Brain response to putative pheromones in lesbian women

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The progesterone 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. In previous positron emission tomography studies, we found that smelling AND and EST activated regions primarily incorporating the sexually dimorphic nuclei of the anterior hypothalamus, that this activation was differentiated with respect to sex and compound, and that homosexual men processed AND congruently with heterosexual women rather than heterosexual men. These observations indicate involvement of the anterior hypothalamus in physiological processes related to sexual orientation in humans. We expand the information on this issue in the present study by performing identical positron emission tomography experiments on 12 lesbian women. In contrast to heterosexual women, lesbian women processed AND stimuli by the olfactory networks and not the anterior hypothalamus. Furthermore, when smelling EST, they partly shared activation of the anterior hypothalamus with heterosexual men. These data support our previous results about differentiated processing of pheromone-like stimuli in humans and further strengthen the notion of a coupling between hypothalamic neuronal circuits and sexual preferences.

hypothalamus | olfaction | positron emission tomography | sexual orientation

In animals, the choice of sexual partner is highly influenced by signals from sex-specific pheromones. These signals are processed by specific nuclei located in the anterior hypothalamus, identified as male and female mating centers (1–5). A lesion of the respective mating center as well as impairment of pheromone transduction may alter the coital approach in a sex-specific way (3–5). For example, electrolytic lesion of the preoptic area is reported to shift the mean preference of male ferrets away from the estrous females to the stud males (3, 5). Male rats are found to reduce their coital behavior after destruction of the preoptic area and show more interest in stimulus males than receptive females (1). Female ferrets, however, preferred females after destruction of the ventromedial hypothalamic nucleus (2) and did not allow males to intromit (4), whereas female rats increased the proportion of female approaches after kindling of the preoptic area (6).

In humans, reproductive functions are mediated by neuronal circuits of the anterior hypothalamus. There is reason to believe that these circuits also participate in the integration of the hormonal and sensory cues that are necessary for our sexual behavior and may also be involved in our sexual preferences (7). The preoptic area of the hypothalamus harbors cells releasing luteinic hormone-releasing hormone (8). These cells develop from the migrating neuroblasts of the olfactory mucosa (9) and mediate estrogen feedback. The estrogen feedback differs between males and females and also is reported to differ between homosexual men (HoM) and heterosexual men (HeM) (10). In addition, the anterior hypothalamus contains neuronal conglomerates (interstitial hypothalamic nuclei), of which two are reported to be sexually dimorphic in humans, and in a single study, one was found to differ in volume between HoM and HeM (10–13). A difference between HoM and HeM has also been found in the volume of suprachiasmatic nucleus (14).

In a previous positron emission tomography (PET) study of regional cerebral blood flow (rCBF) in heterosexual subjects, we found that smelling of two steroids, 4,16-androstadien-3-one (AND) and estra-1,3,5(10),16-tetraen-3-ol (EST), activated the anterior hypothalamus in a sex-differentiated manner (15). AND is a progesterone derivative detected in human sweat in concentrations that are ≈10 times higher in men compared with women (16). EST is an estrogen-like steroid that is detected in the urine of pregnant women (17). Both compounds are reported to induce sex-specific effects on the autonomic nervous system, mood, and context-dependent sexual arousal even without conscious perception (18–24), and both have been proposed as candidate compounds for human pheromones. Notwithstanding that the higher complexity of human behavior precludes direct extrapolations from the animal data to human biology, the colocalization of circuits processing signals from the two putative pheromones with the regions mediating mating behavior raises the question about a possible involvement of these same circuits in the physiology of human sexuality and sexual orientation. This issue is further emphasized by recent findings from HoM. Like heterosexual women (HeW) but unlike HeM, HoM activated the preoptic and ventromedial hypothalamic nuclei when smelling AND (25) but the classical olfactory regions (the amygdala, the piriform cortex, and the anterior insular cortex) (26–32) when smelling EST. The pattern of activation was reciprocal in HeM. Notably, signals from common odorants, such as cedar oil and lavender oil, were processed by the classical olfactory regions in HeM as well as in HoM and HeW (25).

Very little is currently known about the physiology of female homosexuality. However, if the chemosensory processing of AND and EST is related to sexual orientation rather than the biological sex, the pattern of activation in lesbian women would be expected to deviate from that of HeW. To investigate this hypothesis, PET experiments were carried out with measurements of rCBF in 12 lesbian women while they smelled AND, EST, and four ordinary odors (OO). Smelling of odorless air (denoted below as AIR) served as the base-line condition, and activations were defined as increases in rCBF during smelling of AND, EST, and OO in relation to air. The experimental design was identical to that of our previous study (25), and the results were compared with the previously generated data from HeM and HeW (25).

