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Male subjects who could not perceive the pheromone 5a-Androst-16-en-3-one, produced similar orbitofrontal changes on PET compared with perceptible phenylethyl alcohol (rose)*

Valerie Treyer[1], Heinz Koch[2], Hans Rudolf Briner[3], Nick S. Jones[4], Alfred Buck[1], Daniel B Simmen[3]

[1] PET Center, Division of Nuclear Medicine, University Hospital, CH-8091 Zürich, Switzerland
[2] Givaudan Schweiz AG, Fragrance Research, CH-8600 Dübendorf, Switzerland
[3] ORL-Zentrum, The Hirslanden Clinic, Zürich, Switzerland
[4] Department of Otorhinolaryngology, University Hospital, Nottingham, United Kingdom

SUMMARY

***Background**: The aim of this study was to evaluate in how far cerebral blood flow changes in male subjects when exposed to a pheromone that they cannot consciously smell.*

***Methods**: We used a boar taint steroid (5a-Androst-16-en-3-one), which is similar to human axillary sweat but could not be detected by the human volunteers who participated in this study.*

***Results**: The pheromone produced activation of the orbitofrontal and frontal cortex in comparison to a baseline condition. The same regions were activated when the subjects smelled a rose-like odour.*

***Conclusion**: This study shows that a pheromone, which is not consciously detected, can evoke a response in the brain that is similar to a detectable odour.*

Keywords: pheromone, functional imaging, human, orbitofrontal cortex, odour, smell

INTRODUCTION

Although the term "pheromone" is widely used, definitions differ. For this study we define a chemical compound as a pheromone, if it can act as a signal between living organisms. These compounds have been extensively studied in mammals[1]. We investigate whether a pheromone that cannot consciously be detected by the participating subjects, can nevertheless produce cerebral changes and how these compare with changes evoked by an olfactory substance than can consciously be detected.

In this study we use the pheromone 5a-Androst-16-en-3-one, which is a boar taint steroid, a variation (androsta-4,16,-dien-3-one) of which is found in human axillary sweat[2]. Only 5 to 50% of the population can detect this odour[3,4]. They describe the smell as "urine like" or as sweet[5]. It is disputed, whether or not this pheromone is processed by the human vomeronasal organ[6-8]. A recent study revealed that covering of the vomeronasal duct does not alter androstenone detection[9]. Likewise it is not clear if androstenone has an influence on human behavior[10].

Changes in human brain metabolism or blood flow can be measured when different pheromones such as oestra-1,3,5[10], 16-tetraen-3yl acetate[11] or Delta 4,16-androstadien-3-one[12,13] are presented, even when these pheromones are not consciously detectable.

As we are interested in the changes in cerebral blood flow changes when an odour is presented we did not introduce a second task other than to ask the participant to breathe normally and to concentrate on the presented odour regardless of the olfactory perception. In order to compare possible changes resulting from a pheromone we introduced phenylethyl alcohol as a control odour. This is a simple chemical compound that produces a smell similar to a rose.

Positron emission tomography (PET) is the method of choice to measure cerebral blood flow changes in the orbitofrontal cortex a region that is involved in olfaction[14,15]. Measuring changes with fMRI artifacts are present in this region. Even though some techniques exist that diminish artifacts in fMRI[16-18] they are still visible in the raw data and also in signal-to-noise maps[19]. Consequently, we here use H215O PET to test whether or not olfactory processing in the orbitofrontal cortex depends on the conscious percept of the odor.

MATERIALS AND METHODS

Subjects

Nine healthy right-handed male students (aged 20-31) took part in this study, which was approved by the local Ethical Committee. They all gave written consent. None had any nasal disease or a history of head injury or was taking any medication.

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Pheromone test

A transport air-tube was used to guide clean medical grade air to a glass nosepiece at a flow rate of 8 l/min to a supply-pressure of 1 bar. Three small sample vials containing the odorous substances were connected via fused silica-capillaries directly to the nosepiece and the assembly was attached very close to the glass-nose-piece. The sample vials each had an air-inlet that was connected to a computer operated flow control box. A flow rate of 10 ml/min was found to be high enough to ensure supply of the odour without bothering the subjects. Guiding the air through the sample vial ensuring 100% saturation of the headspace by the odorant molecules generated the concentration of odour that was optimal. The odorants were vented with an extraction hood to prevent dilution within the room. Before each subjects examination the tubes were cleared with air and the system was calibrated.

