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## Altered taste sensitivity in obese, prediabetic OLETF rats lacking CCK-1 receptors

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### Abstract

Otsuka Long-Evans Tokushima fatty (OLETF) rats lack the CCK-1 receptor, are hyperphagic, progressively become obese, and develop type-2 diabetes. We recently demonstrated an increased preference for both real and sham feeding of sucrose in this strain, suggesting altered orosensory sensitivity. To investigate taste functions, we used an automated gustometer with 10-s access to different concentrations of various sapid stimuli. Tests were repeated at 10 and 18 wk of age to assess the early and advanced stages of prediabetes, respectively. Compared with age-matched, nonmutant controls, the OLETF rats showed higher avidity for sucrose at both ages. This difference increased as a function of age and tastant concentration. An exaggerated response also occurred for saccharin, alanine, and fructose, but not for Polycose. Similarly, OLETF rats consumed monosodium-glutamate more at the lower concentrations compared with controls, an effect that age also accentuated. In contrast, there was no statistical strain or age differences in responses to NaCl, MgCl<sub>2</sub>, citric acid, quinine-HCl, and the trigeminal stimulus capsaicin. These findings demonstrate that compared with controls, OLETF rats differ in their gustatory functions with an overall augmented sensitivity for sweet that progresses during prediabetes. This effect explains their overconsumption of sweet solutions and may contribute to the overall hyperphagia and obesity in this strain.

### Keywords

Overeating; dietary obesity; insulin resistance; sweet receptors; hedonic coding

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Obesity is a global pandemic that continues to accelerate and increases the risk of multiple medical conditions, such as noninsulin-dependent diabetes mellitus (NIDDM), hypertension, and coronary heart disease (34). Although the etiology of obesity is complex, overconsumption induced by the high palatability of the modern diet, in general, and individual differences in responsivity to palatability, in particular, may contribute to obesity (34,74,87). Meal size is controlled by orosensory stimulatory and postingestive inhibitory feedback (71). Thus increased appetite and overeating can be the result of either an enhanced responsiveness to orosensory stimulatory properties of a meal, a decreased sensitivity to postingestive inhibitory signals, or both. Importantly, the signaling systems that underlie appetite control appear to operate independently from the energy homeostatic regulation resulting in sustained overeating and body weight gain in some individuals and also in animal models of obesity.

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Despite the controversy about the particular contribution of different nutrients to the epidemic of obesity, there is agreement that an increased palatability of foods that taste sweet and contain fats, plays a critical role by increasing the overall calorie intake (40,56). As with humans, high carbohydrate and high fat foods are strongly preferred by rats (59). Postingestive factors play an important role in this preference (63,84), but rats also sham feed sugar and fat solutions (i.e., fatty acids and triglycerides) in a concentration-dependent manner (23,52,71,72,85). These findings demonstrate that the orosensory effects of these nutrients are sufficient to elicit and maintain ingestion. It is well established that a diet rich in highly preferred foods can establish obesity in animals (for review, see Ref. 61). Less is known, however, about the opposite relationship, which is, whether taste functions are different in obese compared with lean individuals.

Over the years, a number of genetically obese models have been developed. Regardless of the strain, hyperphagia occurs in virtually all genetically obese rodents. In some strains however, increased food intake seems to be necessary for the development of obesity, whereas in other strains, obesity develops even when increased food intake is not permitted. For example, whereas the Zucker rat, which carries a recessive mutation of the leptin receptor gene (11), becomes obese in the absence of hyperphagia, the CCK-1 receptor-deficient Otsuka Long-Evans Tokushima fatty (OLETF) rat does not. In other words, Zucker rats become obese even if the obese mutants are pairfed to the ad libitum intake of lean siblings (45). In contrast, OLETF rats pairfed with Long-Evans Tokushima Otsuka (LETO) lean controls normalized weight, body composition, and levels of plasma insulin and leptin (3,45). Therefore, the OLETF rats represent an ideal model to examine mechanisms involved in hyperphagia in the absence of the metabolic derangements associated with hyperphagia.

OLETF is an outbred strain of Long-Evans rat that has been established as an animal model of NIDDM and obesity (38). These rats have a 6.8-kb deletion in the CCK-1 receptor gene, resulting in a failure to produce or express functional CCK-1 receptors (22,78). CCK-1 receptors are the receptor subtype that mediates CCK action in satiety (46). Consistent with this role, OLETF rats have deficits in the control of meal size. The size of spontaneous meals is almost double, and OLETF rats have deficits in responding to CCK and gastric and intestinal preloads (14,47,58). The underlying cause(s) of hyperphagia in this rat model has not been fully revealed. The overall hyperphagia, however, suggests that OLETF rats may have other deficits beyond that of controlling meal size. In addition, obesity as well as NIDDM can be greatly reduced by caloric restriction (53) or exercise (4,67), suggesting that obesity and NIDDM in OLETF rats with CCK-1 receptor deficits are secondary to their hyperphagia. Relevant to this, recently we have demonstrated that, in addition to a diminished sensitivity to postingestive satiation signals, OLETF rats express an increased sham intake of normally preferred sucrose solutions (17). Despite this very strong indication of altered taste sensitivity, we found only one study in the literature that investigated taste functions in this strain. A study by Tsunoda et al. (82) demonstrated enhanced chorda tympani (CT) responses to high concentrations of sucrose (>1 M) in the OLETF rat. The present study was designed to assess taste functions in this strain using an automated lickometer (Davis Rig) with an extended array of sapid chemicals at various concentrations to establish a concentration-preference/aversion function.

With the exception of a taste reactivity test (24), where orofacial expressions to tastants are screened, the investigation of taste preference typically relies on intake as a measure. In the standard intake paradigms, a taste stimulus is presented either alone (one-bottle test) or in conjunction with water or vehicle (two-bottle tests). The relative level of consumption of the taste stimulus is then interpreted as evidence of preference for or aversion to that taste quality at a certain concentration. Intake measures, however, can be influenced by preingestive and postingestive factors (6,12,60). To focus on preingestive factors only, the present study

employed a brief-access paradigm with lick rate analysis to compare taste functions between hyperphagic OLETF and normal control LETO rats. This test permits short access to taste solutions; thus ingestive response can be assessed within the first 10 s of exposure to the taste solution without postingestive influence (8). These tests were repeated at two time points, at ~10 and ~18 wk of age, representing nondiabetic and prediabetic stages, respectively.

A subset of data from this study was presented at the 2005 Annual Meeting of the Association for Chemoreception Sciences (28).

## MATERIALS AND METHODS

### Subjects

Four-week-old male OLETF and LETO rats were obtained as a generous gift of the Tokushima Research Institute, Otsuka Pharmaceutical, Tokushima, Japan. All animals were individually housed in mesh-floored, stainless-steel, hanging cages in a temperature-controlled vivarium while maintained on a constant 12:12-h light-dark cycle (lights on at 0700). Rats were handled daily for a minimum of 1 wk before the onset of experimental procedures. Pelleted normal rat chow (Rodent Diet-W 2018; Harlan Teklad, Madison, WI) was available ad libitum throughout experiments except during taste sessions. Tap water also was available ad libitum, except when taste responses to aversive chemicals were tested. All protocols used were approved by The Pennsylvania State University College of Medicine Institutional Animal Care and Use Committee and were in accordance with NIH Guidelines.

### Taste Assessment

Rats (6 OLETF and 6 LETO) were tested individually using a multibottle gustometer (Davis Rig; DiLog Instruments, Tallahassee, Florida) similar to that described previously (73). The testing apparatus was housed in the animal room, and the testing took place during the light phase starting in the morning (~0800 h). All animals were tested at two time points, between 8–12 and 16–20 wk of age (labeled 10- and 18-wk test, respectively). Between the two tests, animals were maintained on ad libitum food and water access and received no experimental manipulation except the oral glucose tolerance tests (OGTTs) and daily measurement of body weight.

