ORIGINAL CONTRIBUTION

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Olfactory detection thresholds and pleasantness of a food-related and a non-food odour in hunger and satiety*

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SUMMARY

The primary aim of this study was to investigate whether olfactory detection thresholds are dependent on different states of satiety. Using the threshold test of the Sniffin' Sticks test battery (single-staircase, three alternative forced choice procedure), sensitivity to a non-food odour (n-butanol) and a food-related odour (isoamyl acetate) was investigated. Twenty-four healthy, female subjects (mean age 24.2 years, SD 2.7 years) with normal olfactory function performed the tests when hungry and when satiated. Additionally, they rated their emotional condition, arousal, alertness as well as the intensity and pleasantness of both odorants. No significant change in the detection thresholds for the non-food odour n-butanol, but a significant change in detection threshold for the food-related odour isoamyl acetate was found. The detection threshold for isoamyl acetate was significantly lower in the state of satiety compared to the hungry condition. As expected, the perceived pleasantness of isoamyl acetate was significantly lower in satiety. In summary, the results indicate that in our experimental setting the actual state of satiety has effects on detection thresholds of a food-related odour, but not of a non-food odour. Interestingly, the higher sensitivity was found during the state of satiety challenging the current hypothesis that control of food intake is supported by a decrease in sensitivity to food odours. Instead our findings that satiety decreases the pleasantness of a food-related odour support the hypothesis that both odour threshold as well as pleasantness play an important role in the control of food intake.

Key words: olfaction, odour, n-butanol, isoamyl acetate, Sniffin' sticks

INTRODUCTION

For a long time olfactory researchers have been interested in a possible relationship between olfactory sensitivity and food intake in humans, particularly as indicated by changes in odour detection thresholds [1-5]. The detection threshold of an odorant is the lowest odour concentration at which the presence of this odorant can reliably be detected by a subject.

Recently, since the discovery of leptin in the mid '90's [6] a connection between human olfaction and metabolic functions became more evident. Hormones like leptin, orexin, and insulin play a major role in the regulation of body weight homeostasis (for review see Hellstrom et al. [7]). Amongst several other cortical regions of the rat, receptors for leptin, orexin, and insulin have been found to be expressed in the piriform cortex [8-10], which from an evolutionary point of view is a secondary olfactory cortex region although it is commonly named primary olfactory cortex [11, 12]. In mice the results of a study by Getchell et al. [13] suggest that leptin, acting through leptin receptors, modulates olfactory-mediated pre-ingestive behavior. In humans the results of a study by Karlsson et al. [14] gave evidence for a gender-specific relation between leptin and the ability to identify an odour: high odour identification abilities were associated with high serum leptin levels in men and low serum leptin levels in women. The results of these studies demonstrate the strong link between feeding peptide functions and olfactory processing.

Since food intake is an essential human activity regulated by homeostatic and hedonic sensory mechanisms in the brain [15], it is assumed that there is a connection between the actual state of human satiety and olfactory sensitivity i.e. the inhibition of further food intake maybe supported by an increase in the olfactory detection threshold. There are reports in the literature suggesting lower olfactory thresholds before a meal com-

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pared to those after a meal. In these studies olfactory thresholds were measured using blast injection [16] or a variety of sniffing techniques [17-19]. Accordingly, Goetzl et al. [20, 21] found diurnal variations in olfactory thresholds linked to the ingestion of food. They hypothesized that a meal is preceded by a period of decreased olfactory thresholds (increased sensitivity), followed by a period of increased olfactory thresholds (decreased sensitivity). A study by Hammer [22] confirmed these findings. In contrast, Berg et al. [23] as well as Fikentscher [24] gave evidence for higher olfactory sensitivities as measured by lower thresholds for 2-heptanone and phenylethyl alcohol after a satiating meal. Other investigators, however, were not able to demonstrate significant changes in olfactory thresholds depending on the ingestion of food [5, 25-28]. The inconsistency of results in the literature mentioned above maybe either a sign for the weakness of the effect, i.e. the irrelevance of olfactory sensitivity for the control of food intake, or else maybe explained by weaknesses in the methodological approaches.