The subjects were tested for their ability to smell using a standardized clinical screening test (Zürcher Geruchstest[20]) about a week prior to the experiment and all performed normally, detecting all presented odours. In addition subjects were tested to see if they could smell the pheromone or not. Only subjects, who did not smell the pheromone, even at a high concentration, participated in the main experiments.

All subjects were instructed to breathe normally and avoid sniffing. During the experiment the subjects had to judge if they could smell anything, and if they did, to identify the odour. The subjects were exposed to the same concentration of phenylethyl alcohol (condition “rose”), 5a-Androst-16-en-3-one (“pheromone”), then both equally mixed, as well as an empty vial (“blank”). The presentation of the different odours (3 * ”pheromone”, 3 * ”blank”, 2 * ”rose”, 2 * ”mix” (“pheromone+rose”) was counterbalanced except for the first presentation of the pheromone, which was always presented the first time after the “rose” and the “blank” condition to minimize a possible long lasting effect of the “pheromone” in the first-time presentation. The scans were started 42-47 seconds after the onset of the odour admission. There was a 10 minutes break between all scans.

PET scans

PET scans were acquired on a whole-body scanner (Advance GE Medical Systems, Waukesha, WI) in 3D mode with a 15 cm axial field of view. For each scan, 300-350 MBq H215O were administered as a slow bolus with a remotely controlled injection device. PET counts were recorded over 60 sec after the arrival of the bolus in the brain. A ten-minute transmission scan was performed before the measurements for attenuation correction. The data were reconstructed into 35 image planes (Slice thickness; 4.25; Matrix: 128x128; Pixel size: 2.34). The accumulated radioactivity counts over 60 sec were taken as measure for cerebral blood flow.

PET scan statistics

Statistical parametric mapping was performed after head movement correction, spatial normalization into stereotaxic space [Montreal Neurological Institute coordinates (MNI)], smoothing with a gaussian filter of 15 mm FWHM and proportional scaling for global normalization as implemented in the standard statistical parametric mapping software, SPM9921. The difference between conditions was then evaluated voxel by voxel and a second including only the first measurements of each experiment. This analysis was introduced, as it could have been possible that the heavy pheromone molecule persists for longer time in the air, the clothes and especially in the cover of the scanner. We accepted a result as significant when $p \leq 0.001$. To reveal overlapping clusters of the different conditions we showed in the figure activations with a cut-off $p < 0.01$.

RESULTS

When questioned after each scan, all subjects reported that they did not smell anything from the pheromone or the empty vial. The phenylethyl alcohol was perceived as weak decreasing “rose” or “flower like” odour in both the “rose” and the “mixed” condition.

Results over all measurements

The results of the SPM analysis are displayed in Figure 1, overlaid on an averaged anatomical magnetic resonance image. Coordinates of maximal activation and the statistical values

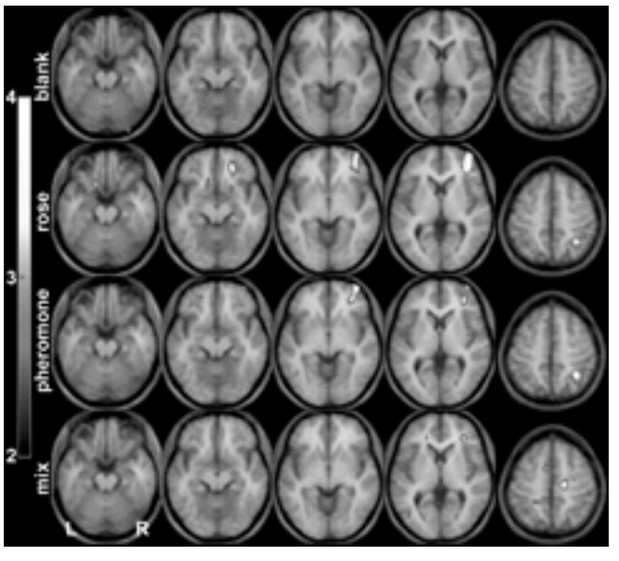

Figure 1. Significantly ($p<0.01$) activated areas related to rose, pheromone or both odours mixed. These results were obtained by summarizing all scans. All conditions were compared with the empty vial (blank) condition. For demonstration purpose also the opposite contrast blank compared to the others is displayed. The statistical parametric maps (with a cut-off $p<0.01$ for display purpose) are overlaid on an averaged T1 magnet resonance image displaying the axial slices z=-24/-16/-8/0/48 (MNI coordinates). The color scale on the left side indicates T-values resulting from the SPM99 statistics.