Results

As opposed to our previous study groups, the lesbian subjects did not show a differentiated pattern of activation with AND and EST;

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Abbreviations: HeM, heterosexual men; HeW, heterosexual women; HoM, homosexual men; AND, 4,16-androstadien-3-one; EST, estra-1,3,5(10),16-tetraen-3-ol; OO, ordinary odors; AIR, odorless air; PET, positron emission tomography; rCBF, regional cerebral blood flow; SPM, statistical parametric mapping; VOI, volume of interest.

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Table 1. Activations

Region	Lesbian women			HeM			HeW		
	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**			
				4.6	5.6	+10, −12, −2			
Anterior cingulate							**4.3**	**0.7**	**−8, +30, +34**
Right amygdala plus piriform cortex							**4.4**	**1.1**	**+34, 0, −14**
							4.6	3.2	+28, −6, −22
Left amygdala plus piriform and insular cortices	**4.0**	**1.1**	**−22, +16, −8**	5.9	7.2	−26, 0, −22	*3.8*	*1.8*	*−24, +2, −22*
	4.0	11.2	−22, +16, −8*						
Left fusiform gyrus and portion of cerebellar hemisphere	4.2	4.6	−24, −54, −32						
AND − AIR									
Hypothalamus							**5.4**	**0.8**	**−6, +0 , −12**
							5.4	2.5	+2, −2, −8
Right amygdala plus piriform and insular cortices				**5.1**	**1.3**	**+30, 0, −12**			
				4.4	**1.1**	**+38, −8, +14**			
				5.1	9.1	+18, −10, −12†			
Left amygdala plus piriform and insular cortices	**4.5**	**1.2**	**−20, +2, −4**	**4.5**	**3.6**	**−10, +30, −2‡**			
	4.5	5.3	−20, +2, 0						
Right lingular gyrus				4.3	4.4	−6, −60, +6			
Right fusiform gyrus							4.6	2.2	+12, −54, −6
Left superior and medial temporal gyrus	**4.4**	**1.3**	**−42, −66, +20**						
Cerebellum	4.0	4.8	−4, −56, −2						
OO − AIR									
Right amygdala plus piriform, insular, anterior cingulate, and orbitofrontal cortices	**5.1**	**1.1**	**+18, −4, −16**	**4.6**	**0.8**	**+22, +4, −12**	**4.9**	**3.2**	**+24, −8, 0**
Left amygdala plus piriform, insular, and anterior cingulate cortices	**4.7**	**2.5**	**−24, +22, −10**	**5.8**	**3.0**	**−18, +4, −13**	**4.5**	**0.9**	**−38, +2, +6**

Activations were calculated with a one-group random-effect analysis (SPM99). Talairach coordinates indicate local maxima. Bold text indicates a significant cluster at $T = 0.001$ (corrected $P < 0.05$); the areas covered by the respective cluster are indicated. Regular text indicates a significant cluster at $T = 0.01$ (corrected $P < 0.05$). Italic text indicates a cluster at $T = 0.01$ (corrected $P < 0.1$). The clusters calculated at $T = 0.01$ were included to illustrate that the distribution of activations of the olfactory circuits during smelling of the two steroids was similar in the three groups.

*This large cluster covered at $T = 0.01$ the amygdala and piriform cortex on both sides, as well as the anterior hypothalamus, with a maximal activation corresponding to tuberomammillary and dorsomedial nuclei (Talairach coordinates 4, −11, −6; *Z* level, 3.8).

†Large cluster that also covered the left amygdala and piriform cortex and a portion of anterior cingulate.

‡This cluster covered a portion of anterior cingulate gyrus.

they engaged the amygdala and the piriform and the insular cortices (the classical odor-processing circuits) when smelling both of these compounds (Table 1 and Fig. 1). In the HeW, however, smelling of AND was processed by the anterior hypothalamus, whereas smelling of EST involved the olfactory regions; the pattern of activation in HeM was reciprocal to the pattern in HeW (Table 1 and Fig. 1). In contrast to the two steroids, and in accordance with several previous studies of odor stimulation (15, 25–32), activation with OO yielded similar clusters in all three groups of subjects, covering the amygdala, the piriform and insular cortices, as well as minor portions of the anterior cingulate and orbitofrontal cortices (Table 1 and Fig. 1).