The aim of this study was to reassess this potential phenomenon and to investigate the relationship between olfactory sensitivity, measured by means of odour detection thresholds, and the actual state of satiety in a well controlled experiment employing a validated measurement technique [29-32] in a controlled study population. The hypothesis of this study was that human olfactory detection thresholds would be different before and after eating a meal to satiety. Specifically it was hypothesized that in the light of support of food intake control, human volunteers have lower olfactory thresholds when hungry compared to being satiated.

MATERIALS AND METHODS

Subjects

Twenty-four healthy female subjects (mean age of 24.2 years, SD 2.7 years) participated in the study. They were non-smokers, reported normal olfactory functions and did not take any medication known to interfere with sensory perception [33-35]. The body mass index (BMI) of the subjects was in a range of 17.4 to 24.9 kg/m^2 (mean 21.0 kg/m^2, SD 1.7 kg/m^2). None of the subjects was suffering from depression (mean 1.0, SD 1.1) as obtained by the Beck Depression Inventory (BDI [36]). Neither during the study (mean 0.2, SD 0.1) nor during their former life (mean 0.4, SD 0.2) were subjects suffering from an anorectic or bulimic eating disorder as obtained by the Self-report Screening Version of the Structured Interview for Anorexic and Bulimic syndromes for DSM-IV and ICD-10 (SIAB-S [37]). To avoid gender effects and effects of smoking on olfactory function a homogenous group of only female non-smokers was recruited. As this group of subject was the control group for a study investigating olfactory performance in anorexic subjects [38] only female subjects were used.

All subjects gave written, informed consent. The protocol was approved by the Medical Ethics Review Committee (IRB) of the Ludwig-Maximilians-University Munich.

Olfactory testing

All experimental sessions were performed between 8 and 10.30 a.m. Subjects were requested to be in a fasting condition i.e. they had not eaten or consumed caloric beverages for a minimum of 10 hours. When subjects arrived for testing, they had to rate their current state of hunger (1 = not hungry at all, 9 = very hungry), their desire for food (1 = very weak, 9 = very strong) and the fullness of their stomach (1 = not full at all, 9 = very full) on a 9-point scale. Olfactory function was assessed by means of the olfactory detection threshold subtest of the Sniffin' Sticks (Burghart Instruments, Wedel, Germany), a test battery that measures nasal chemosensory function using pen-like devices for odour presentation [29,32]. Detection thresholds were determined using a single-staircase, three alternative forced choice (3-AFC) procedure [29,39]. Two different odour qualities were investigated: (1) n-butanol, a non-food odour (chemical smell), and (2) isoamyl acetate representing a food-related odour (banana smell). For the n-butanol threshold the standard Sniffin' Sticks threshold test was applied, whereas isoamyl acetate was presented in a custom-made threshold test using the same dispensing devices which contained 16 dilution steps starting with a 5% isoamyl acetate/propylene glycol solution (Sigma-Aldrich). This first solution was further diluted 15 times in a ratio of 1:2 in propylene glycol. The two tests were applied in pseudo-randomized order.

After each olfactory threshold test, subjects rated their emotional condition (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 n-butanol or isoamyl acetate. For assessment of emotional condition and arousal, as well as pleasantness of the odours the Self-Assessment Manikin (SAM) [40] scale, a pictorial scale, was used. In contrast, common 9-point scales were used for assessment of alertness and the perceived intensity of the odours.

Then, subjects received a breakfast with standardized food including a banana, bread rolls, optionally butter, cheese, chocolate cream, coffee, tea, milk, and/or orange juice. They were instructed to eat until completely satiated. Grams of consumed standard food items were assessed and consumed calories were calculated using calorie tables [41,42].

