Gastric bypass surgery alters behavioral and neural taste functions for sweet taste in obese rats

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Hajnal A, Kovacs P, Ahmed T, Meirelles K, Lynch CJ, Cooney RN. Gastric bypass surgery alters behavioral and neural taste functions for sweet taste in obese rats. Am J Physiol Gastrointest Liver Physiol 299: G967–G979, 2010. First published July 15, 2010; doi:10.1152/ajpgi.00070.2010.—Roux-en-Y gastric bypass surgery (GBS) is the most effective treatment for morbid obesity. GBS is a restrictive malabsorptive procedure, but many patients also report altered taste preferences. This study investigated the effects of GBS or a sham operation (SH) on body weight, glucose tolerance, and behavioral and neuronal taste functions in the obese Otsuka Long-Evans Tokushima Fatty (OLETF) rat. OLETF rats lack CCK-1 receptors and behavioral and neural taste functions in the obese Otsuka Long-Evans Tokushima Fatty (OLETF) rats lacking CCK-1 receptors and lean controls (LETO). OLETF rats lost body weight (−26%) and demonstrated improved glucose tolerance. They also expressed a reduction in 24-h two-bottle preference for sucrose (0.3 and 1.0 M) and decreased 10-s lick responses for sucrose (0.3 through 1.5 M) compared with OLETF-Sh or LETO-GBS. A similar effect was noted for other sweet compounds but not for salty, sour, or bitter tastants. In lean rats, GBS did not alter responses to any stimulus tested. Extracellular recordings from 170 taste-responsive neurons of the pontine parabrachial nucleus revealed a rightward shift in concentration responses to oral sucrose in obese compared with lean rats (OLETF-Sh vs. LETO-Sh): overall increased response magnitudes (above 0.9 M), and maximum responses occurring at higher concentrations (+0.46 M). These effects were reversed by GBS, and neural responses in OLETF-GBS were statistically different from those in any LETO groups. These findings confirm obesity-related alterations in taste functions and demonstrate the ability of GBS to alleviate these impairments. Furthermore, the beneficial effects of GBS appear to be independent of CCK-1 receptor signaling. An understanding of the underlying mechanisms for reduced preferences for sweet taste could help in developing less invasive treatments for obesity.

Overeating; dietary obesity; surgical weight reduction; insulin resistance; palatability; gustatory coding; cholecystokinin-1 receptor

THE SUCCESS OF WEIGHT REDUCTION is a function of the magnitude and longevity of the body weight loss. Weight reduction achieved by dieting or exercise elicits compensatory changes in appetite and energy expenditure (36, 53) that make weight loss of more than 5–10 percent difficult to sustain (3, 52, 63). In contrast, following the commonly performed bariatric procedure, Roux-en-Y gastric bypass surgery (GBS) patients typically lose ~30% of their body weight (50–70% of excess body weight) with a long-term success rate of ~70% (26, 28, 44, 61). Although imperfectly, GBS appears to undermine the normal compensatory physiological responses to energy deficit. This effect is unlikely to result from early satiety due to gastric restriction alone. The success rate of the purely restrictive vertical banded gastroplasty is only 40%, with “failure of weight loss” commonly attributed to increased consumption of sweets (4). Following GBS, patients typically eat less food per meal and fewer snacks per day (25). They voluntarily restrict consumption of calorie-dense, highly palatable foods such as fats, concentrated carbohydrates, ice cream, and sweetened beverages. Many patients also note that they are no longer “hungry all the time” (19, 50). A recent study (6) concluded postoperative (but not preoperative) eating behavior is predictive of weight loss after GBS. Specifically, patients with reduced sweet craving after GBS maintained weight loss greater than 12 mo after surgery. Collectively, these observations suggest GBS not only promotes satiety but reduces appetite driven by orosensory experience (palatability), which is critical for the maintenance of weight loss.

The Otsuka Long-Evans Tokushima Fatty (OLETF) rat has been used by our laboratory to characterize the effects of obesity on taste preference and sucrose reward. The OLETF is an outbred strain of Long-Evans rat that has recently been established as an animal model of obesity and Type 2 diabetes (30). These rats fail to express a functional cholecystokinin (CCK)-1 receptor (16, 60), which mediates CCK’s actions in satiety (43). Spontaneous meal size is almost double in the OLETF rats and they gradually develop Type 2 diabetes during their life span. OLETF rats also exhibit diminished sensitivity to postigestive satiation signals (8, 42) and vagal responses (9), as well as increased avidity to sweet. Compared with lean Long-Evans Tokushima Otsuka (LETO) controls, OLETF rats exhibit increased real and sham intake of normally preferred sucrose solutions with a heightened preference for higher over lower concentrations (1 vs. 0.3 M) (13). The OLETF rat also expresses increased lick responses in brief access tests to sucrose as well as various agents that taste sweet to humans including the noncaloric sweetener saccharin and the amino acid alanine (21). Recently, we found that corresponding with increased behavioral avidity to sweet, the central neural coding for oral sucrose stimulation is also altered in this strain (35). On this basis, and given the similarities with human bariatric patients with respect to behavioral and metabolic profiles, we chose to use this animal model of obesity to test the effects of GBS on sucrose preference and neural taste functions.

