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# Olfactory performance of patients with anorexia nervosa and healthy subjects in hunger and satiety*

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SUMMARY

*The aim of this study was to compare the olfactory performance of anorectic patients and healthy controls with regard to the state of satiety. Using the Sniffin' Sticks, sensitivity to a non-food odor (n-butanol) and to a food-related odor (isoamyl acetate) was assessed in 12 anorectic females and compared with 24 healthy controls. Threshold tests were performed in a hungry as well as in a satiated state, odor discrimination and odor identification only when satiated. Pleasantness of the odors was recorded. In terms of the non-food odor n-butanol, the olfactory sensitivity of anorectic patients and controls did not differ. Patients with anorexia nervosa had a significantly lower detection threshold for the food-related odor, but only in the hungry condition. Anorectic patients showed significant deficits in odor discrimination and identification, and under-evaluated the pleasantness of isoamyl acetate. Our results suggest an impaired projection from secondary to tertiary olfactory structures in anorexia nervosa, based upon the dichotomy of performance between detection threshold and odor discrimination/identification. The reduced pleasantness of isoamyl acetate indicates a decreased olfactory responsiveness to food stimuli in anorexia nervosa.*

*Key words: anorexia nervosa, Sniffin' Sticks, olfaction, threshold, food intake*

## INTRODUCTION

Olfaction and taste are known to be the phylogenetically oldest human senses. Not only do they provide information about the palatability and flavor of foods, but they can warn against dangers like fire, polluted air, or spoiled food [(1)]. In addition, they play an important role in the development of food preferences and the control of food intake [(2)].

Infections of the upper respiratory tract, cranio-facial traumas and sinonasal pathologies have been identified as the most common aetiopathogenetic factors for olfactory dysfunctions [(3)]. Besides, olfactory dysfunction is associated with a number of neuropsychiatric disorders, for example, Parkinson's disease and Alzheimer's disease [(4,5)]. It is well known that anosmia, the complete loss of olfactory function, interferes with patients' quality of life, predominantly in the areas of safety and eating [(6,7)]. Temmel et al. [(7)] demonstrated that younger patients suffered to a greater extend from anosmia than older ones, and women had more complaints than men. Concerning the interaction between olfaction and eating, there is evidence that a decreased or absent olfactory function may lead to reduced food intake [(1,8,9)]. However, investigating the effects of olfactory dysfunction on body weight has produced conflicting results, i. e. both weight gain [(9,10)] and loss of weight [(5,9,10)] have been described.

Regarding the close relationship between olfaction and food intake, the question arises whether anorectic patients could have altered olfactory functions as well. Anorexia nervosa predominantly affects adolescent girls and young women and is characterized by an inappropriately low body weight and disturbed eating behavior. In fact, many anorectic patients report a decreased pleasure in eating and show a reduced hedonic responsiveness to flavor [(11)]. It is still to be clarified if and how olfaction is affected in anorexia nervosa.

Few studies have been conducted on this topic so far and they have produced conflicting results. While some authors were not able to demonstrate any alterations of the olfactory performance of anorectic patients [(12)] or found significant olfactory impairments only in very low-weight patients with anorexia nervosa [(13)], others reported significant deficits regarding odor discrimination and odor detection in anorectic patients [(11)]. In the latest study carried out by Lombion-Pouthier et al. [(14)], patients with anorexia nervosa showed a higher olfactory sensi-

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tivity, over-evaluated the intensity, and under-evaluated the pleasantness of odors compared to healthy control subjects.

Since the results in literature on the olfactory performance of patients with anorexia nervosa are quite inconsistent, the aim of our study was to assess the olfactory function of anorectic patients more precisely. In particular, two new aspects were established in the study design: on one hand, the state of satiety was taken into consideration; on the other hand, a distinction was made between food-related and non-food odors. To reach this end, we augmented the standard set of the Sniffin' Sticks with a custom-made threshold test for a food-related odor and applied the two threshold tests in the hungry as well as in the satiated state. The odor discrimination and the odor identification test were performed only once in the satiated state because they are not suitable for repetition.

## MATERIALS AND METHODS

### *Participants*

Twelve female patients suffering from anorexia nervosa participated in the study. Their age ranged from 17 to 27 years with a mean age of 20.25 ± 3.28 years. Three of them were inpatients of a psychosomatic clinic (Medizinisch-Psychosomatische Klinik Roseneck, Prien, Germany), the remaining nine were recruited from two different self-help groups in Munich (ANAD e.V. pathways and Cinderella e.V.). They all fulfilled the DSM-IV criteria for anorexia nervosa. Using the self-report screening version of the Structured Inventory for Anorexic and Bulimic Eating Disorders (SIAB-S) [15], seven patients were assigned to the restrictive type, five to the binge-eating/purging type of anorexia nervosa. Depressive symptoms were assessed by means of the Beck Depression Inventory (BDI) [16]. The patients' mean BDI score was 15.83 ± 10.92 indicating only a mild depression.

In this context, it should be mentioned that the possibility of an altered odor perception in patients with major depressive disorder (MDD) cannot be excluded even though the results in literature are contradictory. Some authors were able to demonstrate an effect of MDD on olfactory performance [14,17,18], others not [19,20]. However, since eating disorders are associated with a raised incidence of alexithymia and depression [21,22], depressive symptoms are hard to eliminate in patients with anorexia nervosa. Therefore, our primary aim was to choose the cohort such that they had only mild depression.

We calculated a mean current body mass index (BMI) of 16.88 ± 1.26 kg/m$^2$ for the patients. All patients had at some point during their disorder a BMI that was below the 10$^{th}$ age-related percentile, and none of them had a BMI above the 25$^{th}$ percentile at the time of testing. The mean duration of illness was 3.73 ± 2.49 years.