Immediately after having breakfast and at least 60 minutes after the first testing subjects had to perform the threshold tests for both odorants again. The two tests and the subjective ratings were performed in the same order as before. This was followed by the odour discrimination and the odour identification subtest of the Sniffin' Sticks. Odour discrimination was tested using 16 triplets of odorants, again presented as a 3-AFC procedure. The odour identification test consisted of 16 commonly known every day odorants. Using a multiple-choice task, identification of individual odours was performed from lists of four descriptors each. Results of the three Sniffin' Sticks subtests (threshold for isoamyl acetate was not included)

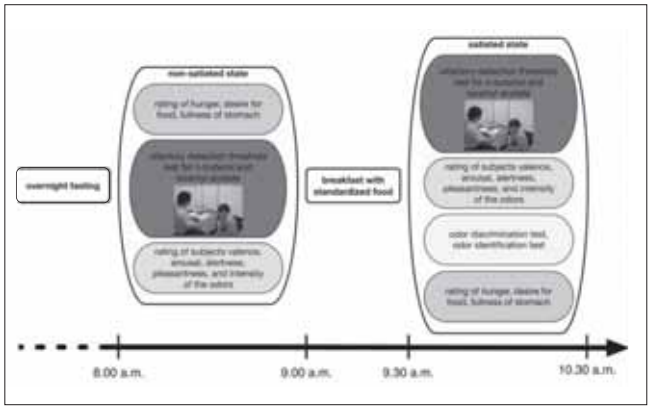

Figure 1. Graphic representation of the experimental design.

were presented as a composite TDI score, which was the sum of the results obtained for olfactory threshold of n-butanol, discrimination, and identification measures [29, 32]. The TDI score was used to determine normal olfactory function. Due to the non-repeatability of the identifcation subtest of the Sniffin' Sticks the TDI score was obtained only once in the satiated state. Odour discrimination and identification tests were accomplished after threshold testing because otherwise subjects would have been influenced by adaptation.

A survey assessing subject's state of satiety (1 = not satiated at all, 9 = very satiated), their actual desire for food (1 = very weak, 9 = very strong), and perceived fullness of their stomach (1 = not full at all, 9 = very full) ended the test session. Subjects were tested individually in a ventilated, illuminated, and quiet room (Figure 1).

Statistics

SPSS program package (version 15.0 for Windows, SPSS Inc, Chicago, IL, USA) was used for statistical evaluation. Means and standard deviations were calculated. Student's t-tests for paired samples were used for comparisons of the data (olfactory detection thresholds for the two odorants, ratings of emotional condition, arousal, alertness of the subjects, ratings of pleasantness and intensity of the two odorants) between the two groups (non-satiated state/satiated state). A Student's t-test for independent samples was used to compare the mean values of the pleasantness of the two odorants independent of the actual state of satiety. The alpha level for all tests was set at 0.05.

RESULTS

At the beginning of the experimental sessions subjects described themselves as moderately hungry (mean 5.4, SD 1.9). They had a moderate desire for food (mean 4.9, SD 1.9) and they described their stomach as being empty (mean 2.9, SD 1.7). The average caloric consumption was 667.5 kilocalories (SD 134.5 kilocalories) per breakfast. After the breakfast subjects felt satiated (mean 7.9, SD 1.1), they felt only weak desire for food (mean 1.4, SD 0.6), and had the feeling of a full stomach (mean 7.0, SD 1.2). Ratings of the state of satiety differed significantly between non-satiated and satiated state (satiety: $t(1,23) = 5.2$, $p < 0.001$, desire for food: $t(1,23) = 8.8$, $p < 0.001$, fullness of stomach: $t(1,23) = 10.5$, $p < 0.001$).

