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Role of Olfaction in the Conditioned Sucrose Preference of Sweet-Ageusic T1R3 Knockout Mice

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Abstract

Prior work has shown that sweet taste–deficient T1R3 knockout (KO) mice developed significant sucrose preferences when given long-term sugar versus water tests. The current study investigated the role of olfaction in this experience-conditioned sucrose preference. T1R3 KO and C57BL/6 wild-type (WT) mice were given 24-h sugar versus water tests with ascending concentrations of sucrose (0.5–32%), after which the mice received olfactory bulbectomy (OBx) or sham surgery. When retested with sucrose, the Sham-KO mice preferred all sugar solutions to water, although their intake and preference were less than those of the Sham-WT mice. The OBx-KO mice, in contrast, showed no or weak preferences for dilute sucrose solutions (0.5–8%) although they strongly preferred concentrated sugar solutions (16–32%). OBx-WT mice displayed only a partial reduction in their sucrose preference. Although the OBx mice of both genotypes underconsumed dilute sucrose solutions relative to Sham mice, they overconsumed concentrated sucrose. These results indicate that olfaction plays a critical role in the conditioned preference of T1R3 KO mice for dilute sugar solutions. Further, the fact that OBx-KO mice preferred concentrated sucrose solutions in the absence of normal sweet taste and olfactory sensations underscores the potency of postoral nutritive signals in promoting ingestion.

Key words: C57BL/6J mice, olfactory bulbectomy, postoral conditioning, preference, sucrose

Introduction

The appetite for sweet foods and drinks begins with the stimulation of sweet taste receptors by natural sugars and artificial sweeteners. In mammals, the primary sweet taste receptor is a heterodimer consisting of T1R2 and T1R3 subunits (Bachmanov and Beauchamp 2007). Knockout (KO) mice with selective deletion of either receptor subunit display greatly attenuated behavioral and gustatory nerve response to sweeteners (Damak et al. 2003; Zhao et al. 2003). Double-KO mice with both T1R2 and T1R3 deleted show no nerve response or behavioral response to concentrated sugar solutions in brief licking tests (Zhao et al. 2003). These results suggest that the small residual response to concentrated sugar solutions observed in single-KO mice is mediated by the remaining T1R2 or T1R3 acting as a homodimer receptor, although only the T1R3 subunit appears to function as a low-affinity sugar receptor in normal mice (Damak et al. 2003; Zhao et al. 2003; Nie et al. 2005).

The magnitude of the behavioral deficit to sugars displayed by T1R3 KO mice depends upon the test method used. In the early studies cited above, T1R3 KO mice displayed no brief licking response, above water baseline, to a dilute sucrose solution (0.1 M or 3.4%) and only a very weak response to a concentrated solution (1 M or 34%) (Zhao et al. 2003). Yet, when given more prolonged exposure in 24-h sugar versus water 2-bottle tests, T1R3 KO mice displayed a robust preference (~90%) for 1 M sucrose that was comparable to that observed in normal wild-type (WT) mice (Damak et al. 2003). A recent study in our laboratory confirmed the importance of test duration and sugar concentration in the sucrose preference of T1R3 KO mice (Zukerman et al. 2009). In 60-s 2-bottle tests, KO mice were indifferent to 4% and 8% sucrose and displayed weak and nonsignificant preferences for 16–32% solutions. In 24-h 2-bottle tests, the T1R3 KO mice were indifferent to dilute (0.5–8%) solutions but displayed significant preferences (>80%) for 16–32% sucrose solutions. A new finding of this study was that, after developing a preference for 16–32% sucrose solutions, the same T1R3 KO mice displayed robust (>80%) preferences for all sucrose solutions (0.5–32%) in a second series of 24-h tests.

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The 24-h preference displayed by KO mice for concentrated sucrose solutions has been attributed to postoral nutritive effects; such effects would be minimal or absent in brief licking or 2-bottle tests because sugar intakes are minimal (Zhao et al. 2003; Delay et al. 2006; Treesukosol et al. 2009; Zukerman et al. 2009). Supporting this interpretation, T1R3 KO mice, like WT mice, learn to prefer a flavored solution (e.g., grape or cherry) that is paired with intragastric (IG) infusions of 16% sucrose (Sclafani et al. 2008). Thus, although they are minimally attracted to sucrose solutions in brief tests, KO mice may learn to associate the orosensory features of concentrated solutions (T1R3-independent taste, texture, odor) with the sugar's postoral reward effects. The significant preference displayed by the KO mice when subsequently tested with dilute sugar solutions may represent a generalization of this learned response. However, it is uncertain what orosensory cues mediate the conditioned preference for dilute sugar solutions. It appears unlikely to be T1R3-independent taste cues because T1R3 KO mice display no gustatory neural response to dilute sucrose solutions (Damak et al. 2003; Zhao et al. 2003; Lemon and Margolskee 2009; Zukerman et al. 2009). It is also questionable whether dilute sugar solutions have detectable textural properties. It is possible, though, that dilute sugar solutions have a detectable odor that can support the conditioned preference. Results obtained with rats indicate that they sense the odor of sucrose solutions (Oakley 1965; Van Buskirk and Erickson 1977; Van Buskirk 1981; Ramirez 1993; Rhinehart-Doty et al. 1994). In particular, measurements of the latency to lick a drinking tube indicate that rats can distinguish the odor of 0.5% and 1% sucrose solutions (Rhinehart-Doty et al. 1994), and experimentally induced anosmia reduces the preference for 0.5% and 1% sucrose solutions in rats (Ramirez 1993).