Four patients were smokers with an average consumption of seven cigarettes per day. Three patients were taking antidepressive medication (Citalopram, Flouxetine), but the time since initiation of treatment did not exceed three weeks. One patient was taking a proton pump inhibitor (Esomeprazole), which is not known to interfere with sensory perception [23]. Exclusion criteria were any oropharyngeal problems or other medical conditions that could affect olfactory function (for example, hyperthyroidism).

All subjects gave their written informed consent. The study was conducted in accordance with the Declaration of Helsinki; ethical approval was obtained from an institutional review board.

In order to compare anorectic patients with healthy control subjects, we used a group of twenty-four healthy females, which we had tested in a similar manner. The results of the control subjects are being published separately [24]; however, we will briefly summarize their characteristics here. The age of control subjects ranged from 20 to 30 years (mean age of 24.17 ± 2.65 years), and their mean BMI was 20.99 ± 1.71 kg/m$^2$. They were all non-smokers, had normal olfactory functions and were not taking any medication. They were bound to the same exclusion criteria as the patients, and none of them had a history of or was currently suffering from an eating disorder which was verified by the SIAB-S. The control group scored 1.00 ± 1.14 on the BDI which is regarded as non-pathological.

### *Olfactory testing*

Olfactory performance was assessed by means of the Sniffin' Sticks (Burghart Instruments, Wedel, Germany), a test battery that uses pen-like odor-dispensing devices for measuring olfactory function [25,26]. Consisting of three subtests (odor threshold, odor discrimination, and odor identification), the Sniffin' Sticks combine quantitative and qualitative measurements of olfaction and can assess a wide range of olfactory disorders [27].

In addition to the standard Sniffin' Sticks threshold test which uses n-butanol, we employed a custom-made threshold test for isoamyl acetate. N-butanol, with its solvent-like odor, served as a non-food stimulus; isoamyl acetate on the other hand has a banana-like odor and was therefore categorized as a food-related stimulus. For the custom-made threshold test, empty Sniffin' Sticks pens were filled with 16 dilution steps of a 5% isoamyl acetate/propylene glycol solution (Sigma-Aldrich). The 5% solution represented the highest concentration of isoamyl acetate and was diluted 15 times in a ratio of 1:2 in propylene glycol. Olfactory detection thresholds were determined using a single-staircase, three alternative forced choice (3-AFC) procedure [25,28].

In the odor discrimination task, 16 triplets of clearly suprathreshold odors were presented. One stick of each triplet smelled different and had to be selected in a 3-AFC procedure. The odor identification test comprised 16 commonly known every day odorants. For each odor to be identified, subjects had to choose one out of four descriptives from a multiple choice template. All subtests were carried out birhinally. The results achieved in the three Sniffin' Sticks subtests (threshold for isoamyl acetate not included) were summed up to the so-called "TDI score", which characterizes the individual olfactory performance as the sum of odor threshold, discrimination and

identification ability [25,26]. According to normative data for the Sniffin' Sticks [29], the mean TDI score of female subjects in the age group of 16 to 35 years is 36.06 ± 4.17.

*Questionnaires and psychometric tests*

Each olfactory threshold test was accompanied by a questionnaire which recorded the subjects' emotional valence (1 = negative, 9 = positive), arousal (1 = calm, 9 = aroused), and alertness (1 = inattentive, 9 = very attentive), as well as the perceived pleasantness (1 = unpleasant, 9 = pleasant), and subjective intensity (1 = very weak, 9 = very strong) of the pen with the highest concentration of either n-butanol or isoamyl acetate. The Self-Assessment Manikin (SAM) [30] combined with a 9-point scale were applied to the dimensions emotional valence, arousal, and pleasantness of the odors, whereas a common 9-point scale was used to assess the subjects' alertness and the intensity of the odors.

To compare the patients' attention capability in states of hunger and satiety, the d2 Test of Attention [31] was applied. This test measures speed and quality of performance in crossing out "d"s with two dashes in rows of similar letters. Measures of performance include the Total Number of Items Processed (TN), the Percentage of Errors (E%), the Total Number of Items Minus Error Scores (TN–E), and the Concentration Performance (CP) derived from the number of correctly crossed out items minus errors of commission. The d2 Test of Attention was performed only by the patients. Since each patient had to complete the test twice (before and after breakfast), the time permitted for each line was shortened from 20 seconds to 15 seconds according to the instructions in the d2 test manual [31]. In doing so, ceiling effects were eliminated.

*Procedure*

Each experimental session started in a fasting condition, i. e. subjects had neither eaten nor consumed caloric beverages for at least 10 hours. In case of the inpatients, the experiments began at 6.00 a.m. because in the psychosomatic clinic, breakfast time was set from 7.00 till 8.00 a.m. Concerning the patients from the self-help groups and the control subjects, the experiments started at 8.00 a.m. Subjects were tested separately in a well-ventilated, light and quiet room.

Upon arrival, subjects rated their state of hunger (1 = not hungry at all, 9 = very hungry), desire for food (1 = very weak, 9 = very strong), and fullness of their stomach (1 = not full at all, 9 = very full) on a 9-point scale. Subsequently, the d2 Test of Attention [31] was performed. Then, the subjects' detection thresholds for n-butanol and isoamyl acetate were determined. The odor threshold tests were applied in a pseudo-randomized order. After each test the aforementioned questionnaire concerning emotional valence, arousal, alertness, pleasantness, and intensity of the odor was completed.

Then, the inpatients consumed the hospital's breakfast, whereas the patients of the self-help groups and the healthy control subjects received a breakfast with standardized food items (rolls, banana, optionally butter, cheese, chocolate hazelnut spread, coffee, tea, milk, and/or orange juice). All subjects were instructed to eat until completely satiated. All of them, including the inpatients, had to finish the meal with one banana. In the group of control subjects, every food was weighed before consumption and calorie intake was calculated from the weight. In the group of patients, the amount of food was noted down in units such as "spoons" or "slices" and calorie intake was estimated.

