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Research report

Changes in rat olfactory detection performance induced by orexin and leptin mimicking fasting and satiation

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Abstract

Numerous peripheral and hypothalamic peptides control food intake. Among these signals are orexin, an orexigenic molecule released into the olfactory bulb by centrifugal hypothalamic fibres and leptin, an anorexigenic molecule that is released peripherally and can pass through the blood–brain barrier. In the present study, we injected either orexin or leptin, intracerebroventricularly, and their effect on olfactory performance was evaluated in two groups of rats, using a behavioral paradigm based on conditioned olfactory aversion. Rats were made aversive to water odorized with isoamyl acetate (ISO) at 10^{-5} (1 μl in 100 ml of water). One group was injected with orexin versus saline and the other with leptin versus saline. They were then presented with different concentrations (lower than 10^{-5}) of ISO-odorized water to compare their ability to avoid the ISO-drink. Orexin decreased ISO-drink consumption, showing increased avoidance of the ISO concentrations tested which ranged from 10^{-9} to 10^{-7}. Conversely, the administration of leptin resulted in a dose dependant increase in the odorized-drink consumption for ISO 10^{-10}. Orexin therefore increases and leptin decreases olfactory sensitivity. Orexin and leptin modulate the olfactory performance in a similar way as do physiological induced fasting and satiation and appear to be important factors in the interdependency of olfaction and food intake.

Keywords: Olfaction; Odor; Feeding; Aversion; Leptin; Orexin

1. Introduction

Olfaction is one of the main external cues which can induce feeding independently from the nutritional cravings of an organism [54]. Most animals including humans use this sense to appreciate food palatability and initiate food intake [14,24,35,36,41–43,59] and impairment of the olfactory signal may affect the control of eating behavior [20,33,39]. Thus, deciphering how olfactory mechanisms are involved in feeding behavior appears to be a relevant way to better define some of the factors responsible for obesity and other eating disorders, whether caused by genetic or environmental factors.

In humans, the feeding state modulates olfactory sensitivity [26–29,35,38,63,68]. In animals, although this modulation had been reported [11], it had never been specifically analyzed until a very recent behavioral experiment by our team where the ability to detect odors was compared in fasted and satiated rats [1]. Fasting and satiation were found to increase and decrease the olfactory detection power of rats.

The findings that the feeding state has a direct influence on olfactory sensitivity raises the question of what the chemical signals involved in such changes in olfactory performance are, *i.e.* in the crosstalk between the hypothalamic feeding network and the olfactory system. Numerous peripheral or hypothalamic molecules have been reported to act in the priming and stopping of food intake [44]. Among these molecules, orexin appears especially interesting since a considerable body of evidence has pointed to it being a stimulator of food intake [15,34,44,47,55,57]. In the central nervous system, orexin is synthesized and secreted by hypothalamic neurons [13,58] and is released into the olfactory bulb (OB) and cortices from centrifugal fibers originating directly from the lateral hypothalamic

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areas [8,12,13,52,58]. It is thus possible that orexin is involved in the signaling between the hypothalamus and olfactory centers.

One of the most potent among satiety signals, leptin [5,16,17,22] is synthesized peripherally by the adipocytes and secreted in direct proportion to the degree of adiposity [46] acting on the hypothalamic feeding networks which in turn regulate food intake by initiating satiety [46,48,64]. Leptin is involved in fat regulation, insulin signaling, glucose homeostasis and bone remodeling and appears to be of great interest for a therapeutic approach to obesity [9]. It has also been proposed that leptin is one of the major signal mediators involved in modulating performance of the olfactory system in relation to the alimentary status [37] and indeed, leptin receptor expression has been shown to increase in the olfactory epithelium of fasted rats [4].

Intracerebroventricular (icv) administration of orexin has been demonstrated to increase food intake in rats [15,56,58,66] and mice [47] and icv administration of leptin to decrease food intake in both rats [16,34,50,61] and humans [19,32,49] which led us to hypothesize that these two molecules should be capable of mimicking very closely the feeding states of physiological fasting and satiation.

In order to decipher some of the mechanisms underlying the influence of the feeding state on olfactory sensitivity, the present study addresses the question of the action of orexin and leptin on olfactory perception. The aim was to provide experimental evidence of the involvement of orexin and leptin in the crosstalk between hypothalamic and olfactory structures by showing that orexin and leptin act on olfactory sensitivity in a very similar way to that of fasting and satiety. This was done by pairing the behavioral paradigm based on conditioned olfactory aversion (COA) with icv injections of orexin and leptin in rats placed under a strictly controlled food-intake regimen.

2. Materials and methods

2.1. Animals

The experiments were carried out in accordance with the European Community Council Directive of November 24, 1986 (86/609/EEC), for the care and use of laboratory animals and every effort was made to minimize animal suffering and keep to a minimum the number of animals used. Experimental protocols were approved by the Comité d'Expérimentation Animale of the Université Claude Bernard, Lyon 1.