The present study investigated the role of olfaction in the experience-induced sucrose preference displayed by T1R3 KO mice. This is of importance because little attention has been focused on nongustatory stimuli that promote sugar intake. The fact that KO mice with substantially reduced sweet taste sensitivity develop strong preferences for sugar solutions clearly demonstrates that sweet taste is not the only flavor element that influences sugar intake. As in our prior study, KO and WT mice were first given a series of 24-h preference tests with sucrose solutions at ascending concentrations (0.5–32%). Some of the mice were then given olfactory bulbectomy (OBx), whereas others had sham surgery. Following surgery, the animals were given a second series of preference tests with 0.5–32% sucrose solutions. We predicted that the OBx-KO mice, unlike OBx-WT mice and Sham-KO and WT mice, would fail to prefer dilute sucrose solutions during the second test series. In view of reports that olfactory function may return in bulbectomized mice (Wright and Harding 1982), at the end of the sugar preference tests the olfactory abilities of the mice were evaluated using a conditioned odor avoidance task.

Materials and methods

Animals

T1R3 KO mice were derived from mice produced by homologous recombination in C57BL/6J embryonic stem cells and maintained on this background (Damak et al. 2003). C57BL/6J WT (B6 WT) mice were derived from mice obtained from the Jackson Laboratories. Ten-week-old female mice of each genotype (n = 24 per genotype) were studied. The animals were singly housed in plastic tub cages with ad libitum access to chow (5001, PMI Nutrition International) and deionized water in a room maintained at 22 °C with a 12:12 h light:dark cycle. Experimental protocols were approved by the Institutional Animal Care and Use Committee at Brooklyn College and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Surgery

Mice were anesthetized with 2.5% avertin (0.3 ml/30 g, intraperitoneally) diluted in 0.9% saline and fixed in a stereotaxic instrument with an incisor bar and blunt ear bars. An incision was made in the skin covering the skull, 2 burr holes were drilled to expose the olfactory bulbs, and the bulbs were removed with aspiration. The brain cavity was filled with hemostatic sponge, and the skin was sutured closed. Sham operations were performed in the same way but with the skull and bulbs left intact. At the end of the study, mice were euthanized (pentobarbital), and the brains were removed. The extent of damage to the olfactory bulbs was assessed by visual inspection using a stereo binocular microscope. The aspiration boundaries of each OBx brain were drawn on separate sheets with an outline of an intact brain. Composite figures with the extent of the lesions for the OBx-KO and OBx-WT mice were prepared from these drawings. In addition, a behavioral test of anosmia was conducted at the end of the experiment (see below). One OBx-WT mouse died during behavioral testing, and one OBx-KO mouse failed the behavioral test of anosmia. Final group sizes were OBx-KO (n = 12), OBx-WT (n = 12), Sham-KO (n = 11), and Sham-WT (n = 11).

Test solutions

Preference tests were conducted using food-grade sucrose (Domino Foods, Inc), reagent-grade sucrose (Sigma Chemical Co), and sodium saccharin (Sigma) dissolved in deionized water. The solutions were formulated on a w/w basis because intakes were measured by weight. Odor avoidance tests were conducted using 0.001% ethyl acetate and 0.001% propyl acetate (Sigma) dissolved in 0.12 M lithium chloride (LiCl, Sigma) or 0.12 M sodium chloride (NaCl, Fisher Scientific).

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Apparatus

The 24-h 2-bottle tests were conducted in the animal's home cage. Fluid was available through sipper spouts (1.5-mm diameter spout opening, OT-100.5SP, Ancare) attached to 50- or 80-ml plastic tubes that were placed on the top of the cage. The sipper spouts were inserted through holes positioned 3.7 cm apart in a stainless steel plate, and the drinking tubes were fixed in place with clips. Fluid intakes were measured to the nearest 0.1 g by weighing the drinking tubes on an electronic balance interfaced to a laptop computer. Daily fluid spillage was estimated by recording the change in weight of 2 drinking tubes that were placed on an empty cage. The estimated spill throughout the experiment was approximately 0.2 g, and intake measures were corrected by the spillage amount.

Procedure

KO mice were given a 24-h 2-bottle preference test for 2 days with 0.2% saccharin (ca. 10 mM) versus water in their home cages to confirm their phenotype; WT mice were not tested with saccharin so that they would remain naive to sweet taste prior to sucrose testing. All mice were then given a series of 24-h 2-bottle tests with food-grade sucrose at ascending concentrations of 0.5%, 1%, 2%, 4%, 8%, 16%, and 32% (ca. 0.015–0.9 M, Test 1). The solutions were presented on 2 successive days at each concentration with the position of the sucrose and water bottles alternated daily. (This procedure is referred elsewhere as a 48-h test, but this term is not used here because the results are expressed as intake per day.) Following Test 1, the mice were divided into OBx and Sham groups equated for sucrose intake and body weight. Following surgery and 10 days after the end of Test 1, the mice were tested again (Test 2) with 0.5–32% sucrose solutions versus water as in the first series. They were given water only for 6 days and then additional 0.5% sucrose versus water tests (2 days each) using food-grade sucrose and then reagent-grade sucrose (Test 3). The latter test was conducted to determine if the purity level of sucrose altered sugar preference (see Rhinehart-Doty et al. 1994).

The mice were next given 3 days of water only followed by a conditioned odor avoidance test to evaluate the anosmia of the OBx mice (Test 4). Over 4 consecutive 1-bottle training days, an odorized 0.12 M NaCl (days 1 and 3) or 0.12 M LiCl solution (days 2 and 4) was available 24 h/day as the only drinking fluid. For half of the mice, the odor (CS+) added to the LiCl solution was 0.001% ethyl acetate and the odor (CS–) added to the NaCl solution was 0.001% propyl acetate; the odor–salt solution pairs were reversed for the remaining animals. Water only was available on day 5, and then a 2-bottle test (days 6 and 7) was conducted with the CS+ and CS– odors presented in NaCl solutions. The mice were given chow ad libitum throughout the test. This procedure was based on a prior rat study using orally presented LiCl and NaCl solutions (Ramirez 1991). The solutions share a common salty taste, so that the animals attribute the visceral malaise induced by the consumed LiCl to its paired odor rather than to its salty taste; note that T1R3 KO mice are normal in their salt taste (Damak et al. 2003; Zhao et al. 2003; Lemon and Margolskee 2009). The 0.12 M LiCl and NaCl concentrations, which are higher than those used with rats (Ramirez 1991), were based on pilot work; mice in general are less responsive to LiCl than are rats (Rowland et al. 2002). Ethyl acetate and propyl acetate and the aqueous 0.001% concentration were used based on prior odor discrimination studies (Slotnick et al. 1997; Slotnick 2007).