After breakfast, patients performed the d2 Test of Attention [31] a second time. For all subjects, detection thresholds for n-butanol and isoamyl acetate were determined once more in the satiated state. Odor threshold tests and corresponding questionnaires were completed in the same order as before. Thereafter, subjects rated their state of satiety (1 = not satiated at all, 9 = very satiated), actual desire for food (1 = very weak, 9 = very strong), and perceived fullness of their stomach (1 = not full at all, 9 = very full) on a 9-point scale. Then, the odor discrimination test and the odor identification test were carried out. Following olfactory testing, subjects filled in the SIAB-S [15] and the BDI [16]. Finally, height and weight of each subject were determined.

*Statistics*

SPSS for Windows (version 14.0, SPSS Inc, Chicago, IL, USA) was used for the statistical evaluation. In a first step, patients were analyzed separately. For the comparison between the non-satiated and the satiated condition, a Student's t-test for paired samples was applied. A Student's t-test for independent samples was used to compare patients' mean pleasantness ratings of the two odorants independently of the actual state of satiety. To test for effects of smoking on olfactory function, patients were divided into four smokers and eight non-smokers. In this particular case, the non-parametrical Mann-Whitney *U* test was applied to test for differences between groups as the small sample sizes prohibited using a t-test. In a second step, anorectic patients and healthy controls were compared to one another using a Student's t-test for independent samples. For this comparison, two additional variables were defined: the absolute difference of olfactory thresholds in hunger and satiety, and the threshold alteration expressed as a percentage of the detection threshold in the non-satiated state. Moreover, the 16 items of the odor identification test were assigned to the categories food-related odors (13 items) and non-food odors (3 items). The results of patients and control subjects calculated for each category were compared within groups as well as between groups by means of Mann-Whitney *U* tests. The alpha level was set at 0.05 for all tests.

## RESULTS

### Part I: Patients with anorexia nervosa

*Questionnaires and psychometric tests*

At the beginning of the experimental session, patients reported that they felt moderately hungry (mean 4.58 ± 2.54). They had

a moderate desire for food (mean 4.50 ± 2.51), and an empty stomach (mean 2.25 ± 1.66). After the breakfast during which they had consumed an average of 429.42 ± 149.21 kilocalories, patients described themselves as satiated (mean 7.38 ± 1.26); they felt a weak desire for food (mean 1.92 ± 0.90) and characterized their stomach as being moderately full (mean 6.67 ± 1.43). In the categories desire for food and fullness of the stomach, ratings revealed a significant difference between the non-satiated and the satiated state ($t(11) = 4.43$, $p = 0.001$ and $t(11) = -6.85$, $p < 0.001$, respectively).

Ratings of emotional valence, arousal, alertness, intensity, and pleasantness of the odors did not show significant differences between the two conditions. However, we found a significant difference between the pleasantness ratings of n-butanol and isoamyl acetate if regarded independently of the actual state of satiety: the smell of isoamyl acetate was judged significantly more pleasant than the odor of n-butanol (mean isoamyl acetate 5.21 ± 1.91 vs. mean n-butanol 3.54 ± 1.93, $t(46) = -3.00$, $p = 0.004$).

The patients' attentional capabilities showed a significant difference between the hungry and the satiated condition as quantified by the d2 Test of Attention: in the satiated state, patients scored significantly higher on all the measures of performance except for E% (Table 1).

Table 1. Results of anorectic patients (n = 12) in the d2 Test of Attention performed in a non-satiated state (after overnight fasting) and in a satiated state (after controlled breakfast).

| | non-satiated state (mean ± SD) | satiated state (mean ± SD) | t (df) | p-value (2-tailed) |
|---|---|---|---|---|
| TN | 386.58 ± 55.73 | 436.42 ± 61.76 | -8.61 (11) | < 0.001 |
| E % | 4.81 ± 8.04 | 3.82 ± 7.88 | 2.10 (11) | ns |
| TN–E | 369.75 ± 68.63 | 421.50 ± 75.28 | -8.17 (11) | < 0.001 |
| CP | 149.25 ± 40.86 | 171.58 ± 45.24 | -6.71 (11) | < 0.001 |

Note: TN = Total Number of Items Processed, E% = Percentage of Errors, TN–E = Total Number of Items Minus Error Scores, CP = Concentration Performance.

*Olfactory testing*

Neither the odor detection threshold for n-butanol nor the odor detection threshold for isoamyl acetate differed significantly between the satiated and the non-satiated state (n-butanol: non-satiated state: mean 10.16 ± 2.09, satiated state: mean 9.54 ± 1.93, $t(11) = 0.72$, $p = 0.49$; isoamyl acetate: non-satiated state: mean 13.50 ± 1.59, satiated state: mean 12.87 ± 2.45, $t(11) = 0.97$, $p = 0.35$).

Odor discrimination and odor identification were only performed once, in the satiated condition. In the former, patients scored 12.50 ± 1.57 on average, in the latter, they achieved a mean score of 13.33 ± 1.30. Adding the olfactory detection threshold for n-butanol in the satiated state, this resulted in a mean TDI score of 35.38 ± 3.88.

No adverse effects of smoking on olfactory function could be demonstrated. The Mann-Whitney U test did not reveal any significant difference in the olfactory performance of smokers and non-smokers (Table 2).

Part II: Patients with anorexia nervosa vs. healthy control subjects

In the following, the results of the anorectic patients compared to the healthy control subjects will be mentioned. A more detailed information on the performance of the control group is given in Albrecht et al. [24].

*Questionnaires and psychometric tests*

Both the healthy controls and the patients with anorexia nervosa were female and within an age range of 17 to 30 years. Nevertheless, the control subjects (mean 24.17 ± 2.65 years) were slightly older than the anorectic patients (mean 20.25 ± 3.28 years; $t(34) = -3.86$, $p < 0.001$). As expected, the patients' mean BMI (16.88 ± 1.26 kg/m$^2$) was significantly lower than that of the controls (20.99 ± 1.71 kg/m$^2$; $t(34) = -7.35$, $p < 0.001$). In the BDI patients scored significantly higher (mean 15.83 ± 10.92) than the healthy control subjects (mean 1.00 ± 1.14; $t(11.12) = 4.69$, $p = 0.001$).