The orexin and leptin experiments were performed on two different groups of 12 naive male Wistar rats. The rats (2 months old and weighing 220/240 g on receipt) were purchased from Charles River Laboratories (France). On arrival, they were housed individually in Plexiglas chambers at constant temperature and relative humidity ($22 \pm 0.5\,^{\circ}C$ and $50 \pm 5\%$). All rats were kept under a 12 h light–12 h dark cycle and were weighted daily at 09:00 a.m.

2.2. Surgery

The surgical procedures we used have been described in detail by Mistry et al. [48]. Drugs were icv administered through cannula (Plastic One, 22-gauge stainless steel guide) implanted in the left lateral cerebral ventricle. The correct positioning of the cannula was verified by an intense drinking response to an icv injection of angiotensin II (100 ng; Sigma–Aldrich, France) after which the rats were allowed to recover for 7 days in their home cage with free access to food and water before the water-restriction pre-study phase began. During this period, they were handled daily and the dummy cannula inserted in the icv cannula was carefully removed and replaced.

2.3. Drugs

Orexin A was purchased from Bachem AG (Switzerland) and leptin from Sigma–Aldrich (France). The orexin was administrated at a dose of 10.7 μg/rat (3 nmol) and leptin at 30 or 90 ng/rat (18.75 and 56.25 pmol). The two drugs were delivered in 3 μl of saline vehicle (NaCl 9‰) over 60 s. Neither the orexin [31] nor the leptin [45] injected at these doses were found to quantitatively change the food intake.

2.4. Behavioral protocol

2.4.1. Common features of the experiments

The behavioral tests utilized in the two experiments were based on beverage consumption. One week prior to testing the rats were habituated to a 22 h/day water restriction schedule which continued throughout the experiments.

The behavioral tests were conducted in parallel in groups of four rats in individual Plexiglas operant chambers (330 mm × 210 mm × 180 mm) details of which can be found elsewhere [1]. Two plastic tubes were mounted on opposite sides of the flat ceiling of each chamber. Each tube was connected to a custom-made capacitance circuit which allowed the experimenter to measure the amount of liquid consumed by the rat and to record its licks using a CED 1401 interface (CED, Cambridge) connected to a computer.

The behavioral experiments are schematically illustrated in Fig. 1A and B. They are based on conditioned olfactory aversion. Rats were first trained to drink tap water in the operant chambers for 3 days (not shown). During the following 4 days, corresponding to COA establishment, the rats only had access to water odorized with ISO at 10^{-5} (corresponding to 1 μl of ISO in 100 ml of water at which dilution ISO is only perceived olfactorily) [62]. On the first day, the isoamyl acetate was paired with an intraperitoneal injection of LiCl (10 ml/kg at 0.15 M) 15 min later to make the animals sick. Then only those which drank more than 0.5 ml of ISO during any of the subsequent 3 days were injected with LiCl. Once COA was established, the aversion was tested on day 0 (D0) of each experiment by giving the animals the choice between tap water and water odorized with ISO 10^{-5}. During the test period the rats were offered a choice between tap water and water odorized with ISO at different concentrations which were chosen according to the results of our preceding experiment [1]. Indeed, using the same test, we had shown that olfactory detection performances differed significantly between physiologically fasted and satiated rats for ISO at 10^{-9} and 10^{-8}. The present concentration range used in orexin and leptin experiments includes these values. At the end of the two experiments, 7 days later (D7), COA stability was checked by giving rats the choice between ISO at 10^{-5} and tap water (aversion retest).

When the tubes contained two different drinks, their right–left position was systematically interchanged across sessions. At the beginning of each session, rats were intentionally placed under the tube containing the ISO-odorized water.

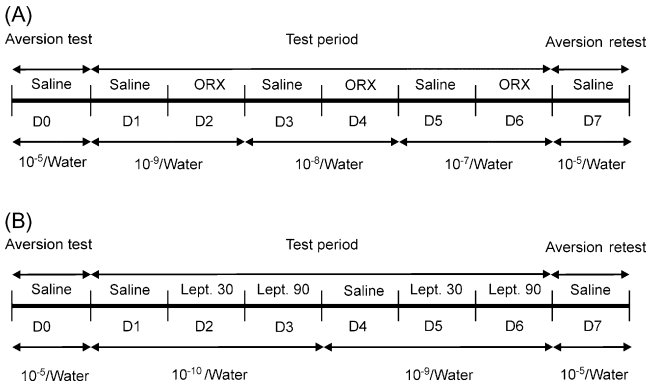

Fig. 1. Schematic representation of the overall course of the orexin (A) and leptin (B) experiments. COA was tested on D0 (aversion test) and tested again on D7 (aversion retest) where the rats had a choice between ISO 10^{-5} and tap water. In both experiments the test period ran from D1 to D6. The nature of the icv injection and the ISO concentration are given for each day. Orexin (ORX) was icv injected at 10.7 μg/rat and leptin (Lept) at either 30 or 90 ng/rat.