Data analysis

Daily solution and water intakes were averaged over the 2 days at each solution concentration. Sucrose preferences were also expressed as percent sugar solution intakes (sucrose solution intake/total intake × 100). Overall, the WT mice weighed more than the KO mice at the start and end of sucrose testing (24.8 ± 0.3 vs. 22.5 ± 0.4 g, $t_{44} = 4.21$, $P < 0.01$). Preliminary analyses of sucrose intakes expressed as intake per mouse and intake per 30 g body weight, as in previous studies (Bachmanov et al. 2001), produced very similar results, and therefore, intakes are reported as intake per mouse (Zukerman et al. 2009). Genotype differences in sucrose intakes and preferences were evaluated using separate mixed model analyses of variance (ANOVAs) with genotype and sugar concentration as between-group and within-group factors, respectively. Additional ANOVAs examined sucrose intake and preference within a genotype. The significance of the sucrose preference at each concentration was evaluated within each group by comparing sucrose and water intakes using paired *t*-tests corrected for multiple comparisons using the Bonferroni procedure.

Results

Saccharin pretest

As expected, the KO mice failed to prefer 0.2% saccharin in the initial 2-bottle test; in fact, they drank less saccharin than water (2.0 ± 0.1 vs. 2.6 ± 0.1 g/day, $t_{22} = 3.91$, $P < 0.01$). This confirms prior work and is attributed to the KO mice avoiding the bitter taste component of saccharin (Blednov et al. 2008; Zukerman et al. 2009).

Test 1: sucrose versus water

The results of the sucrose Tests 1 and 2 are presented for the Sham and OBx groups in Figures 1 and 2, respectively. In Test 1 conducted prior to surgery, the Sham-KO mice consumed less sucrose overall than did the Sham-WT mice (3.6 ± 0.2 vs. 10.7 ± 0.7 g/day, $F_{1,20} = 88.5$, $P < 0.001$), and the genotype differences were significant at all concentrations except 0.5% and 1% (genotype × concentration interaction, $F_{6,120} = 57.0$, $P < 0.001$). With respect to sucrose

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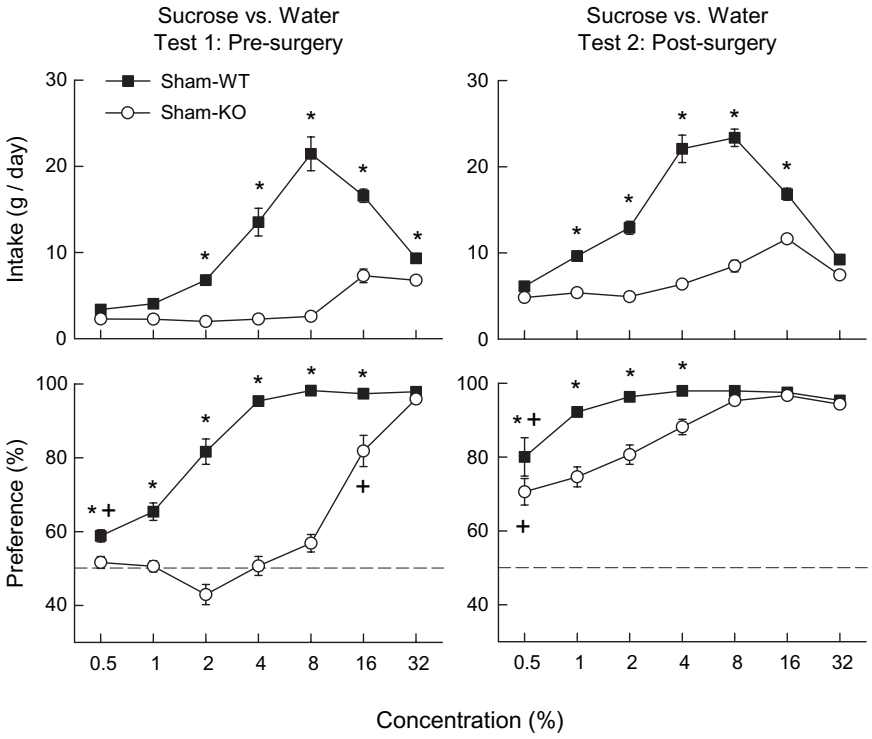

Figure 1 Sucrose solution intake (±standard error of the mean) (top panel) and percent sucrose preference (bottom panel) of Sham-KO and Sham-WT mice during sucrose versus water 2-bottle Tests 1 and 2. Water intakes are not shown. Ten days intervened between the 2 tests during which sham surgery was performed. Significant ($P < 0.05$) genotype differences at individual concentrations are indicated by an asterisk. The lowest concentration at which sucrose was significantly preferred to water is indicated by a plus sign.

