Brain response to putative pheromones in homosexual men

Ivanka Savic*†‡, Hans Berglund§, and Per Lindström*

Departments of *Clinical Neuroscience and §Medicine, Karolinska University Hospital, 171 76 Stockholm, Sweden; and †Department of Neuroscience, Center for Gender-Related Medicine, Karolinska Institute, 171 77 Stockholm, Sweden

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The testosterone derivative 4,16-androstadien-3-one (AND) and the estrogen-like steroid estra-1,3,5(10),16-tetraen-3-ol (EST) are candidate compounds for human pheromones. AND is detected primarily in male sweat, whereas EST has been found in female urine. In a previous positron emission tomography study, we found that smelling AND and EST activated regions covering sexually dimorphic nuclei of the anterior hypothalamus, and that this activation was differentiated with respect to sex and compound. In the present study, the pattern of activation induced by AND and EST was compared among homosexual men, heterosexual men, and heterosexual women. In contrast to heterosexual men, and in congruence with heterosexual women, homosexual men displayed hypothalamic activation in response to AND. Maximal activation was observed in the medial preoptic area/anterior hypothalamus, which, according to animal studies, is highly involved in sexual behavior. As opposed to putative pheromones, common odors were processed similarly in all three groups of subjects and engaged only the olfactory brain (amygdala, piriform, orbitofrontal, and insular cortex). These findings show that our brain reacts differently to the two putative pheromones compared with common odors, and suggest a link between sexual orientation and hypothalamic neuronal processes.

olfaction | positron emission tomography | hypothalamus | homosexual males

According to animal studies, the choice of sexual partner is highly influenced by sex-specific pheromone signals, which are processed by male and female mating centers located in the anterior hypothalamus (1–3). A lesion of the respective mating center, as well as impairment of pheromone transduction, may alter the coital approach in a sex-specific way (3, 4).

In a majority of animals, pheromone signals are transferred to the hypothalamus from the vomeronasal organ via the accessory olfactory nerve (5). Because our vomeronasal pit lacks neuronal connections to the brain (5, 6), the occurrence of pheromone transduction has long been questioned in humans. Several recent observations, however, suggest that this type of chemical communication cannot be ruled out. Sex steroid-derived compounds such as 4,16-androstadien-3-one (AND) and, less consistently, estra-1,3,5(10),16-tetraen-3-ol (EST) have been reported to induce sex-specific effects on the autonomic nervous system, mood, and context-dependent sexual arousal (7–12). The exact effects of AND and EST vary with the administered dose and experimental design, but, nevertheless, they seem to be sex-differentiated (especially with respect to AND), and thus to differ from the effects of ordinary odors. Studies with positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have shown that smelling both AND and of EST activates the human brain (13–15), even in nonodorous concentrations (14, 15). AND is a derivative of testosterone and is primarily produced in male sweat (16), whereas EST is an estrogen-resembling steroid that has been detected in the urine of pregnant women (17). Male sweat has recently been reported to alter the pulsative hypothalamic release of luteinic hormone in females in an ovulation-promoting way (18). Thus, although it is premature to classify AND and EST as pheromones, the data suggest that they may function as chemosignals.

In a previous PET study of regional cerebral blood flow (rCBF) in heterosexual subjects (13), we found that smelling AND and EST caused a sex-differentiated activation of the anterior hypothalamus. In women, AND activated the preoptic area and ventromedial nuclei, whereas, in men, activation by EST involved an area covering the paraventricular and dorsomedial nuclei. In contrast, when men smelled AND and women EST, activations were found only in amygdala plus piriform cortex, anterior insular cortex, orbitofrontal cortex, and anterior cingulate cortex. These areas are reported to process the signals of common odors (19, 20, 21), and were possibly recruited by the odor components of AND and EST. Our interpretation of this sex-differentiated pattern of activation was that the two steroid compounds may act bimodally, both as pheromones and odors. We proposed the hypothesis that the anterior hypothalamus primarily processed signals from the pheromone-like component of AND and EST, whereas the olfactory brain primarily mediated the signals of their odor component. Depending on the sex of the responder in relation to the specific compound (AND or EST), one pathway dominated, whereas the other was suppressed. This hypothesis was based upon observations of a similar phenomenon in studies of other bimodal odorants (for example, acetone) (22).

