Brain response to putative pheromones in homosexual men

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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.

Methods
Thirty-six healthy, unmedicated, right-handed, and HIV-negative HeM, HeW, and HoM (12 in each group), who were osmotic 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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measurements of rCBF with $^{15}$O H2O. The resolution of the PET
elsewhere (13, 19, 21). In summary, it included MRI scans, and PET
menters. The experimental protocol has been described in detail
standardized (23°C, 997 hPa) (21). The three groups of subjects were
purity was 98%, as tested repetitively at our doping laboratory
form (200 mg, Steraloids, Newport, RI) during PET scans. The
respective compound was presented in crystalline and odorous
odors were undiluted. All of the odor concentrations were thus
butanol was administered at a concentration of 10%; the other
consisted of lavender oil, cedar oil, eugenol, and butanol. The
vapor was administered at a concentration of 10%; the other
odors were undiluted. All of the odor concentrations were thus
supratresholded. 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).

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
supratresholded. 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 stan-
dardized (23°C, 997 hPa) (21). The three groups of subjects were
investigated over the same time period and by the same experi-
menters. 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 H2O. 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, smel-
lng 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
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 be-
fore, 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 reform-
matted 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 map-
ing 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
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 hypo-
thalamus, 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 30 ml/min per 100 g.
The mean CBF 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
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
significantly different when $P < 0.05$.

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

<table>
<thead>
<tr>
<th>Region</th>
<th>z level</th>
<th>Size, cm³</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>HoM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EST-AIR Hypothalamus</td>
<td>4.4</td>
<td>0.3</td>
<td>+24, 0, -6*</td>
</tr>
<tr>
<td>R amygdala plus piriform cortex</td>
<td>4.2</td>
<td>0.7</td>
<td>+4, -14, -2</td>
</tr>
<tr>
<td>L amygdala plus piriform cortex</td>
<td>4.5</td>
<td>1.4</td>
<td>-22, -4, -2</td>
</tr>
<tr>
<td>Cingulate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HoM</td>
<td>5.5</td>
<td>1.0</td>
<td>+8, -2, -2</td>
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<tr>
<td>R amygdala plus piriform cortex</td>
<td>5.1</td>
<td>1.3</td>
<td>+30, 0, -12</td>
</tr>
<tr>
<td>L amygdala plus piriform cortex</td>
<td>4.4</td>
<td>1.1</td>
<td>+38, -8, +14</td>
</tr>
<tr>
<td>Cingulate</td>
<td>4.5</td>
<td>0.6</td>
<td>-10, +30, -2*</td>
</tr>
<tr>
<td>OO-AIR Hypothalamus</td>
<td>4.2</td>
<td>0.9</td>
<td>+18, +4, -16</td>
</tr>
<tr>
<td>R amygdala plus piriform cortex</td>
<td>4.6</td>
<td>0.8</td>
<td>+22, +4, -12</td>
</tr>
<tr>
<td>L amygdala plus piriform cortex</td>
<td>4.6</td>
<td>3.4</td>
<td>-26, -2, -10</td>
</tr>
</tbody>
</table>

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.

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### Table 2. Conjunctural clusters

<table>
<thead>
<tr>
<th>Region</th>
<th>HoM and HeW</th>
<th>HoM and HeM</th>
<th>HeM and HeW</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>z level</td>
<td>Size, cm³</td>
<td>Coordinates</td>
</tr>
<tr>
<td>EST vs. AIR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R amygdala plus piriform plus insular cortex</td>
<td>4.9</td>
<td>1.0</td>
<td>+26, −6, −12</td>
</tr>
<tr>
<td>L amygdala plus piriform plus insular cortex</td>
<td>4.4</td>
<td>1.5</td>
<td>−24, −2, −8</td>
</tr>
<tr>
<td>AND vs. AIR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>4.0</td>
<td>0.9</td>
<td>−6, −2, −12</td>
</tr>
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<td>R amygdala plus piriform plus insular cortex</td>
<td></td>
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<tr>
<td>OO vs. AIR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R amygdala plus piriform plus insular cortex</td>
<td>6.4</td>
<td>6.4</td>
<td>+22, −2, −12</td>
</tr>
<tr>
<td>L amygdala plus piriform plus insular cortex</td>
<td>6.6</td>
<td>6.6</td>
<td>−20, −2, −10</td>
</tr>
</tbody>
</table>

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 HeW, 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.](image-url)
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 cm³; 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 cm³) 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).

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
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 tuberomammillary 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 than 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 HeW 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. Conjunctural clusters for AND and EST were found in the amygdala,

**Table 3. Hormone levels**

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

DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; LH, luteinizing hormone.
Discussed (32–34). Their size in humans (with increased serum concentrations of luteinic hormone (positive mucosa (30) and mediate estrogen feedback (31). According to a releasing luteinic hormone-releasing hormone (29). In humans, maximum in the preoptic area. Rather, it indicates that an area of 10 mm around this activated. It is important to emphasize that the finding of a local maximum it is of HoM differed from HeM and resembled HeW given the small size of the individual hypothalamic nuclei, however, it is important to emphasize that the finding of a local maximum not only conclude that HoM differed from HeM and resembled HeW in that their hypothalamic nuclei 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 mm³) 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 hypothalamic in HoM, leading to an altered response pattern. Alternatively, it could reflect an acquired sensitization to AND stimuli in the hypothalamic 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 absorption 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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