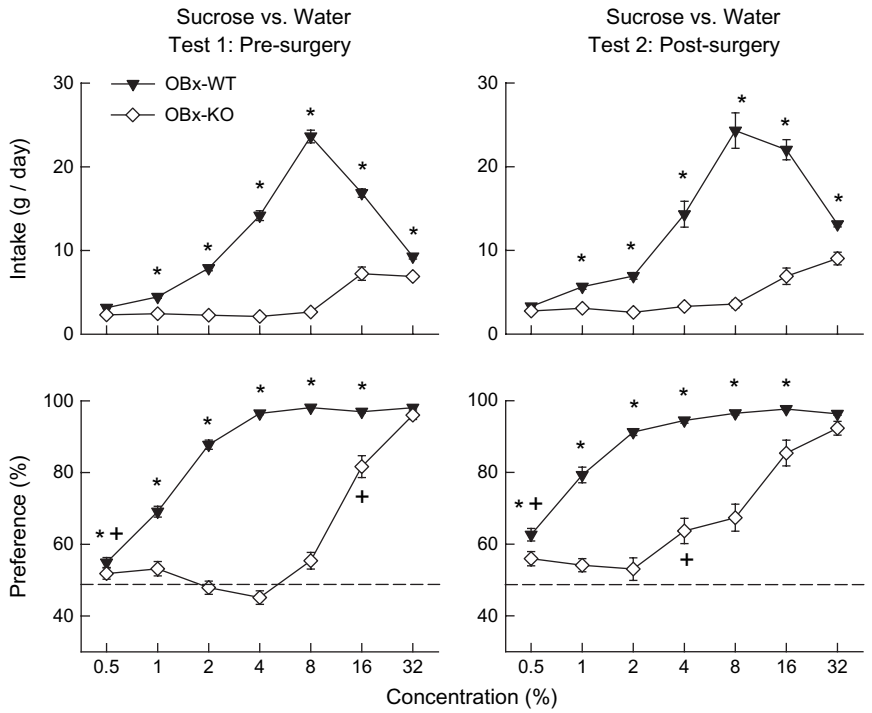

Figure 2 Sucrose solution intake (±standard error of the mean) (top panel) and percent sucrose preference (bottom panel) of OBx-KO and OBx-WT mice during sucrose versus water 2-bottle Tests 1 and 2. Water intakes are not shown. Ten days intervened between the 2 tests during which OBx surgery was performed. Significant ($P < 0.05$) genotype differences at individual concentrations are indicated by an asterisk. The lowest concentration at which sucrose was significantly preferred to water is indicated by a plus sign.

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versus water preference, the Sham-KO mice were indifferent to 0.5–8% sucrose and only at 16% and 32% concentrations did they drink more ($P < 0.001$) sugar solution than water. In contrast, Sham-WT mice preferred ($P < 0.001$) all sucrose solutions to water. The percent sucrose intakes of Sham-KO mice were less than those of the Sham-WT mice at all concentrations except 32% (genotype × concentration interaction, $F_{6,120} = 34.4$, $P < 0.001$). As illustrated in Figure 2, the presurgery sucrose intakes and preferences for the OBx-KO and OBx-WT groups were nearly identical to those of the Sham groups, and the statistical analyses are not presented.

Test 2: sucrose versus water

In the second sucrose test conducted after sham surgery (Figure 1), the Sham-KO mice continued to consume less sucrose than did Sham-WT mice (7.0 ± 0.3 vs. 14.3 ± 0.7 g/day, ($F_{1,20} = 101.2$, $P < 0.001$), and the genotype differences were significant at 1–16% concentrations (genotype × concentration interaction, $F_{6,120} = 77.3$, $P < 0.001$). In this test, however, the Sham-KO, like the Sham-WT mice, consumed more ($P < 0.001$) sucrose than water at all concentrations. The percent sucrose intakes of the Sham-KO mice were less ($P < 0.05$) than those of Sham-WT mice at 0.5–4% concentrations (genotype × concentration interaction, $F_{6,120} = 6.4$, $P < 0.001$).

Following OBx (Figure 2), the OBx-KO mice consumed less sucrose than the OBx-WT mice (4.5 ± 0.3 vs. 12.8 ± 0.8 g/day, $F_{1,22} = 128.3$, $P < 0.001$), and the genotype differences were significant at 1–32% concentrations (genotype × concentration interaction, $F_{6,132} = 49.2$, $P < 0.001$). In this test the OBx-KO were indifferent to 0.5–2% sucrose and consumed more ($P < 0.003$) 4–32% sucrose than water. In contrast, the OBx-WT preferred sucrose to water at all concentrations tested. The percent sucrose intakes of the OBx-KO mice were less than those of OBx-WT mice at 0.5–16% concentrations (genotype × concentration interaction, $F_{6,132} = 21.8$, $P < 0.001$).

Figure 3 compares within-genotype sucrose data for the OBx and Sham groups in Test 2. The OBx-WT mice consumed less ($P < 0.05$) 1–4% sucrose than Sham-WT mice (and marginally so 0.5% sucrose, $P = 0.0507$), but they consumed more ($P < 0.05$) 16% and 32% sucrose (surgery × concentration interaction, $F_{6,126} = 17.9$, $P < 0.001$). The percent sucrose intakes of OBx-WT mice were less than those of the Sham-WT mice at 0.5–2% concentrations (surgery × concentration interaction, $F_{6,126} = 9.87$, $P < 0.001$). Within-group analyses indicated that Sham-WT mice increased sucrose intake from Test 1 to Test 2 ($F_{1,10} = 126.2$, $P < 0.001$) with the increase being significant at 0.5–8% concentrations (test × concentration interaction, $F_{6,60} = 29.6$, $P < 0.001$). Their preference increased at 0.5–2% concentrations (test × concentration interaction, $F_{6,60} = 18.6$, $P < 0.001$). The OBx-WT mice only increased their intakes of 16% and 32% sucrose from Test 1 to 2 (test × concentration interaction, $F_{6,66} = 3.5$, $P = 0.005$). On the other hand, their percent sucrose