In the present study, we investigated the question whether the pattern of activation induced by AND and EST could be related to sexual orientation rather than to the biological sex. We therefore compared the pattern of activation between homosexual men and heterosexual men and women. The activations were induced by smelling AND, EST, and ordinary odors (here denoted as OO). Smelling of odorless air served as the baseline condition. The following issues were addressed in particular: (*i*) In homosexual men (HoM), is the hypothalamus activated by AND, EST, or both? (*ii*) Is the pattern of activation in HoM similar to that in heterosexual men (HeM) and that in heterosexual women (HeW), or are entirely different regions involved in HoM? (*iii*) If there are group differences, are they confined to the pheromone-like compounds, or do they occur also with OO?

Methods

Thirty-six healthy, unmedicated, right-handed, and HIV-negative HeM, HeW, and HoM (12 in each group), who were osmic for both AND and EST and had normal MRI of the brain, participated in the study. The groups were matched for age (28 ± 2, 26 ± 2, and 33 ± 7 yr) and educational level, and differed only with respect to biological sex and sexual orientation. All HeW

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Abbreviations: HoM, homosexual men; HeM, heterosexual men; HeW, heterosexual women; AND, 4,16-androstadien-3-one; EST, estra-1,3,5(10),16-tetraen-3-ol; AIR, odorless air; OO, ordinary odors; PET, positron emission tomography; rCBF, regional cerebral blood flow; MRI, magnetic resonance imaging; ROI, region of interest.

‡To whom correspondence should be addressed at: Department of Neuroscience, Karolinska Institute, Retzius väg 8, 171 77 Stockholm, Sweden. E-mail: ivanka.savic-berglund@neuro.ki.se.

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and HeM scored 0, whereas all HoM scored 6 on the Kinsey heterosexual/homosexual scale (0 = maximally heterosexual, 6 = maximally homosexual) (23). Seven HeW, six HeM, and six HoM had a stable sexual partner. Women were investigated during the second to third week of the menstrual cycle.

Before PET experiments, we tested odor thresholds in each subject with the butanol test (21). Using an identical approach, we also tested odor thresholds to AND and EST. For this purpose, the two compounds were diluted in odorless mineral oil. At about 10 a.m. on the day of scanning, blood samples were drawn from each subject for measurements of the concentrations of the following hormones: dehydroepiandrosterone sulfate, follicle-stimulating hormone, luteinizing hormone, bound and free testosterone, prolactin, and androstenedione.

During PET experiments, all of the stimuli were presented in glass bottles at a distance of 10 mm from the nose. The OO consisted of lavender oil, cedar oil, eugenol, and butanol. The butanol was administered at a concentration of 10%; the other odors were undiluted. All of the odor concentrations were thus suprathresholded. Because, theoretically, dissolving of AND and EST could change their possible pheromone properties (24), the respective compound was presented in crystalline and odorous form (200 mg, Steraloids, Newport, RI) during PET scans. The purity was 98%, as tested repetitively at our doping laboratory (Department of Pharmacology, Karolinska University Hospital).

PET measurements were carried out at the same time of day. Furthermore, the room temperature and air pressure were standardized (23°C, 997 hPa) (21). The three groups of subjects were investigated over the same time period and by the same experimenters. The experimental protocol has been described in detail elsewhere (13, 19, 21). In summary, it included MRI scans, and PET measurements of rCBF with ^{15}O H_2O. The resolution of the PET scanner was 3.8 mm. Four conditions were used: smelling odorless air (baseline condition, denoted here as AIR), smelling AND, smelling EST, and smelling OO. There were 12 scans per person (three scans per condition, balanced and randomly interleaved). During AND, EST, and AIR scans, the same item was presented four times for 15 s per time, with 5 s in between, in an on-off mode. During the OO scans, separate odors were presented consecutively with an identical scheme. The subjects were instructed to breathe normally. They were informed that they would smell odor or odorless air, without knowing the type or order of items.

Respiratory movements were recorded continuously 2 min before, and during each scan, by using a strain gauge around the lower thorax connected to a graph (Comair, Stockholm). After the PET scans, the participating subjects rated AND, EST, and OO for pleasantness, irritability, intensity, and familiarity, using a 100-mm bipolar visual-analogue scale (21).

Image Analysis. The individual MRI and PET images were reformatted into a common space (standard brain) and filtered with 10-mm Gaussian kernel as described (13, 19, 21). Significant activations were then determined with statistical parametric mapping statistics (SPM99, Wellcome Foundation, London) (25), by using the contrasts AND-AIR, EST-AIR, and OO-AIR. Three types of analyses were performed.

