Enhanced initial and sustained intake of sucrose solution in mice with an oxytocin gene deletion

Janet A. Amico,1,2 Regis R. Vollmer,2 Hou-ming Cai,2 Julie A. Miedlar,2 and Linda Rinaman3

Departments of 1Medicine, 2Pharmaceutical Sciences, and 3Neuroscience, University of Pittsburgh, Pittsburgh, Pennsylvania

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Amico, Janet A., Regis R. Vollmer, Hou-ming Cai, Julie A. Miedlar, and Linda Rinaman. Enhanced initial and sustained intake of sucrose solution in mice with an oxytocin gene deletion. Am J Physiol Regul Integr Comp Physiol 289: R1798–R1806, 2005. First published September 8, 2005; doi:10.1152/ajpregu.00558.2005.—Laboratory mice drink little sucrose solution on initial exposure, but later develop a strong preference for sucrose over water that plateaus after a few days. Both the initial neophobia and later plateau of sucrose intake may involve central oxytocin (OT) signaling pathways. If so, then mice that lack the gene for OT [OT knockout (KO)] should exhibit enhanced initial and sustained sucrose intake compared with wild-type (WT) cohorts. To test this hypothesis, female OT KO and WT mice (11–13 mo old) were given a two-bottle choice between 10% sucrose and water available ad libitum for 4 days. On the first day, sucrose intake was 20-fold greater in OT KO mice compared with WT cohorts. The avid sucrose consumption by OT KO mice increased further on day 2 and was sustained at significantly higher levels than intake by WT mice. Enhanced initial and sustained sucrose intake also was observed in 5- to 7-mo-old male OT KO mice. The absence of OT in mice is associated with enhanced initial and sustained sucrose intake compared with WT mice (33), as in rats (14, 16, 17, 32), although both rodent species have a neophobic response to sucrose solution upon initial exposure. We hypothesized that, compared with WT mice, OT KO mice with a congenital absence of OT should release less neophobia to sucrose solution on initial exposure, and 2) higher levels of sustained intake of sucrose solution during continued exposure.

MATERIALS AND METHODS

Animals. Female and male mice unable to synthesize OT (OT KO) and WT mice, each of C57BL/6 background strain, were bred and housed in a virally free animal facility at the University of Pittsburgh. Founder breeding mice were purchased from Jackson Laboratories from a line originally generated by Dr. Scott Young (46). Female OT KO mice from the F8 generation and age-matched WT controls were studied at 11–13 mo of age (F8 females). Female and male OT KO mice from the F9 generation and age-matched WT controls were studied at 5–7 mo of age (F9 males and females). Before experiments, mice were group housed (4 per cage, single sex) in a temperature- and light-controlled room (12 h dark; 12 h light, on at 0700). Mice were housed individually in the same environment beginning 72 h before and during experiments. Mouse chow (Purina Prolab RMH 3000: 1% sucrose, 0.1% glucose, 0.2% fructose, 0% lactose by weight) and water were provided ad libitum. Experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh and were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals. Mice were tested as paired cohorts, according to genotype and treatment.

RESULTS FROM STUDIES USING RATS AND MICE TO SUPPORT THE VIEW THAT OXYTOCIN (OT) ACTS CENTRALLY TO INHIBIT INTAKE OF FOOD AND OTHER SOLUTES (I.E., SODIUM CHLORIDE, NaCl SOLUTION) UNDER CERTAIN EXPERIMENTAL CONDITIONS. FOR EXAMPLE, FASTING-INDUCED FOOD INTAKE IS SUPPRESSED AFTER CENTRAL INFUSION OF SYNTHETIC OT IN RATS (3, 27), AND OT RECEPTOR BLOCKADE REDUCES THE ANOREXIC RESPONSE TO SYSTEMICALLY ADMINISTERED HYPERTONIC SALINE OR TO CENTRALLY INFUSED CORTICOTROPIN-RELEASING FACTOR (26, 28, 39–43). SIMILARLY, DEHYDRATION-INDUCED ANOREXIA IS MARKEDLY ATTENUATED IN MICE WITH AN OT GENE DELETION [OT KNOCKOUT (KO)] (36), AND OT KO MICE CONSUME MORE NaCl SOLUTION THAN WT-MICE AFTERT AVOIDING FLUID DEPRIVATION (2) OR AFTER EXPOSURE TO MILD ENVIRONMENTAL STRESS (34). HOWEVER, WT AND OT KO MICE INGEST SIMILAR AMOUNTS OF STANDARD CHOW UNDER AD LIBITUM BASELINE CONDITIONS, AFTER OVERNIGHT FOOD DEPRIVATION WHEN DRINKING WATER IS AVAILABLE, AND AFTER SYSTEMIC ADMINISTRATION OF EITHER CHOLECYSTOKININ OCTAPEPTIDE OR D-FENFLURAMINE (18, 36). THESE FINDINGS INDICATE THAT NEUROCHEMICAL SYSTEMS OTHER THAN OT ARE SUFFICIENT TO LIMIT OR SUPPRESS FOOD INTAKE IN MICE UNDER SUCH CONDITIONS.