The odour detection threshold for n-butanol showed no significant difference between the non-satiated and the satiated state (non-satiated state: mean 10.2, SD 1.9; satiated state: 10.3, SD 1.9; $t(1,23) = 0.21$, $p = 0.84$). Sensitivity for isoamyl acetate was significantly higher after the meal (non-satiated state: mean 11.4, SD 2.5; satiated state: 12.5, SD 2.7; $t(1,23) = 2.37$, $p = 0.03$) (Figure 2). Interestingly, pleasantness of isoamyl acetate was significantly lower (non-satiated state: mean 7.7, SD 1.0, satiated state: mean 7.3, SD 1.1; $t(1,23) = 2.58$,

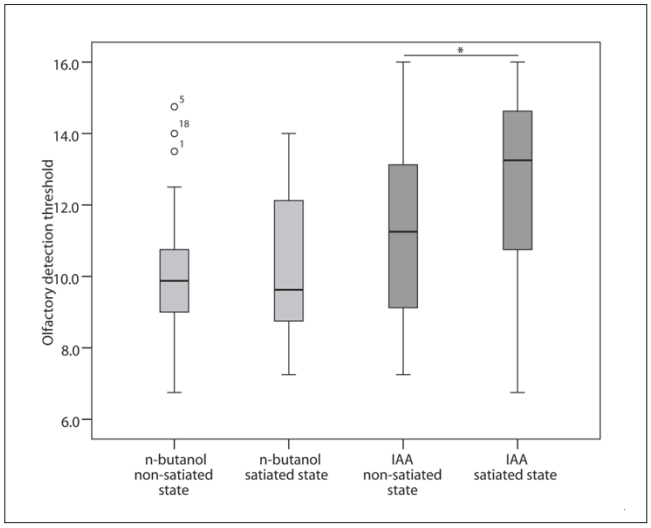

Figure 2. Box plots of the olfactory detection thresholds for n-butanol and isoamyl acetate (IAA) in the non-satiated and satiated state (n = 24) (* significantly different with p = 0.03 (paired t-test)).

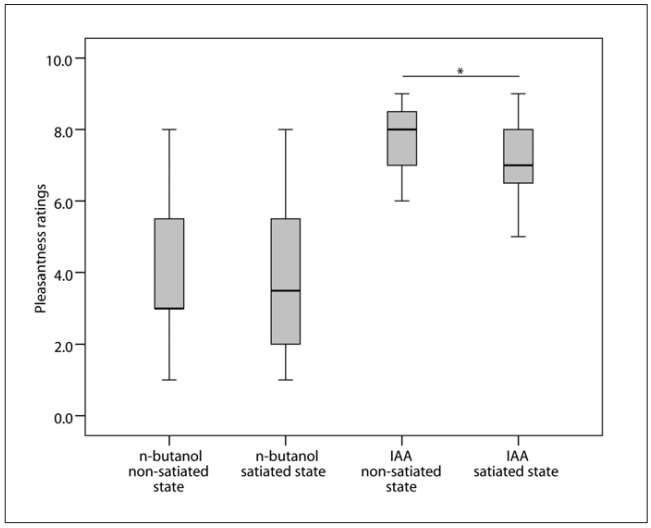

Figure 3. Box plots of the subjective ratings regarding the pleasantness of n-butanol and isoamyl acetate (IAA) in the non-satiated and satiated state (n = 24) (* significantly different with p = 0.02 (paired t-test)).

Table 1. Olfactory detection thresholds of isoamyl acetate and n-butanol and the subsequent subjective ratings in the non-satiated and satiated state (paired t-test).