Table 2. Olfactory performance of smoking (n = 4) and non-smoking (n = 8) patients with anorexia nervosa.

| | Smokers (n = 4) | | Non-smokers (n = 8) | | Analysis | | |
|---|---|---|---|---|---|---|---|
| | Mean rank | Sum of ranks | Mean rank | Sum of ranks | Mann-Whitney U | Z | Exact Sig.[a] |
| n-butanol$_1$ | 6.75 | 27.00 | 6.38 | 51.00 | 15.00 | -0.17 | 0.93 |
| n-butanol$_2$ | 6.88 | 27.50 | 6.31 | 50.50 | 14.50 | -0.26 | 0.81 |
| iaa$_1$ | 6.25 | 25.00 | 6.63 | 53.00 | 15.00 | -0.17 | 0.93 |
| iaa$_2$ | 6.75 | 27.00 | 6.38 | 51.00 | 15.00 | -0.17 | 0.93 |
| discrimination | 6.25 | 25.00 | 6.63 | 53.00 | 15.00 | -0.17 | 0.93 |
| identification | 8.25 | 33.00 | 5.36 | 45.00 | 9.00 | -1.28 | 0.28 |
| TDI-score | 7.25 | 29.00 | 6.13 | 49.00 | 13.00 | -0.51 | 0.68 |

Note: n-butanol$_1$ = olfactory detection threshold for n-butanol in the non-satiated state, n-butanol$_2$ = olfactory detection threshold for n-butanol in the satiated state, iaa$_1$ = olfactory detection threshold for isoamyl acetate in the non-satiated state, iaa$_2$ = olfactory detection threshold for isoamyl acetate in the satiated state, discrimination = odor discrimination score, identification = odor identification score, TDI-score = sum of olfactory detection threshold for n-butanol in the satiated state, odor discrimination score, and odor identification score

[a] Exact significance [2*(1-tailed)]

Table 3. Olfactory detection thresholds for n-butanol (non-food odor) and isoamyl acetate (food-related odor), and the corresponding subjective ratings of anorectic patients (n = 12) and healthy controls (n = 24) in the non-satiated state (after overnight fasting).

| | Anorexics (mean ± SD) | Controls (mean ± SD) | t (df) | p-value (2-tailed) |
|---|---|---|---|---|
| State of hunger | 4.58 ± 2.54 | 5.38 ± 1.86 | -1.06 (34) | ns |
| Desire for food | 4.50 ± 2.51 | 4.92 ± 1.91 | -0.56 (34) | ns |
| Fullness of stomach | 2.25 ± 1.66 | 2.88 ± 1.68 | -1.06 (34) | ns |
| Olfactory detection threshold for n-butanol | 10.17 ± 2.09 | 10.20 ± 1.90 | -0.05 (34) | ns |
| Emotional valence during n-butanol test | 4.54 ± 1.83 | 6.21 ± 1.47 | -2.95 (34) | 0.01 |
| Arousal during n-butanol test | 3.33 ± 1.83 | 2.63 ± 1.91 | 1.07 (34) | ns |
| Alertness during n-butanol test | 5.92 ± 2.02 | 7.42 ± 1.14 | -2.39 (14.60)* | 0.03* |
| Pleasantness of n-butanol | 3.67 ± 1.92 | 4.04 ± 2.07 | -0.52 (34) | ns |
| Intensity of n-butanol | 7.00 ± 2.17 | 7.63 ± 1.21 | -0.93 (14.50)* | ns* |
| Olfactory detection threshold for isoamyl acetate | 13.50 ± 1.59 | 11.36 ± 2.51 | 2.68 (34) | 0.01 |
| Emotional valence during isoamyl acetate test | 5.83 ± 1.95 | 6.79 ± 1.67 | -1.54 (34) | ns |
| Arousal during isoamyl acetate test | 3.79 ± 2.52 | 2.46 ± 1.53 | 1.69 (15.20)* | ns* |
| Alertness during isoamyl acetate test | 6.25 ± 2.01 | 7.63 ± 0.97 | -2.25 (13.63)* | 0.04* |
| Pleasantness of isoamyl acetate | 5.33 ± 2.10 | 7.71 ± 1.00 | -3.71 (13.54)* | 0.002* |
| Intensity of isoamyl acetate | 7.75 ± 1.66 | 7.13 ± 1.70 | 1.05 (34) | ns |

* equal variances not assumed

During breakfast, patients consumed significantly less calories than the control subjects (patients: 429.42 ± 149.21 kcal, controls: 667.50 ± 134.52 kcal, t (34) = -4.83, p < 0.001). However, patients and controls did not differ in their ratings of hunger and satiety. Except for one olfactory threshold determination, control subjects felt significantly more positive during the threshold tests, and they always characterized themselves as significantly more attentive than the patients (Table 3, Table 4). There were no significant differences between the two groups in the categories arousal, subjective intensity of the two odors, and pleasantness of n-butanol (Table 3, Table 4). By contrast, patients perceived the odor of isoamyl acetate as significantly less pleasant than the healthy control subjects in the hungry as well as in the satiated state (non-satiated state: patients: mean 5.33 ± 2.10, controls: mean 7.71 ± 1.00, t (13.54) = -3.71, p = 0.002; satiated state: patients: mean 5.08 ± 1.78, controls: mean 7.33 ± 1.13, t (34) = -4.63, p < 0.001).