The centers of the hypothalamic clusters in HeM and HeW were ≈10 mm apart. Because registration and repositioning of PET clusters on individual reformatted magnetic resonance images revealed similar locations in all subjects and no systematic shifts between the groups, attention was paid to the more precise location of the respective local maxima. It should, however, be emphasized that their relationship to the specific hypothalamic nuclei should be viewed with caution and that the localization of atlas coordinates to 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.

The hypothalamic activation in HeM covered the dorsomedial and paraventricular nuclei. In HeW, it covered the preoptic area and the ventromedial and tuberomammillary nuclei (Table 1).

The group comparisons showed that lesbian subjects differed only from HeW and that the difference was constituted by the absence of preoptic activation with AND in lesbian women and its presence in HeW. The HeW − lesbian women contrast for AND − AIR showed a cluster with local maxima corresponding to Talairach coordinates −6, −8, −10 and −2, −2, 0 (*Z* level, 3.6; cluster size, 2.0 cm^3; corrected $P = 0.016$) (Fig. 1). According to the atlas of Schaltenbrand (33), this cluster primarily covered the preoptic and ventromedial hypothalamic nuclei. No group differences were observed in OO − AIR.

Also, the two heterosexual groups differed from each other only in their hypothalamic activations. The difference was constituted by the higher AND − AIR activation in HeW and the higher EST − AIR activation in HeM. The peak coordinate for HeW − HeM for AND − AIR corresponded to the preoptic area: Talairach coordinates +3, +2, −13 (*Z* level, 4.2; cluster size, 0.8 cm^3). The HeM − HeW contrast for EST − AIR showed a cluster with a peak coordinate corresponding to the dorsomedial hypothalamic nucleus (Talairach coordinates +6, −8, +2; *Z* level, 4.0; cluster size, 0.4; height threshold at $T = 0.001$; corrected $P < 0.05$) (Fig. 2).

We also carried out conjunctional analysis for evaluation of possible common activations. All three groups shared clusters in odor-processing regions, regardless of whether the odorous stimulus was AND, EST, or OO (Table 2, Fig. 2). In addition, the lesbian women shared a cluster with HeM in the anterior hypothalamus

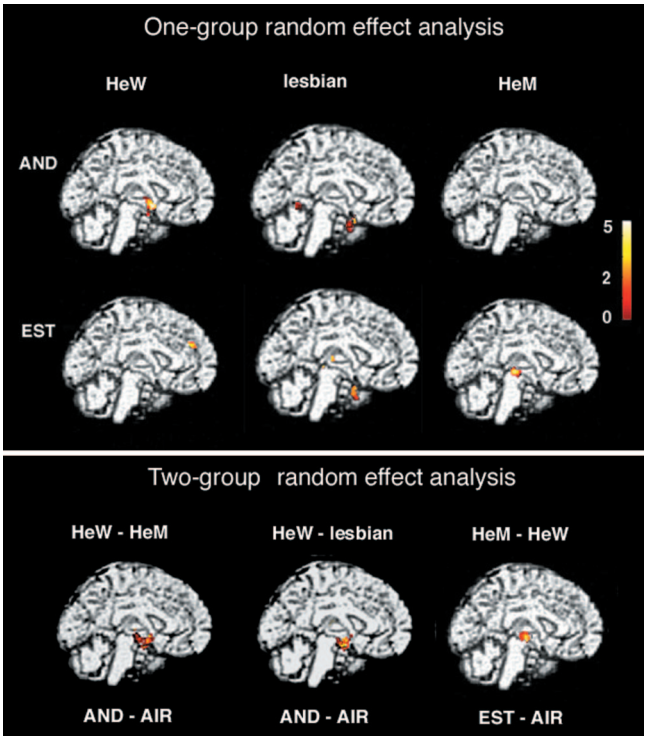

Fig. 1. Illustration of group-specific activations with the putative pheromones. The Sokoloff color scale illustrates *Z* values reflecting the degree of activation (0.0–5.0). Because the same brain section is chosen, the figures do not always illustrate maximal activation for each condition. (*Upper*) Cerebral activation during smelling of AND and EST. Clusters of activated regions are superimposed on the standard MRI brain (midsagittal plane). (*Lower*) Significant differences among the different groups. Shown are the clusters calculated with two-group random-effect analysis. Only significant activations are shown.