On the basis of previous findings in heterosexual subjects (13), we first investigated whether the hypothalamus in HoM was activated in congruence with that in HeW or HeM. For this purpose, a region of interest (ROI) analysis was carried out. Because of the difficulty in determining the anatomical boundaries of the anterior hypothalamus, the ROIs were defined from hypothalamic activations by AND in HeW (covering the preoptic plus ventromedial nuclei) and by EST in HeM (covering the dorsomedial plus paraventricular nuclei), generated earlier (13). The rCBF was in all subjects first normalized to the global mean in the brain of 50 ml/min per 100 g. The mean rCBF of the three scans per condition (13) was then calculated in each ROI and subject for AIR, AND, and EST. Differences between HoM, HeW, and HeM on the basis of AND-AIR and EST-AIR in each predetermined ROI were tested with two-way repeated measure ANOVA with subject group as the between factor and the type of steroid as within factor. In case of a significant interaction in a main effect, the results were further explored with appropriate contrast. P values were considered significant when <0.05.

There is a possibility, however, that HoM might have an entirely different pattern of activation in extrahypothalamic structures. The statistical evaluation therefore relied primarily on explorative and

Table 1. Activations

	HoM			HeM			HeW		
Region	*z* level	Size, cm^3	Coordinates	*z* level	Size, cm^3	Coordinates	*z* level	Size, cm^3	Coordinates
EST-AIR									
Hypothalamus				4.2	0.7	+4, −14, −2			
R amygdala plus piriform cortex	*4.4*	*0.3*	*+24, 0, −6**				4.4	1.1	+34, 0, −14
L amygdala plus piriform cortex	4.5	1.4	−22, −4, −2				*4.3*	*0.3*	*−20, −24, −8**
Cingulate							4.0	0.6	−8, +30, +34
AND-AIR									
Hypothalamus	5.5	1.0	+8, −2, −2				5.4	0.8	−6, 0, −12
R amygdala plus piriform plus insular cortex				5.1	1.3	+30, 0, −12			
				4.4	1.1	+38, −8, +14			
L amygdala plus piriform plus insular cortex									
Cingulate				*4.5*	*0.6*	*−10, +30, −2**			
OO-AIR									
R amygdala plus piriform plus insular cortex	4.2	0.9	+18, +4, −16	4.6	0.8	+22, +4, −12	4.9	3.2	+24, −8, 0
L amygdala plus piriform plus insular cortex	4.6	3.4	−26, −2, −10	5.8	3.0	−18, +4, −13	4.5	0.9	−38, +2, +6

Activations calculated with one-random effect analysis (SPM99). All the significant clusters, calculated with T-threshold at $P = 0.001$ (a corrected $P < 0.05$), are included. Talairach coordinates indicate local maxima. The OO clusters also covered minor portions of anterior cingulate. R, right; L, left.

*Italics indicate subsignificant clusters calculated at T-threshold at $P = 0.001$ and with a corrected $P < 0.1$. These clusters were included to illustrate that the distributions of activations of the olfactory circuits during smelling of the two steroids were similar in the three groups.

Table 2. Conjunctional clusters

Region	HoM and HeW			HoM and HeM			HeM and HeW		
	z level	Size, cm^3	Coordinates	*z* level	Size, cm^3	Coordinates	*z* level	Size, cm^3	Coordinates
EST vs. AIR									
R amygdala plus piriform plus insular cortex	4.9	1.0	+26, −6, −12				4.1	0.4	+34, −10, −8
L amygdala plus piriform plus insular cortex	4.4	1.5	−24, −2, −8	5.1	1.0	−20, +4, −16	4.0	0.8	−24, 0, −12
AND vs. AIR									
Hypothalamus	4.0	0.9	−6, −2, −12						
R amygdala plus piriform cortex				3.7	0.9	+16, +6, −10			
L amygdala plus piriform plus insular cortex							3.6	0.9	−26, +2, −8
OO vs. AIR									
R amygdala plus piriform plus insular plus cortex	6.4	6.4	+22, −2, −12	5.6	5.5	+20, +2, −12	6.3	5.5	+22, 0, −14
L amygdala plus piriform plus insular cortex	6.6	6.6	−20, −2, −10	5.7	5.9	−24, 0, −14	6.4	5.1	−18, −2, −14

Activations calculated with conjunctional analysis (SPM99). T-threshold at $P = 0.001$ (corrected $P < 0.05$). R, right; L, left.

user-independent analysis with statistical parametric mapping (SPM99) (25), using the entire brain as search space. Significant activations were first tested in each separate group with one-group random effect analysis (SPM99 basic model, height threshold at $P = 0.001$, corrected $P < 0.05$). Next, we used conjunctional analysis (25, 26) to investigate which activations, if any, were common to the two or more groups. Finally, we tested whether there were any differences between HoM, HeM, and HeW with two-group random effect analysis (SPM99, basic models, two-group *t* test; height threshold at $P = 0.001$, corrected $P < 0.05$) (25).