OT NEURONS ARE ACTIVATED BY A VARIETY OF STRESSFUL AND ANXIENDIC STIMULI IN RATS (9, 13, 21, 22, 24, 44, 45) AND MICE (1, 20, 25). CENTRAL OT PATHWAYS MAY CONTRIBUTE TO A LARGER SET OF NEURAL SYSTEMS THAT SERVE TO LIMIT FOOD AND SUCROSE INTAKE DURING STRESS, ANXIETY, AND MALAISE, INCLUDING SITUATIONS IN WHICH EXCESSIVE INTAKE BEGINS TO CHALLENGE PHYSIOLOGICAL HOMEOSTASIS. THEREFORE, CENTRAL OT SYSTEMS MIGHT PROVIDE AN INHIBITORY CONTROL THAT LIMITS FOOD AND SUCROSE INTAKE UNDER CERTAIN CONDITIONS, E.G., DURING ANXIENDIC SITUATIONS, OR IN SITUATIONS IN WHICH EXCESSIVE INTAKE OF A HIGHLY PALATABLE FOOD BEGINS TO PRODUCE MALAISE. IF SO, THEN AN ABSENCE OF OT SHOULD RELENT THE INHIBITION AT LEAST PARCEL, AND THEREBY PROMOTE EXCESSIVE INTAKE UNDER SUCH CONDITIONS. THE PRESENT STUDY WAS DESIGNED TO TEST THIS HYPOTHESIS BY COMPARING INITIAL AND SUSTAINED AD LIBITUM INTAKE OF PALATABLE SUCROSE SOLUTION AND SUCROSE-ENRICHED FOOD IN WT AND OT KO MICE. PREVIOUS STUDIES HAVE REPORTED THAT PALATABILITY PROMOTES PREFERENTIAL INGESTION OF SUCROSE SOLUTION OVER WATER IN WT MICE (33), AS IN RATS (14, 16, 17, 32), ALTHOUGH BOTH RODENT SPECIES HAVE A NEOPHOBIC RESPONSE TO SUCROSE SOLUTION UPON INITIAL EXPOSURE. WE HYPOTHEZIZED THAT, COMPARED WITH WT MICE, OT KO MICE WITH A CONGENITAL ABSENCE OF OT SIGNALING PATHWAYS WOULD DISPLAY 1) LESS NEOPHOBIA TO SUCROSE SOLUTION ON INITIAL EXPOSURE, AND 2) HIGHER LEVELS OF SUSTAINED INTAKE OF SUCROSE SOLUTION DURING CONTINUED EXPOSURE.
Experiment 1. Intake of sucrose solution and water in female mice. Experimentally naive F8 female mice (n = 8 OT KO, n = 8 WT) were given a two-bottle choice between tap water and 10% sucrose solution, both available ad libitum, in drinking pipettes calibrated at 0.25-ml increments. Bottle positions remained constant in this and subsequent experiments. Fresh sucrose solution was prepared each day. The 10% concentration was selected based on a report that C57BL/6J mice prefer sucrose solution in this concentration range (33), and maximal intakes were desired. Cumulative water and sucrose intakes were recorded daily for 4 days. Food was available ad libitum, but food intake was not recorded in this experiment (however, see experiment 3 below). Body weights were recorded at the beginning and end of the 4-day sucrose exposure period.