*Olfactory testing*

Detection thresholds for the non-food odor n-butanol did not differ significantly between patients and controls, neither in the hungry (Table 3, Figure 1) nor in the satiated state (Table 4, Figure 2). Detection thresholds for isoamyl acetate in the satiated state also revealed no significant difference between

Table 4. Olfactory detection thresholds for n-butanol (non-food odour) and isoamyl acetate (food-related odour), and the corresponding subjective ratings of anorectic patients (n = 12) and healthy controls (n = 24) in the satiated state (after controlled breakfast).

| | Anorexics (mean ± SD) | Controls (mean ± SD) | t (df) | p-value (2-tailed) |
|---|---|---|---|---|
| State of satiety | 7. 38 ± 1. 26 | 7. 92 ± 1. 06 | -1. 36 (34) | ns |
| Desire for food | 1. 92 ± 0. 90 | 1. 42 ± 0. 58 | 1. 75 (15, 77)* | ns* |
| Fullness of stomach | 6. 67 ± 1. 44 | 7. 04 ± 1. 20 | -0. 83 (34) | ns |
| Olfactory detection threshold for n-butanol | 9. 54 ± 1. 93 | 10. 30 ± 1. 90 | -1. 13 (34) | ns |
| Emotional valence during n-butanol test | 4. 92 ± 1. 73 | 6. 38 ± 1. 61 | -2. 50 (34) | 0. 02 |
| Arousal during n-butanol test | 3. 67 ± 2. 54 | 2. 33 ± 1. 69 | 1. 65 (16. 03)* | ns* |
| Alertness during n-butanol test | 5. 67 ± 1. 72 | 7. 46 ± 1. 44 | -3. 29 (34) | 0. 002 |
| Pleasantness of n-butanol | 3. 42 ± 2. 02 | 3. 92 ± 2. 06 | -0. 69 (34) | ns |
| Intensity of n-butanol | 7. 17 ± 1. 34 | 7. 38 ± 1. 47 | -0. 41 (34) | ns |
| Olfactory detection threshold for isoamyl acetate | 12. 88 ± 2. 46 | 12. 50 ± 2. 66 | 0. 41 (34) | ns |
| Emotional valence during isoamyl acetate test | 4. 83 ± 1. 80 | 6. 71 ± 1. 27 | -3. 63 (34) | 0. 001 |
| Arousal during isoamyl acetate test | 3. 50 ± 2. 11 | 2. 33 ± 1. 34 | 1. 75 (15. 58)* | ns* |
| Alertness during isoamyl acetate test | 6. 00 ± 1. 76 | 7. 25 ± 1. 33 | -2. 39 (34) | 0. 02 |
| Pleasantness of isoamyl acetate | 5. 08 ± 1. 78 | 7. 33 ± 1. 13 | -4.63 (34) | < 0.001 |
| Intensity of isoamyl acetate | 7. 67 ± 1. 16 | 7. 13 ± 1. 36 | 1. 18 (34) | ns |

* equal variances not assumed

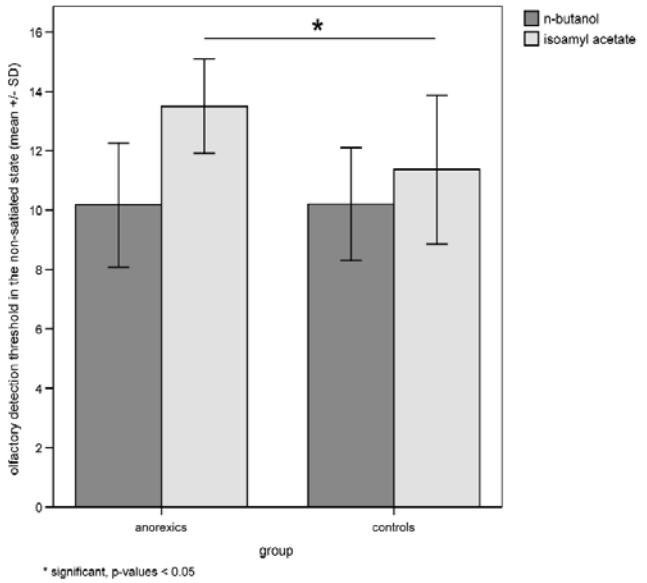


Figure 1. Olfactory detection thresholds for n-butanol (non-food odour) and isoamyl acetate (food-related odour) of anorectic patients (n = 12) and healthy controls (n = 24) in the non-satiated state (after overnight fasting).

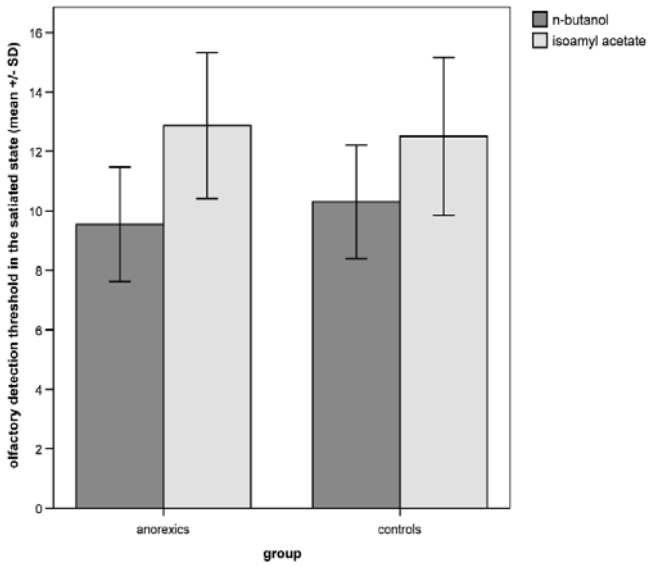


Figure 2. Olfactory detection thresholds for n-butanol (non-food odour) and isoamyl acetate (food-related odour) of anorectic patients (n = 12) and healthy controls (n = 24) in the satiated state (after controlled breakfast).

patients and control subjects (Table 4, Figure 2). However, in the non-satiated state, patients could detect the odor of isoamyl acetate significantly better than controls, i. e. patients had a significantly lower threshold for the food-related odor (patients: mean 13.50 ± 1.59, controls: 11.36 ± 2.51, t (34) = 2.68, p = 0.01) (Table 3, Figure 1). The absolute and the relative alteration of detection thresholds for isoamyl acetate between hunger and satiety differed significantly between patients and controls (absolute alteration: patients: mean –0.63 ± 2.23, controls: mean 1.14 ± 2.35, t (34) = -2.15, p = 0.04; relative alteration: patients: mean –0.04 ± 0.18, controls: mean 0.12 ± 0.24, t (34) = 2.07, p = 0.046). After ingestion, we recorded a significant increase in olfactory sensitivity for isoamyl acetate in healthy subjects [24] and a trend towards lower olfactory sensitivity for isoamyl acetate in patients with anorexia nervosa (see results, part I). Regarding n-butanol, the absolute and the relative alteration of detection thresholds did not show any significant differences between the two groups.