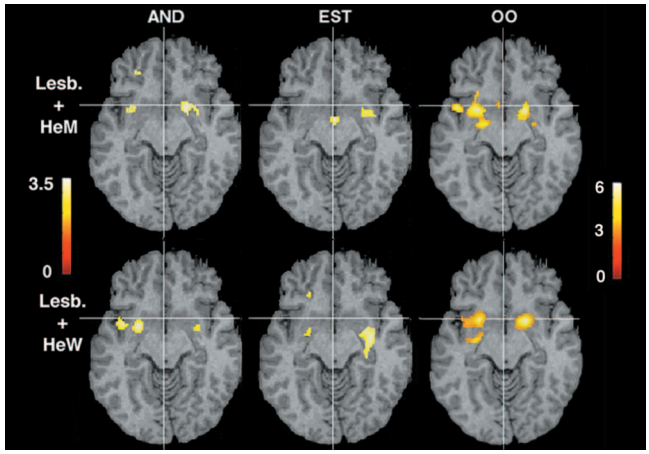

Fig. 2. Common activations between the groups. Shown are conjunctional clusters in different groups of subjects, superimposed on the standard brain. All images show horizontal level at $Z = -8$ according to the atlas of Talairach and Tournoux (51). The Sokoloff color scale illustrates *Z* values (0.0–3.5 for AND and EST and 0.0–6.0 for OO). Because the same brain section is chosen, figures do not always illustrate maximal activation for each condition. The subject's right side is to the right. The right cluster in the lesbian woman plus HeW (Lesb+HeW) for AND − AIR did not pass the level of significance; the level of significance for the hypothalamic cluster for EST − AIR in Lesb+HeM was 0.06.

during smelling of EST, albeit only at a subsignificant level ($T = 0.001$; corrected $P = 0.06$). The Talairach coordinates for this cluster were, +6, −16, −6 and corresponded to the location of the dorsomedial hypothalamic nucleus (Table 2 and Fig. 2).

The statistical parametric mapping (SPM) statistics are rather conservative and carry a risk that physiologically relevant changes in rCBF can remain undetected (34, 35). To secure identification of possible qualitative similarities between the groups, we examined the results from the explorative analysis also at $T = 0.01$. Table 1 shows activations at both $T = 0.001$ and $T = 0.01$. Notably, when $T = 0.01$ was used, EST − AIR incorporated in lesbian women an area corresponding to the location of the dorsomedial hypothalamic nucleus (Talairach coordinate for maximal activation was + 4, −11, −6; *Z* level, 3.8). Conversely, when running the EST − AIR contrast at $T = 0.01$ in HeM, activation was observed not only in the hypothalamus but also in the left amygdala, piriform cortex, and insular cortex (Table 1). However, HeW showed a restricted activation of the hypothalamus with AND and a restricted activation of the amygdala and piriform cortex with EST, independent of the significance level. Thus, by lowering the level of significance, the activated fields in HeM and lesbian women became more similar,

Table 2. Conjunctional clusters

Region	Lesbian women and HeW			Lesbian women and HeM			HeW and HeM		
	Z level	Size, cm^3	Coordinates	*Z* level	Size, cm^3	Coordinates	*Z* level	Size, cm^3	Coordinates
EST − AIR									
Left amygdala plus piriform and insular cortices	4.2	1.3	−12, +14, +2 −36, +4, −2				4.0	0.8	−24, 0, −12
Right amygdala plus piriform cortex and anterior hypothalamus				4.4	1.2	+18, +2, −10 +6, −16, −6*			
Right piriform and insular cortices	4.4	2.0	+30, −6, −2	4.3	0.5	+20, 2, −10	4.1	0.4	+34, −10, −8
AND − AIR									
Right amygdala plus piriform and insular cortices				4.7	2.0	+24, +2, −18			
Left amygdala plus piriform cortex	4.5	1.0	−24, +2, −10	3.9	0.8	−22, +4, −6	3.6	0.9	−26, +2, −8
OO − AIR									
Right amygdala plus piriform, insular, and anterior cingulate cortices	5.2	3.4	+22, +2, −12	5.6	2.2	+22, +4, −14	6.3	5.5	+22, 0, −14
Left inferior frontal gyrus	4.8	0.5	−46, +30, +20				6.4	5.1	−18, −2, −14
Left amygdala plus piriform, insular, and anterior cingulate cortices	5.3	8.0	−22, 0, −6	5.1	4.0	−28, +6, −2			

Activations shared by the respective groups were calculated with conjunctional analysis (SPM99). Talairach coordinates indicate local maxima. Calculations at $T = 0.001$ are shown (corrected $P < 0.05$; *, corrected $P = 0.06$).

whereas the dissimilarity between lesbian women and HeW remained.