Experiment 2. Intake of sucrose-enriched chow vs. standard chow in female mice. Results from experiment 1 indicated that female OT KO mice consume significantly more sucrose solution compared with WT cohorts (see RESULTS). Experiment 2 sought to determine whether female OT KO mice also consume more sucrose-enriched solid food compared with WT controls. One week after the end of experiment 1, the same F8 female mice (n = 8 OT KO, n = 8 WT) were acclimated to ad libitum access to standard mouse chow presented in a powdered form. Methods for presenting powdered chow and accurately recording intake in mice have been reported (18). Fresh food was provided daily, and cumulative 24-h intake was recorded. Tap water was available ad libitum with daily water intake recorded. Stable daily food intakes were achieved within 3 days. On the following day (experimental day 1), mice were given a two-dish choice between standard powdered chow and standard powdered chow mixed with powdered sucrose to achieve a mixture comprising 30% sucrose by weight. Intake from each dish was recorded daily for 4 days; feeding dish positions remained constant during the experiment. The 30% sucrose composition of the powdered diet was selected based on results from experiment 1, in which female OT−/− mice consumed ~1.5 g of sucrose in the form of 10% solution each day (see RESULTS). Adult OT KO and WT mice in our colony typically eat ~5 g of powdered chow each day, thus the 1.5 of every 5 g of powdered chow (i.e., 30%) were replaced with powdered sucrose, so that the expected daily sucrose intake (in grams) would be generally similar.

Experiment 3. Food and fluid intake in female mice during reexposure to 10% sucrose solution. This experiment sought to determine initial and sustained daily intake of 10% sucrose solution in “sucrose-experienced” OT KO and WT female mice (i.e., the same F8 mice used in experiments 1 and 2, n = 8 per genotype) and to determine whether OT KO and WT mice differentially alter their daily intake of standard powdered chow to compensate for the excess calories provided by sucrose solution. The experiment began 2 wk after the end of experiment 2. Tap water, 10% sucrose solution, and powdered chow were available ad libitum for 4 days, with intakes of each recorded daily. Total caloric intake was calculated as the sum of calories ingested as food or as sucrose solution. The caloric content of Prolab RMH 3000 chow or sucrose is 4.1 kcal/g. Body weights were recorded at the beginning and end of the 4-day experiment.

Experiment 4. Intake of sucrose solution and water in male mice. This experiment sought to determine whether initial and sustained daily intake of 10% sucrose solution in 5- to 7-mo-old male mice is similar to that observed in 11- to 13-mo-old female mice. For this purpose, the design of experiment 1 was repeated using male experimentally naive F9 mice (n = 8 OT KO; n = 8 WT). Body weights were recorded at the beginning and end of the 4-day sucrose exposure period.

Experiment 5. Concentration-dependent effects on intake of sucrose solution in male and female mice. This experiment was performed in F9 male and female mice to determine whether there is any effect of sex or genotype on preference for different concentrations of sucrose solution. Three weeks after completing experiment 4 (i.e., two-bottle choice between 10% sucrose solution and water), the same F9 male mice (n = 8 per genotype) were given a two-bottle choice between water and 5% sucrose solution available ad libitum for 4 days, followed by 1 wk of water access only, followed by a two-bottle choice between water and 2.5% sucrose solution available ad libitum for 4 days. Age-matched female F9 cohorts (n = 8 per genotype) were similarly tested to determine their preference for 10, 5, and 2.5% sucrose solution in consecutive 4-day two-bottle choice tests, with 1 wk of access to water only between each sucrose exposure period. Intakes of water and sucrose solution were recorded daily. Standard pelleted chow was available ad libitum throughout the experiment; food intake was not recorded.

Experiment 6. Time-dependent effects on intake of sucrose solution in female mice. Results from experiments 1, 3, and 4 indicated that OT KO mice consumed significantly more sucrose solution than WT mice during a 4-day exposure (see RESULTS). This experiment sought to determine whether these differences persist during a continuous 8-day exposure period. The design of experiment 1 was repeated, but for 8 days in sucrose-experienced female F8 OT KO and WT mice (i.e., the same mice used in experiments 1, 2, and 3). Body weights were recorded at the beginning and end of the 8-day exposure period.

Statistical analyses. Data are presented as group means ± SE. Daily measures of water, sucrose, and chow intakes were analyzed by one- or two-way repeated-measures ANOVA. When F ratios indicated significant effects of genotype and/or ingesta type on cumulative intake values, Bonferroni t-tests were used for post hoc multiple comparisons between groups. Simple comparisons evaluating the effect of a single independent variable on a single dependent measure were conducted using a two-tailed t-test. Differences were considered significant when P < 0.05.