Table 1. Results summarising all scans.
SPM results with $p<0.001$ of the results summarizing all the scans. The results of the pheromone, rose and both mixed compared with the empty vial are displayed. The x, y, and z values indicate the MNI coordinates of the clusters' maximal activity. T-values with stars indicate a significance level $p<0.000$. BA denotes the number of the Brodmann Area the cluster lies within.

			pheromone-base				**rose-base**				**mix-base**			
regions	BA	side	coord.			T	coord.			T	coord.			T
			x	y	z		x	y	z		x	y	z	
frontal and limbic regions														
orbitofrontal cortex	11	R					22	42	-20	3.50*				
med. frontal gyrus	47/25	L					-12	16	-20	3.24				
mid. frontal gyrus	10	R	42	62	-4	4.04*	38	42	8	4.34*	36	36	0	3.22
sup. frontal gyrus	10	R	22	70	8	3.16								
inf. frontal gyrus	46/45	R	54	26	16	3.45*	52	18	20	3.54*				
cingulate gyrus	24/32	L									-14	-2	28	3.90*
med. frontal g./precentral g.	6	R									18	-18	56	4.07*
mid. frontal gyrus	6	R					22	8	64	3.47*				
precentral gyrus	6/9	R	54	0	28	4.01*					48	4	24	3.97*
other regions														
inf. parietal Lobule	40	R	36	-52	52	4.06*	32	-54	40	4.31*				
mid. occipital gyrus	37	L									-34	-66	12	3.34
precuneus	7	L									-14	-70	32	3.07

provided by SPM99 are shown in Table 1. All the scans revealed increased activation in the middle frontal gyrus (BA 10) compared with the baseline. The contrast "rose minus baseline" showed in addition an increase in activation in the orbitofrontal cortex (BA 11). Whereas the combined presentation of "pheromone" and "rose" showed higher regional cerebral blood flow in the precentral gyrus and visual cortices as compared to the empty vial.

Results of the first measurements

The task design minimized the potential for the first presentation of the "rose" to confound the result. Hence, we analyze the first presentations separately. Comparing the first presentation of the "pheromone" to the empty vial, there was a change in the regional cerebral blood flow in the orbitofrontal as well as anterior prefrontal cortex. Comparing "rose" with the baseline and the mixture of "rose" and "pheromone" with the baseline produced the same activation (Table 2 and Figure 2).

The "pheromone", "rose" as well as the "mixed" condition showed higher blood flow values in the parietal cortex than baseline. The "pheromone" and "rose" condition revealed differences in cerebral blood flow of the mediotemporal lobe in the left hemisphere when the pheromone was presented and changes were found in the right side with exposure to "rose" when compared to baseline. The cingulate gyrus exhibited an increased signal only when "pheromone" and baseline were compared.

DISCUSSION

This study demonstrated a change in regional cerebral blood flow in the orbito- and prefrontal cortex of humans when they were initially exposed to 5a-Androst-16-en-3-one, a pheromone that the subjects were unable to detect consciously. A similar but stronger result was obtained with phenylethyl alcohol, an odour that the subjects could detect. When both odours were presented at the same time the precentral gyrus also showed some activation. While a definite interpretation of the latter finding will require additional experimental investigations beyond the scope of the present study, the result that the combination of a scented odour together with a pheromone can cause a distinct pattern of activation compared to their isolated

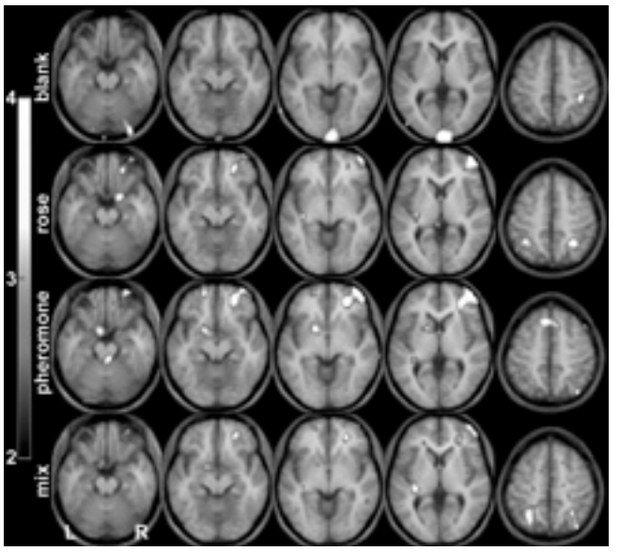

Figure 2. Significantly ($p<0.01$) activated areas related to rose, pheromone or both odours mixed. For this statistics only the first scans were included. All conditions were compared with the empty vial (blank) condition. All conditions were compared with the blank condition and displayed in the same way as in Figure 1.