In line with a previous observation (15), lowering of the *T* level led to the appearance of additional clusters in the lingular and fusiform gyri in all groups (Table 1).

According to the Kinsey scores, the lesbian population was less homogeneous than the two heterosexual populations. To investigate whether this difference could have influenced the results, all of the calculations were repeated with the data restricted to the nine lesbian women who scored Kinsey 6. The results remained unchanged; only the conjunctional hypothalamic EST − AIR cluster for lesbian women and HeM now passed the level of significance.

To specifically investigate a possible congruence between the lesbian women and HeM with respect to the hypothalamic engagement, we carried out a post hoc volume of interest (VOI) analysis. The underlying hypothesis was that lesbian women recruited selected hypothalamic networks when smelling EST but not AND and thus processed the two putative pheromones as HeM rather than HeW. Because the anatomical boundaries of the anterior hypothalamus are difficult to determine with MRI, we used two functionally generated VOIs. They were defined from the AND − AIR activation in HeW and EST − AIR activation in HeM, generated in an earlier study (15). The AND − AIR VOI measured 1.4 ml and was centered to the preoptic and ventromedial nuclei; the EST − AIR measured 0.9 ml and covered the dorsomedial and paraventricular nuclei (see Fig. 3, which is published as supporting information on the PNAS web site). These VOIs were generated by images reformatted to the same standard brain (15) as the present PET images. Both VOIs were, therefore, directly transferred to the individual PET images from heterosexual and lesbian subjects. In these images, the rCBF was normalized to 50 ml/min per 100 g. The rCBF was extracted for each VOI, and the mean rCBF of the three scans per condition was calculated in each subject. The mean rCBF for AIR, AND, EST, and OO was then compared in each subject group in separate repeated-measures ANOVAs (one for each VOI) (15, 25). The df was 3. If there was a significant interaction at this level, appropriate contrasts were calculated. In addition, we tested possible differences among lesbian women, HeW, and HeM in OO − AIR, AND − AIR, and EST − AIR in each predetermined VOI by means of two-way repeated-measures ANOVAs with subject group as the between-subjects factor and the type of odorant as the within-subjects factor. This analysis yielded statistical data on the main effects: group, type of odorant, and the group by odorant interaction. Because the variable of interest, the group by odorant interaction, was significant in the preoptic and ventromedial VOI ($F = 3.56$; $P = 0.01$; df = 4) as well as in the dorsomedial and paraventricular VOI ($F = 3.79$; $P = 0.008$; df = 4), the results were further explored with contrast (df = 1) to determine which specific group and type of odorant determined the observed interaction. *P* values were considered significant when <0.05.

In lesbian women, the VOI analysis yielded a significant interaction among the three stimuli and air only in the VOI covering the dorsomedial and paraventricular hypothalamus ($F = 3.3$; $P = 0.03$; df = 3). This interaction was constituted only by EST − AIR ($F = 11.0$; $P = 0.002$; df = 1). This activation was significant in relation to HeW ($F = 7.4$; $P = 0.01$; df = 1). Also, the HeM activated only the dorsomedial and paraventricular hypothalamic VOI with EST ($P = 0.002$; $F = 10.8$, df = 1), significantly more than seen for the HeW ($F = 7.8$; $P = 0.01$; df = 1). The HeW, however, increased rCBF in the preoptic VOI, but only when smelling AND ($F = 12.3$; $P = 0.0.01$; df = 1). This increase was significant in relation to both the lesbian women ($F = 16.7$; $P < 0.001$; df = 1) and the HeM ($F = 11.3$; $P = 0.002$; df = 1). No difference was detected between the lesbian women and HeM in any of the VOIs. In contrast to AND and EST, OO showed no significant activation in any of the VOIs or subject groups.

Table 4. Hormone levels in lesbian women

Hormone	Level
Plasma LH, units/liter	5.6 ± 3.1
Plasma FSH, units/liter	4.9 ± 3.0
Plasma prolactine, μg/liter	13.3 ± 4.5
Plasma testosterone, free nmol/liter	0.7 ± 0.2
Plasma testosterone, nmol/liter	2.0 ± 0.7

LH, luteinizing hormone; FSH, follicle-stimulating hormone.