RESULTS

Experiment 1. Intake of sucrose solution and water in female mice. Experimentally naive OT KO female mice manifested a robust preference for novel 10% sucrose solution over water that was evident on the first day of sucrose exposure, when sucrose solution accounted for 95 ± 1.5% of total fluid intake (Fig. 1). Conversely, experimentally naive WT cohorts did not prefer sucrose solution over water on exposure day 1, when sucrose solution accounted for only 14 ± 7% of total fluid intake (Fig. 1). Day 1 intake of 10% sucrose solution was 20-fold greater in OT KO mice (10.0 ± 1.5 ml) compared with WT cohorts (0.4 ± 0.1 ml). OT KO mice further increased their sucrose intake on day 2 (16.0 ± 1.3 ml, representing 98 ± 0.2% of total fluid intake), and then maintained similar daily sucrose intake volumes and preference values on days 3 and 4 (Fig. 1). Thus the initial high preference for novel sucrose solution manifested by OT KO mice (i.e., ~95%) increased only slightly on subsequent exposure days. Conversely, WT cohorts significantly increased their intake of (and preference for) sucrose solution after the first day of exposure. On day 2, sucrose accounted for 33 ± 11% of total fluid intake by WT mice, 40 ± 16% on day 3, and 50 ± 17% on day 4 (Fig. 1). On each exposure day, cumulative sucrose intake volumes and preference values were significantly greater in OT KO mice than in WT cohorts [F(1,14) = 25.65, P = 0.001; post hoc Bonferroni t-test, P < 0.05 on each day]. Although OT KO mice consumed significantly less water each day compared with WT cohorts [F(1,14) = 41.50, P < 0.001; post hoc Bonferroni t-test, P < 0.05 on each day; Fig. 1], the enhanced sucrose drinking manifested by OT KO mice led to greater total daily fluid intake compared with total fluid intake by WT mice [F(1,14) = 41.50, P = 0.001; post hoc Bonferroni t-test, P < 0.05 on each day; Fig. 1]. There was no effect of genotype on body weights measured at the beginning or end of the 4-day
Pre- and postexposure body weights of OT KO mice were 25.5 \pm 0.5 and 25.0 \pm 0.5 g, and for WT mice were 26.9 \pm 0.9 and 26.8 \pm 0.9 g, respectively.

**Experiment 2. Intake of sucrose-enriched chow vs. standard chow in female mice.** When presented with a continuous two-dish choice between sucrose-enriched powdered chow and standard powdered chow, female OT KO and WT mice consumed similar amounts of each type on days 1 and 2, but consumed significantly more of the sucrose-enriched chow by days 3 and 4 [overall $F(1,13) = 17.9, P < 0.001$; $P < 0.05$ only on days 3 and 4 for each genotype; Fig. 2]. On days 3 and 4, the sucrose-enriched chow represented >75% of the total food ingested by mice of both genotypes. There was no significant effect of genotype on cumulative intake of either sucrose-enriched or standard chow on total food intake across each of the 4 days of the experiment (g/day; Fig. 2) or on daily water intake (4.1 \pm 0.2 ml in OT KO mice vs. 3.8 \pm 0.2 ml in WT mice).

**Experiment 3. Food and fluid intake in female mice during reexposure to 10% sucrose solution.** Two weeks after the end of experiment 2, the same female OT KO and WT mice were reexposed to 10% sucrose solution. As expected, day 1 intake of familiar sucrose solution by OT KO and WT mice was greater than day 1 intake of novel sucrose solution by the same mice in experiment 1 (compare Figs. 1 and 3). As in experiment 1, OT KO mice consumed significantly more sucrose solution [$F(1,14) = 5.45, P = 0.035$] (Fig. 3A) and significantly less water [$F(1,14) = 6.99, P = 0.019$] (Fig. 3B) on each day compared with WT cohorts (Fig. 3). Differences between genotypes in total daily fluid intake approached but did not achieve statistical significance [$F(1,14) = 4.21, P = 0.059$; Fig. 3C].
Female OT KO mice consumed significantly less powdered standard chow compared with WT cohorts \([F(1,14) = 5.94, P = 0.029]\) during concurrent exposure to 10% sucrose solution (Fig. 3D). The differences in cumulative food intake between genotypes were statistically significant on days 2 and 3 \((P < 0.05)\). However, total daily caloric intake derived from both sucrose solution and chow did not differ between genotypes (Fig. 3E). There was no effect of genotype on body weights measured at the beginning or end of the experiment. Pre- and postexposure body weights of OT KO mice were 24.9 ± 0.4 and 25.3 ± 0.6 g, and for WT mice were 25.2 ± 0.6 and 26.2 ± 0.8 g, respectively.