To locate the hypothalamic clusters more precisely, the coordinates of Talairach's atlas were translated to those of Schaltenbrant's atlas (27, 28), which visualizes the hypothalamic nuclei in detail.

Comparisons with Psychophysical Parameters and Hormone Levels. The mean respiratory amplitude and frequency were first calculated during each prescan and scan period. The percentage difference between the scan and prescan value was then compared among HoM, HeM, and HeW with respect to AIR, AND, EST, and OO by using a two-way ANOVA, factoring for subject group and stimulus type including AIR, as described (13, 19). A two-way ANOVA was used also to test group differences in odor ratings, but the stimuli were AND, EST, and OO, because AIR was perceived as odorless. Finally, the group differences in hormone levels and odor thresholds were tested with separate one-way ANOVAs. The significance level was 0.05 for all comparisons.

Results

The hypothesis-based ROI analysis showed that the HoM processed AND congruently with HeW rather than with HeM. As in HeW, in HoM, rCBF increased significantly in the preoptic plus ventromedial ROI during smelling of AND ($P = 0.03$), but not of EST ($P = 0.05$). The AND-induced activation was significant compared with AND-AIR in HeM ($P = 0.03$). EST also induced an increase in rCBF in HoM, but in the dorsomedial plus paraventricular ROI ($P = 0.0003$). As in HeM, this increase was significant compared with EST-AIR in HeW ($P = 0.01$). No other differences between homosexual and heterosexual subjects were observed.

The explorative statistical parametric mapping analysis confirmed the previously reported (13) dissociation of activations by AND and EST, in that HeW showed activation of the anterior hypothalamus with AND, whereas, in HeM, this area was recruited during smelling of EST (Tables 1 and 2 and Fig. 1 *Upper*). As in HeW but not in HeM, in HoM, the anterior hypothalamus was activated with AND. When HoM smelled EST, the left amygdala and piriform cortex were primarily recruited (although with inclusion of a minor portion of the anterior hypothalamus) (Table 1 and

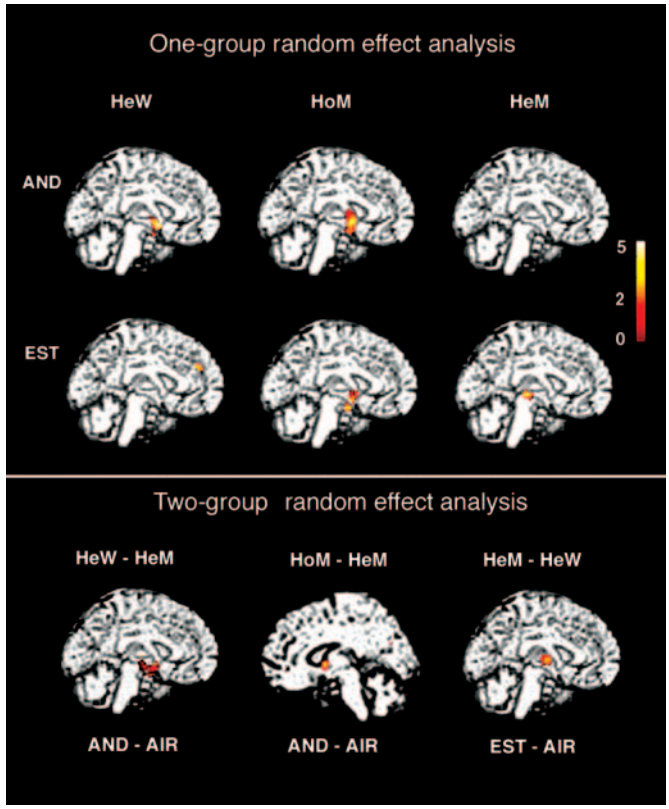

Fig. 1. Illustration of group-specific activations with the putative pheromones. (*Upper*) Cerebral activation during smelling of AND and EST. Clusters of activated regions are superimposed on the standard MRI brain (SPM99), midsagittal plane. The inferior portion of the EST cluster in homosexual men is in the amygdala and piriform cortex. (*Lower*) Significant differences between the groups. Shown are the clusters calculated with two-group random effect analysis. The Sokoloff color scale illustrates *z* values reflecting the degree of activation. Only significant activations are shown. Because the same brain section is chosen, the figures do not always illustrate maximal activation for each condition.

