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Research report

Rat strains with different metabolic statuses differ in food olfactory-driven behavior

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HIGHLIGHTS

- Chronic energy imbalance impacts olfactory-driven behaviors in response to food.
- Fed obese rat strains respond to food odor in the same way as when they are fasted.
- Fasting induces alterations in food olfactory-driven behaviors in lean strains only.
- Expression of olfactory-related metabolic genes varies in different strains.

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ABSTRACT

In most species, food intake is influenced by olfactory cues and metabolic status can affect the olfactory function of animals and regulate feeding-related behaviors. We investigated whether modulation of the endocrine system that regulates or modifies energy balance affected the olfactory system by examining four rat strains, obese Zucker and obesity-resistant Lou/C rats and their counterparts. Such models were chosen because they differ largely in their energy status and in their insulin and leptin blood levels, two hormones known to impact olfactory behaviors. After evaluation of the main metabolic parameters, we analyzed the food-driven olfactory behaviors of the four strains by measuring general activity time and sniffing time in response to food cues together with food reward localization performances in fed and fasted states. In fed conditions, obese Zucker and Wistar rats exhibited a great interest for food odor, which was not enhanced by fasting, in contrast to Lou/C and Zucker lean rats. All strains, except Lou/C, showed decreased latencies to find a hidden food reward with time, whereas a 24-h fasting was necessary to improve food search performances in Lou/C. These metabolic and behavioral changes were partly associated with variations in the transcription profiles of leptin, insulin and orexin and their receptors in the hypothalamus and olfactory system. The results show that variations in metabolic-related genes expression along the olfactory pathways comes with obesity in influencing food odors-driven behaviors. Our data indicate that food-olfactory driven behaviors are clearly affected by the long-term metabolic status.

1. Introduction

Food intake relies on the interaction between homeostatic regulation of energy balance and hedonic sensations [1,2]. Various factors influence hedonic sensations, among which olfaction is a major sensory determinant of food choice and ultimately intake. In humans, food palatability and hedonic ratings for food odors are modulated according to the short-term variation of the satiety status: they are higher in a hunger state than in a satiated state [3,4] and are restricted to food-related odors [5,6]. Recent data show that olfactory performances and acuity for neutral odors are greater in a severe hunger state [7,4], whereas olfactory acuity for food-related odors increases after a satiating meal [7,8]. Thus, during food intake, both modulation of olfactory sensitivity and decrease of hedonic rating for food odors reduce the overall intake of nutrients by eliminating those that are no longer needed.

Abbreviations: OM, olfactory mucosa; OB, olfactory bulb; HO, hypothalamus; Ob-Ra, leptin receptor short form; Ob-Rb, leptin receptor long form; InsR+11, insulin receptor long form; InsR−11, insulin receptor short form; OxRs, orexins receptors; OxR1, orexin R1 receptor; OxR2, orexin R2 receptor; Ox, orexins.

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Long-term modifications of the metabolic status due to positive and negative energy imbalances are also associated with modifications of food intake, which could be linked to modified olfactory behaviors. In common obesity, i.e. for which no abnormal gene associated with the pathology is detected, compulsive food intake is often observed. In this case, hedonic signals override homeostatic satiety signals and this leads to over-consumption of highly palatable foods. The influence of the obese status on olfactory behaviors has been explored in humans and has shown that other factors such as the nature of the stimulus, the satiety context or the age of the subjects are important to consider. During the satiated state after lunch, subjects with a high body mass index (BMI) are able to discriminate better between food odors than those with a low BMI, whereas non-satiated subjects with a high or a low BMI do not. In the case of a neutral odor, the high BMI group displays a lower sensitivity than the low BMI group whatever the satiated state [7] and this relationship seems to be age-dependent [9]. An early attenuation of the olfactory perception is described in children suffering from common obesity, since they display significantly lowered thresholds of identification and detection of odors [10]. In extreme obesity or morbid obesity cases, a diminution of olfactory acuity is observed in smell identification tests, suggesting that olfactory dysfunction may be a contributing factor for the development of obesity [11,12].

Many patients with anorexia nervosa experience less pleasure in eating and show a reduced hedonic responsiveness to flavor, which could be linked to a modification of the olfactory performances. The results described so far are not always consistent as different groups of people and different methods have been used. Some studies reported that, despite similar identification capacities, anorectic subjects have a reduced sensitivity and discrimination abilities to neutral odors than control subjects [13,14], while other studies showed that anorectic patients with a lower identification capacity had similar discrimination capacity and sensitivity [15]. Finally, it becomes clear that such differences in odor perception seem to depend both on the nature of the stimulus and of the nutritional state, since Schreder et al., using food- and non-food-related odors found lower detection thresholds only for the food-related odors in anorectic fasted patients [16]. Taken together, these data indicate that long-term metabolic imbalances have a significant impact on olfactory capabilities via the complex integration of numerous neural and hormonal signals that reflect the interaction between internal state and environmental cues. Any increase in the understanding of the mechanisms underlying the relationship between eating disorders and olfaction appears to be critical to unravel feeding behaviors.