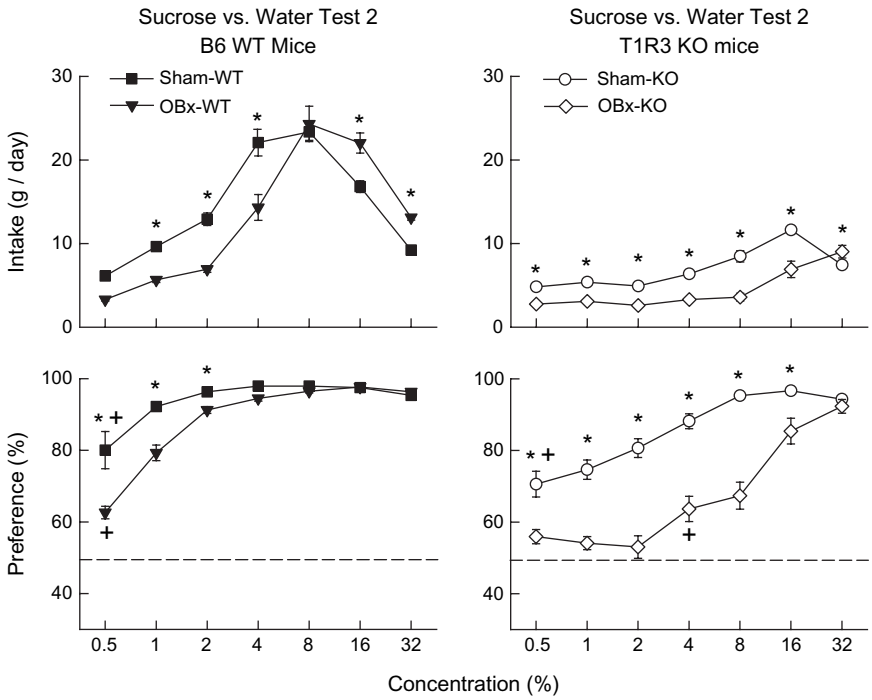

Figure 3 Sucrose solution intake (±standard error of the mean) (top panels) and percent sucrose preference (bottom panels) of Sham-WT and OBx-WT mice (left panels) and Sham-KO and OBx-KO mice (right panels) during sucrose versus water 2-bottle Test 2. Water intakes are not shown. Significant ($P < 0.05$) within-genotype differences at individual concentrations are indicated by an asterisk. The lowest concentration at which sucrose was significantly preferred to water is indicated by a plus sign.

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intakes increased at 0.5–2% concentrations (test × concentration interaction, $F_{6,66} = 10.1$, $P = 0.001$).

The OBx-KO mice consumed less sucrose than Sham-KO mice at 0.5–16% concentrations, but they consumed more sucrose at the 32% concentration (surgery × concentration interaction, $F_{6,126} = 16.5$, $P < 0.001$). The percent sucrose intakes of the OBx-KO mice were less than those of the Sham-KO mice at 0.5–16% concentrations (surgery × concentration interaction, $F_{6,126} = 8.7$, $P < 0.001$). The Sham-KO mice increased their absolute and percent intakes of 0.5–16% sucrose from Test 1 to 2 (test × concentration interaction, $F_{6,60} = 13.8$ and 20.5, respectively, $P < 0.001$). The OBx-KO mice, in contrast, increased their sucrose intake from Test 1 to 2 only at 32% concentration (test × concentration interaction, $F_{6,60} = 2.78$, $P < 0.018$) and their percent intake only at 4% and 8% concentrations (test × concentration interaction, $F_{6,66} = 5.49$, $P < 0.001$).

Test 3: 0.5% sucrose versus water

Because the 2 OBx groups consumed more 32% sucrose than the 2 Sham groups at the end of Test 2, the mice were further tested with 0.5% sucrose to determine if their experience with the concentrated sugar altered their preference for dilute sucrose. Also, the effect of sugar purity on preference was evaluated by comparing food-grade and reagent-grade sucrose in Test 3. As illustrated in Figure 4, overall, the OBx groups consumed less 0.5% sucrose than did the Sham groups ($F_{1,42} = 7.03$, $P < 0.05$), and the KO mice consumed less than the WT mice ($F_{1,42} = 75.82$, $P < 0.001$). Overall, the type of sucrose used did not alter intake although there was a 3-way interaction of genotype × surgery × test ($F_{1,42} = 7.35$, $P < 0.01$). This was due to the Sham-KO mice reducing their sucrose intake when tested with reagent-grade sugar compared with the Sham-WT mice. With respect to sucrose versus water intake, all groups except the OBx-KO consumed significantly ($P < 0.001$) more 0.5% sucrose than water. With both types of sugar, the percent intakes were lower in OBx mice than Sham mice ($F_{1,42} = 16.94$, $P < 0.001$) and in KO mice than in WT mice ($F_{1,42} = 36.36$, $P < 0.001$). The preference for reagent-grade sugar was marginally less than that for food-grade sugar ($F_{1,42} = 4.00$, $P < 0.06$).

Test 4: conditioned odor avoidance

During odor avoidance training, overall, the mice consumed less of the CS+/LiCl solution than of the CS–/NaCl solution ($F_{1,42} = 725.2$, $P < 0.001$) and the KO mice consumed less than the WT mice ($F_{1,42} = 20.7$, $P < 0.001$) (Figure 5). In addition, there was a solution × surgery interaction, and the OBx mice consumed more LiCl and less NaCl than did the Sham mice ($F_{1,42} = 73.6$, $P < 0.001$). In the 2-bottle choice test with both odors presented in NaCl solutions, the 2 Sham groups strongly avoided the CS+/NaCl solution whereas the 2 OBx groups consumed comparable amounts of the CS+/NaCl and CS–/NaCl solutions (surgery × CS interaction,

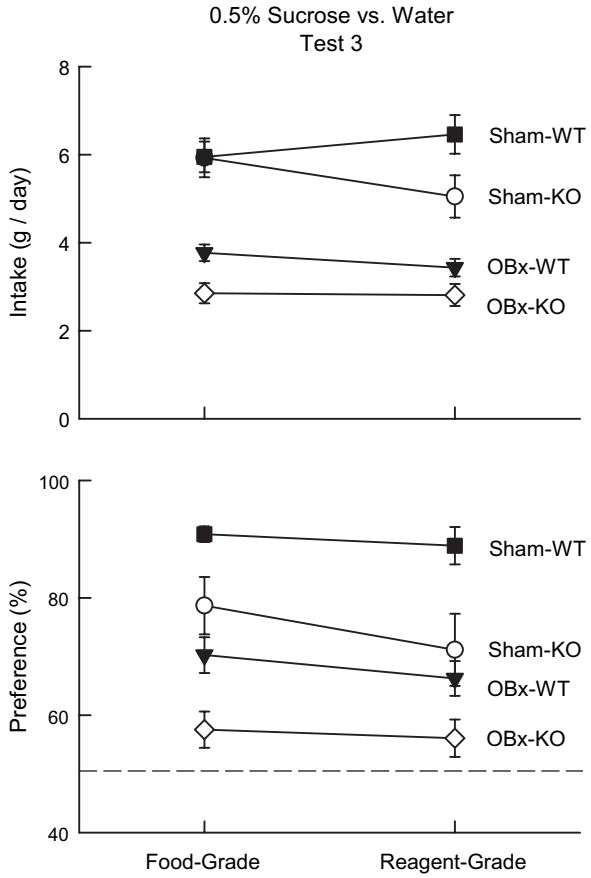

Figure 4 Sucrose solution intake (±standard error of the mean) (top panel) and percent sucrose preference (bottom panel) of OBx-KO, OBx-WT, Sham-KO, and Sham-WT mice during 24-h 0.5% sucrose versus water 2-bottle Test 3 using food-grade and reagent-grade sugar. Water intakes are not shown.