**Experiment 4. Intake of sucrose solution and water in male mice.** Similar to results in F8 female mice, experimentally naive male F9 OT KO mice manifested an avid preference for 10% sucrose solution over water that was evident on day 1, when sucrose solution accounted for 97.8 ± 0.6% of total fluid intake. Conversely, sucrose solution accounted for only 32.3 ± 15.6% of total fluid intake in age-matched male WT cohorts (Fig. 4). Male OT KO mice consumed fourfold greater volumes of 10% sucrose solution on day 1 \((16.5 ± 2.0 \text{ ml})\) compared with sucrose intake by WT mice \((4.4 ± 2.4 \text{ ml}; P < 0.05)\). Both OT KO and WT mice increased their cumulative intake of sucrose solution on day 2 and then maintained similar levels of sucrose intake within each genotype on days 3 and 4 (Fig. 4). In OT KO mice, sucrose solution accounted for 98 ± 1.1% of total volume consumed on day 2, 99.4 ± 0.2% on day 3, and 99 ± 0.1% on day 4. In WT mice, sucrose solution accounted for 61 ± 15.6% of total volume consumed on day 2, 58 ± 17.4% on day 3, and 62 ± 17.3% on day 4. On each day, male OT KO mice consumed significantly greater volumes of 10% sucrose compared with WT cohorts \([F(1,14) = 14.09, P = 0.002;\text{ post hoc Bonferroni }t\text{-test}, P < 0.05\) on each day]. Although OT KO mice consumed significantly less water each day compared with WT cohorts \([F(1,14) = 7.71, P = 0.015;\text{ Fig. 4}]\), the enhanced sucrose intake in OT KO mice led to significantly greater total daily fluid intake (i.e., approximately double) compared with total fluid intake by WT mice \([F(1,14) = 13.94, P = 0.002;\text{ Fig. 4}]\). Body weights within each genotype did not change significantly over the 4-day experiment (pre- and postexposure body weights were 31.3 ± 0.9 and 32 ± 1.1 g in OT KO mice, and 28.3 ± 0.6 and 28.4 ± 0.6 g in WT mice, respectively). The male OT KO mice weighed slightly but significantly more than the age-matched male WT mice in this experiment \((t\text{-test}, P < 0.01)\). However, the small group differences in body weight (i.e., 9.5–11.2%)
were proportionately much smaller than the group differences in sucrose intake, indicating that body weight differences did not account for the effect of genotype on sucrose intake.

**Experiment 5. Concentration-dependent effects on intake of sucrose solution in male and female mice.** Male F9 mice previously exposed to 10% sucrose solution (experiment 4; n = 8 per genotype) were used in additional two-bottle choice tests between water and 5% sucrose solution, and then between water and 2.5% sucrose solution (4 days per test) in experiment 5. Using a similar approach, F9 female mice (n = 8 per genotype) were first tested for 10% sucrose preference, followed by additional two-bottle choice tests between water and 5% sucrose solution and then between water and 2.5% sucrose solution. Figure 5 shows cumulative daily intakes of water and sucrose solution on the final day of each 4-day choice test (left panels = male mice; right panels = female mice) and the actual grams of sucrose consumed. In both sexes, a main effect of genotype on sucrose intake (in ml) was found across all three sucrose concentrations, such that OT KO mice consumed significantly more (i.e., approximately twice as much) sucrose on day 4 of each test compared with WT cohorts [males: F(1,14) = 14.753, P = 0.002; females: F(1,14) = 30.145, P = 0.001]. Water intakes were consistently low during the 4th day of exposure to each sucrose concentration and did not differ between sexes or genotypes. In both sexes, a main effect of genotype on sucrose intake (grains) was found across all three sucrose concentrations, such that OT KO mice consumed significantly more (i.e., approximately twice as much) sucrose solution on the final day of each 4-day choice test [males: F(1,14) = 13.587, P = 0.002; females: F(1,14) = 21.043, P < 0.001]. In both genotypes, the total amount of sucrose ingested (in grams) on day 4 of each test were significantly greater when mice consumed 10 or 5% sucrose solution compared with 2.5% sucrose solution (Bonferroni t-test, P < 0.001).