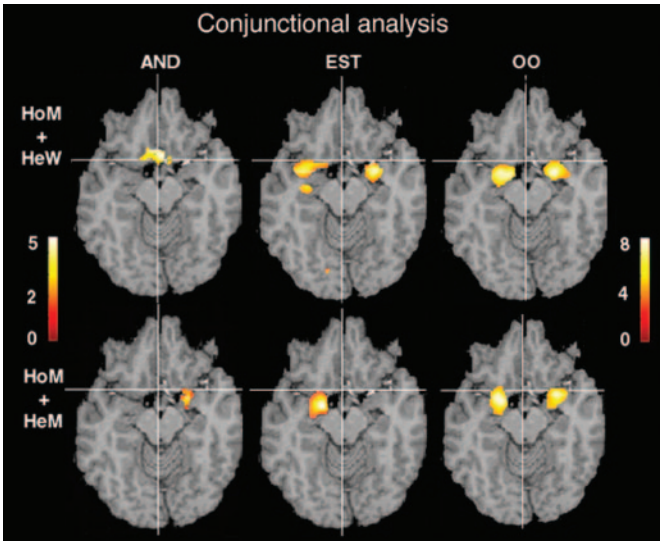

Fig. 2. Common activations. Shown are conjunctional clusters in different groups of subjects, superimposed on the standard brain. All images show horizontal level at $z = -12$ according to Talairach's atlas. The Sokoloff color scale in illustrates z values, which are 0.0–5.0 for activations with AND and EST, and 0.0–8.0 for activations with OO. Only significant activations are shown. Because the same brain section is chosen, the figures do not always illustrate maximal activation for each condition. The subject's right side is to the right.

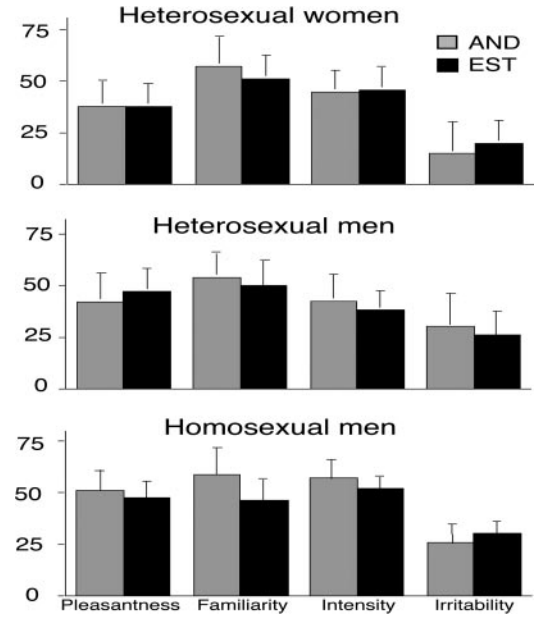

Fig. 4. Odor ratings for AND and EST. The vertical axis indicates a visual analogue scale in millimeters (mean ± SEM). (*Top*) Heterosexual women. (*Middle*) Heterosexual men. (*Bottom*) Homosexual men. None of the ratings differed between the three groups of subjects.

Fig. 1 *Upper*). There was also a subsignificant activation of the right amygdala and piriform cortex. Thus, the HoM showed a pattern of activation that resembled that of HeW rather than of HeM. In contrast to the two steroids, OO yielded similar activations in all three groups. Furthermore, these activations were confined to the olfactory brain (19–21) (Table 1).

The results (based on one-group random effect analysis) were in accordance with those from conjunctional analysis. HoM shared hypothalamic activation only with HeW, and only when smelling AND (Fig. 2 and Table 2). HoM thus showed no hypothalamic involvement in common with HeM. In contrast, they shared clusters with the HeM in the amygdala plus piriform plus insular cortex. Conjunctional clusters in these classical odor-processing regions were observed in all three groups, and independently of the type of odorous stimulus (Table 2 and Fig. 2).

The group comparisons (two-group random effect analysis) showed that HoM differed only from HeM. This difference consisted in AND-induced activation of the hypothalamus in HoM (Talairach's coordinates in the random effect analysis contrasting HoM–HeM with respect to AND-AIR were +6, +2, +2; $z = 4.7$; size 0.6 cm^3, height threshold $P = 0.001$; corrected $P < 0.05$) (Fig. 1). Random effect analysis also showed differences between HeW and HeM. The peak coordinates for this comparison were +3, +2, −13 (yielded by the contrast HeW–HeM with respect to AND-AIR; $z = 4.2$, cluster size 0.8 cm^3) and +6, −8, +2 (yielded by the contrast HeM–HeW with respect to EST-AIR; $z = 4.0$, cluster size 0.4; height threshold at $P = 0.001$, corrected $P < 0.05$) (Fig. 1). Thus, as reported (13), HeW and HeM differed only regarding the hypothalamic activation by AND and EST (Table 1).