There were no significant group by odor interactions for any of the odor-rating variables. Mean values are illustrated in Figs. 4 and 5, which are published as supporting information on the PNAS web site, is a "within-subject" comparison (as scattergrams). We also did not find any group by stimulus interaction in respiratory amplitude or frequency (see Fig. 6, which is published as supporting information on the PNAS web site). No group differences were observed in odor thresholds (Table 3), and the measured hormone levels were normal in lesbian women (Table 4).

As opposed to the explorative whole-brain analysis, which showed no significant difference between AND and EST and vice versa in any of the three groups of subjects, several differences appeared in the VOI analysis: AND − EST revealed an increase in normalized rCBF in the preoptic VOI in HeW ($P = 0.01$; $F = 7.4$; df = 1), whereas EST − AND showed an increase in the dorsomedial and paraventricular VOI in HeM ($P = 0.046$; $F = 4.3$; df = 1) and a similar trend in the lesbian women ($P = 0.07$; $F = 3.5$; df = 1). No other differences between AND and EST were observed.

Discussion

The main observation in the present study is that lesbian women differed from HeW in that they did not activate the preoptic hypothalamus with AND. Furthermore, the lesbian women shared a hypothalamic cluster with the HeM when smelling EST. Finally, when restricting the search volume to predetermined hypothalamic VOIs, lesbian women showed activation of the dorsomedial and paraventricular hypothalamic VOI with EST, like the HeM and unlike the HeW. Together, these data suggest that lesbian women processed AND and EST more congruently with HeM than HeW.

Several previous observations add support for the view that the present findings may have biological relevance: AND seems to possess certain pheromone-like properties [i.e., it is produced by the human body, shows sex-differentiated detection thresholds (16, 23, 24), and induces sex-differentiated changes in autonomic function and mood (18–22)]. Moreover, several independent studies indicate that signals from AND activate a region covering the preoptic hypothalamus (15, 25). A lack of activation of this particular region with AND in lesbian women is therefore unlikely to be accidental. Rather, it could be expected, when considering that the preoptic area participates in the integration of hormonal and sensory cues that are necessary for sexual behavior. Together with animal experiments indicating that the preoptic region is involved in the

Table 3. Olfactory thresholds

Group	Butanol, M	AND, M	EST, M
Lesbian women	$3.0 \times 10^{-5} \pm 4.5 \times 10^{-5}$	$2.2 \times 10^{-4} \pm 3.8 \times 10^{-4}$	$8.6 \times 10^{-5} \pm 3.6 \times 10^{-5}$
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	$5.0 \times 10^{-5} \pm 1.0 \times 10^{-5}$	$1.0 \times 10^{-4} \pm 1.5 \times 10^{-4}$	$2.0 \times 10^{-4} \pm 2 \times 10^{-4}$

choice of sexual partner, these data suggest that the observed difference between lesbian women and HeW reflects a physiological process. The nature of this process is not evident from the present experiments, and at least three alternative explanations should be considered. One possibility is that detection of AND was associated with sexual arousal in HeW but not lesbian women. However, as discussed in the study of HoM (25), sexual arousal seems to engage several cerebral structures outside the hypothalamus (36, 37), which were not activated in the present study. In addition, none of our subjects reported sexual arousal.

Another tentative explanation is that the observed activation with AND in HeW reflected an acquired sensitization to its stimuli in the hypothalamus or its centrifugal networks due to the repeated exposure to men through life sexual experience (38). However, sensitization can be acquired to several odorants and more easily in women (39). Consequently, it could be expected also *vis-à-vis* EST and perhaps even more readily in lesbian women than HeM. This scenario is difficult to reconcile with the less-prominent hypothalamic activation by EST in lesbian women and the pronounced activation in HeM. Although we made no measures of sexual activity, it is of note that the number of stable sexual partners was comparable (seven in the group of lesbian women and six in the group of HeM).

A third explanation is that some aspects of the differentiation of neuronal circuits in the anterior hypothalamus or the signal transduction of these circuits in lesbian women could vary from that in the HeW, indicating that our lesbian subjects process the stimuli from AND as odors rather than pheromones. The three tentative mechanisms are not mutually exclusive, nor can they be discriminated on the basis of the present PET data.