	non-satiated state (mean ± SD)	*satiated state (mean ± SD)*	*t-value (df)*	*p-value (2- tailed)*
n-butanol (non-food odour):				
olfactory detection threshold	10.2 ± 1.9	10.3 ± 1.9	0.21 (23)	NS
emotional condition	6.2 ± 1.5	6.4 ± 1.6	0.89 (23)	NS
arousal	2.6 ± 1.9	2.3 ± 1.7	1.77 (23)	NS
alertness	7.4 ± 1.1	7.5 ± 1.4	0.23 (23)	NS
pleasantness	4.0 ± 2.1	3.9 ± 2.1	0.57 (23)	NS
intensity	7.6 ± 1.2	7.4 ± 1.5	1.45 (23)	NS
isoamyl acetate (food odour):				
olfactory detection threshold	11.4 ± 2.5	12.5 ± 2.7	2.37 (23)	0.03
emotional condition	6.8 ± 1.7	6.7 ± 1.3	0.33 (23)	NS
arousal	2.5 ± 1.5	2.3 ± 1.3	0.51 (23)	NS
alertness	7.6 ± 1.0	7.3 ± 1.3	1.81 (23)	NS
pleasantness	7.7 ± 1.0	7.3 ± 1.1	2.58 (23)	0.02
intensity	7.1 ± 1.7	7.1 ± 1.4	0.00 (23)	NS

NS = not significant, SD = standard deviation, df = degrees of freedom. N = 24.

Table 2. Results of the ratings regarding the current feeling of hunger/satiety, desire for food, and fullness of stomach in the non-satiated and satiated state (paired t-test).

	non-satiated state (mean ± SD)	*satiated state (mean ± SD)*	*t-value (df)*	*p-value (2- tailed)*
hungriness/satiety	5.4 ± 1.9	7.9 ± 1.1	5.2 (23)	< 0.001
desire for food	4.9 ± 1.9	1.4 ± 0.6	8.8 (23)	< 0.001
fullness of stomach	2.9 ± 1.7	7.0 ± 1.2	10.5 (23)	< 0.001

NS = not significant, SD = standard deviation, df = degrees of freedom. N = 24.

$p = 0.02$) in the satiated state (Figure 3). Subjects' ratings regarding emotional condition, arousal, and alertness as well as intensity ratings of the odours did not reveal any significant difference between the hungry and the satiated condition (Table 1).

Independent of the state of satiety the experiment also demonstrated a significant difference between pleasantness ratings of n-butanol compared to that of isoamyl acetate. Subjects rated the smell of isoamyl acetate more pleasant than the smell of n-butanol (n-butanol mean 4.0, SD 2.0; isoamyl acetate mean 7.5, SD 1.0, $t(1,47) = 9.7$, $p < 0.001$).

The TDI score (the sum of the threshold of n-butanol, discrimination, and identification measures [29,32]) was only obtained once, in the satiated state. Mean value of the olfactory detection threshold test of n-butanol was 10.3, (SD 1.9), mean value of the odour discrimination test was 13.8 (SD 1.4), and 14.4 (SD 0.8) in the odour identification test, resulting in a mean TDI score of 38.5 (SD 4.1) In the group of subjects aged between 16 and 35 years the sum of the results of the threshold, discrimination, and identification subtests (TDI score) separating hyposmia and normosmia is 30.3 [30]. Thus the mean TDI score of our subjects indicated normosmia in all subjects.

DISCUSSION

This study aimed to prove the hypothesis that human olfactory sensitivity changes with the actual state of satiety. Specifically it was hypothesized that humans have lower olfactory detection thresholds when hungry compared to being satiated, which would support the notion that a decrease in sensitivity is part of the food intake control mechanism. As outlined in the introduction, there are several reports on the relationship between food intake and olfactory thresholds but the results are contradictory. Since this may well be due to methodological issues, the aim of this study was to apply a more rigorous study design by choosing a well defined study population, validated methods for measurement of olfactory detection thresholds, and a controlled food intake.

For evaluation of olfactory performance the Sniffin' Sticks test battery was employed because of its established test-retest reliability and validity [29-32,42]. For practical reasons subjects were always tested initially in the hunger condition and subsequently in the satiety condition. The high test-retest reliability of the olfactory detection threshold test of the Sniffin' Sticks [29,42] excludes effects of repeated measurements on olfactory sensitivity.