Given that homosexual behavior can be induced in male ferrets, rats, and mice by damage to the preoptic nucleus (1–3), it was of interest to locate the hypothalamic clusters more precisely. An exact

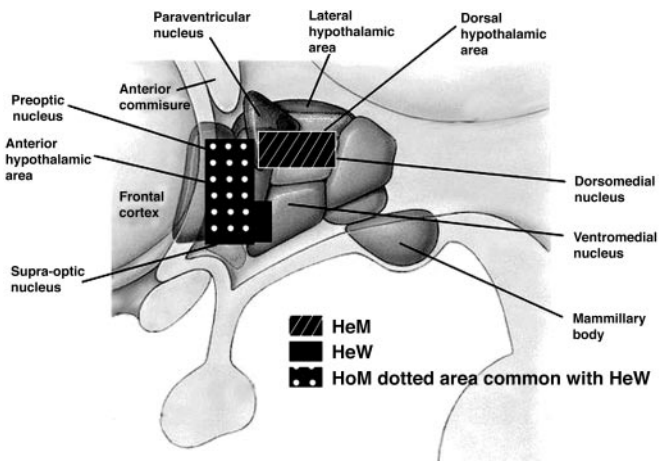

Fig. 3. A schematic presentation of the areas covered by the hypothalamic clusters.

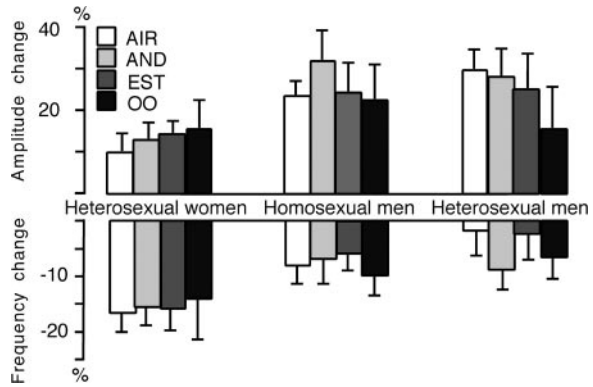

Fig. 5. Respiratory movements. The vertical axis shows percentage change in mean amplitude and frequency in relation to the mean baseline value. All of the groups showed an increase in amplitude and a decrease in frequency during scans compared with the respective prescan baselines, independently of stimulus type. Data are expressed as mean and SEM. No significant differences were observed between the groups irrespective of the stimulus type. As previously reported, the variation within each group was relatively high.

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Table 3. Hormone levels

Group	DHEAS, μmol/liter	S-testosterone, free, nmol/liter	S-testosterone, nmol/liter	S-prolactin, μg/liter	S-FSH, units/liter	S-LH, units/liter	S-androstendione, nmol/liter
HoM	9.8 ± 4.3	11.6 ± 5.8	17.2 ± 6.8	4.4 ± 1.4	4.8 ± 4.7	3.8 ± 2.3	5.9 ± 1.1
HeM	9.8 ± 2.6	11.7 ± 3.6	21.7 ± 7.0	3.8 ± 1.6	3.8 ± 5.5	3.5 ± 1.5	5.9 ± 0.7

DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

localization was considered relevant despite the 10-mm image filtering, because the clusters were at least 10 mm apart. To justify such an evaluation, we first tested whether the hypothalamic clusters obtained at group level showed a similar localization in each individual of the respective group. Coregistration and repositioning of PET clusters on individual reformatted MRI images revealed similar cluster locations in different subjects, without any systematic shifts between the groups. In all HoM, as in all HeW, the AND cluster incorporated an area corresponding to the preoptic, ventromedial, and tuberomamillary nuclei (Table 1). The EST cluster covered the dorsomedial and paraventricular nuclei in HeM (Table 1). In HoM, the EST cluster showed a local maximum in the amygdala plus piriform cortex, but encompassed a minor portion of the hypothalamus. Because this portion was anterior to the EST-related cluster of HeM, we hypothesized that the possible EST-induced hypothalamic activation in HoM differed from that in HeM. To test this possible difference, a separate post hoc random effect analysis was performed with a reduced search volume, defined with a manually drawn rectangular mask incorporating only the hypothalamus, fornix, and medial amygdalae (Talairach's coordinates: $x = -20$ to $+20$; $y = +20$ to -40; $z = -13$ to $+5$). A significant difference was found; as expected, this difference consisted in more pronounced activation in HeM, the maximum corresponding to the location of the dorsomedial nucleus (Talairach's coordinates $+8, -10, -2$; $z = 5.4$, uncorrected $P = 0.013$). The respective hypothalamic clusters are shown schematically in Fig. 3.