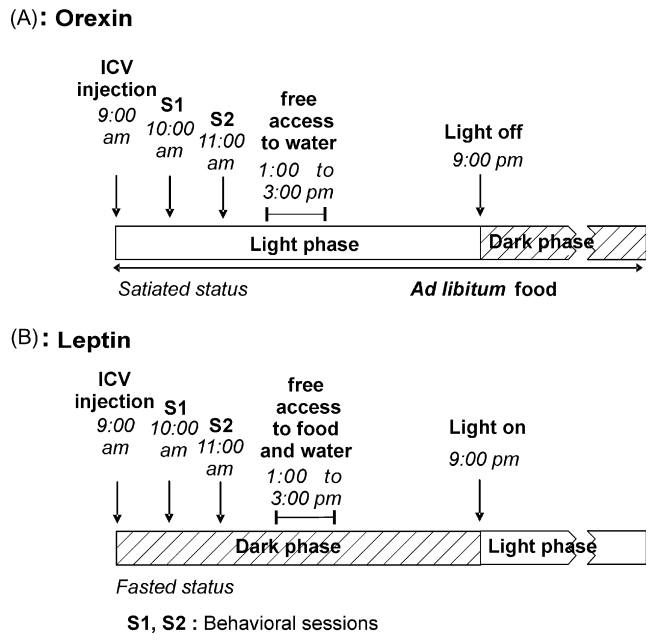

Fig. 2. Schematic representation of the daily schedule of the orexin (A) and leptin (B) experiments. For both experiments, the behavioral tests comprised two sessions, S1 and S2. Orexin experiments were done on satiated rats, at the beginning of the light phase, the animals having *ad libitum* access to food. Leptin experiments were done in fasted rats at the beginning of the dark phase, the animals having access to food for 2 h/day from 01:00 to 03:00 p.m.

Rat olfactory sensitivity for ISO was thus assessed using a forced-choice task, and not by using a simple choice task, since the thirsty rats were forced to smell the odorized tube first. This procedure was chosen to avoid the possibility that the rats, highly motivated by thirst, would go by chance to the pure water tube first, drink only water, and not sample the ISO tube.

2.4.2. Specifics of the orexin experiment

The colony lighting was controlled according to a normal 12 h light–12 h dark cycle with light being turned on at 09:00 a.m. (Fig. 2A). The rats had *ad libitum* access to food, so they were satiated at the beginning of the test, and had access to water only from 1:00 to 3:00 p.m. They were submitted to two 5-min daily test sessions performed at 10:00 a.m. (S1) and 11:00 a.m. (S2). As shown in Fig. 1A, the test schedule ran over 6 days (D1–D6). Each daily test period started at 9:00 a.m. with an icv injection of saline (D1, D3 and D5) or orexin (D2, D3 and D4). Then during the two 5-min daily test sessions, the rats were offered the choice between tap water and water odorized at different ISO concentrations: ISO at 10^{-9} (D1–D2), 10^{-8} (D3–D4) and 10^{-7} (D5–D6).

2.4.3. Specifics of the leptin experiment

The colony lighting was controlled according to an inverted 12 h light–12 h dark cycle with light being turned off at 09:00 a.m. (Fig. 2B). The rats were habituated and kept on a 22 h food- and water-restriction schedule. As they had access to food and water only from 01:00 to 03:00 p.m., they were fasted at the beginning of the test. The detection test comprised two 5-min sessions: S1 at 10:00 a.m. and S2 at 11:00 a.m. As shown in Fig. 1B the test schedule ran over 6 days (D1–D6). Each daily test period started at 9:00 a.m. with an icv injection of saline (D1 and D4) or leptin (30 ng on D2 and D5; 90 ng on D3 and D6). Then during the two 5-min daily test sessions, the rats were offered the choice between tap water and water odorized at different ISO concentrations: ISO at 10^{-10} (D1–D3) and ISO at 10^{-9} (D4–D6).

2.4.4. Data processing and statistical analysis

The raw data collected during each test session were the volumes of tap and odorized water consumed by the rats and the sequences of licks recorded at each tube by the detectors connected to the CED 1401 apparatus. The volumes were utilized to calculate the proportion of odorized water consumed with respect to the total beverage intake. The licking recording sequences were processed to determine the number of side (tube) changes during each session.

All statistical procedures were performed using SAS software. Variable distribution was first tested for normality using Shapiro–Wilk and Kolmogorov–Smirnov tests. If the studied variable followed a normal distribution, data analysis was performed using the ANOVA method. Otherwise, the drugs' effects on behavior or ISO intake were analyzed by using the non-parametric Wilcoxon two-sample test. P-values < 0.05 were considered as statistically significant.

3. Results

During the orexin and leptin experiments, several physiological and behavioral parameters were controlled (Table 1). The weight of rats remains stable over the orexin and leptin experiments. Although orexin or leptin have not been reported to change food intake at the utilized doses, food intake was measured in the leptin experiments because, first the animals were kept on a 22 h food-restriction schedule (versus an *ad libitum* regimen for orexin experiment) and second, their restrained access to food occurred in the hours following the tests (Fig. 2). In Table 1, it can be seen that food intake is not affected by leptin. The drinking behavior in the operant chamber was analyzed, for drug versus saline, by comparing the amounts of fluid consumed (tap water + odorized water). It is shown that the orexin injections significantly increased the liquid intake ($P < 0.005$). In contrast, no significant change was observed for leptin regardless of the dose injected. To evaluate whether the drugs changed the locomotor activity of the rats inside the test apparatus, the numbers of side changes during the sessions were compared. Neither leptin nor orexin were found to have a significant influence on the number of side changes.