**Experiment 6. Time-dependent effects on intake of sucrose solution in female mice.** Female F8 mice (n = 8 per genotype) were reexposed to a two-bottle choice between water and 10% sucrose solution for 8 days. Mice of both genotypes consumed significantly more sucrose (i.e., approximately twice as much) sucrose solution on the first day of each test compared with WT cohorts [males: F(1,14) = 13.80, P < 0.01]. On each day, OT KO mice drank significantly more sucrose (i.e., approximately twice as much) compared with intake by WT cohorts [F(1,14) = 11.03, P = 0.005] (Fig. 6). Water intakes were consistently low across the 8 days, with no significant differences between genotypes.

**DISCUSSION**

We report that initial and sustained ad libitum intake of sucrose solution is markedly enhanced in OT KO mice compared with WT cohorts of C57BL/6 background strain. The effect of OT gene deletion to significantly increase sucrose intake was observed in 11- to 13-mo-old female mice and also in 5- to 7-mo-old male and female mice. The effect was immediately evident on the first day of exposure to novel 10% sucrose solution, was maintained during subsequent 4-day repeated exposures, and was maintained over 8 days of continual exposure. The effect also was observed over a range of sucrose concentrations (i.e., 2.5, 5, and 10%). These results support the view that endogenous OT signaling pathways provide inhibitory control over the initial intake of a novel, sweet, and palatable solution in WT mice, and that OT pathways also provide inhibitory control over intake of the same familiar solution in WT mice during sustained ad libitum access. However, the stimuli that presumably recruit central OT signaling pathways in each situation are probably distinct, as discussed further, below.

**Reduced neophobia in OT KO mice.** Male and female WT mice consumed less 10% sucrose than water on their 1st day of
exposure to sucrose solution. A preference for sucrose over water emerged by days 2 and 3 in WT mice and was maintained in subsequent testing periods. These findings are consistent with a previous report using WT mice of the same background strain (33). In striking contrast, male and female OT KO mice avidly consumed significantly more novel sucrose solution than water on the 1st day of exposure.

In the animal behavior literature, neophobia refers to the tendency of an animal to avoid or retreat from an unfamiliar object or situation. Neophobia commonly is observed in rats and mice upon initial presentation of novel ingesta, even those that come to be highly preferred. Neophobia to novel foods and fluids is an adaptive behavioral response that serves to limit initial intake of potentially harmful substances by rats and mice, which cannot vomit, and, therefore, cannot quickly expel ingested substances from the body after they are swallowed. Neophobic responses to novel ingesta and/or environments often are used to index anxiety in rats and mice, in which neophobia is attenuated by anxiolytics and exacerbated by anxiogenic drugs and treatments. Novelty itself is a mild stressor and promotes anxiety in rodent species. The markedly suppressed neophobic response to novel sucrose solution displayed by OT KO mice in this study suggests that endogenous OT signaling pathways contribute to neophobia in WT mice. As novelty decreased during sustained exposure or upon reexposure to sucrose solution in “experienced” WT mice, the now familiar sucrose cues (i.e., presence of the bottle, taste of the solution) presumably lost their anxiogenic effect and thus no longer promoted recruitment of the central OT signaling pathways that restrained initial intake of sucrose solution when it was novel.

It would be premature to conclude that neophobia to sucrose solution is completely absent in OT KO mice, because these mice did consume more 10% sucrose solution on the 2nd day of exposure compared with the 1st. However, increased sucrose intake over the first 2–3 days of exposure in both genotypes also reflects the emergence of a conditioned preference for sucrose solution that is based on its positively reinforcing oral and postabsorptive sensory qualities (38). Thus the relative extent to which loss of neophobia and emergence of conditioned preference each contribute to increased sucrose intake over the first few days of exposure cannot be ascertained from the present results.