Table 2. Result of first scnas only.

SPM results with p<0.001 of the results of the first scans only. The contrasts of the 3 conditions (pheromone, rose and both mixed) against the empty vial are displayed in columns. The x, y, and z values indicate the MNI coordinates of the clusters' maximal activity. T-values with stars indicate a significance level p<0.000. BA denotes the number of the Brodmann Area the cluster lies within.

			pheromone-base				**rose-base**				**mix-base**			
regions	BA	side	coord.			T	coord.			T	coord.			T
			x	y	z		x	y	z		x	y	z	
frontal and limbic regions														
orbitofrontal cortex	11	L	-16	56	-16	3.58								
orbitofrontal cortex	11	R					22	42	-20	4.49*	28	44	-12	3.80*
inf. frontal gyrus	10/46	R	52	28	20	5.24*	52	30	20	5.31*	54	46	8	4.86*
med. frontal gyrus	8/32	L	-6	24	44	4.39*								
inf. frontal gyrus	9	R	54	4	36	4.16*	56	6	40	3.36				
head of hippocampus/ amygdala		L	-20	2	-44	3.65								
uncus	34	R					20	2	-24	3.72				
ant. cingulate	24	R	2	30	12	3.94*								
cingulate gyrus	32	R	12	22	40	3.72								
other regions														
putamen		L	-16	6	-12	3.90*	-30	-20	4	3.76*				
inf. parietal lobule	40	R					34	-54	44	5.79*				
inf. parietal lobule	41	R					52	-38	36	4.19*				
inf. parietal lobule	40	L					-40	-54	56	4.29*				
inf. parietal lobule	7	R	40	-76	48	3.59					34	-56	48	3.54
sup. parietal lobule	7	L									-28	-62	48	4.05*
subcallosal gyrus	34	L	-6	4	-24	3.40								

presentation may be of relevance for the design of imaging studies of olfactory stimuli. The activation in the inferior frontal gyrus as well as the orbitofrontal gyrus is in line with the results of Sobel et al.[11]. They used an oestra-1,3,5(10),16-tetraen-3yl acetate, a probable human pheromone at high and low concentration. Subjects reported that they could not detect these odours but they showed at high concentrations a better than chance detection rate in a forced choice paradigm. We presented the pheromone at a similarly high concentration as that of the "rose" odour. As the subjects did not perceive any odour of the pheromone even at highest concentration we classified them as anosmic for this compound. We cannot exclude the possibility that some of them would detect the pheromone in a forced choice paradigm and should therefore be categorized as hyposmics[4]. Jacob et al.[12] revealed similar results measuring glucose consumption changes in the prefrontal cortex, cingulate gyrus and hippocampal region during pheromone (androstadienone) presentation when subjects performed a visual discrimination task.

This study did not combine olfaction with other tasks involving memory, detection, or qualitative tasks as was done in some studies[17,22], but nevertheless it demonstrated orbitofrontal activation with both "rose" and undetected "pheromone". The orbitofrontal cortex is known to be important for taste and olfaction, for example as a converging multimodal region[23], or for taste and olfaction as reward bearers[14,24] and also it is known to be involved in reward processing per se [25,26]. In this context, it is remarkable that the activation of the orbitofrontal cortex was independent of the perception of the odor.

CONCLUSION

Irrespective as to whether there are specific human pheromones, or if the human VNO is responsible for their detection, we found activation in the odour-relevant regions of the human brain when healthy human subjects were challenged with a boar pheromone which they could not smell, even at high concentration levels.

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REFERENCES

1. Zufall F, Kelliher KR, and Leinders-Zufall T. Pheromone detection by mammalian vomeronasal neurons. Microsc Res Tech 2002; 58: 251-260.
2. Grosser BI, Monti-Bloch L, Jennings-White C, and Berliner DL. Behavioral and electrophysiological effects of androstadienone, a human pheromone. Psychoneuroendocrinology 2000; 25: 289-299.
3. Dorries KM, Schmidt HJ, Beauchamp GK, and Wysocki CJ. Changes in sensitivity to the odor of androstenone during adolescence. Dev Psychobiol 1989; 22: 423-435.
4. Bremner EA, Mainland JD, Khan RM, and Sobel N. The prevalence of androstenone anosmia. Chem Senses 2003; 28: 423-432.
5. Araneda RC, and Firestein S. The scents of androstenone in humans. J Physiol 2004; 554: 1.