Patients' performance was significantly lower than that of controls for both odor discrimination (patients: mean 12.50 ± 1.57, controls: mean 13.75 ± 1.42, t (34) = -2.41, p = 0.02) and odor identification (patients: mean 13.33 ± 1.30, controls: mean 14.42 ± 0.78, t (34) = -3.13, p = 0.004). Additionally, patients had a significantly lower mean TDI score than controls (patients: mean 35.38 ± 3.88, controls: mean 38.47 ± 2.50, t (34) = -2.90, p = 0.01).

After dividing the 16 items of the odor identification test into food-related and non-food odors, no differences in performance within groups were found. In the between-group analysis however, patients scored significantly lower on the food-related odors than control subjects (n = 13; rank sum 150 for patients vs. rank sum 516 for controls, U = 72, Z = -2.58, p = 0.02). No significant differences could be found between groups for the non-food odors (n = 3; rank sum 218.5 for patients vs. rank sum 447.5 for controls, U = 140, Z = -0.13, p = 0.91). Therefore, the significant difference between patients and control subjects in the odor identification task was due to differences in the food-related odors.

## DISCUSSION

The aim of this study was not only to compare the olfactory performance of anorectic patients and healthy controls but also to examine patients' olfactory sensitivity for food-related and non-food odors in states of hunger and satiety. Regarding the non-food odor n-butanol, in this study, the detection thresholds of anorectic patients and healthy controls did not differ significantly in the hungry or in the satiated state. All of the differences between patients and control subjects were found with food-related stimuli. Patients perceived the food-related odor of isoamyl acetate at significantly lower concentrations than control subjects but only in the non-satiated state. Ingestion led to a significantly increased olfactory sensitivity to isoamyl acetate in healthy subjects [24] but to a trend towards lower olfactory sensitivity in anorectic patients. The divergence of the results leads to the hypothesis that there is a food-specific alteration of olfactory detection thresholds in patients with anorexia nervosa. Moreover, our results emphasize the importance of differentiating between food-related and non-food odors as well as hunger and satiety in examining olfactory sensitivity.

In this study, patients with anorexia nervosa had normal (or even lowered) odor detection thresholds, but decreased odor discrimination and odor identification capacity. Olfactory detection is a precondition for odor discrimination and odor identification, and can therefore be characterized as the more basic process [32]. Neuroimaging studies were able to show that the intensity of odors is mainly processed in the piriform cortex [33] and amygdala [34]. By contrast, odor discrimination and identification are represented in the orbitofrontal cortex [35-37]. According to recent classifications [38,39], the olfactory bulb is the primary olfactory cortex. All regions receiving direct projections from the olfactory bulb such as the piriform cortex and the amygdala are considered secondary olfactory areas. The tertiary olfactory cortex consists among other regions of the orbitofrontal cortex, the anterior insula, and the cingulate gyrus. On the basis of this categorization, it can be concluded that the intensity of odors is processed in parts of the secondary olfactory cortex, whereas odor discrimination and odor identification are functions of tertiary olfactory regions. Since the anorectic patients scored less only in the odor discrimination and identification task, we suggest that there is either a dysfunction of tertiary olfactory structures or an impaired projection from secondary to tertiary olfactory regions in patients with anorexia nervosa. Such an impairment would be consistent with the disease pattern of anorexia nervosa as the orbitofrontal cortex is known to play an important role in the control of appetite and food-intake [40].

This was the first study to determine TDI scores for anorectic patients. Compared to healthy control subjects, patients with anorexia nervosa had a significantly lower mean TDI score. However, this result has to be interpreted with caution as the performance of the patients cannot be considered pathological if compared to normative data for the Sniffin' Sticks [29].
The aforementioned food-specific alteration of olfactory detection thresholds was observed in the pleasantness ratings of the two odorants as well: there were no differences in the perceived pleasantness of the non-food stimulus between patients and control group, but the food-related odor was evaluated as significantly less pleasant by anorectic patients than by control subjects in the hungry as well as in the satiated state. Furthermore, the healthy control subjects tested in our study perceived the odor of isoamyl acetate as significantly more pleasant in the hungry compared to the satiated state [24], whereas the patients' estimates of isoamyl acetate did not change. As far as n-butanol is concerned, none of the groups showed significant differences in their ratings of pleasantness between hunger and satiety. Since all of the subjects had to consume one banana and both of the groups described themselves as satiated after breakfast, we would have expected a decrease in pleasantness of isoamyl acetate in patients with anorexia nervosa as well. Interestingly, a neuroimaging study [41] in which anorectic patients and healthy controls had to rate the pleasantness of visual food and non-food stimuli in hunger and satiety found similar results. The authors suggested an altered cognitive processing of food cues in anorectic patients, and claimed that this reduced somatosensory-gustatory responsiveness may facilitate fasting in anorexia nervosa. The results of our study expand these findings indicating that anorectic patients also suffer from a reduced olfactory responsiveness towards food stimuli.