These data raised the question whether the direct contrasts between the effects of the two steroids also would differ between homo- and heterosexual subjects. When the entire brain was used as a search space, no clusters were observed for AND-EST and vice versa in any group. However, when applying the rectangular mask described in the previous paragraph, the HeW showed a hypothalamic cluster for AND-EST ($+8, -2, -8$; z score 4.1, with a second peak corresponding to Talairach's coordinates of $-18, -20, -8$; corrected $P < 0.05$); in contrast, a cluster was detected in the amygdala and piriform cortex for EST-AND ($+12, +20, -20$ and $-18, +20, -16$; $z = 4.2$). HeM displayed significant EST-AND activation at corrected $P = 0.08$ with a peak coordinate of $+4, 0, -2$ ($z = 4.1$); they also displayed AND-EST activations, but in the olfactory circuits: the insula and piriform cortex ($+30, -2, -2$; $z = 3.8$) and the anterior cingulate cortex ($-10, +32, +6$ and $+22, +40, +2$; $z = 4$). In HoM, clusters were found only at a corrected P of <0.1. AND-EST showed a cluster corresponding to the fornix ($+16, -20, -8$; $z = 4.1$), whereas the EST-AND cluster covered the amygdala and piriform cortex ($-24, -6, -14$; $z = 3.7$). No other clusters were found. Together, these data suggest the occurrence of partly overlapping activations induced by AND and EST (see *Discussion*).

There were no significant group–odor interactions for any of the rating parameters (Fig. 4). Neither did we find any group–stimulus interaction in respiratory amplitude or frequency (Fig. 5).

No group differences were observed either in odor thresholds or plasma concentrations of the tested hormones (Tables 3 and 4).

Discussion

As discussed (13), signals from AND and EST seem to be bimodal, and primarily mediated either by the hypothalamus or by the olfactory regions. Consistent with the fact that both compounds were odorous, the conjunctional analysis showed involvement of olfactory areas even when the hypothalamic pathway predominated (Table 2).

The major finding in the present study was that the preferred pathway in relation to the presented compound was associated with the responder's sexual orientation (at least in men) rather then the biological sex. This finding was based on an objective and user-independent state-of-the-art method, consistent across several types of analysis. According to the method applied, the material was sufficient to generate inference at group level, implying that each subject was representative of his or her designated group (25, 26).

The odors presented in this study have been used in several of our previous experiments (13, 19, 21, 22). To avoid the possibility that the results would rely on one specific odor, four different smells were used during the OO scans. In contrast, AND and EST were presented four times during the same scan. It might be claimed that the OO condition could produce greater activity in odor-processing areas than the pheromone conditions just because novel smells were presented during the OO-scans. However, a previous activation study with vanillin, presented in the same manner as AND and EST, showed no significant difference in the pattern or degree of activation compared with OO (19). Furthermore, the presently observed distribution and order of magnitude of the activation of olfactory regions by EST in HoM and HeW, and by AND in HeM were not consistently different from those resulting from OO. It is thus conceivable that the on-off mode of stimulus presentation prevented habituation, at least to a certain degree, thereby minimizing a potential bias due to presentation of one versus several compounds during the respective scans.

Another issue requiring clarification is that no significant clusters were found in the olfactory brain when HoM and HeW smelled AND, or when HeM smelled EST, although both compounds were clearly perceived as odorous. That these circuits were indeed involved is, however, indicated by the emergence of clusters in the olfactory regions also in conditions showing the hypothalamic activation in the between-group conjunctional analysis (Table 2). To address this issue more specifically, we conducted a post hoc test analyzing which activations were common for AND and EST (in relation to AIR) within each separate group of subjects. Conjunctional clusters for AND and EST were found in the amygdala,