$F_{1,42} = 160.5$, $P < 0.001$). As noted above, this was not true for one OBx-KO mouse that strongly avoided the CS+/NaCl solution. Thus, except for this mouse, which was eliminated from the study, the remaining 24 OBx animals were anosmic as measured by the odor avoidance test. Overall, the KO mice consumed less solution than did the WT mice during the 2-bottle test ($F_{1,42} = 7.08$, $P < 0.05$).

The aspiration boundaries of the 12 OBx-KO and 12 OBx-WT mice are summarized in Figure 6. All OBx mice sustained total or near-total bilateral removal of the olfactory bulbs. Some damage to the rostral anterior olfactory nucleus (AON) was observed in 10 OBx-KO and 6 OBx-WT mice. Five OBx-KO and 5 OBx-WT mice had some marginal damage to the frontal cortex. There were no statistical differences in the sucrose preferences of the OBx-KO or OBx-WT mice with and without frontal cortex damage or between OBx-WT mice with and without AON damage.

Discussion

This study investigated the involvement of the olfactory system in the experience-conditioned sucrose preference

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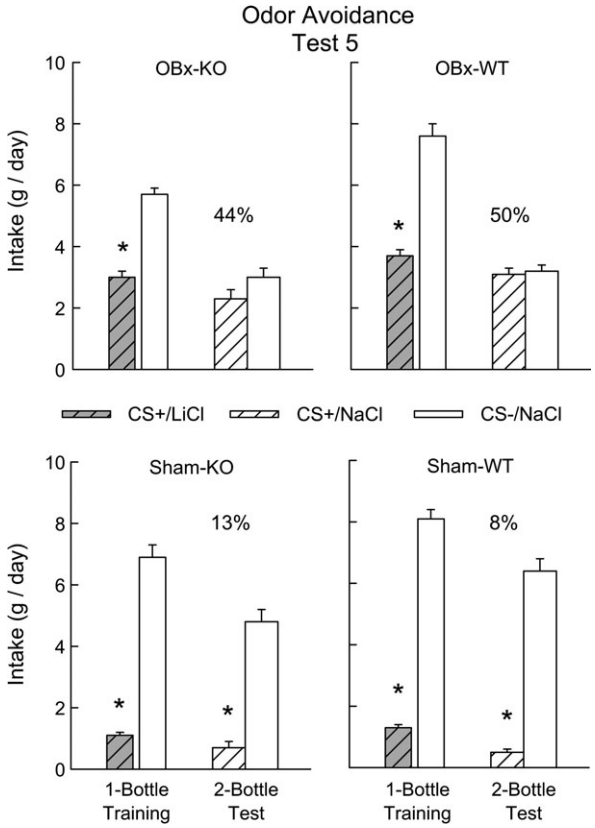

Figure 5 Odor avoidance Test 5. CS+/LiCl and CS−/NaCl solution intakes (+standard error of the mean) during 1-bottle training and CS+/NaCl and CS−/NaCl intake during 2-bottle test of OBx-KO, OBx-WT, Sham-KO, and Sham-WT mice. CS+ refers to the odor added to LiCl during training but added to NaCl during testing. CS- refers to the odor added to NaCl during training and testing. Significant ($P < 0.05$) differences between CS+ versus CS− odorized solutions are indicated by an asterisk. Numbers atop bars in 2-bottle tests are the percent CS+/NaCl.

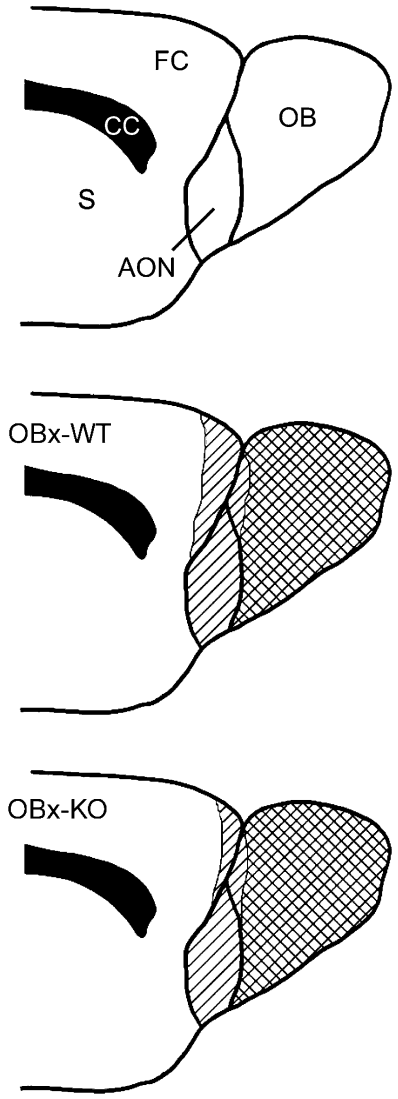

Figure 6 Schematic representation of the extent of the smallest (cross-hatched) and largest (hatched) OBx lesions in the OBx-KO and OBx-WT groups. Anatomical landmarks of the intact brain are indicated in the top diagram: AON; cc, corpus callosum; FC, frontal cortex; OB, olfactory bulb; S, striatum.

displayed by sweet taste–impaired T1R3 KO mice. The findings revealed that loss of olfaction substantially reduced the conditioned sucrose preference in KO mice, whereas it had much less impact on sucrose preference in WT mice. These data are consistent with the hypothesis that experienced KO mice display preferences for dilute sucrose solutions based, in part, on a learned association between the odor and postoral nutritive effects of sucrose (Zukerman et al. 2009).