Furthermore, dividing the 16 items of the odor identification test into food-related and non-food odors provided corresponding findings: Patients with anorexia nervosa scored significantly lower than control subjects when identifying food-related odors, but there were no inter-group differences in case of non-food odors. Thus, we were able to show that the patients' deficits in identifying odors apply primarily to food-related odors. Although one has to consider that the Sniffin' Sticks might not be the most sophisticated instrument for this kind of question as only three non-food odors are tested, these findings corroborate the theory of a reduced olfactory responsiveness to food stimuli. It is still to be clarified whether this insensitivity towards food cues has to be regarded as cause or consequence of the disease.
Four anorectic patients were included in this study in spite of being smokers. It is still a matter of controversy whether smoking influences olfactory performance. Some studies were able to demonstrate adverse effects of smoking on olfaction [42,43], others not [44]. The most sophisticated experiments on this topic were conducted by Frye et al. [45] who did not only distinguish between non-smokers, previous and current smokers but also counted pack years. They found that smoking was adversely associated with odor identification ability but this effect was highly dose-related. The four smoking patients with anorexia nervosa tested in this study consumed an average of 7 cigarettes per day. Considering their young age (17 to 25 years) and their low rate of consumption, they would not have reached a critical dose of smoking at the time they were tested. In addition, our results show that the smokers outperformed non-smokers in three out of four threshold tests and in the odor identification test, and they had a higher TDI score than the non-smokers. However, these differences were not significant (see Table 2). Therefore, the inclusion of anorectic patients in this study who were smokers appears justified.

The d2 Test of Attention was administered in the hungry as well as in the satiated state to monitor the patients' attentiveness. It was suspected that after breakfast, patients might be distracted by thinking about the previous meal and the consumed calories instead of focussing on the tests. Surprisingly, patients performed better after the meal than before. On the one hand, this result could be due to higher attentional capabilities because of nutrient uptake. On the other hand, repetition of a test of attention can lead to an improvement in performance by training even though this should be prevented by the modified test instructions we used (see methods).

Nevertheless, an effect of training can never be completely excluded and constitutes the most likely explanation for the patients' improved performance after breakfast.

In comparison with the pre-existent literature, our study confirms the results of Lombion-Pouthier et al. [14] who reported that patients with anorexia nervosa had a higher olfactory sensitivity and tended to under-evaluate the pleasantness of odors. However, we were able to demonstrate that these findings only apply to food-related odors and that the patients' detection threshold is decreased only in a hungry condition. Unlike Lombion-Pouthier et al. [14], we did not observe an over-evaluation of the intensity of odors in anorectic patients. Furthermore, our results are consistent with Roessner et al. [11] insofar as patients with anorexia nervosa show deficits in the subtest odor discrimination of the Sniffin' Sticks. Yet, we cannot affirm the authors' statement that anorectic patients have a higher olfactory detection threshold for n-butanol. Finally, we neither agree with Kopala et al. [12] reporting that patients with anorexia nervosa have intact olfactory function nor with Fedoroff et al. [13] suggesting that olfactory impairments only appear in very low-weight anorectic patients.

If future studies are able to confirm our findings, this might provide a useful supplement to the therapy of anorexia nervosa. Deficits in identifying odors and in discriminating among suprathreshold stimuli have also been observed in the elderly, for example, where they lead to reduced appetite, inadequate food choices, and diminished nutrient intake [46]. Several studies were able to demonstrate that flavor enhancers can not only improve food palatability and acceptance [46] but also increase dietary intake and body weight [47] in elderly nursing home residents. Therefore, flavor enhancement of food might be considered for the therapy of anorexia nervosa as well. At least at the beginning of treatment, it could increase the enjoyment of food and thus raise the willingness to eat.