Table 1
Physiological and behavioral parameters measured in rats included in the orexin and leptin experiments

	Orexin experiment		Leptin experiment		
	Saline	Orexin	Saline	Leptin 30	Leptin 90
Weight (g)	317.77 ± 5.9	318.88 ± 5.48	291.58 ± 6.69	290.83 ± 7	293.12 ± 7.45
Mean food intake (g)	–	–	19.52 ± 0.98	21.28 ± 0.12	19.56 ± 1.09
Mean fluid intake (ml)	7.82 ± 0.96	9.64*** ± 0.89	6.10 ± 0.69	6.97 ± 0.62	6.14 ± 0.70
Number of side changes	5.04 ± 0.48	5.67 ± 0.45	4.92 ± 0.32	5 ± 0.58	4.5 ± 0.47

For each measure, comparisons were made inside each experimental group, orexin and leptin, between saline and drug injection. The weight remains stable over orexin and leptin experiments as well as the mean food intake for the rat injected with leptin. The mean fluid intake is significantly increased in rats receiving orexin (***$P < 0.005$). The numbers of side changes during the test did not significantly differ in any condition. ±: S.E.M.

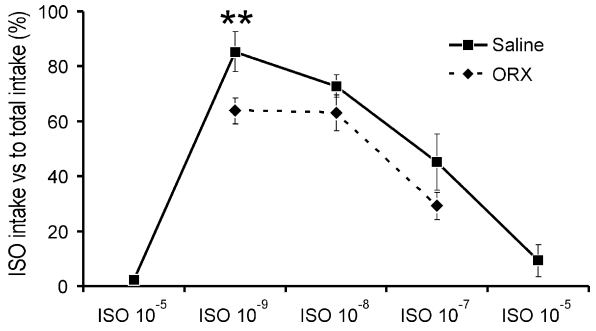

Fig. 3. Effect of orexin on ISO-drink consumption as a function of concentrations. The rats decreased their ISO consumption under orexin as compared to saline, regardless of the concentration; this decrease was highly significant for ISO 10^{-9} (**$P<0.01$).

In both experiments, COA establishment was checked on D0 by giving the rats the choice between ISO 10^{-5} and tap water (Fig. 1). In both groups of rats, COA was perfectly established since they consumed less than 0.5 ml of ISO 10^{-5} odorized water. COA was tested again at the end of each experiment on D7 when ISO intake was not found to significantly differ from that on D0, indicating that there was no COA attenuation.

In Fig. 3, the two curves give the proportion of ISO intake as a function of the concentration in the control (NaCl) and orexin injected rats. The amounts consumed per rat in each test sessions (S1 and S2) were averaged. For 10^{-9}, 10^{-8} and 10^{-7}-odorized water, the two curves show a significantly higher consumption than that observed for 10^{-5} ($P<0.005$), and inversely proportional to the ISO concentration. The curves show that the rats decreased their ISO consumption when under orexin compared with saline whatever the concentration, and that this decrease was highly significant for ISO 10^{-9} ($P<0.01$). Since it has been shown that the ability of rats to manifest COA by avoiding ISO is an appropriate index of their olfactory perception [1], these results indicate that orexin increases olfactory sensitivity in the rat similarly to physiological fasting.

The three curves in Fig. 4 give the proportion of odorized fluid intake as a function of ISO concentration after saline, leptin 30 ng or leptin 90 ng injections. In this experiment, the control rats were physiologically fasted. Consequently whatever the ISO

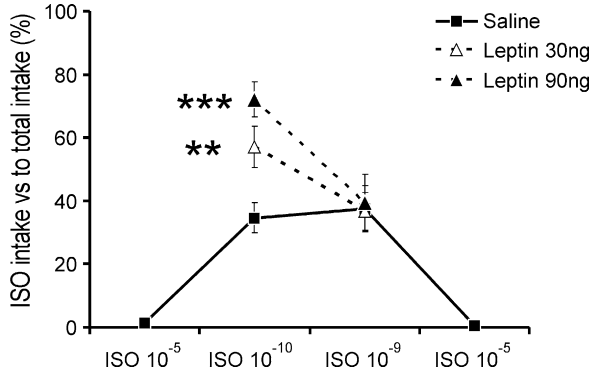

Fig. 4. Effect of leptin on ISO-drink consumption as a function of concentrations. Both doses of leptin increased the rat's ISO-drink consumption at 10^{-10} (leptin 30 ng: **$P<0.005$; leptin 90 ng: ***$P<0.0001$); the highest dose reflected a more potent effect.