Despite identical experimental designs and use of identical control data, the congruence with the opposite sex was weaker in the present study of lesbian women compared with previous findings in HoM. When only taking into account the explorative first-level analysis, HoM activated the hypothalamus with AND and the olfactory regions with EST, as did the HeW (25). Lesbian women, in contrast, showed activations of the olfactory regions with both AND and EST, which differs from HeM, who primarily engaged the anterior hypothalamus when smelling EST. The poorer congruence with the opposite sex in the lesbian group is unlikely to be an effect of the higher inhomogeneity with respect to Kinsey scorings, because the results of the explorative analysis remained unchanged when only taking into account the data from subjects scoring Kinsey 6. The weaker expression of homotypical pattern could, theoretically, be an effect of the compound. The high congruence between HoM and HeW in hypothalamic activation was related to AND, which has been more thoroughly investigated than EST and seems to have more prominent pheromone-like features (18–22). Consequently, the hypothesized dichotomy and interaction in the processing of pheromone and odor signals, discussed in our earlier publications (15, 25), could be less pronounced with EST than AND. Another explanation is that female homosexuality differs from male homosexuality. Indeed, the observations from several comparative studies between the HoM and lesbian women favor this view. HoM are reported to have a later birth order relative to HeM, whereas no significant birth order has been reported in lesbian women (40). According to gene mapping, the genetic influence is significantly higher in male compared with female homosexuals (41). HoM are found to perform akin to HeW on certain verbal and mental rotation tasks, whereas lesbians appear to perform more in a sex-typical manner (42, 43). Finally, change in sexual orientation after some form of "reparative therapy" seems to occur more frequently in lesbians than in HoM (44). Lesbian women are suggested to have a more "sex-flexible" behavior, which is in accordance with our difficulties recruiting lesbian women scoring 6 on the Kinsey scale (45, 46).

It should be noted that according to the method applied, the material was sufficient to generate inference at group level, implying that each individual was representative of his/her designated group (34, 35). The method is, however, not informative about the separate individuals belonging to a group. Another methodological issue needing attention is assignment to the specific hypothalamic nuclei. Because the distance between centers of the hypothalamic clusters was just at the limit of scanner resolution and filtering, no firm statements can be made about which specific nuclei were activated. Nevertheless, we choose to report cluster locations in relation to the various nuclei because they provide support for the observed sex-atypical pattern activation in the lesbian women, just as they did in our previous study of HoM. In the absence of identical studies with higher-resolution techniques, these data should, however, be interpreted with caution.

The present study adds no further information about the possible pathways for signal transduction of AND and EST. The various alternatives have been discussed in detail in our previous studies (15, 25) and will not be repeated here. In short, we proposed that in humans, signals from AND and EST could be transduced by the olfactory mucosa (47). We also suggested that these compounds could be processed bimodally, as odors and pheromones, and that the odor and pheromone signaling could interact in accordance with the trigeminal and odor signaling in trigeminal odorants (15, 25, 47). Thus, the pheromone-related hypothalamic activation could be associated with a reduced (but not abolished) odor activation of olfactory circuits. In this context, the lesbian woman in the present study seem to have perceived AND primarily as an odor. However, additional and specifically designed studies are necessary to investigate pathways for AND and EST signaling in detail, which until then remain speculative.

Independently of the exact pathways, the presented data demonstrate that lesbian women process the two putative pheromones AND and EST differently from HeW and in partial congruence with HeM. The data support the notion of a coupling between hypothalamic neuronal circuits and sexual preferences and encourage further evaluation of the possible neurobiology of homosexuality and human sexuality in general.