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## REFERENCES

1. Bromley SM. Smell and taste disorders: a primary care approach. Am Fam Physician 2000; 61: 427-436, 438.
2. Burdach KJ, Doty RL. The effects of mouth movements, swallowing, and spitting on retronasal odour perception. Physiol Behav 1987; 41: 353-356.
3. Ciofalo A, Filiaci F, Romeo R, Zambetti G, Vestri AR. Epidemiological aspects of olfactory dysfunction. Rhinology 2006; 44: 78-82.
4. Doty RL, Deems DA, Stellar S. Olfactory dysfunction in parkinsonism: a general deficit unrelated to neurologic signs, disease stage, or disease duration. Neurology 1988; 38: 1237-1244.
5. Deems DA, Doty RL, Settle RG, et al. Smell and taste disorders, a study of 750 patients from the University of Pennsylvania Smell and Taste Center. Arch Otolaryngol Head Neck Surg 1991; 117: 519-528.
6. Miwa T, Furukawa M, Tsukatani T, Costanzo RM, DiNardo LJ, Reiter ER. Impact of olfactory impairment on quality of life and disability. Arch Otolaryngol Head Neck Surg 2001; 127: 497-503.
7. Temmel AF, Quint C, Schickinger-Fischer B, Klimek L, Stoller E, Hummel T. Characteristics of Olfactory Disorders in Relation to Major Causes of Olfactory Loss. Arch Otolaryngol Head Neck Surg 2002; 128: 635-641.
8. Robert-Koch-Institut. Gesundheitsberichterstattung des Bundes: Gesundheit in Deutschland. Berlin: Statistisches Bundesamt; 2006: 112-115.
9. Aschenbrenner K, Hummel C, Teszmer K, et al. The Influence of Olfactory Loss on Dietary Behaviors. Laryngoscope 2008; 118: 135-144.
10. Mattes RD, Cowart BJ. Dietary assessment of patients with chemosensory disorders. J Am Diet Assoc 1994; 94: 50-56.
11. Roessner V, Bleich S, Banaschewski T, Rothenberger A. Olfactory deficits in anorexia nervosa. Eur Arch Psychiatry Clin Neurosci 2005; 255: 6-9.
12. Kopala LC, Good K, Goldner EM, Birmingham CL. Olfactory identification ability in anorexia nervosa. J Psychiatry Neurosci 1995; 20: 283-286.
13. Fedoroff IC, Stoner SA, Andersen AE, Doty RL, Rolls BJ. Olfactory dysfunction in anorexia and bulimia nervosa. Int J Eat Disord 1995; 18: 71-77.
14. Lombion-Pouthier S, Vandel P, Nezelof S, Haffen E, Millot JL. Odour perception in patients with mood disorders. J Affect Disord 2006; 90: 187-191.
15. Fichter MM, Quadflieg N. Comparing self- and expert rating: a self-report screening version (SIAB-S) of the structured interview for anorexic and bulimic syndromes for DSM-IV and ICD-10 (SIAB-EX). Eur Arch Psychiatry Clin Neurosci 2000; 250: 175-185.
16. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961; 4: 561-571.
17. Pause BM, Miranda A, Goder R, Aldenhoff JB, Ferstl R. Reduced olfactory performance in patients with major depression. J Psychiatr Res 2001; 35: 271-277.
18. Serby M, Larson P, Kalkstein D. Olfactory sense in psychoses. Biol Psychiatry 1990; 28: 830.
19. Amsterdam JD, Settle RG, Doty RL, Abelman E, Winokur A. Taste and smell perception in depression. Biol Psychiatry 1987; 22: 1481-1485.
20. Warner MD, Peabody CA, Csernansky JG. Olfactory functioning in schizophrenia and depression. Biol Psychiatry 1990; 27: 457-458.
21. Corcos M, Guilbaud O, Speranza M, et al. Alexithymia and depression in eating disorders. Psychiatry Res 2000; 93: 263-266.
22. Speranza M, Corcos M, Loas G, et al. Depressive personality dimensions and alexithymia in eating disorders. Psychiatry Res 2005; 135: 153-163.
23. Doty RL, Bromley SM. Effects of drugs on olfaction and taste. Otolaryngol Clin North Am 2004; 37: 1229-1254.
24. Albrecht J, Schreder T, Kleemann AM, et al., Olfactory detection thresholds of food-related and non-food odours in hunger and satiety, submitted.
25. Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G. 'Sniffin' sticks': olfactory performance assessed by the combined testing of odour identification, odour discrimination and olfactory threshold. Chem Senses 1997; 22: 39-52.
26. Kobal G, Hummel T, Sekinger B, Barz S, Roscher S, Wolf S. "Sniffin' sticks": screening of olfactory performance. Rhinology 1996; 34: 222-226.
27. Simmen D, Briner HR. Olfaction in rhinology--methods of assessing the sense of smell. Rhinology 2006; 44: 98-101.
28. Doty RL, Olfactory system. In: Getchell TV, Doty RL, Bartoshuk LM,Snow JB, Jr, editors. Smell and taste in health and disease. New York: Raven Press; 1991.
29. Hummel T, Kobal G, Gudziol H, Mackay-Sim A. Normative data

for the "Sniffin' Sticks" including tests of odour identification, odour discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. Eur Arch Otorhinolaryngol 2007; 264: 237-243.
30. Bradley MM, Lang PJ. Measuring emotion: the Self-Assessment Manikin and the Semantic Differential. J Behav Ther Exp Psychiatry 1994; 25: 49-59.
31. Brickenkamp R, Zillmer E, d2 Test of Attention. Goettingen: Hogrefe; 1998.
32. Daum RF, Sekinger B, Kobal G, Lang CJ. [Olfactory testing with "sniffin' sticks" for clinical diagnosis of Parkinson disease]. Nervenarzt 2000; 71: 643-650.
33. Rolls ET, Kringelbach ML, de Araujo IE. Different representations of pleasant and unpleasant odours in the human brain. Eur J Neurosci 2003; 18: 695-703.
34. Anderson AK, Christoff K, Stappen I, et al. Dissociated neural representations of intensity and valence in human olfaction. Nat Neurosci 2003; 6: 196-202.
35. Jones-Gotman M, Zatorre RJ. Olfactory identification deficits in patients with focal cerebral excision. Neuropsychologia 1988; 26: 387-400.
36. Takagi SF. The olfactory nervous system of the old world monkey. Jpn J Physiol 1984; 34: 561-573.
37. Tanabe T, Iino M, Takagi SF. Discrimination of odours in olfactory bulb, pyriform-amygdaloid areas, and orbitofrontal cortex of the monkey. J Neurophysiol 1975; 38: 1284-1296.
38. Cleland TA, Linster C, Central olfactory structures. In: Doty RL, editor. Handbook of olfaction and gustation. New York: Marcel Dekker; 2003.
39. Wiesmann M, Kopietz R, Albrecht J, et al. Eye closure in darkness animates olfactory and gustatory cortical areas. Neuroimage 2006; 32: 293-300.
40. Rolls ET. Taste, olfactory, and food texture processing in the brain, and the control of food intake. Physiol Behav 2005; 85: 45-56.
41. Santel S, Baving L, Krauel K, Munte TF, Rotte M. Hunger and satiety in anorexia nervosa: fMRI during cognitive processing of food pictures. Brain Res 2006; 1114: 138-148.
42. Ishimaru T, Fujii M. Effects of smoking on odour identification in Japanese subjects. Rhinology 2007; 45: 224-228.
43. Katotomichelakis M, Balatsouras D, Tripsianis G, et al. The effect of smoking on the olfactory function. Rhinology 2007; 45: 273-280.
44. Hubert HB, Fabsitz RR, Feinleib M, Brown KS. Olfactory sensitivity in humans: genetic versus environmental control. Science 1980; 208: 607-609.
45. Frye RE, Schwartz BS, Doty RL. Dose-related effects of cigarette smoking on olfactory function. Jama 1990; 263: 1233-1236.
46. Schiffman SS, Graham BG. Taste and smell perception affect appetite and immunity in the elderly. Eur J Clin Nutr 2000; 54 Suppl 3: S54-63.
47. Mathey MF, Siebelink E, de Graaf C, Van Staveren WA. Flavor enhancement of food improves dietary intake and nutritional status of elderly nursing home residents. J Gerontol A Biol Sci Med Sci 2001; 56: M200-205.

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