The standard protocol of training and brief-access tests used in our laboratory are described elsewhere (70). Briefly, rats were dehydrated when licking behavior was evaluated for the normally aversive chemicals and rehydrated when licking behavior was evaluated for the normally preferred chemicals. Twenty-four hours before water training, water cylinders were removed from the home cages of all rats. On *days 1–5*, each rat was placed in the gustometer and allowed to lick the drinking spout for water for a 20-min period. Each rat was tested twice for a tastant, in a counterbalanced fashion (i.e., the same stimulus was never presented on two consecutive days), but only one stimulus a day was given. In a daily session, 5–6 concentrations of the same tastant with water were available for 10-s access periods in a randomized order during 20 min. During this period, all concentrations were presented in equal number. On *days 6–15*, the rats received water and various concentrations of normally aversive solutions, but only one chemical was tested a day: citric acid (0.001, 0.003, 0.006, 0.01, 0.03, 0.1 M), quinine-HCl (10<sup>-5</sup>, 3 × 10<sup>-5</sup>, 10<sup>-4</sup>, 3 × 10<sup>-4</sup>, 10<sup>-3</sup>, 3 × 10<sup>-3</sup> M), MgCl<sub>2</sub> (0.01, 0.03, 0.1, 0.3, 1.0 M), and the nontaste (i.e., trigeminal) stimulus capsaicin (10<sup>-5</sup>, 3 × 10<sup>-5</sup>, 10<sup>-4</sup>, 3 × 10<sup>-4</sup>, 10<sup>-3</sup> M). To maintain proper hydration, all rats received an additional 120-min access period to water in the home cage following each daily session (1500–1700 h). At the completion of the testing session on *day 15*, water bottles were returned to the home cages, and the rats were maintained on free water for the remainder of the experiment. On *days 16 and 17* the rats were not tested. On *days 18–31*, they were tested in the gustometer with water or various

concentrations of more palatable sapid solutions. For testing responsiveness to sweetness and saltiness, the following taste stimuli were used: sugars: sucrose (disaccharide, 0.01, 0.03, 0.10, 0.3, 1.0, 1.5 M), fructose (monosaccharide, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 M), and Polycose (polysaccharide, 0.01, 0.03, 0.06, 0.1, 0.2, 0.4 M); nonsugar stimuli: sodium saccharin (0.05, 0.1, 0.2, 0.4, 0.8, 1.6% or ~2.3, 4.5, 9, 18, 36, 45 mM), alanine (0.01, 0.03, 0.06, 0.1, 0.3, 1.0 M), monosodium L-glutamate (MSG: 0.01, 0.03, 0.06, 0.1, 0.3, 1.0 M), and NaCl (0.01, 0.03, 0.06, 0.1, 0.3, 1.0 M). Again, all sapid stimuli concentrations were randomly presented, and only one tastant was tested per day. A trial was initiated when a rat made a lick within 10 s. The minimum intertrial interval (i.e., between solutions) was 5 s, the amount of time required for the shutter operation and the rig to change positions. The session length was 20 min, given the 80 presentations and 15-s trials including the intertrial intervals. Data only from the second sessions with each chemical were used for the statistical analysis included in this paper. The rationale for this was to minimize the effect of novelty and experience factors in the first test.

### Chemicals Used As Oral Stimuli

All chemicals used in the gustometer were dissolved in filtered tap water from a source identical to the maintenance water available to the animals in their home cages. All taste stimuli were prepared freshly and presented at room temperature. Chemical purity was at least 95% and purchased from standard vendors, except for MSG that was obtained from a local grocery store. Specifically, quinine-HCl and capsaicin were from Sigma (St. Louis, MO), Polycose from the Abbott Laboratories (Columbus, OH), and sucrose, fructose, Polycose, Na-saccharin [1,2-benzisothiazol-3(2H)-one,1,1-dioxide, sodium salt], citric acid, MgCl<sub>2</sub>, NaCl, and alanine were all from Fisher Scientific (Fair Lawn, NJ).

### OGTTs

OGTT were performed once after each gustometer test, at 12 and 20 wk of age and at least 2 days after the last taste session. Before the tests, the rats were fasted overnight (minimum 16 h). Glucose 2 g/kg (500 g/l) was administered orally using a 8-Fr intragastric tube, and blood was taken from the tail vein without anesthesia at 0, 30, 60, and 120 min for measurement of blood glucose levels. Blood glucose was determined with a glucometer (Elite Glucometer; Bayer, Elkhart, IN). Animals were classified as diabetic if the peak level of plasma glucose at any time point was 16.8 mmol/l (300 mg/dl) or glucose level at 120 min > 11.2 mmol/l (200 mg/dl) (38).

### Blood Glucose and Plasma Hormone Assays

After completion of the experiments, rats now 22 wk old were euthanized and trunk blood was collected for plasma glucose and insulin measurements. A separate set of animals ( $n = 10$  per strain) from a parallel histological study were killed at 14 wk and plasma was collected for leptin measurements. Approximately 3 ml of trunk blood was taken from each decapitated rat and collected in an EDTA (K3, 15%) vacutainer tube (Becton Dickinson, Franklin Lakes, NY). From this sample, 20 $\mu$ l was removed for blood glucose assay (Elite Glucometer, Bayer), the remaining plasma was stored at  $-80^{\circ}\text{C}$  until the day of the assay. Standard radioimmunoassays from Linco Research (St. Charles, MO) were used to determine insulin (sensitivity: 0.1 ng/ml) and leptin (sensitivity: 0.5 ng/ml) plasma concentrations.

### Statistical Analysis

Licks elicited during each 10-s trial were measured, and the mean number of licks for water and for each concentration of chemical was computed for each rat. These means were then used as average total licks or to calculate the difference score between licks made for a given concentration of chemical and those made for water for each rat: lick difference score (chemical  $x$ ) = licks (chemical  $x$ ) - licks (water). ANOVA (main factors: age, strain) with repeated

measures (on concentrations) was conducted on both total licks and the lick difference score for each tastant. The results included in the figures are presented as total licks. Because the objective of the experiments was to investigate strain and age interactions, RESULTS reports statistical data exclusively from these relevant comparisons. Post hoc tests were conducted, when appropriate, using Fisher's least significant difference tests. Data from OGTT tests were analyzed by two-way ANOVA (strain and age as main factors) and, the area under the curve (AUC) was calculated for each group and compared between strains at each time point using a Student's *t*-test. Body weight and data from the hormone assays were expressed as means  $\pm$  SE and were analyzed by independent *t*-tests. In all analyses, significance was set at  $\alpha = 0.05$  and Statistica 6.0 software for PC was used (Tulsa, OK).

## RESULTS

### Body Weight

At the beginning of the first taste assessment, i.e., at 8 wk of age, OLETF and LETO rats weighed  $172.7 \pm 4.9$  and  $148.5 \pm 2.8$  g, respectively. By the time of the second taste testing, i.e., over a period of 8 wk, OLETF gained to  $455.2 \pm 8.0$  g, whereas LETO gained to  $396.5 \pm 9.3$  g. Differences between strains were statistically significant at both ages [ $t(1,5) = 4.04$ ,  $P < 0.01$ ;  $t(1,5) = 4.29$ ,  $P < 0.01$ , for 8 and 16 wk, respectively]. At the end of the experiment (22 wk of age) the OLETF group weighed  $619.4 \pm 15.1$  g, whereas the LETO group weighed  $444.2 \pm 17.6$  g; again the difference being statistically significant [ $t(1,4) = 7.16$ ,  $P < 0.01$ ]. Rats used for the 14th wk leptin and insulin assays weighed  $375 \pm 13.2$  and  $305.5 \pm 12.8$  g, for OLETF and LETO, respectively. The body weights within strains were not statistically different from the body weight of the animals included in the taste experiment at 14 wk of age ( $390.3 \pm 8.7$  and  $323.3 \pm 2.8$  g, for OLETF and LETO, respectively).