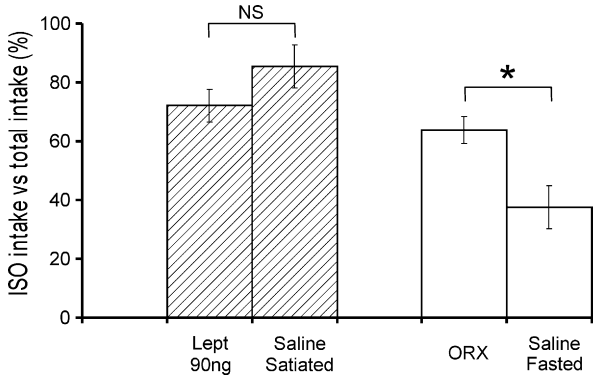

Fig. 5. Parallel between the physiologically induced and the drug-mimicked feeding states: comparison of ISO-drink consumptions. The physiologically satiated rats correspond to the control rats injected with saline during the orexin experiment. The physiologically fasted rats correspond to the control rats injected with saline during the leptin experiment. The physiologically fasted and ORX rats consumed significantly less ISO-drink than the physiologically satiated and Lept rats ($P<0.05$). The physiologically fasted rats consumed less ISO-drink than orexin fasted rats (*$P<0.05$). The physiologically satiated rats and leptin rats did not consume significantly different amounts of the ISO-odorized drink.

concentration, their consumption of the ISO-drink was very low, and lower than that of the control rats in the orexin experiments (Fig. 3), which were physiologically satiated. This low initial consumption makes it all the more obvious that both doses of leptin increased ISO-drink consumption at 10^{-10} (leptin 30 ng: $P<0.005$; leptin 90 ng: $P<0.0001$), the highest dose reflecting a more potent effect. These results indicate that leptin decreases olfactory sensitivity similarly to physiological satiation [1], in a dose dependant manner.

Because our experiments were based on the assumption that orexin and leptin are two important agents linking the fasted and satiated states to olfactory perception, Fig. 5 focuses on the parallel between the physiologically induced and the drug-mimicked feeding states. We expected that the physiologically fasted control rats (of the leptin experiment) and the *ad libitum* fed rats injected with orexin would demonstrate similar ISO-drink consumption. This was the case as in both experiments the rats consumed significantly less ISO-drink than the satiated ones, *i.e.* the physiologically satiated rats under saline (of the orexin experiment) and the leptin satiated rats ($P<0.05$). However, the physiologically fasted control rats consumed even less ISO-drink than did the orexin fasted rats ($P<0.05$). Likewise, the physiologically satiated control rats and the leptin satiated rats were expected to consume similar proportions of ISO. Again this proved to be true since the leptin rats (90 ng) and physiologically satiated rats consumed significantly more ISO-drink than did the others although the relative amounts consumed were not significantly different.

4. Discussion

By studying the general drinking behavior of rats under drugs, orexin was found to significantly increase the overall drink consumption. This confirms the previously reported results of Kunii et al. [40]. Orexin is furthermore well known to influence loco-

motor activity and plays an important role in arousal [7,65]. Similarly, leptin has also been stated to exert a positive influence on locomotor activity [10] and reported to normalize locomotion in hypoactive ob/ob mice [51]. In our paradigm, neither orexin nor leptin induced significant changes in locomotor activity as measured by the number of side changes. This allows us to exclude that the effects of orexin and leptin on ISO-drink avoidance or intake are attributable, even in part, to their effect on locomotor activity or arousal.

Using the same COA paradigm we showed in a previous experiment that fasted rats avoid ISO-drink at very low concentrations which are undetectable when rats are satiated and concluded that fasting increases olfactory sensitivity [1]. In order to mimic fasting, orexin was administered during the early light phase (at 09:00 a.m.) when its effect on feeding has been said to be at a maximum [18,31]. Under these conditions, rats were found to avoid low concentrations of ISO, despite the global influence of orexin which tends to increase the total drink consumption and orexin clearly appears to be able to modulate olfactory perception in exactly the same direction as fasting does. This supports the idea that orexin has an important role in the fasted status, at least regarding changes in olfactory perception. Orexin can be assumed to act directly at the level of the OB network where it is released by hypothalamic fibers [8,12,13,52,58]. In recent electrophysiological studies it was shown that orexin modulates the electrophysiological activity of the OB network both in vitro [30] and in vivo [2] by acting on orexin receptors types localized on different neuronal subpopulations [30]. Hardy et al. [30] also reported that orexin could change mitral cell excitability to electrical shocks applied on the olfactory nerve. Given the present results, we assume that the centrifugal action of orexin might modulate the sensitivity of OB neurons to the sensory input.

By inducing a dose dependant increase in ISO-drink consumption, and thus a decrease in ISO-drink avoidance, leptin produces an effect close to that of physiological satiation [1]. To prove that leptin changes ISO-drink intake, we needed both to increase the leptin dose and to decrease ISO concentrations (by comparison with our preceding experiments [1]). Because leptin injections were done in physiologically fasted rats to mimic satiation, leptin had to counteract the action of endogen peptides including that of orexin which has been shown to have a very potent effect on olfactory performance, and which has a very high level during the dark period when animals are active [23,67]. As shown by the saline curve, the physiologically fasted rats consumed little ISO-drink, even at the lowest concentration. In these conditions, leptin had to counterbalance the high sensitivity accompanying the fasted state [1]. Leptin did this for ISO 10^{-10}, thus significantly decreasing the olfactory sensitivity in the same direction as for physiological satiation. This is consistent with the finding that leptin injections in ob/ob mice decrease olfactory performance when food seeking, lowering their sensitivity to a level similar to that of wild-type mice [25]. The effect of leptin on olfactory performance is anatomically supported by the fact that, along with the hypothalamus, the olfactory system is also a major target for leptin, as leptin receptors are strongly expressed in both the piriform cortex [6] and the OB [60]. Globally, our results point to an important role for leptin in the satiated state at least regarding the changes in olfactory perception. Further in vitro electrophysiological studies are in progress to determine the precise action of leptin on OB neurons.

