology*, 19(5–7), 673–686.
- Nicholson, B. (1984). Does kissing aid human bonding by semiochemical addition? *British Journal of Dermatology*, 111, 623–627.
- Nixon, A., Mallet, A. I., & Gower, D. B. (1988). Simultaneous quantification of five odorous steroids (16-androstenes) in the axillary hair of men. *Journal of Steroid Biochemistry*, 29(5), 505–510.
- Pause, B. M., Sojka, B., Krauel, K., Fehm-Wolfsdorf, G., & Ferstl, R. (1996). Olfactory information processing during the course of the menstrual cycle. *Biological Psychology*, 44(1), 31–54.
- Piferi, R. L., Kline, K. A., Younger, J., & Lawler, K. A. (2000). An alternative approach for achieving cardiovascular baseline: Viewing an aquatic video. *International Journal of Psychophysiology*, 37(2), 207–217.
- Preti, G., Wysocki, C. J., Barnhart, K. T., Sondheimer, S. J., & Leyden, J. J. (2003). Male axillary extracts contain pheromones that affect pulsatile secretion of luteinizing hormone and mood in women recipients. *Biology of Reproduction*, 68, 2107–2113.
- Rothardt, G., & Beier, K. (2001). Peroxisomes in the apocrine sweat glands of the human axilla and their putative role in pheromone production. *Cellular and Molecular Life Sciences*, 58, 1344–1349.
- Savic, I., Berglund, H., Gulyas, B., & Roland, P. (2001). Smelling of odorous sex hormone-like compounds causes sex-differentiated hypothalamic activations in humans. *Neuron*, 31, 661–668.
- Shinohara, K., Morofushi, M., Funabashi, T., & Kimura, F. (2001). Axillary pheromones modulate pulsatile LH secretion in humans. *NeuroReport*, 12, 893–895.
- Smith, T. E., Tomlinson, A. J., Mlotkiewicz, J. A., & Abbott, D. H. (2001). Female marmoset monkeys (*Callithrix jacchus*) can be identified from the chemical composition of their scent marks. *Chemical Senses*, 26(5), 449–458.
- Sobel, N., & Brown, W. M. (2001). The scented brain: Pheromonal responses in humans. *Neuron*, 31, 512–514.
- Sobel, N., Prabhakaran, V., Hartley, C. A., Desmond, J. E., Glover, G. H., Sullivan, E. V., et al. (1999). Blind smell: Brain activation induced by an undetected air-borne chemical. *Brain*, 122(Pt. 2), 209–217.
- Thysen, B., Elliott, W. H., & Katzman, P. A. (1968). Identification of estra-1,3,5(10),16-tetraen-3-ol (estratetraenol) from the urine of pregnant women (1). *Steroids*, 11(1), 73–87.
- von Euler, C., Wexler, I., & Herrero, F. (1970). Control mechanisms determining rate and depth of respiratory movements. *Respiration Physiology*, 10(1), 93–108.

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Correction to Wang and Hsiao (2003)

In the article “Amphetamine Sensitization: Nonassociative and Associative Components,” by Ying-Chou Wang and Sigmund Hsiao (*Behavioral Neuroscience*, 2003, Vol. 117, No. 5, pp. 961–969), the institutional affiliation for Ying-Chou Wang is incomplete. The correct affiliation is Ching Kuo Institute of Management and Health and National Chung Cheng University.