### OGTTs

Fasting blood glucose levels and responses to intragastric glucose load were tested twice, at 12 and 20 wk of age, at the end of each taste test (Fig. 1). The AUC above fasting plasma glucose concentrations was calculated for each group and is depicted in Fig. 1, *insets*. Repeated-measures ANOVA revealed a significant effect of age times strain interaction for blood glucose levels [ $F(1,12) = 5.96$ ,  $P < 0.04$ ]. Whereas at 12 wk, fasting blood glucose levels were normal and did not differ between strains, at 20 wk, glucose levels significantly increased in OLETF rats ( $6.16 \pm 0.38$  mmol/l), compared with their own 12-wk baseline ( $3.87 \pm 0.06$  mmol/l) and with the 20-wk levels in LETO controls ( $4.67 \pm 0.16$  mmol/l) ( $P < 0.01$ ). Further analysis demonstrated that glucose tolerance was significantly impaired in OLETF rats compared with age-matched LETO at both 12 and 20 wk of age ( $P < 0.05$ ,  $P < 0.01$ , respectively). At the older age, however, glucose intolerance was significantly augmented in OLETF compared with LETO rats with a 90% increase in AUC by *week 20* compared with 48% by *week 12* (Fig. 1, *insets*). This age effect was statistically significant for both the peak value as well as AUC ( $P < 0.05$ ,  $P < 0.01$ , respectively). This finding indicates that, despite continuous increased glucose intolerance, OLETF did not meet the criteria for clinical diabetes even at the time of the second taste testing (i.e., *weeks 8–12*).

### Water Baseline During Training Sessions

As part of the standard protocol during habituation to the gustometer, all rats received several days (5–6) of training with water only (Fig. 2). We noticed a significant increase in water licks of OLETF compared with LETO rats [ $F(1,20) = 48.58$ ,  $P < 0.01$ ]. This significant difference was observed at both ages (10 wk:  $P < 0.05$ ; 18 wk:  $P < 0.01$ , post hoc tests between strains). Differences in learning abilities or factors related to neophobia were less likely contributors because the strain difference was present during the first test and persisted during subsequent

tests. Although the exact nature of the increased water licks in OLETF rats remains unknown, several possibilities are addressed in the discussion.

### Concentration-Response Functions

Concentration-response functions are shown in Figs. 3–5. These figures depict the average total licks ( $\pm$ SE) during the 10-s period in response to a range of concentrations of tastants at 10 and 18 wk of age. The first data points on the x-axis (0 concentration) represent intratrial water responses. Although the average lick difference score (i.e., taste licks – water licks) was calculated for each tastant, only those significantly different from the absolute lick scores are included in RESULTS. This is because with the exception where aversive chemicals were tested, and unlike water training during habituation, there were no significant strain differences in the number of water licks (see DISCUSSION).

### Responses to Aversive Chemicals

**Citric acid**—Concentration-response function generated for citric acid was not different between OLETF and control LETO rats [strain  $\times$  concentration:  $F(5,100) = 1.04$ ,  $P = 0.4$ , not significant (NS); Fig. 3A] but differed across time points [age  $\times$  concentration:  $F(5,100) = 8.04$ ,  $P < 0.001$ ]. A post hoc test showed that this effect was carried by the difference in responses to the lowest concentration ( $P < 0.01$ ). Thus there was a one-third log-shift sensitivity increase in both strains at the 10-wk test (see Fig. 3A, *left*). Total licks and lick difference scores for all other concentrations were not statistically different ( $P > 0.05$ ).

**Quinine-HCl**—Interaction for strain times concentration revealed no strain difference in responses to quinine-HCl [ $F(6,120) = 13.22$ ,  $P = 0.97$ , NS; Fig. 3B]. However, there was a significant interaction for age times concentration [ $F(6,20) = 11.34$ ,  $P < 0.001$ ]. At 10 wk, both strains expressed an increased sensitivity to the lowest concentrations of quinine-HCl (see Fig. 3B, *left*), whereas at 18 wk, the concentration-response function was more similar to what is expected for quinine-HCl in wild-type rat strains, i.e., an aversion function at concentrations above  $10^{-4}$  M (see Fig. 3B, *right*). Thus post hoc tests confirmed a significant age effect at the lowest concentration tested ( $1 \times 10^{-5}$  M:  $P < 0.01$ ), an effect similar to citric acid.

**MgCl<sub>2</sub>**—The strain times concentration interaction was significant for total licks [ $F(5,90) = 3.07$ ,  $P < 0.02$ ], but not for the lick difference scores [ $F(4,72) = 2.29$ ,  $P = 0.67$ , NS]. Post hoc analyses of the total licks showed that OLETF rats at both ages made more licks for water and the lowest concentration of MgCl<sub>2</sub> ( $P < 0.05$ ; Fig. 3C).

**Capsaicin**—The strain times concentration interaction was not significant when either total licks [ $F(5,85) = 1.21$ ,  $P = 0.31$ , NS, Fig. 3D] or lick difference score [ $F(4,68) = 0.79$ ,  $P = 0.53$ , NS] was assessed. Similarly, there was no reliable age times concentration effect observed for either measures [ $F(5,85) = 0.84$ ,  $P = 0.52$ , NS,  $F(4,68) = 0.92$ ,  $P = 0.46$ , NS]. Despite this, there was a nonsignificant trend for strain effect at 10 wk of age indicated by post hoc tests on total licks ( $P = 0.08$ ) carried by differences in responses by OLETF rats for water and the second weakest concentration of capsaicin ( $P = 0.07$ ,  $P = 0.08$ , respectively; see Fig. 3D, *left*).

### Responses to Carbohydrate Solutions

**Sucrose**—There was a significant effect of both the strain times concentration interaction, as well as of age times concentration [ $F(6,120) = 3.02$ ,  $P < 0.01$ ,  $F(6,120) = 2.61$ ,  $P < 0.03$ , respectively]. Whereas at 10 wk, OLETF rats made more total licks for the weaker solutions (0.03, 0.1, 0.3 M,  $P < 0.05$  for all; see Fig. 4A, *left*), at 18 wk, they preferred the highest concentrations more than the age-matched LETO controls (0.3, 1.0, 1.5 M,  $P < 0.01$  for all;

see Fig. 4A, *right*). In contrast, no difference in concentration-response function was revealed between younger and older LETO rats. In addition to the strain differences, at 18 wk, OLETF had increased responses to the two highest concentrations of sucrose (1.0, 1.5 M) compared with the same rats' responses at younger age ( $P < 0.05$  for both concentrations; see Fig. 4A, *right*).

**Fructose**—As with sucrose, the concentration-response function for fructose differed between OLETF and LETO {strain  $\times$  concentration interaction for total licks: [ $F(6,120) = 2.54, P < 0.03$ ]}. There were also a significant effect of age times concentration interaction for total licks: [ $F(6,120) = 3.92, P < 0.01$ ]. Specifically, 10-wk OLETF showed increased responses to weaker concentrations (0.05, 0.4 M,  $P < 0.05$  for both concentrations, see Fig. 4B, *left*), whereas 18-wk OLETF showed a stronger increase for all concentrations above 0.05 M (for 0.1 through 0.8 M,  $P < 0.01$ ; for 1.6 M,  $P < 0.05$ ; see Fig. 4B, *right*).