Our results are consistent with the conclusion that orexin and leptin probably modify processing in the olfactory bulb by increasing or decreasing the olfactory detection power of rats for a neutral odor. It is remarkable that their action appears to be more especially linked with low concentrations, with 10^{-9} for orexin and 10^{-10} for leptin. Such a pharmacological action on olfactory performance, linked with concentration of the stimulus has been already described by Pho et al. [53] who showed that intraperitoneal injections of rolipram impaired odor detection in mice specifically at low concentrations. In the olfactory bulb, orexin has been experimentally demonstrated to act on the network, by activating periglomerular and granular GABAergic neurons or mitral cells [30]. Such an action may result in a modification in activation of the global network and thus the activated glomerular map which depends on both odor quality and intensity [21]. Thus, it may be suggested that the action of orexin or leptin could come into play at low levels of intensity, probably by enhancing activation of the neuron network set in motion at threshold concentrations of odorants.

The parallel made between the physiological and the drug-induced fasting and satiation reinforces the argument that orexin and leptin are important agents in the hypothalamic feeding network/olfactory structure crosstalk. However physiological fasting appears to be more effective than orexin-induced "fasting" in altering olfactory perception. This is probably due, even in part, to the fact that the rats receiving orexin were fed *ad libitum* while the physiologically fasted rats were fed only during the same 2-h period each day. The *ad libitum* fed rats therefore would have had different food intakes and probably had higher degrees or at least different degrees of satiation. In addition, orexin injections in *ad libitum* fed rats probably had to counteract high level of endogenous circulating leptin. Taken together, these hypotheses probably account for the difference in ISO avoidance and/or olfactory perception observed with physiological and orexin-induced fasting. Furthermore, physiological fasting consists in a far more complex chemical environment than does a simple increase in orexin levels and the same remark is valid for physiological versus leptin-induced "satiety". Indeed, numerous peripheral and central chemical signals are involved in controlling the two feeding states and could also intervene to modulate olfactory processing ([44], for a review). Thus, it is perfectly consistent that orexin and leptin influence the olfactory sensitivity in a similar direction to that of physiological fasting and satiety, but that they cannot be as potent as fasting and satiety are.

The results lie within the scope of our experimental approach concerning the role of olfaction on feeding behavior [1–3,30] and enrich our previous data showing that olfactory sensitivity is modulated by the physiological feeding state [1]. We now have further insight into the signals underlying the interaction between feeding state and olfactory sensitivity by showing that orexin and leptin are important agents in the hypothala-

mic feeding network/olfactory pathway crosstalk. These results open important perspectives for evaluating the links between the olfactory performance of some rat strains and their metabolic specificities leading to a tendency or not towards obesity and the specific links with their relative rates of orexin or leptin endogen circulation. Such studies will provide important insights into the understanding of the involvement of molecular signal deficit or abundance in the development of pathophysiological metabolic states and their relationship with olfaction.