Confirming our recent report and consistent with earlier findings (Damak et al. 2003; Zhao et al. 2003; Zukerman et al. 2009), naive T1R3 KO mice were indifferent to 0.5–8% sucrose solutions but developed significant preferences for 16–32% sucrose during 24-h tests with ascending sugar concentrations. When retested with the same solutions, the Sham-KO mice preferred all sucrose solutions although their preferences for 0.5–4% sucrose remained weaker than those of the WT mice, and they consumed less at 1–16% sucrose than did WT mice. The Test 2 results obtained with the Sham-KO and Sham-WT mice paralleled those obtained in our earlier study (Zukerman et al. 2009) that is noteworthy because the mice in the present experiment were subjected to sham surgery and a longer interval between Tests 1 and 2 (10 vs. 4 days). Because T1R3 KO mice display no or minimal licking responses to sucrose in 5-s 1-bottle or 60-s 2-bottle tests, their preference in 24-h tests is attributed to a learned response reinforced by the postoral nutritive actions of the sugar (Zhao et al. 2003; Treesukosol et al. 2009; Zukerman et al. 2009). Direct support for this inference is provided by the observation that T1R3 KO mice, like WT mice, learn to prefer an arbitrary flavored solution (e.g., grape) that is paired with intragastric infusions of 16% sucrose over another flavored solution (e.g., cherry) that is paired with IG water infusions during 24-h training sessions (Sclafani and Glendinning 2005; Sclafani et al. 2008).

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Note that although T1R3 KO mice show little or no attraction to concentrated sucrose solutions in brief access tests (Zhao et al. 2003; Treesukosol et al. 2009; Zukerman et al. 2009), it is conceivable that over a 24-h period sugar solutions provide sufficient oral reward to produce a preference independent of postoral effects. However, we observed that naive T1R3 KO mice ($n = 7$) given 32% sucrose versus water for 2 days showed no sugar preference during the first day (57 ± 10.7%) but a strong preference the second day (95 ± 1.4%; Zukerman S. and Sclafani A., unpublished data). This delayed preference suggests that KO mice are not inherently attracted to the orosensory sensations provided by the sucrose solution but rather acquire a preference for these sensations based on a learned association with the postoral actions of the sugar. In Test 1 of the present experiment, the KO mice displayed strong preferences (96 ± 0.9%) for 32% sucrose on the first and second days of the 2-bottle test, which can be attributed to their prior experience with the ascending sugar concentrations.

The OBx findings indicate that odor cues contribute to the learned preference T1R3 KO mice acquire for orally consumed sucrose solutions. Unlike the Sham-KO mice that preferred all sucrose solutions in Test 2, the OBx-KO mice were indifferent to 0.5–2% solutions and displayed weaker preferences for 4–16% sucrose solutions compared with both Sham-KO and OBx-WT type mice. Only at the 32% sugar concentrations, did the OBx-KO mice display a strong preference that was comparable to the other groups. In the absence of olfaction, the sucrose preferences displayed by the T1R3 KO mice were presumably mediated by T1R3-independent taste and/or texture cues. Studies with T1R2 + T1R3 double-KO mice with and without OBx would be informative on the role of the T1R2 receptor subunit on the residual sugar preference displayed by anosmic KO mice. Solution texture effects could be explored by testing anosmic KO mice with sucrose solutions versus gum solutions matched in viscosity.

Although sugar-naive T1R3 KO mice fail to show appetitive responses to dilute sucrose solutions, their sucrose detection threshold is reported to be essentially the same as WT mice when measured in a shock avoidance licking task (Delay et al. 2006). This residual capacity of T1R3 KO mice to detect dilute sucrose solutions is consistent with the preference response displayed by the sugar-experienced KO mice with an intact olfactory system in the present and prior studies (Zukerman et al. 2009). As discussed by Delay et al. (2006), the sucrose discriminative ability of T1R3 KO mice may be mediated by the remaining T1R2 receptor subunit or by non-T1R taste receptors that respond to sucrose. The present findings also suggest the possibility that olfactory cues contribute to sucrose detection by T1R3 KO mice. It would be of interest, therefore, to compare sucrose detection thresholds in Sham-KO and OBx-KO mice.

Compared with the OBx-KO mice, the OBx-WT mice displayed much smaller deficits in sucrose preference. The OBx-WT mice preferred all sucrose concentrations to water although their percent sucrose intakes were reduced, relative to the Sham-WT mice, at 0.5–2% concentrations and their absolute sugar intakes at 0.5–4% concentrations. There are, to our knowledge, no prior studies of sugar intake in bulbectomized mice. Zinc sulfate–induced anosmia, however, was reported to block the preference for 17% (0.5 M) sucrose in mice of the Slc:ICR strain (Uebayashi et al. 2001). This is surprising given the strong preference displayed by the OBx-WT mice for 16% sucrose in the present study. Differences in strain, prior sucrose experience, and method of inducing anosmia presumably account for the discrepant results. In rats, Ramirez (1993) reported that OBx reduced but did not abolish the preference for 0.5–1% sucrose as well as for other carbohydrates (Polycose, corn starch). Reduced intakes of dilute (0.8–1%) sucrose solutions have been reported in some but not all OBx rat studies (Stock et al. 2000; Primeaux et al. 2003; Chambliss et al. 2004; Slattery et al. 2007). In apparently the only rat study that investigated a range of sucrose concentrations (1–30%), OBx rats consumed less sucrose than controls at all concentrations (particularly 5% and 10%), but the differences were not significant perhaps because of small group sizes ($n = 5$) (Vance 1967).