**Polycose**—There was a significant effect of strain times concentration as well as age times concentration interaction for total licks [ $F(6,120) = 2.76, P < 0.02, F(6,120) = 6.77, P < 0.0001$ , respectively]. Further analysis of the age effect demonstrated that for OLETFs, the concentration-response functions for Polycose differed from responses to other carbohydrates. For sugars, i.e., mono- and disaccharides, OLETF rats increased the number of total licks as a function of concentration and age, whereas for Polycose there were no strain times age effect between OLETF and LETO ( $P = 0.12$ ). Thus both strains showed a mild increase in intake with increasing concentration of Polycose above 0.03 M with a peak at 0.1 M (Fig. 4C, *right*).

### Responses to Other Preferred, Noncarbohydrate Stimuli

**NaCl**—The strain times concentration interaction was not significant for the NaCl concentration-response function [ $F(6,108) = 0.96, P = 0.46, NS$ ]. Nevertheless, both strains demonstrated a reliable concentration-response at 18 wk of age revealed by a highly significant age times concentration interaction [ $F(6,108) = 5.6, P = 0.0001$ ]. In fact, at the younger age, both strains showed an overall flat dose-response function to NaCl. Although the reason for this is not known, there was an indication for a reduced preference of OLETF to the highest concentrations of NaCl solutions. At 10 wk, post hoc tests revealed that, whereas the total lick numbers did not differ significantly between strains at any concentration ( $P > 0.05$ ), the differential lick score yielded a significantly reduced preference in OLETFs for 0.06, 0.1, and 1.0 M concentrations ( $P < 0.01$ , for all comparisons; see Fig. 5A, *left*).

**MSG**—The concentration-response function for MSG differed between strains and age reliably as revealed by a highly significant strain times concentration and age times concentration interaction for total licks [ $F(6,120) = 12.58, P < 0.0001, F(6,120) = 5.03, P < 0.001$ , respectively] and lick difference scores [ $F(5,100) = 13.74, P < 0.0001, F(5,100) = 4.63, P < 0.001$ , respectively]. Post hoc tests revealed that, whereas younger OLETF rats frolicked more at the lower concentrations compared with LETO (0.01, 0.03, and 0.06 M,  $P < 0.05$ , for all comparisons; see Fig. 5B, *left*), by 18 wk, they also ingested more water and 0.1 M MSG. In fact, by 18 wk, OLETF responses to 0.06 and 0.1 M MSG were significantly higher than for the same stimuli at the younger age ( $P < 0.05, P < 0.02$ , respectively). Also, at 18 wk, OLETF showed a decreased response for the highest concentration (1.0 M,  $P < 0.05$ ; see Fig. 5B, *right*). Likewise, LETO rats exhibited a somewhat increased response to concentrations above 0.06 M at an older age, but this difference did not reach significance.

**Na-saccharin**—Unlike carbohydrates, the concentration-response function for the noncaloric sweetener Na-saccharin did not differ between strains {strain  $\times$  concentration interaction for total licks [ $F(6,120) = 1.23, P = 0.3, NS$ ; Fig. 5C]}. Nevertheless, there was an

increased response across all concentrations in both strains when tested at 18 wk {age  $\times$  concentration interaction for total licks [ $F(6,120) = 4.53, P = 0.001$ ; Fig. 5C, right]}. Post hoc analysis revealed that this effect was carried by both strains with an accentuated peak response to 0.2% solution by both OLETF and LETO ( $P < 0.05$ ) and to 0.4% Na-saccharin by OLETF rats ( $P < 0.05$ ). In addition, 18-wk OLETF preferred more Na-saccharin at 0.1 and 1.6% concentrations ( $P < 0.05, P < 0.01$ , respectively) than LETO.

**Alanine**—Similar to preferred carbohydrates, the concentration-response function for alanine differed between strains {strain  $\times$  concentration interaction: [ $F(6,84) = 2.22, P < 0.05$ ]}, and age [age  $\times$  concentration interaction:  $F(6,84) = 2.37, P < 0.01$ ]. Whereas at 10 wk, OLETF showed a flat concentration-function for alanine, at 18 wk, all rats demonstrated a significant concentration-response function. Post hoc tests revealed that 10-wk old OLETF rats had an increased response to the weaker 0.03 M alanine solution ( $P < 0.05$ , see Fig. 5D, left), whereas 18-wk old OLETF had a significantly increased response to all concentrations starting with the weakest concentration tested (for 0.01–0.3 M,  $P < 0.01$ ; see Fig. 4B, right).

### Blood Glucose, Plasma Insulin, and Leptin Levels

Blood glucose levels were significantly higher in OLETF ( $6.78 \pm 0.13$  mmol/l) compared with LETO ( $5.43 \pm 0.20$  mmol/l) group ( $P < 0.05$ ). Plasma insulin levels also were significantly higher in OLETF ( $1.18 \pm 0.10$  ng/ml) compared with LETO ( $0.64 \pm 0.08$  ng/ml) groups ( $P < 0.01$ ). As expected, leptin levels were significantly elevated in OLETF ( $4.00 \pm 0.74$  ng/ml) compared with LETO ( $2.45 \pm 0.11$  ng/ml) group ( $P < 0.05$ ).

## DISCUSSION

### Summary of Results with Relevance to Model

In the present experiment, we used lick counts in brief access to taste stimuli to measure taste sensitivity. This measurement has been shown to vary directly with the concentration of a preferred tastant (13,15,75). The present results show enhanced behavioral (i.e., lick) responses in prediabetic OLETF rats compared with age-matched lean LETO controls to sweet substances and MSG, whereas responses to sour, salty, and bitter substances remained unaffected. With the exception of Polycose, this effect was augmented by age (i.e., 10 vs. 18 wk). In addition, OLETF rats became obese as a function of age and developed hyperglycemia, hyperinsulinemia, and hyperleptinemia. Nonetheless, all reported differences in taste functions were apparent in the prediabetic stage because rats did not meet the criteria for definition of clinical diabetes by the conclusion of the taste assessment studies (22 wk).

The broader aim of this study was to investigate the relationship between taste sensitivity and overeating characteristic of animal models and humans preceding the development of NIDDM. We chose OLETF rats because unlike other genetically obese rodent models, in this strain, increased food intake is necessary for the development of obesity, suggesting that the NIDDM is secondary to the prediabetic hyperphagia. The underlying cause of this chronic hyperphagia is still unknown, although deficits within meal satiety pathways (14,58), disruption in central pathways critical to overall energy balance (5), and increased preference for palatable foods (17) have been reported as likely contributors. We have recently reported that 14-wk-old prediabetic OLETF rats showed an increased two-bottle preference for sham feeding of sucrose (17). This suggests that dysfunctions in the central pathways critical to control for the hedonic valence of food may be responsible for this effect.

The major finding of the present study is that increased taste preference in OLETF rats is specific to chemicals that taste sweet to humans. Specifically, in addition to sucrose and fructose, alanine was also highly preferred by OLETFs at both ages particularly at lower



concentrations. The observation that the noncaloric sweetener Na-saccharin was also more preferred by 18-wk-old OLETFs indicates that this preference is not primarily driven by postabsorptive effect of the stimulus.

In contrast, responsiveness to Polycose was somewhat different. At 10 wk, there was a clear preference for weaker Polycose solutions in OLETF rats; a difference that disappeared by 18 wk of age. Several lines of evidences have suggested multiple receptor sites for carbohydrates in taste cells (36,79). Recently, receptors for sweet taste stimuli including sugars were identified as T1Rs (41,49). However, the T1Rs family is small and the only one form of functional sweet receptor is known to be a heterodimer of T1R2 and T1R3 (41,49). Thus the molecular basis for the multiple receptor sites for sugars is not so well established. For example, although it is known that rats, unlike humans, are very attracted to the taste of starch-derived polysaccharides such as Polycose, a glucose polymer, the receptor mechanism(s) mediating its effect remain unknown (64). Our finding in OLETF rats showing differential responses to Polycose compared with tastants that are all known to stimulate classic sweet receptors adds to a growing body of data supporting the quest for a hypothesized polysaccharide taste receptor (62). Nevertheless, whether hyperglycemia and/or hyperinsulinemia may alter preference for polysaccharides or whether this effect is due to lack of CCK-1 receptor or to the metabolic alterations is not known.