Among the metabolic factors that may be involved in the modulation of olfaction, the role of metabolic-related hormones has been investigated in a few behavioral studies using genetic or diet-based animal models. Mutant obese mice with targeted deletion of the *leptin* gene (ob/ob) or its receptor gene (db/db), i.e. without leptin signaling, take less time to localize a hidden fatty cookie covered with peanut butter, but if the ob/ob mice are supplemented with exogenous leptin, this performance is reversed [17]. Recently, Tucker et al. studied two other models of obesity, a diet-induced model (C57BL6/J mice) and a gene-targeted deletion model (melanocortin receptor null mice, MC4R-null) and showed that they needed more time to localize a fatty scented cracker than their respective lean controls [18]. In contrast, chronic food restriction is associated with an increase in food odor sniffing, which suggests that olfactory behaviors are sensitive to metabolic status. In that case, the number of insulin receptors is decreased in the OM [19]. Finally, among others, we have demonstrated a direct role for orexin, leptin and insulin on olfactory behaviors linked to food stimulus using rodent models. In rat, short-term fasting increases the animal's interest for isoamyl acetate and food odors [20] and results in an increased sensitivity for isoamyl acetate [21] that is mimicked by orexin administration [22,20]. Olfactory behaviors are also decreased after administering leptin, insulin and orexin-receptor antagonists that mimic the fed state [22,20,23]. Therefore, food-driven olfactory behaviors are sensitive to variations in metabolic signals that exist in physiological situations, such as fasting or metabolic disorders.

Recent results have concluded that these metabolic factors can alter odor processing in the olfactory mucosa (OM) and bulb (OB), which are the two first steps in the treatment of an olfactory signal, i.e. initiation and transfer to the brain. Receptors for orexin, leptin and insulin are expressed in the OB and OM and their genes or protein expression levels are modulated after a 48-h period of fasting or after long-term food restriction [24,25,19]. Orexin, leptin and insulin are also locally synthesized in the OM [26,24,25]. In parallel, application of insulin/leptin or orexin decreases or increases, respectively, the amplitude of olfactory neuron responses to an odorant in the OM measured by electro-olfactogram [25,27] (P. Congar, personal communication) and modulates c-fos response to food odor in the OB [20].

Here, our aim was to compare food olfactory-driven behaviors in lean and fat male adult rats in fed or fasted states. We hypothesized that different metabolic contexts that are associated to insulin and leptin variations in blood and to modulations of orexins in the hypothalamus (HO), which all act on the olfactory message, could modify food-related olfactory behaviors and thus participate in the deregulation of food intake. Lou/C lean rats and Zucker obese rats (and their counterparts) were chosen for their metabolic features: Lou/C rat is an inbred strain of Wistar origin that does not develop obesity with age or when fed a high-fat diet [28,29]. Compared to Wistar rats, Lou/C rats exhibit a reduced body weight, a stable fat pad mass [30] and increased locomotor activity [31]. Zucker rats are obese because of a mutation in the *leptin receptor* gene and exhibit increased food intake and fat pad accumulation compared to their lean counterparts [32]; reviewed in [33]. After evaluation of the main metabolic parameters for the four strains of rats in our experimental conditions, we recorded their olfactory-based behaviors in response to the presentation of food or hedonic cookies, in fed and fasted conditions. We hypothesized that compared to individuals in a fed state, fasted individuals would demonstrate olfactory performance changes, the amplitude of these changes depending on their basal energy state. In addition, the expression of insulin, leptin, orexins and their receptors, all known to modulate the olfactory message and to vary according to the nutritional status [34,24,25,27,20,19] were evaluated in the OM, OB and hypothalamus to determine whether strain-specific regulation of these key genes was associated to their food-related behavioral phenotype.

2. Materials and methods

All experiments were carried out in accordance with the recommendations provided by the European Communities Council Directive of 24 November 1986 (86/609/EEC). C.B. holds the Individual Authorization for Performing Experiments in Animals, including the animal experiments used in the present study, provided by the Préfecture des Yvelines (France), according to French and European laws (agreement #78-65). Since the project was initiated with support from the French National Research Agency ANR in 2006–2009, the local institutional animal committee approval was not required to perform behavioral experiments. However, every effort was made to minimize the number of rats used in this study and their suffering. Euthanasia was properly conducted by skilled staff using well-maintained equipment that ensured rapid death.