Acknowledgments

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References

[1] Aimé P, Duchamp-Viret P, Chaput MA, Savigner A, Mahfouz M, Julliard AK. Hunger increases and satiation decreases rat olfactory detection performances for a neutral odor. Behav Brain Res 2007;179:258–64.
[2] Apelbaum A, Perrut A, Chaput M. Orexin A effects on the olfactory bulb spontaneous activity and odor responsiveness in freely breathing rats. Regul Pept 2005;129:49–61.
[3] Apelbaum AF, Chaput MA. Rats habituated to chronic feeding restriction show a smaller increase in olfactory bulb reactivity compared to newly fasted rats. Chem Senses 2003;28:389–95.
[4] Baly C, Aioun J, Badonnel K, Lacroix MC, Durieux D, Schlegel C, et al. Leptin and its receptors are present in the rat olfactory mucosa and modulated by the nutritional status. Brain Res 2007;1129:130–41.
[5] Bates SH, Myers Jr MG. The role of leptin receptor signaling in feeding and neuroendocrine function. Trends Endocrinol Metab 2003;14:447–52.
[6] Bennett PA, Lindell K, Karlsson C, Robinson IC, Carlsson LM, Carlsson B. Differential expression and regulation of leptin receptor isoforms in the rat brain: effects of fasting and oestrogen. Neuroendocrinology 1998;67:29–36.
[7] Butler AA, Marks DL, Fan W, Kuhn CM, Bartolome M, Cone RD. Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. Nat Neurosci 2001;4:605–11.
[8] Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 1999;98:437–51.
[9] Cohen Jr MM. Role of leptin in regulating appetite, neuroendocrine function, and bone remodeling. Am J Med Genet A 2006;140:515–24.
[10] Coppari R, Ichinose M, Lee CE, Pullen AE, Kenny CD, McGovern RA, et al. The hypothalamic arcuate nucleus: a key site for mediating leptin's effects on glucose homeostasis and locomotor activity. Cell Metab 2005;1:63–72.
[11] Cunningham GB, Van Buskirk RW, Bonadonna F, Weimerskirch H, Nevitt GA. A comparison of the olfactory abilities of three species of procellariiform chicks. J Exp Biol 2003;206:1615–20.
[12] Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukara S, Kangawa K, et al. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. PNAS 1999;96:748–53.
[13] De Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, et al. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA 1998;95:322–7.
[14] Duclaux R, Feisthauer J, Cabanac M. Effects of a meal on the pleasantness of food and nonfood odors in man. Physiol Behav 1973;10:1029–33.
[15] Edwards CM, Abusnana S, Sunter D, Murphy KG, Ghatei MA, Bloom SR. The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. J Endocrinol 1999;160:R7–12.
[16] Elmquist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. Neuron 1999;22:221–32.
[17] Elmquist JK, Maratos-Flier E, Saper CB, Flier JS. Unraveling the central nervous system pathways underlying responses to leptin. Nat Neurosci 1998;1:445–50.
[18] Espana RA, Plahn S, Berridge CW. Circadian-dependent and circadian-independent behavioral actions of hypocretin/orexin. Brain Res 2002;943:224–36.
[19] Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, et al. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med 1999;341:879–84.
[20] Fedoroff IC, Stoner SA, Andersen AE, Doty RL, Rolls BJ. Olfactory dysfunction in anorexia and bulimia nervosa. Int J Eat Disord 1995;18:71–7.
[21] Fried HU, Fuss SH, Korsching SI. Selective imaging of presynaptic activity in the mouse olfactory bulb shows concentration and structure dependence of odor responses in identified glomeruli. Proc Natl Acad Sci USA 2002;99:3222–7.
[22] Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–70.
[23] Fujiki N, Yoshida Y, Ripley B, Honda K, Mignot E, Nishino S. Changes in CSF hypocretin-1 (orexin A) levels in rats across 24 h and in response to food deprivation. Neuroreport 2001;12:993–7.
[24] Geiselman PJ. Control of food intake. A physiologically complex, motivated behavioral system. Endocrinol Metab Clin N Am 1996;25:815–29.
[25] Getchell TV, Kwong K, Saunders CP, Stromberg AJ, Getchell ML. Leptin regulates olfactory-mediated behavior in ob/ob mice. Physiol Behav 2006;87:848–56.
[26] Goetzl FR, Abel MS, Ahokas AJ. Occurrence in normal individuals of diurnal variations in olfactory acuity. J Appl Physiol 1950;2:553–62.
[27] Goetzl FR, Stone F. Diurnal variations in acuity of olfaction and food intake. Gastroenterology 1947;9:444–53.
[28] Goetzl FR, Stone F. The influence of amphetamine sulfate upon olfactory acuity and appetite. Gastroenterology 1948;10:708–13.
[29] Hammer FJ. The relation of odor, taste, and flicker-fusion thresholds to food intake. J Comp Physiol Psychol 1951;44:03–11.
[30] Hardy AB, Aioun J, Baly C, Julliard KA, Caillol M, Salesse R, et al. Orexin A modulates mitral cell activity in the rat olfactory bulb: patch-clamp study on slices and immunocytochemical localization of orexin receptors. Endocrinology 2005;146:4042–53.
[31] Haynes AC, Jackson B, Overend P, Buckingham RE, Wilson S, Tadayyon M, et al. Effects of single and chronic intracerebroventricular administration of the orexins on feeding in the rat. Peptides 1999;20:1099–105.
[32] Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, et al. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA 1999;282:1568–75.
[33] Hunter-Smith DG, Kessel K, Grant M, Piotrowski ZH. Association between elevated body-mass index and impaired sense of smell in older people. J Am Geriatr Soc 1996;44:100–1.
[34] Ida T, Nakahara K, Katayama T, Murakami N, Nakazato M. Effect of lateral cerebroventricular injection of the appetite-stimulating neuropeptide, orexin and neuropeptide Y, on the various behavioral activities of rats. Brain Res 1999;821:526–9.
[35] Janowitz HD, Grossman MI. Gusto-olfactory thresholds in relation to appetite and hunger sensations. J Appl Physiol 1949;2:217–22.
[36] Janowitz HD, Hollander F, Marshar RH. The effect of tween-65 and tween-80 on gastrointestinal motility in man. Gastroenterology 1953;24:510–6.
[37] Karlsson AC, Lindroos AK, Lissner L, Torgerson JS, Carlsson B, Carlsson LM, et al. Evidence for gender-specific associations between leptin and olfaction. J Gend Specif Med 2002;5:25–32.