Although the OBx-WT mice drank less of the dilute sucrose solutions than did Sham-WT mice, they overconsumed the 16% and 32% sucrose solutions in Test 2. The OBx-KO mice also consumed more 32% sucrose than did the Sham-KO mice. Yet, when retested with 0.5% sucrose in Test 3, the OBx mice again underconsumed the dilute solution compared with Sham mice (and the OBx-KO mice failed to prefer it to water), indicating that they had not recovered from their bulbectomy-induced deficit with respect to dilute sugar solutions. The failure of the OBx mice to acquire an LiCl-conditioned odor avoidance at the end of the study clearly documents their persistent anosmia. Why the OBx mice overconsumed the concentrated sucrose solutions is not clear. It may be that in the first test series the mice associated the odor of the concentrated sugar solutions with the sugar's postoral satiating effects. The OBx mice may have therefore overconsumed the concentrated sugar solutions in Test 2 because they failed to detect the "satiating" conditioned odor cue. This interpretation is suggested by an early report that removing an odor component of a familiar maintenance diet produced a transient overeating response in rats (Le Magnen 1956). In the present study, the OBx mice overconsumed 32% sucrose on both test days, and it is not known how long this overdrinking response would have continued. OBx has been found to produce long-lasting increases in food intake in some rat obesity models (Larue and Le Magnen 1970; Primeaux et al. 2007); this may be of relevance to the B6 mouse strain used in the present study that is prone to diet-induced obesity (Collins et al. 2004).

The finding that bulbectomy reduced the intake of dilute sucrose solutions in WT mice and even more so in T1R3 KO mice supports the idea that sucrose solutions have

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a detectable odor to mice as well as to rats. Different lines of evidence support the existence of a sucrose odor. Rats trained to drink sucrose solutions from a recessed licking spout displayed decreased latencies to their first lick as sugar concentration increased (Rhinehart-Doty et al. 1994). Rats trained to lever press for sucrose solutions increased their rate of responding during extinction sessions when they could smell the sucrose solution in the test chamber (Oakley 1965). Rats trained to avoid a sucrose solution by pairing its intake with LiCl displayed a more persistent avoidance when sucrose odor cues were available (Van Buskirk 1981). Electophysiological data also suggest that some sucrose-best neurons in the nucleus of the solitary tract increased their firing rate to lingual stimulation when the animal's nose was exposed to the odor of a saturated sucrose solution (Van Buskirk and Erickson 1977). Human subjects are reported to rate the taste of a sucrose solution as less intense when olfactory cues are reduced by a nose clip (Mojet et al. 2005). Because sugar is not volatile, the exact source of sucrose solution odor is not certain, and impurities in the solution and/or oxidation products have been mentioned as possibilities (Van Buskirk 1981; Ramirez 1993). In Test 3 of the present study, the 3 groups of mice showing preferences for 0.5% sucrose solution responded to reagent-grade as well as food-grade sugar, although Sham-KO mice showed a somewhat reduced response to reagent-grade sucrose. Lick latency responses in rats were found to be similar to food-grade and reagent-grade sucrose (Rhinehart-Doty et al. 1994).

It has long been known that OBx produces complex behavioral and physiological changes that extend beyond anosmia (Alberts 1974; Brunjes 1992). Relevant to the present discussion are reports that bulbectomy in rats produces symptoms of major depression including anhedonia, one index of which is reduced sucrose intake (Stock et al. 2000; Primeaux et al. 2003; Chambliss et al. 2004; Song and Leonard 2005). However, the reliability of reduced sucrose intake as a measure of anhedonia has been questioned (Slattery et al. 2007) as has the reliability of the OBx mouse as a model of depression (Cryan and Mombereau 2004). Furthermore, in view of the evidence that rodents can smell sucrose, the possibility that reduced sucrose intake following bulbectomy may be due to anosmia rather than, or in addition to, anhedonia needs to be considered. Nevertheless, it is possible that bulbectomy-induced motivational changes contributed to the reduced intake of dilute sucrose solutions observed in the present study. One approach to dissociate the behavioral effects of anosmia per se and central olfactory damage involves comparisons between zinc sulfate treatment and OBx (Alberts 1974; Katkov et al. 1994; Calcagnetti et al. 1996;). This approach has not been used in the study of sucrose intake, although the intake of another appetitive stimulus in rats (1% corn starch) is suppressed by both zinc sulfate and bulbectomy treatments (Ramirez 1993). In view of the report that zinc sulfate blocked the preference for 0.5 M sucrose in mice (Uebayashi et al. 2001), the effects of this treatment, which has its own limitations (Alberts 1974), must be interpreted with caution.

In conclusion, mice missing the T1R3 sweet taste receptor subunit, which show little or no gustatory neural or brief licking responses to sucrose (Damak et al. 2003; Zhao et al. 2003; Lemon and Margolskee 2009; Treesukosol et al. 2009; Zukerman et al. 2009), nevertheless develop strong sucrose preferences in 24-h 2-bottle tests. This appears to be a conditioned response to the T1R3-independent orosensory properties of the sugar reinforced by postoral nutritive effects. Removal of the olfactory bulbs substantially reduced the conditioned sucrose preference indicating that the mice used odor cues to discriminate dilute sucrose solutions from water. Bulbectomy-induced motivational changes may also contribute to the reduced sugar intake of OBx mice. Sweet taste and olfactory impaired OBx-KO mice strongly (>90%) preferred and overconsumed concentrated (32%) sucrose suggesting the potency of the postoral actions of sugar to promote ingestion even in the absence of normal orosensory experience (Sclafani et al. 2007; de Araujo et al. 2008; Zukerman et al. 2009). Finally, the present findings along with prior work indicating that rodents can smell solutions containing sugars and other tastants (NaCl, quinine, saccharin) (Benjamin 1960; Oakley 1965; Miller and Erickson 1966; Van Buskirk and Erickson 1977; Van Buskirk 1981; Ramirez 1993; Rhinehart-Doty et al. 1994; Capaldi et al. 2004) should be considered in studies of the gustatory abilities of animals and taste KO mice in particular.

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