6. Griffiths NM, and Patterson RL. Human olfactory responses to 5-alpha-androst-16-en-3-one--principal component of boar taint. Journal of the Science of Food & Agriculture. 1970; 21: 4-6.
7. Claus R, and Alsing W. Occurrence of 5alpha-androst-16en-3-one, a boar pheromone, in man and its relationship to testosterone. Journal of Endocrinology. 1976; 68: 483-484.
8. Monti-Bloch L, Jennings-White C, Dolberg DS, and Berliner DL. The human vomeronasal system. Psychoneuroendocrinology 1994; 19: 673-686.
9. Knecht M, Lundstrom JN, Witt M, Huttenbrink KB, Heilmann S, and Hummel T. Assessment of olfactory function and androstenone odor thresholds in humans with or without functional occlusion of the vomeronasal duct. Behav Neurosci 2003; 117: 1135-1141.
10. Pause BM. Are androgen steroids acting as pheromones in humans? Physiol Behav 2004; 83: 21-29.
11. Sobel N, Prabhakaran V, Hartley CA, Desmond JE, Glover GH, Sullivan EV, et al. Blind smell: brain activation induced by an undetected air-borne chemical. Brain 1999; 122: 209-217.
12. Jacob S, Kinnunen LH, Metz J, Cooper M, and McClintock MK. Sustained human chemosignal unconsciously alters brain function. Neuroreport 2001; 12: 2391-2394.
13. Savic I, Berglund H, Gulyas B, and Roland P. Smelling of odorous sex hormone-like compounds causes sex-differentiated hypothalamic activations in humans. Neuron 2001; 31: 661-668.
14. Rolls ET, Critchley HD, and Treves A. Representation of olfactory information in the primate orbitofrontal cortex. J Neurophysiol 1996; 75: 1982-1296.
15. Rolls ET. The rules of formation of the olfactory representations found in the orbitofrontal cortex olfactory areas in primates. Chem Senses 2001; 26: 595-604.
16. Glover GH, and Law CS. Spiral-in/out BOLD fMRI for increased SNR and reduced susceptibility artifacts. Magn Reson Med 2001; 46: 515-522.
17. Weismann M, Yousry I, Heuberger E, Nolte A, Ilmberger J, Kobal G, et al. Functional magnetic resonance imaging of human olfaction. Neuroimaging Clin N Am 2001; 11: 237-250, viii.
18. Deichmann R, Josephs O, Hutton C, Corfield DR, and Turner R. Compensation of susceptibility-induced BOLD sensitivity losses in echo-planar fMRI imaging. Neuroimage 2002; 15: 120-135.
19. LaBar KS, Gitelman DR, Mesulam MM, and Parrish TB. Impact of signal-to-noise on functional MRI of the human amygdala. Neuroreport 2001; 12: 3461-3464.
20. Briner HR, and Simmen D. Smell diskettes as screening test of olfaction. Rhinology 1999; 37: 145-148.
21. Friston KJ, Holmes AP, Worsley KJ, Poline JB, Frith CD, and Frackowiak RSJ. Statistical Parametric Maps in functional imaging: A general linear approach. Human Brain Mapping 1995; 2: 189-210.
22. Gottfried JA, Deichmann R, Winston JS, and Dolan RJ. Functional heterogeneity in human olfactory cortex: an event-related functional magnetic resonance imaging study. J Neurosci 2002; 22: 10819-10828.
23. Rolls ET, and Baylis LL. Gustatory, olfactory, and visual convergence within the primate orbitofrontal cortex. J Neurosci 1994; 14: 5437-5452.
24. O'Doherty J, Rolls ET, Francis S, Bowtell R, and McGlone F. Representation of pleasant and aversive taste in the human brain. J Neurophysiol 2001; 85: 1315-1321.
25. Phillips AG, Mora F, and Rolls ET. Intracranial self-stimulation in orbitofrontal cortex and caudate nucleus of rhesus monkey: effects of apomorphine, pimozide, and spiroperidol. Psychopharmacology (Berl) 1979; 62: 79-82.
26. Tremblay L, and Schultz W. Relative reward preference in primate orbitofrontal cortex. Nature 1999; 398: 704-708.

Valerie Treyer
Dept. of Nuclear Medicine
University Hospital Zurich
Raemistr. 100
CH-8091 Zurich
Switzerland

Tel.: +41-44-255 3108
Fax: +41-44-255 4414
E-mail: Valerie.Treyer@usz.ch