[38] Koelega HS. Diurnal variations in olfactory sensitivity and the relationship to food intake. Percept Mot Skills 1994;78:215–26.
[39] Kopala LC, Good K, Goldner EM, Birmingham CL. Olfactory identification ability in anorexia nervosa. J Psychiatry Neurosci 1995;20:283–6.
[40] Kunii K, Yamanaka A, Nambu T, Matsuzaki I, Goto K, Sakurai T. Orexins/hypocretins regulate drinking behaviour. Brain Res 1999;842:256–61.
[41] Le Magnen J. The role of olfacto-gustatory stimuli in the regulation of the alimentary behavior of the mammal. J Psychol Norm Pathol (Paris) 1959;56:137–60.
[42] Le Magnen J. In: Solms J, Hall RL, editors. Neurophysiological basis for sensory mediated food selection. Criteria of food acceptance. Zurich: Forster Verlag; 1981. p. 268–80.
[43] Le Magnen J. My scientific life: 40 years at the College de France. Neurosci Biobehav Rev 2001;25:375–94.
[44] Leibowitz SF, Wortley KE. Hypothalamic control of energy balance: different peptides, different functions. Peptides 2004;25:473–504.
[45] Liu L, Karkanias GB, Morales JC, Hawkins M, Barzilai N, Wang J, et al. Intracerebroventricular leptin modulates hepatic but not peripheral glucose fluxes. J Biol Chem 1998;273:31160–7.
[46] Loftus TM. An adipocyte-central nervous system regulatory loop in the control of adipose homeostasis. Semin Cell Dev Biol 1999;10:11–8.
[47] Lubkin M, Stricker-Krongrad A. Independent feeding and metabolic actions of orexins in mice. Biochem Biophys Res Commun 1998;253: 241–5.
[48] Mistry AM, Swick AG, Romsos DR. Leptin rapidly lowers food intake and elevates metabolic rates in lean and ob/ob mice. J Nutr 1997;127: 2065–72.
[49] Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 1997;387:903–8.
[50] Nagamori K, Ishibashi M, Shiraishi T, Oomura Y, Sasaki K. Effects of leptin on hypothalamic arcuate neurons in Wistar and Zucker rats: an in vitro study. Exp Biol Med 2003;228:1162–7.
[51] Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995;269:540–3.
[52] Peyron C, Tighe D, Vanden Pol AN, De Lecea L, Heller HC, Sutcliffe JG, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci 1998;18:9996–10015.
[53] Pho V, Butman ML, Cherry JA. Type 4 phosphodiesterase inhibition impairs detection of low odor concentrations in mice. Behav Brain Res 2005;161:245–53.
[54] Pinel JP, Assanand S, Lehman DR. Hunger, eating, and ill health. Am Psychol 2000;55:1105–16.
[55] Rodgers RJ, Ishii Y, Halford JC, Blundell JE. Orexins and appetite regulation. Neuropeptides 2002;36:303–25.
[56] Sakurai T. Orexins and orexin receptors: implication in feeding behavior. Regul Pept 1999;85:25–30.
[57] Sakurai T. Roles of orexin/hypocretin in regulation of sleep/wakefulness and energy homeostasis. Sleep Med Rev 2005;9:231–41.
[58] Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 1992:573–85.
[59] Share I, Martyniuk E, Grossman MI. Effect of prolonged intragastric feeding on oral food intake in dogs. Am J Physiol 1952;169:229–35.
[60] Shioda S, Funahashi H, Nakajo S, Yada T, Maruta O, Nakai Y. Immunohistochemical localization of leptin receptor in the rat brain. Neurosci Lett 1998;243:41–4.
[61] Shiraishi T, Oomura Y, Sasaki K, Wayner MJ. Effects of leptin and orexin-A on food intake and feeding related hypothalamic neurons. Physiol Behav 2000;71:251–61.
[62] Slotnick BM, Westbrook F, Darling FMC. What the rat's nose tells the rat's mouth: long delay aversion conditioning with aqueous odors and potentiation of taste by odors. Anim Learn Behav 1997;25:357–69.
[63] Turner P, Patterson DS. Smell threshold as a test of central nervous function. Acta Otolaryngol 1967;62:149–56.
[64] Wang L, Barachina MD, Martinez V, Wei JY, Tache Y. Synergistic interaction between CCK and leptin to regulate food intake. Regul Pept 2000;92:79–85.
[65] Wortley KE, Chang GQ, Davydova Z, Leibowitz SF. Peptides that regulate food intake: orexin gene expression is increased during states of hypertriglyceridemia. Am J Physiol Regul Integr Comp Physiol 2003;284: R1454–65.
[66] Yamanaka A, Sakurai T, Katsumoto T, Yanagisawa M, Goto K. Chronic intracerebroventricular administration of orexin-A to rats increases food intake in daytime, but has no effect on body weight. Brain Res 1999;849:248–52.
[67] Yoshida Y, Fujiki N, Nakajima T, Ripley B, Matsumura H, Yoneda H, et al. Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light-dark cycle and sleep-wake activities. Eur J Neurosci 2001;14:1075–81.
[68] Zilstorff-Pedersen K. Olfactory threshold determinations in relation to food intake. Acta Otolaryngol 1955;45:86–90.