Another major finding of the present study was a significantly increased response to MSG in OLETF rats. This effect was exclusive for the lower concentrations at both ages with a marked aversion for the highest concentration at 18 wk, indicating increased taste acuity in addition to an overall increased avidity. Results from preference tests indicate that the ingestion of MSG is correlated with dietary protein intake (48). Similarly, data on the taste perception of MSG strongly suggest that such “umami” taste stimuli describe a basic taste category for protein intake (7,48,81) that is characteristic to saltiness and sweetness (51,80). These findings together with our observation that OLETF rats responded more to MSG, sucrose as well as alanine, but not salts (sodium or nonsodium moieties, a taste category for mineral intake), indicate that the augmented ingestive response to MSG is likely to be the result rather of the generalization to sweet (a taste category for energy intake). This notion is further supported by a recent study in rat showing that conditioned aversions to MSG cross generalize to sucrose and other sweeteners (33). This cross generalization may also explain our observation and reinforce our conclusion that taste preference in OLETF was indeed altered in ways specific to mechanisms that code for sweet. Nevertheless, the possibility that OLETF rats have a special avidity for proteins augmented by diabetic conditions also cannot be excluded.

In summary, the results showed that both sensitivity for and intake of sweet tastants are altered in obese OLETF rats. Whereas at a younger age, overall responses to sucrose in OLETF compared with age-matched lean controls was augmented across all concentration tested, at an older age, the same rats showed a right-shifted concentration-response function with an exaggerated intake of the highest concentrations. The two effects may represent different underlying mechanisms and etiology. An overall upright shift in the concentration-response function often occurs when sensory threshold is altered, whereas a reduced avidity at the lower end combined with augmented responses to higher concentrations is more characteristic of change in motivational modulation. Therefore, one may assume that younger OLETF rats have a preexisting condition of altered taste sensitivity and that over time, an additional effect becomes superimposed secondary to the developing metabolic and hormonal conditions resulting from increasing obesity. It is important to note that although the present study was not designed to evaluate perithreshold taste sensitivity, hence detection thresholds were not directly assessed, an increased responsiveness in younger OLETF to lower sucrose concentrations may be inferred from a reduced threshold. Lack of a similar effect in responses to other tastants, however, mitigates against this notion. We noticed an exceptionally high

sensitivity only to quinine-HCl at 10 wk of age, but since all rats reduced the intake of the most dilute solutions equally, this effect is less likely to be related to strain differences. Nonetheless, by 18 wk of age this increased sensitivity to quinine-HCl disappeared with no difference between strains. In addition, a one-log shift, slight reduction in sensitivity to the nontaste, trigeminal stimulus capsaicin was noticed in 10-wk-old OLETF. This difference was statistically significant, however, only when water was not included in the concentration-response functions due to the higher number of water licks in 10-wk-old OLETF rats. Moreover, there was no statistical difference in trigeminal responsiveness at 18 wk, indicating that a potential effect from neuropathy due to the developing diabetes played no role in overall neural responsiveness (39).

Regarding the increased within-trial water intake in OLETF rats, a similar effect was not apparent when palatable solutions were tested. This observation, together with an increased water intake by OLETF rats observed in water training trials (i.e., *days 1–5*) suggests that a differential water response in the test trials may represent a contrast effect whereby OLETF rats suppress intake of water in favor of more palatable solutions, and vice versa, increase it when water is presented with aversive chemicals. In general, an increased avidity of OLETF to consume more water could be inferred from an overall increased consummatory motivation. In fact, it has been shown in normal rats that a longer food deprivation (48 but not 24-h deprivation) increased hedonic reactivity that was not restricted to sweet tastes, but also extended to the palatability of water (1). Therefore, it is plausible to assume that OLETF rats express an overall reduced reward threshold, or chronic state of “hedonic deprivation” (42) that, in turn, results in increased hedonic reactions to preferred stimuli. The observation that OLETF rats showed unaltered responses to less preferred or aversive chemicals (i.e., bitter and sour tastants) is in concert with data in the literature showing that only the hedonic reactions to taste were changed by hunger or satiety, whereas taste aversion was not altered (1).

### Potential Role for Altered Taste Receptor Functions

The primary site of effect that may influence taste threshold and sensitivity is the taste receptor cells (TRCs). Mammalian TRCs express neuropeptides that may serve as signaling agents in conjunction with classic neurotransmitters. CCK, in particular, has been observed to modulate electrical excitability in TRCs (32). CCK, acting specifically through the CCK-1 receptor subtype, inhibits the inwardly rectifying potassium current and increases intracellular  $\text{Ca}^{2+}$  concentration. TRCs that respond to CCK with elevations of intracellular calcium are those that are also highly responsive to the bitter stimulants quinine and caffeine (43). Thus one may suppose that in rats lacking CCK-1 receptor responses to quinine-HCl were diminished. In contrast, we observed no differences in responses to quinine-HCl in OLETF at any age investigated. One possible explanation for this discrepancy is that the CCK-1 receptor does not act alone in modulating TRC responsiveness, hence alterations in other modulatory neuropeptides secondary to the mutant phenotype may compensate for the deficit. Indeed, studies demonstrating a typical coexpression pattern of peptides and signaling molecules in TRCs, showed CCK-1 receptors being coexpressed with vasoactive intestinal peptide and neuropeptide-Y (30–32,65). Of particular interest, neuropeptide-Y and CCK act via the same transduction mechanism (31), i.e., G-protein-dependent modulation of inwardly rectifying potassium current, but exercise antagonistic effect on excitability of T1R2 TRCs identified as bitter taste receptors (9). However, until modulation of TRCs by these neuropeptides is directly assessed in OLETF, this notion remains highly theoretical.

An additional link has been proposed to exist between function of the TRCs and obesity. Shimizu et al. (68) demonstrated enhanced responses of the CT nerve to sugars in obese rats with lesions of the ventromedial hypothalamus. Genetically diabetic db/db mice, high-fat diet-induced obese rats, and streptozotocin-diabetic rats also showed greater CT nerve responses

to sugars (50). A similar result has been obtained in OLETF rat (82). Thus since the enhanced CT nerve responsiveness to sugars seems to be present among most obese and diabetic models tested, one might assume that some common metabolic impairments could be related to this effect. The most obvious one is hyperleptinemia. Recently, it has been demonstrated that TRCs express the leptin receptor and that leptin directly increases K<sup>+</sup> conductance, resulting in hyperpolarization and reduction of cell excitability (37). This would explain the enhanced CT nerve response to sugars in db/db mice which express a mutant leptin receptor (10). In contrast, one would predict rather a decreased taste receptor sensitivity in nonmutant obese rats with high levels of leptin. In diet-induced obesity models, prolonged hyperleptinemia, however, may lead to downregulation of leptin receptors on the TRCs, an effect similar to the one observed in skeletal muscle and hypothalamic neurons in response to increased leptin levels during development of obesity (18,76). This would explain not only the altered taste sensitivity in obese models but also the enhancement of that sensitivity as obesity develops. This is in line with our findings, demonstrating an increase in taste sensitivity in OLETF rats as a function of obesity.