Methods

Thirty-six healthy, unmedicated, right-handed, HIV-negative HeM, HeW, and lesbian women (12 in each group) who were osmic for both AND and EST and had normal MRI of the brain participated in the study. The 12 lesbian women were selected from a group of 35 in an attempt to recruit subjects who rated at the extreme end of the Kinsey heterosexual/homosexual scale (0 = maximally heterosexual, 6 = maximally homosexual) (48). Because it was important to collect the data from both homosexual and heterosexual subjects within the same period and because of the reportedly higher sexual fluidity in lesbian women (a strictly homosexual choice of sexual partner seems more unusual among the lesbian women than HoM) (44–46), the study group consisted of lesbian women who rated >5 on the Kinsey scale). Thus, three of the lesbian women reported occasional sexual encounters with men, although they regarded themselves as fully lesbian (they rated between 5 and 6 on the Kinsey scale). The remaining nine lesbian subjects either had no sexual experience with men or reported heterosexual sex on one or two occasions, usually before realizing that they were lesbian (they classified themselves as Kinsey 6). In addition to scoring themselves on the Kinsey scale (which is based on self-identification), the subjects also participated in interviews regarding three dimensions of sexual orientation (fantasy, romantic attraction, and sexual behavior) over consecutive 5-year historical time periods, from age 16 to the present (49). All of the lesbian women reported homosexual fantasies and attractions. The heterosexual subjects all rated 0 on the Kinsey scale and had exclusively heterosexual fantasies and romantic attractions. Seven HeW, seven lesbian women, and six HeM had a stable sexual partner at the time of experiments. The three groups were matched for age (26 ± 2, 33 ± 6, and 28 ± 2 years) and educational level and differed only

with respect to sexual orientation. All of the lesbian subjects had normal hormone levels (Table 4). Women were investigated during the second to third week of the menstrual cycle.

As in our previous studies, the activation condition consisted of passive smelling (not sniffing) of AND, EST, and four different odors, denoted as OO (25). The OO were lavender oil, cedar oil, eugenol, and butanol. Although the butanol was administered in a 10% concentration, the other odors were undiluted. As previously, AND and EST were presented in crystalline and odorous form (200 mg; Steraloids Inc., Newport, RI) during the PET scans. In contrast, for testing of the detection threshold of the respective odor (50), both were solved in odorless mineral oil. The purity of AND and EST was tested by our doping laboratory and assessed to be 98%.

PET Experiments and Image Data Analysis. PET measurements were carried out at the same time of day. Furthermore, the room temperature and air pressure were standardized (23°C, 997 hPa) (15, 25). The different subjects were investigated by the same experimenters and over the same time period [which overlapped with the period of scanning of HoM in our earlier reported study (25)]. The experimental protocol and its justification have been described in detail elsewhere (15, 25, 26, 27). In summary, the protocol included MRI scans and PET (full width at half maximum, 3.8 mm) measurements of rCBF with ^{15}O H_2O during three stimulus conditions (smelling of AND, EST, and OO, respectively) and the base-line condition (smelling of room air, which was kept odorless by a suction devise in the scanner room). All of the stimuli (including room air) were presented in a glass bottle at a distance of 10 mm from the nose (15). There were 12 scans per person (three scans per condition, balanced and randomly interleaved). During the scans, subjects were unaware of the identity of items and were instructed not to sniff or judge the odorants.

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) (15, 25). After the PET scans, subjects rated each odorant for pleasantness, irritability, intensity, and familiarity using a 100-mm visual analog scale (15, 25–27).

The individual magnetic resonance images and PET images were reformatted into a common space (standard brain) and filtered with a 10-mm Gaussian kernel (25–27, 33, 34). Significant activations were determined with SPM statistics (SPM99; www.fil.ion.ucl.ac.uk/spm/; Wellcome Foundation, London) (33, 34) by using the contrasts AND − AIR, EST − AIR, and OO − AIR, as well as AND − EST and EST − AND. Significant activations were first evaluated in each separate group with a one-group random-effect analysis, by using the entire brain as search space. Next, a two-group random-effect analysis was applied to test group differences. Finally, possible common activations among several groups were tested with conjunctional analysis. With few exceptions, which are described separately, the significance level was $T = 0.001$ (corrected $P < 0.05$).

Given that the previously detected hypothalamic clusters were small, the location of hypothalamic activations was analyzed with special care. First, we investigated whether there were any systematical errors during the process of normalization to the common space. The hypothalamic clusters obtained in the group analysis were therefore superimposed on each subject's set of reformatted magnetic resonance images to assess whether the location of hypothalamic activations was misplaced (indicating a poor normalization) in any subject. Second, the precise location of the hypothalamic clusters was determined by translation of the Talairach coordinates (51) to those of the atlas of Schaltenbrand (33).

Comparisons of 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 lesbian women, HeM, and HeW with respect to AIR, AND, EST, and OO by using a two-way ANOVA, factoring for subject group and stimulus type, as described previously (15, 25). A separate ANOVA was conducted for each measure (familiarity, irritability, intensity, and pleasantness) to test for group differences in odor ratings, but the stimuli included in the within factor were AND, EST, and OO, because AIR was perceived as odorless. The significance level was 0.05 in all comparisons.

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