Within this range of normal olfactory performance and contrary to our hypothesis results demonstrated a significant change in odour threshold suggesting a higher sensitivity, i.e. a lower threshold, for the food-related odour isoamyl acetate (banana smell) after the meal. However, banana odour was rated significantly less pleasant in the satiated state. This observation of a decreased pleasantness when subjects had eaten until feeling completely satiated is in line with the results of other studies [44,45], supporting the hypothesis that perception of food changes with the state of satiety contributing to the control mechanism for food intake.

In contrast, no significant change of the olfactory detection threshold and the pleasantness for the non-food odour n-butanol was detected. The results regarding the threshold for n-butanol confirm and extend findings of a previous study [23] showing no satiety-related change in olfactory sensitivity for a non-food odour.

In line with the general aim of the study, but contrary to our hypothesis, this study indicates increased sensitivity for the food-related odour isoamyl acetate in the satiety condition. However, higher olfactory sensitivity, meaning lower olfactory threshold for a food-related odour in the state of satiety, has

also been found in a previous study [23]. The assumption that sensitivity to food-related odours change in the way that after eating sensitivity decreases, indicating a less intense perception of the food odour, is in all likelihood a false assumption. Instead, if control of food intake is governed by the pleasantness of food odours [46], it would make perfectly sense that after eating, the unpleasantly perceived food odour is also perceived at lower concentrations making the repellent effect more effective. Consequently, the inhibition of food intake would be more effective as well.

The current study presents several limitations. Firstly, only one gender was chosen to be included in the study population. This confines the generality of our results on the overall effect of the state of satiety on olfactory sensitivity to the two specific odorants. Secondly, although the analyzed sample size is larger compared to previous studies in this field, the sample size might still not be large enough to find significant changes in the detection threshold for the unspecific odorant. Thirdly, because isoamyl acetate was the only food-related odour used in this study, a clear statement on a specific effect of isoamyl acetate compared to any food-related odour cannot be given. Future studies should investigate and compare more odorants, including odours, which are not perceived during the satiating meal and could be used as a control condition. Also the usage of natural flavor mixtures instead of pure odourous compounds should be considered. Finally, it is possible that there are differences in olfactory performance related to satiety other than those measured in this study. Assuming that odour thresholds at least partly reflect the functionality of the peripheral olfactory system [47-49], higher functions of olfactory information processing (e.g., odour discrimination or odour identification) may be affected more intensely [50]. Different, more specific methods might be necessary to give insight into olfactory processing related to food intake than quantifying odour detection thresholds. It may be speculated that interactions between food intake and olfaction are mediated by factors other than subjectively perceived satiety, desire for food, or fullness of stomach. These factors may also differ in their time course. In this study measurements of olfactory detection threshold were performed immediately after ingestion of food. One could argue that more time for ingestion is needed to induce satiety specific circuits. Therefore future studies should include repetitive measurements of olfactory performance over a longer time period after a satiating meal. Koelega et al. [5] hypothesized that the expected decrease in sensitivity after a meal may take place at different times for different individuals, possibly related to the amount of food eaten, the caloric content, and body weight. The combination of the individual time courses then would rather mask the effect instead of showing it. Possibly modern neuroimaging techniques will shed more light into the various homeostatic and hedonic sensory mechanisms that underlie regulation of hunger and satiety and the involvement of the olfactory system. Changes in olfactory function depending on the state of satiety do not even need to be restricted to conscious perception. Anatomical data suggest that olfactory information can be processed independently from conscious perception (for review see Cleland et al. [11] and Wiesmann et al. [51]) and there are preliminary neuroimaging results which support this theory [52].

In conclusion, our results clearly demonstrate a change in olfactory detection threshold and pleasantness of a food-related odour, but no significant threshold and pleasantness changes in a non-food odour in different states of satiety in humans. We propose an explanation for the unexpected behavior of the olfactory threshold to the food-related odour emphasizing that an increased sensitivity would enhance the repellent effect of food odours that have become less pleasant and thus more effectively support the control of food intake

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