### Participation of the Reward System

In addition to the potential role of receptor mechanisms discussed above, an enhanced sensitivity of the reward system can also result in a selective increase in responsiveness to sweet, as observed in this study. Although its exact relationship to behavioral reward remains controversial (2,55), dopamine (DA) activity, particularly in the mesolimbic system, does increase when normally preferred stimuli are encountered (86). We have previously shown that, compared with water, licking sucrose solutions raises DA overflow in the nucleus accumbens in a concentration-dependent manner even when metabolic feedback is prevented and the amount of fluid ingested is held constant (29). A large body of evidence have demonstrated the converse relationship that both the intake of and the preference for sucrose can be altered by dopaminergic manipulations [e.g., dopaminergic stimulation (27,77), DA lesions (69), systemic, or intra-accumbens administration of DA receptor antagonists (27,35, 57,88)]. Although there is no direct evidence for altered DA responses in obese animals in response to palatable taste stimuli, chronic food restriction has been shown to reduce basal DA levels in rats and increase consummatory responses to both food and drug rewards (54), a similar manipulation that also increases bingeing on palatable meals (25). In fact, alterations in DA neurotransmission also have been implicated in the pathology of obesity (83). In particular, a decrease in striatal D2 receptor availability and function that negatively correlates with body mass index (83) have been demonstrated in severely obese individuals. The low D2 receptor expression may be cause for an exaggerated DA release observed in obese subjects (21). Relevant to this, we have demonstrated an altered D2 receptor function and a lower D2 receptor binding in the striatum of obese, 14-wk-old OLETF rats (44). In addition, there is accumulating evidence in support of the notion that OLETF rats have an altered