Enhanced sustained intake of sucrose solution in OT KO mice. Mature WT and OT KO mice in our colony typically consume ~4–5 ml of water each day (2). When sucrose solution and water were available simultaneously in the present study, OT KO mice drank relatively little water but consumed

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Fig. 5. Intakes of sucrose solution and water (top) in male and female 5-mo-old WT and OT KO mice given a two-bottle choice between water and 2.5, 5.0, or 10% sucrose solution, available ad libitum. There is a significant main effect of genotype on the volume (ml) of sucrose ingested (top) across the full concentration range (males: P = 0.002; females: P = 0.001, ANOVA). *At each sucrose concentration, OT KO mice of both sexes consumed significantly more sucrose solution than WT mice (P < 0.05, Bonferroni t-test). Water intake was negligible and was not different between genotypes. Bottom: total amount of sucrose (grams) consumed at each concentration of sucrose solution. There is a significant main effect of genotype on amount of sucrose ingested (grams) (males: P = 0.002; females: P < 0.001, ANOVA) and significant differences between genotypes at each concentration (*P < 0.05, Bonferroni t-test). There also was a significant interaction between genotype and concentration on the total amount (grams) of sucrose consumed (P = 0.005, ANOVA). Values are means ± SE.

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Fig. 6. Daily fluid intakes of WT and OT KO female mice that were reexposed to a two-bottle choice of water and 10% sucrose solution available ad libitum for 8 days. OT KO mice consumed significantly more sucrose solution than WT mice (P = 0.005, ANOVA). Values are means ± SE. *Significant differences between genotypes on each day of the study (P < 0.05, Bonferroni t-test).
15–25 ml of sucrose solution daily. Conversely, although male and female WT mice also displayed a preference for sucrose solution over water that emerged by the 2nd or 3rd day of exposure, their maximal daily sucrose intakes were at least 50% lower than those in OT KO cohorts matched by age and sex. As an initial test of the hypothesis that excessive intake of 10% sucrose solution by OT KO mice might be due to a shifted sucrose concentration preference, we also exposed male and female WT and OT KO mice to lower concentrations of sucrose solution (i.e., 2.5 and 5%). As with the 10% solution, OT KO mice consumed approximately twice as much 2.5 and 5% sucrose solution as WT cohorts. Thus there was no apparent effect of genotype on preference for these different sucrose concentrations that could account for the excessive intake by OT KO mice. It remains possible that a genotype-related effect on preference curves would be observed at higher concentrations of sucrose solution than those examined here.

Potential mechanisms underlying enhanced, sustained intake of sucrose solution in OT KO mice. Sucrose solution is rewarding in mice and rats, and the neural reward mechanism appears to involve recruitment of central opioid signaling pathways (12, 16, 17, 32). Acute sucrose ingestion induces an immediate release of endogenous opioids, which act to inhibit OT neurons. Thus one reason that voluntary intake of highly palatable, rewarding ingesta often far exceeds intake of standard fare may be due to recruitment of opioid pathways, which suppress OT pathways and thereby remove an inhibitory control over intake. In other words, highly palatable, rewarding ingesta may promote overconsumption by recruiting neural pathways that blunt or override the usual satiety signals and allow intake to proceed to a higher level. Under normal circumstances, cessation of intake of even highly preferred ingesta eventually occurs when negative feedback cues related to intake (i.e., satiety) become sufficient to override the intake-promoting cues. Such negative feedback cues may progressively begin to recruit OT signaling pathways during sustained intake and/or release OT signaling pathways from inhibition by signals that promote intake. The congenital absence of OT pathways in OT KO mice may account for the higher volumes of sucrose solution that could be consumed before cessation of intake finally occurred.

Our findings are consistent with a theory put forth by Olszewski and colleagues (29, 30), in which the endogenous opioid system is proposed to increase or prolong food intake in certain situations by suppressing the activity of central “satiety” circuits, including those that encompass centrally projecting OT neurons. In support of this theory is evidence that pharmacological blockade of opioid signaling pathways potently decreases intake of preferred sweet-tasting ingesta (cf. Refs. 17, 38) and also exacerbates the anorexigenic effects of certain experimental treatments (5, 8, 11, 14, 31). The behavioral effects of opioid receptor blockade may involve a release of OT neurons from inhibition by endogenous opioids. The opioid antagonist naltrexone produces anorectic effects that appear to be specific to intake of preferred ingesta, such as sucrose solution (7, 14, 38).