Table 4. Olfactory thresholds

Group	Butanol, M	AND, M	EST, M
HoM	$2.4 \times 10^{-5} \pm 4.0 \times 10^{-5}$	$1.5 \times 10^{-4} \pm 1.0 \times 10^{-4}$	$3.5 \times 10^{-4} \pm 2 \times 10^{-4}$
HeM	$5.0 \times 10^{-5} \pm 5.0 \times 10^{-5}$	$1.0 \times 10^{-4} \pm 0.5 \times 10^{-4}$	$1.0 \times 10^{-4} \pm 2 \times 10^{-4}$
HeW	$5.0 \times 10^{-5} \pm 1.0 \times 10^{-5}$	$1.0 \times 10^{-4} \pm 1.5 \times 10^{-4}$	$2.0 \times 10^{-4} \pm 2 \times 10^{-4}$

piriform cortex, and a minor portion of the anterior insula in all of the groups (Talairach's coordinates for local maxima were −22, +4, −14 and +4, −16, +4 in HeM; +26 −2, −8 and +20, −14, −14 in HeW; and −20, −4, −16 in HoM).

Together, these data support the previously proposed hypothesis that odorous pheromones may act bimodally, and use two different pathways (see ref. 13 and the introduction). Furthermore, they imply that activations induced by AND and EST are partially overlapping. This overlap could explain the lack of significant clusters when contrasting the two steroids with each other and using the whole brain as search space. It applies especially to HoM, whose amygdala plus piriform cluster generated by EST also covered a minor portion of the anterior hypothalamus.

The maximal activations with AND and EST were clearly separable, reproducible (ref. 13, published data), and different from activations caused by common odors (13, 19–21). They were assessed with statistical parametric mapping statistics, which is conservative with respect to type 1 error. Furthermore, the experimental conditions were standardized and identical in all subjects. When adding to that the improbability of chance activation by AND in HoM in the brain area very similar to that in HeW, it seems convincing that we detected an undistorted physiological response. Given the small size of the individual hypothalamic nuclei, however, it is important to emphasize that the finding of a local maximum with atlas coordinates corresponding to the location of a specific hypothalamic nucleus does not imply that only this nucleus was activated. Rather, it indicates that an area of 10 mm around this coordinate was maximally involved. At present, therefore, we can only conclude that HoM differed from HeM and resembled HeW in that their hypothalamus was activated by AND, and with the maximum in the preoptic area.

The preoptic area participates in the integration of hormonal and sensory cues that are necessary for sexual behavior. It harbors cells releasing luteinic hormone-releasing hormone (29). In humans, these cells develop from the migrating neuroblasts of olfactory mucosa (30) and mediate estrogen feedback (31). According to a study by Dorner *et al*. (31), HoM respond to oestrogen injections with increased serum concentrations of luteinic hormone (positive estrogen feedback), thus like HeW and not HeM (31). The preoptic area also harbors neuronal conglomerates (interstitial hypothalamic nuclei) whose possible sexual dimorphism in humans has been discussed (32–34). Their size in humans (<1 mm^3) precludes, however, further argumentation about their relevance for the present results.

The difference between HoM and HeM could reflect a variant differentiation of the anterior hypothalamus in HoM, leading to an altered response pattern. Alternatively, it could reflect an acquired sensitization to AND stimuli in the hypothalamus or its centrifugal networks, due to repeated sexual exposure to men (35). A third possibility is that HeW and HoM associated AND with sex, whereas HeM made a similar association with EST. These tentative mechanisms are not mutually exclusive, nor can they be discriminated on the basis of the present PET data.

Whether the concentrations of AND and EST used during the present experiments are relevant for physiological conditions is at present uncertain. It has been reported, however, that the neuronal response to pheromones becomes saturated already at 10^{-8} M, and that the tuning curve does not broaden with increasing concentrations (36). Thus, the response may be similar in high and normal environmental concentrations. Finally, it is important to emphasize that the present study was not designed to address the issue of olfactory pathways. This said, when considering the short time course of the rCBF increase and the longer time course with humoral distribution of AND during experiments with boars (37), a chemical-sensing pathway seems much more probable than absorbation into the blood stream. As to the discussion concerning the locus for nasal detection, it is of interest to note that some recent preliminary observations suggest a possibility of pheromone signal transduction through the olfactory mucosa (38, 39). A further clarification of this matter needs much more extensive investigation. Nevertheless, the differentiated pattern of cerebral activation with AND and EST compared with OO observed in the present study offers argument for the singularity of these two compounds, and strengthens the notion that signal responses from putative pheromones could operate in humans also. In addition, the colocalization of hypothalamic responses with brain circuits that are involved in human reproduction and that in animals are designed to recognize sex further indicates hypothalamic involvement in physiological processes related to sexual orientation in humans.

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