DA function characteristic of a reduced basal DA tone and increased responsiveness to phasic DA stimulation by natural and psychostimulant reward, i.e., sucrose or amphetamine (20,26,66). The net effect of this regulatory deficit in OLETFs is apparent in higher DA functions, such as the startle response and sensorimotor gating (16,19), and may also contribute to an increased preference for, and intake of palatable meals and contribute to chronic overeating that is characteristic of the prediabetic behavioral phenotype.

### Perspectives

This study is the first detailed taste analysis in the obese OLETF rats. Our results showed that 1) in brief-access lick tests, OLETF rats exhibit an increased response to sucrose and other tastants that taste sweet to humans including noncaloric saccharin, but unaltered responses to salty, sour, and bitter chemicals, and that 2) this effect is enhanced in older rats with increasing insulin resistance. Although the exact mechanisms remain unclear, the strain differences could

reflect the absence of a CCK-1 receptor-mediated effect on the taste receptors or on processing in the taste code upstream from the receptors. In addition, an altered motivational control for taste preferences or for behavioral rewards in general, secondary to the impaired CCK-1 regulation cannot be excluded. The primary deficit may be also complicated by developing metabolic conditions related to obesity in the prediabetic stages. Regardless of the underlying mechanism(s), the observed differences in taste sensitivity, in turn, may contribute to increased preference for, and intake of, palatable meals and to a faulty compensatory regulation resulting in sustained overeating and body weight gain in this strain.

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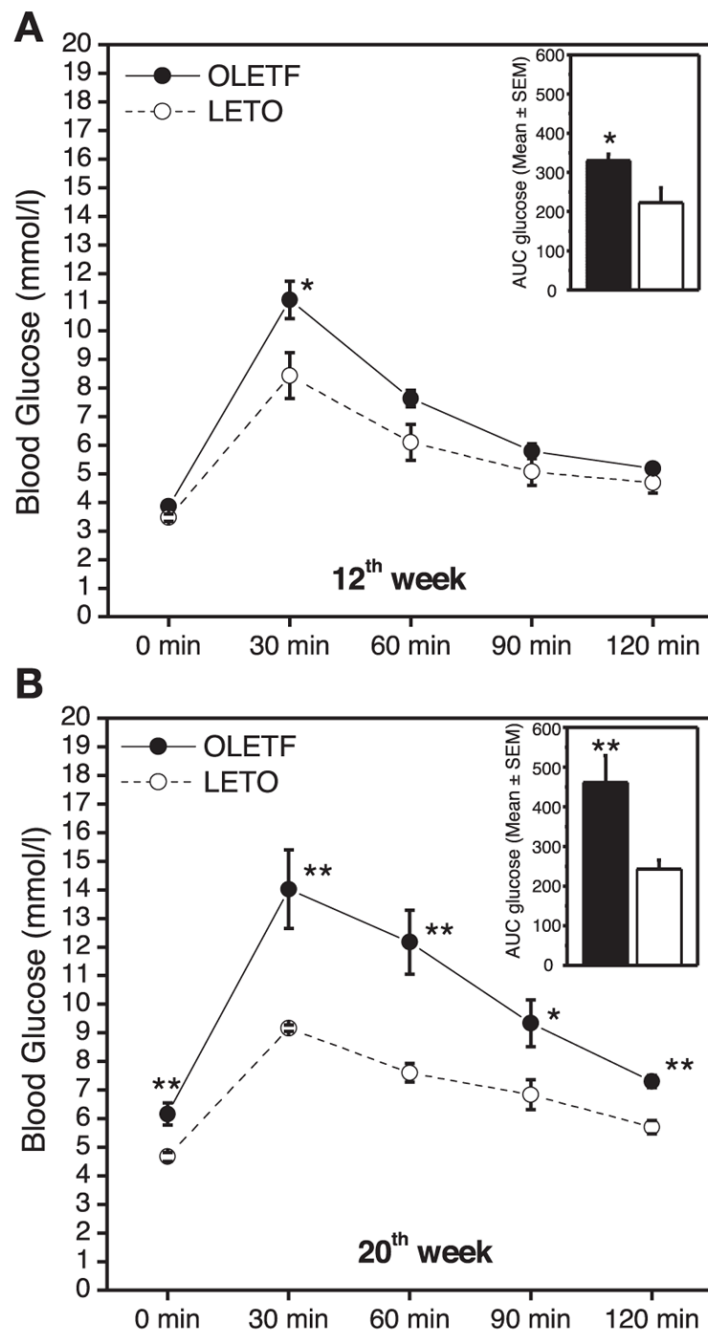
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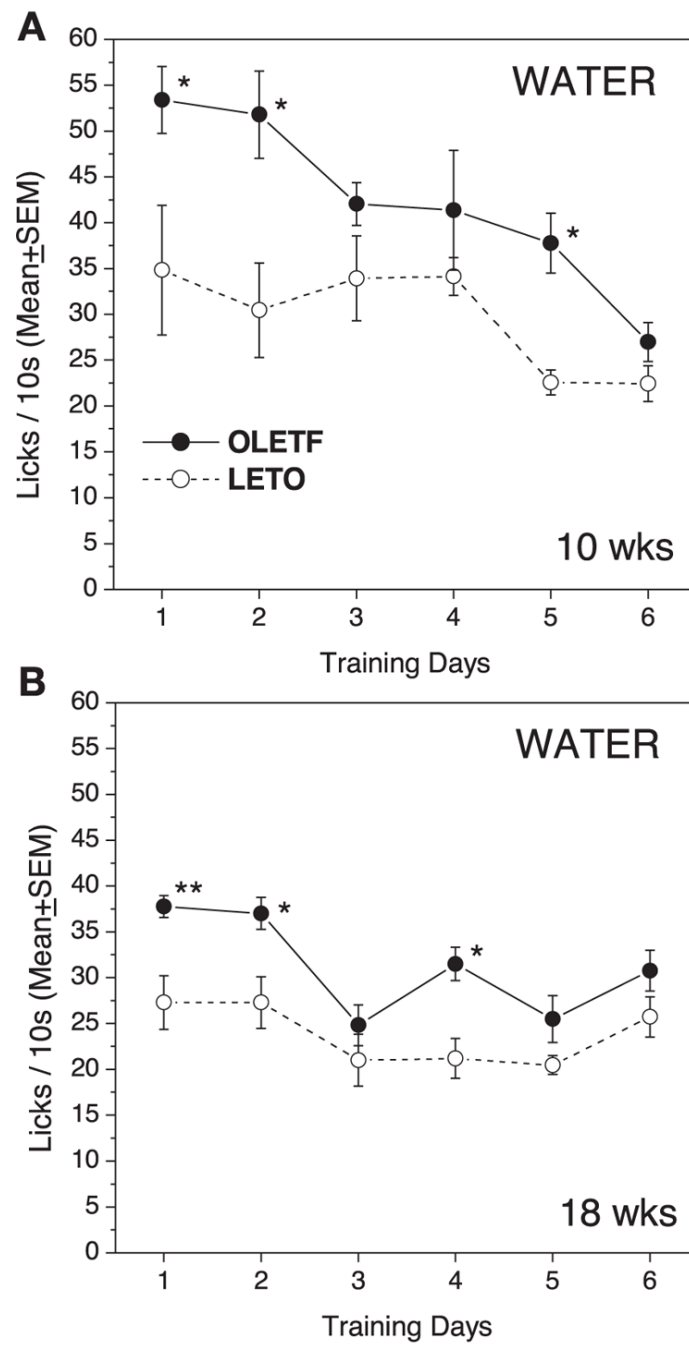
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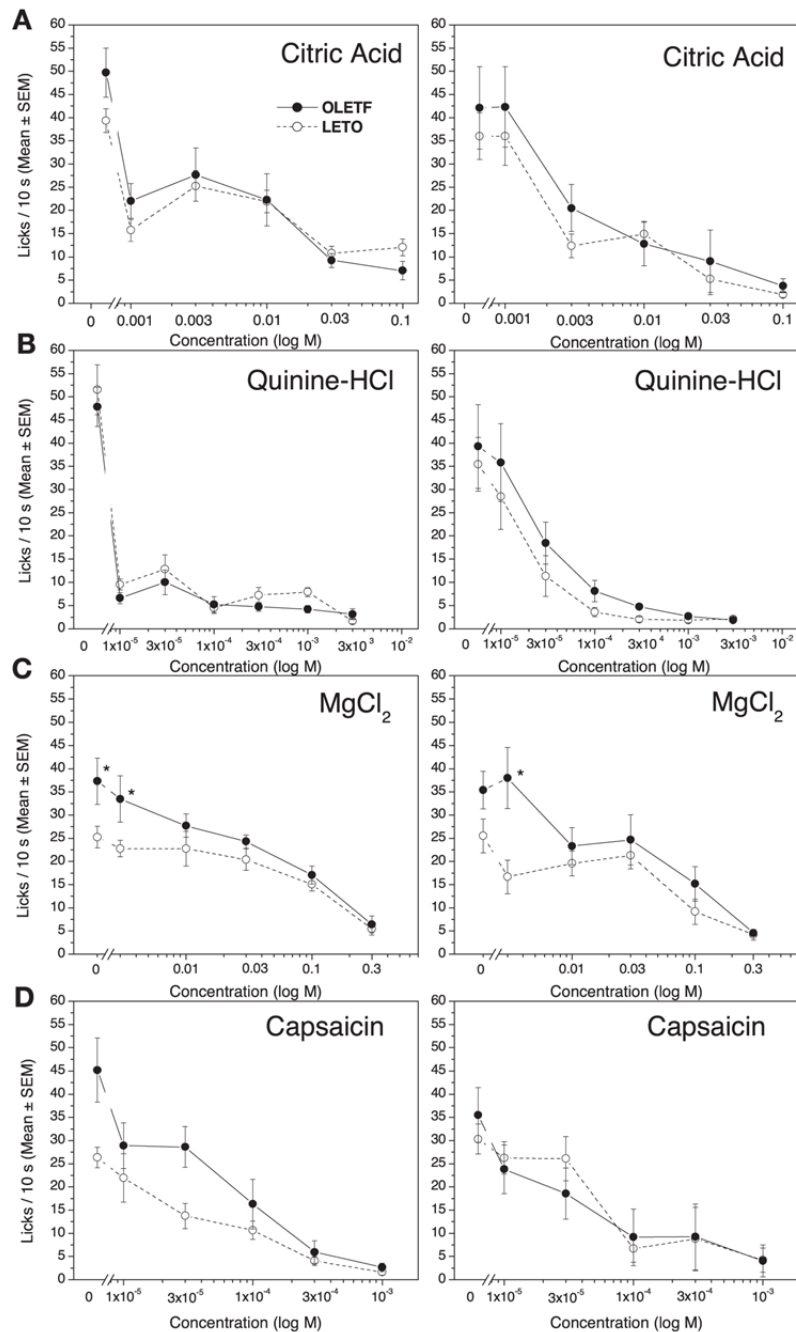