No effect of OT gene deletion on intake of powdered chow. Despite the significantly enhanced intake of sucrose solution by OT KO mice compared with WT cohorts, there was no effect of genotype on ad libitum intake of either standard or sucrose-enriched powdered chow. When given a choice between the two types of chow, mice of both genotypes came to strongly prefer the sucrose-enriched version by the 3rd day of exposure. However, total food intake did not change significantly during the 4-day experiment in either OT KO or WT mice. Interestingly, when standard powdered chow was offered along with 10% sucrose solution, mice of both genotypes adjusted their chow intake according to their intake of sucrose solution. OT KO mice drank more sucrose solution and correspondingly ate less powdered chow compared with WT mice, such that total daily caloric intake did not differ between genotypes and did not change over the course of the 4-day experiment. These findings suggest that neural signaling pathways other than OT are sufficient to regulate food intake and maintain caloric homeostasis under these experimental conditions. Indeed, body weights before and after each experiment did not differ between genotypes or as a result of diet.

It remains unclear why OT KO mice in these experiments consumed significantly more sucrose solution than WT cohorts, but did not consume more sucrose-enriched chow. Perhaps chow consumption generates additional negative feedback signals that do not require central OT neural pathways to promote satiety. Potential postsabosptive satiating signals derived from chow include protein, fat, carbohydrates, and effective osmoles, whereas sucrose solution provides only carbohydrates and water. Chow consumption also takes more time and effort on the part of the animal, which periodically must stop eating to drink water during the meal. In addition, consumed chow likely remains within the stomach and upper digestive tract for a longer period of time compared with sucrose solution; chow, therefore, would generate higher levels of distension-related negative feedback signals to the brain compared with sucrose solution.

Integration with other findings in OT KO mice. To date, three different laboratories have reported the development of mice with an OT gene deletion. Two of the lines are derived from C57BL/6 background (46) (used in the present study) and (23) and the other from a Swiss Black background (15). Various behavioral abnormalities have been reported among these lines. Of particular relevance to the present results, indicating reduced neophobia in OT KO mice of C57BL/6 background, are findings that OT KO mice of the same background strain display altered behavioral and endocrine responses to stressful and anxiogenic stimuli in several experimental paradigms (1, 19, 20).

Our laboratory previously reported the behavior of mice from our colony during two-bottle choice tests between water and hypertonic (0.5 M) NaCl solution (2). As would be expected, WT mice drink plenty of water but relatively little hypertonic NaCl solution after overnight water deprivation. However, male OT KO mice drank significantly more NaCl solution than WT mice after overnight water deprivation, despite attaining equivalent levels of hyperosmolality and hypovolemia (2). Interestingly, OT KO mice also drink more NaCl solution than WT mice after the mild stress caused by transfer to a novel environment. Thus endogenous OT signaling pathways appear to inhibit or restrain intake of hypertonic NaCl solution in WT mice, as this inhibition or restraint is diminished in OT KO mice. As discussed above, a generally similar phenomenon (i.e., reduced intake inhibition) may account for the excessive intake of sucrose solution observed in
OT KO mice in the present study. The neuroanatomical locus (or loci) at which OT acts to exert its apparent inhibitory control over intake during initial and sustained exposure to sucrose solution remains to be determined. However, prior studies in rats and mice suggest that OT-containing projections from the paraventricular nucleus of the hypothalamus to the hindbrain dorsal vagal complex may be particularly relevant (6, 35–37).

Perspectives. As is the case with other types of highly palatable ingesta, sweetened fluids such as soda or juice frequently are consumed in quantities well beyond those needed to satisfy homeostatic needs (4, 10). In humans, the ability to refrain from excess consumption of highly palatable foods and fluids is influenced both by biological and social factors (10). Ingestive controls presumably are far less complex in animals whose behavior is not shaped or constrained by society, but in which intake, nevertheless, is regulated via neural signaling pathways that presumably are similar to those at work in humans. OT is among many neuropeptides implicated in the central control of ingestive behaviors in experimental animals. Continuing studies examining food and fluid intake in OT KO mice should improve our understanding of how the brain regulates these behaviors and may provide a foundation for future clinical studies to explore the potential manipulation of OT pathways to restrain excessive consumption of highly palatable ingesta.

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REFERENCES


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