**Fig 1.**

Oral glucose tolerance tests (2 g/kg) in 12 (A)- and 20 (B)-week-old rats representing time points for the taste testing. Glycemic excursions for Otsuka Long-Evans Tokushima fatty (OLETF; closed symbols and straight lines) and Long-Evans Tokushima Otsuka (LETO; open symbols and dashed lines) rats are expressed as means  $\pm$  SE. *Insets*, quantification of the glycemic excursions as area under the curve. Closed columns, OLETF; open columns, LETO. Statistical symbols represent post hoc comparisons between strains: \* $P < 0.05$ , \*\* $P < 0.01$ . For more details and statistics, see RESULTS.

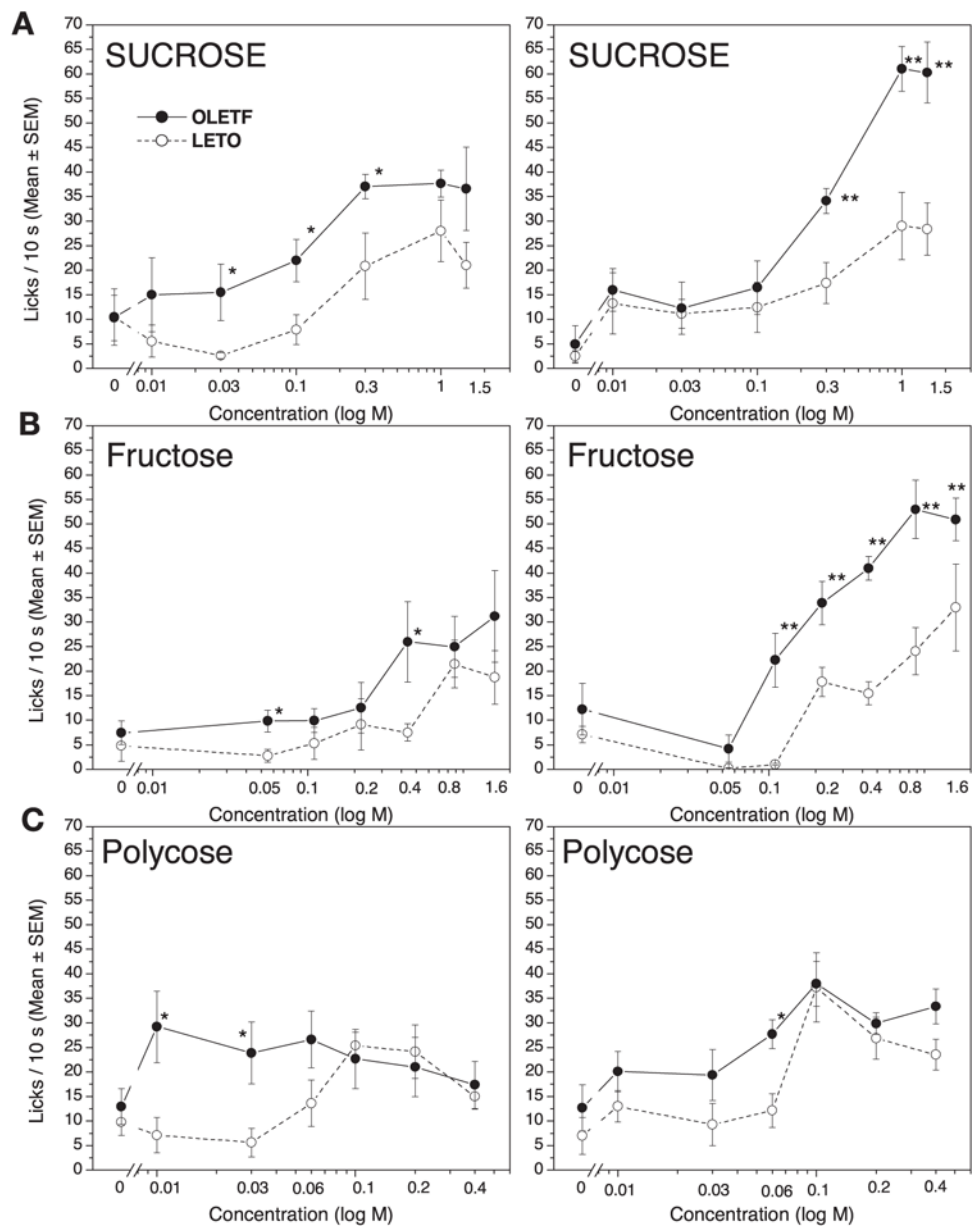


**Fig 2.** Mean number of licks/10 s made for water in the training sessions preceding taste tests at 10 and 18 wk of age. On the *x*-axis, data are depicted from *days 1–6* for OLETF (closed circles, *n* = 6) and LETO (open circles, *n* = 6). Statistical symbols represent post hoc comparisons between strains: \**P* < 0.05. For more details and statistics, see RESULTS.

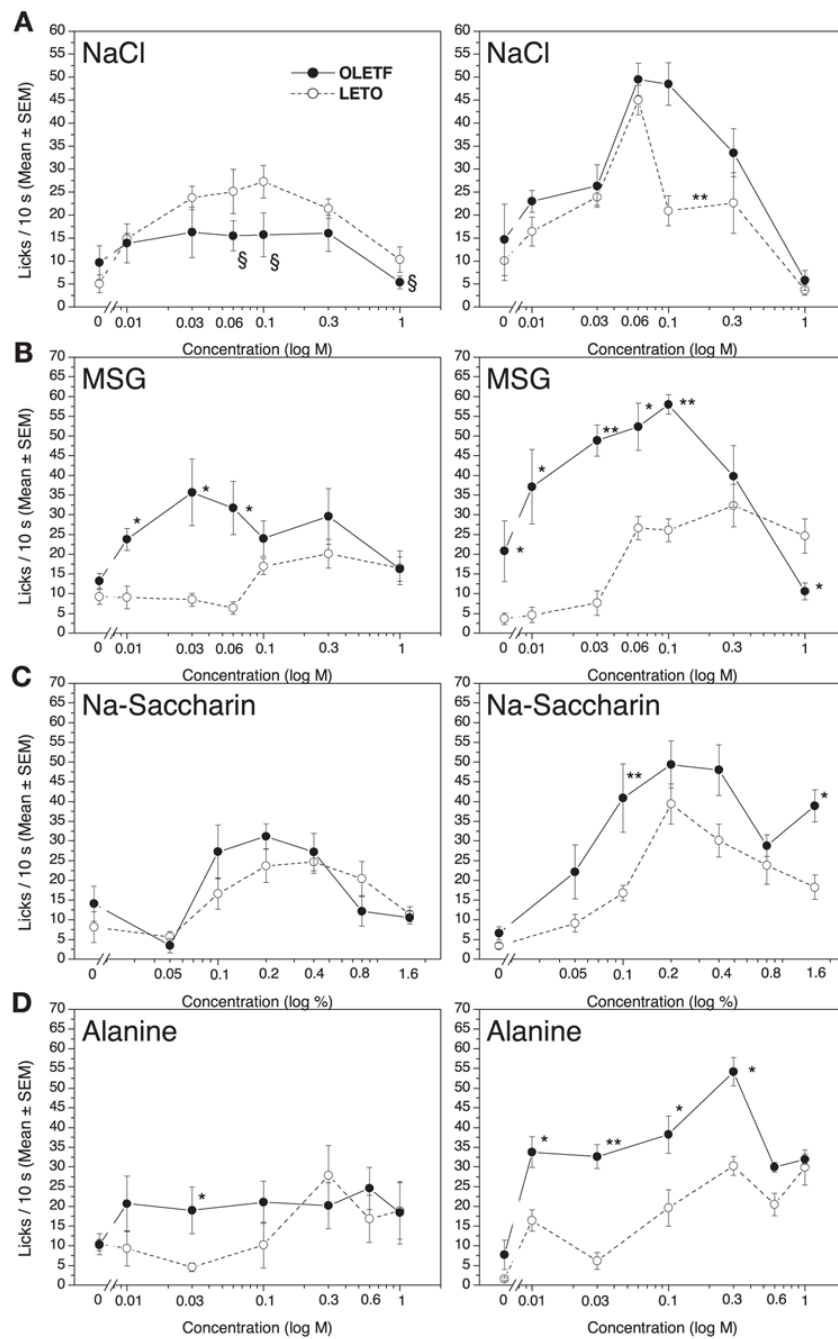


**Fig 3.**

Responses to aversive chemicals. Mean number of licks/10 s made for water (0 on the  $x$ -axis) and increasing concentrations of less preferred or aversive chemicals by OLETF (closed circles,  $n = 6$ ) and LETO rats (open circles,  $n = 6$ ). *Left*: results from test at 10 wk of age (10 wk). *Right*: results from repeated tests at 18 wk of age in the same rats (18 wk). *A*: responses to sour citric acid. *B*: responses to bitter quinine-HCl. *C*: responses to nonsodium bitter salt, MgCl<sub>2</sub>. *D*: responses to the nontaste, i.e., trigeminal stimulus, capsaicin. Statistical symbols represent post hoc comparisons between strains. Statistical comparisons on total licks: \* $P < 0.05$ . For more details and statistics, see RESULTS.



**Fig 4.** Responses to palatable carbohydrate solutions. Mean number of licks/10 s made for water (0 on the x-axis) and increasing concentrations of sugar-based sweet tastants by OLETF (closed circles,  $n = 6$ ) and LETO rats (open circles,  $n = 6$ ). *Left*: results from test at 10 wk of age (10 wk). *Right*: results from repeated tests at 18 wk of age in the same rats (18 wk). *A*: responses to sucrose. *B*: responses to fructose. *C*: responses to Polycose. Statistical symbols represent post hoc comparisons between strains. Statistical comparisons on total licks: \* $P < 0.05$ , \*\* $P < 0.01$ .



**Fig 5.**

Responses to palatable noncarbohydrate stimuli. Mean number of licks/10 s made for water (0 on the x-axis) and increasing concentrations less preferred or aversive chemicals by OLETF (closed circles,  $n = 6$ ) and LETO rats (open circles,  $n = 6$ ). *Left*: results from test at 10 wk of age (10 wk). *Right*: results from repeated tests at 18 wk of age in the same rats (18 wk). *A*: responses to NaCl. *B*: responses to monosodium glutamate (MSG). *C*: responses to sodium saccharin (Na-saccharin). *D*: responses to alanine. Statistical symbols represent post hoc comparisons between strains. Statistical comparisons on total licks: \* $P < 0.05$ , \*\* $P < 0.01$ . For more details and statistics, see RESULTS.