The ability to perform GBS in rat models of obesity (10, 40, 41) provides us with a unique opportunity to investigate the effects of GBS on taste functions. First we assessed brief (10-s) lick responses to sucrose and various tastants, followed by a 24-h two-bottle choice preference test for sucrose after GBS.
Next, we performed extracellular single neuron recordings in the pontine parabrachial nucleus (PBN) to investigate whether GBS affects central taste processing. The PBN is the second central gustatory relay (39, 48), which plays an important role in motivational-hedonic integration of taste functions in the rat (23, 46, 47). The present study reinforced previous findings demonstrating increased behavioral avidity and neuronal responses to sucrose in this obese model (34, 35) and revealed the ability of GBS to reverse these deficits. In contrast to the effects of GBS in obese rats, GBS had no effect on taste preferences and neural responses for sucrose in lean rats. Whereas the underlying mechanisms remain unknown and warrant future investigations, it is feasible that similar effects may contribute to the beneficial changes in eating behavior observed in GBS patients.

MATERIALS AND METHODS

General Methods

Animal care. Four-week-old male OLETF and LETO rats were 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 prior to the onset of experimental procedures. Pelleted normal rat chow (Rodent Diet-W 2018, Harlan Teklad, Madison, WI) was available ad libitum throughout experiments except when otherwise stated. Tap water also was available ad libitum except when taste responses to aversive chemicals were tested. All protocols were approved by The Pennsylvania State University College of Medicine Institutional Animal Care and Use Committee and are in accordance with National Institutes of Health Guidelines.

In experiment 1 (behavioral study), 10 OLETF and 10 LETO age-matched (20 wk at the beginning of the experiment) rats were used. Six rats of each strain received GBS and the remaining four were subject to sham operation (SH). The OLETF-SH were pair fed to the OLETF-GBS and the LETO-SH were pair fed to the LETO-GBS rats, i.e., given the same amount of food as the ad libitum-fed GBS rats consumed. The rationale for pair feeding was to control for potential effects of post-GBS reduction in calorie intake on the behavioral expression of sucrose preference.

In experiment 2 (electrophysiological study), a separate set of eight OLETF and eight LETO rats was divided in equal number to receive GBS or SH (n = 4). Electrophysiological recordings started 3–4 wk following the abdominal surgeries after at least 1 wk recovery from the cranial surgeries. In contrast to experiment 1, the control animals in experiment 2 were not pair fed to compare the effect of GBS weight loss on neural responses in the obese vs. the lean rats.

Roux-en-Y GBS. A detailed description of the surgical technique, surgical controls, and perioperative care for GBS has been published elsewhere (41). Briefly, rats were fasted overnight except for water the night before the surgery. Following randomization, rats were weighed, then anesthetized with isoflurane (3% for induction, 1.5% for maintenance). Ceftriaxone 100 mg/kg im (Roche, Nutley, NJ) was given as a prophylactic antibiotic. Under sterile conditions a midline abdominal incision was made. In the GBS groups, the stomach was divided to create a 15-cm biliopancreatic limb and a 10-cm alimentary (Roux) limb, and the remaining bowel (75 cm) formed the common channel. The gastrojejunal and jejunojejunal anastomoses were performed using interrupted 5-0 silk sutures, followed by abdominal closure using 3-0 silk and 5-0 prolene. Surgical incisions were injected with 0.5 ml of 0.25% bupivacaine to minimize postoperative pain. All rats were injected subcutaneously with normal saline (50 ml/kg, prior to the start of surgery, immediately after surgery, and again on postoperative day 1). Animals also received buprenorphine (0.5 mg/kg im) as needed for pain. Animals were housed individually, body weight and food consumption were monitored daily. To allow the surgical anastomoses to heal, animals were not allowed to eat or drink until 24 h after surgery. Approximately 24 h after surgery, animals were started on a liquid diet consisting of BOOST (Nestle Nutrition, Minneapolis, MN) and access to water ad libitum. Regular chow was started on postoperative day 3.

Surgical controls received the same protocols except that intestinal manipulation was performed instead of GBS followed by abdominal closure. We recognize that there are many possible controls for these studies. Our control was chosen to draw conclusions about taste based on our existing animal model (41) and prior to that for several other reasons: 1) The laparotomy control with intestinal manipulation is a frequently used control for other surgical studies (5, 49, 56). 2) Early in our experience we performed an isolated enterotomy in the sham animals with reanastomosis of the ligation and used this as a control. We stopped doing this since it had no effect on any of the parameters we studied compared with bowel manipulation alone. This observation has been repeated and confirmed by several other groups. Of particular relevance, a recent study by Strader et al. (57) has demonstrated that ileal interposition a surgical manipulation that entails three enterotomies in the jejunum and ileum (similar what we could do as a possible control for the gastric bypass) had no effect on food intake or body weight similar to surgical controls where enterotomies were reanastomosed instead of transposing the ileal segment to the jejunum.

Body weight and adiposity. Body weight was measured every morning with gram accuracy throughout the whole study period. However, because of the need to stagger body weight affecting interventions in experiment 2, only daily body weight data from experiment 1 are shown and discussed. For experiment 2 only initial and final body weights are provided (see Table 1).

To demonstrate the presence of obesity in the OLETF rats we have obtained from the Tokushima Research Institute, H-NMR body composition analysis was performed (Bruker LF90 proton-NMR Minispec; Bruker Optics, Woodlands, TX) in a separate set of age-matched littermates (n = 5 each strain).

OGTT. Oral glucose tolerance tests (OGTT) were performed on postoperative weeks 2 and 7. Before the tests, the rats were fasted overnight (minimum 16 h). Glucose 2 g/kg (500 g/l) was administered by gastric gavage, and blood was taken from the tail vein 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) (30).

Experiment 1: Behavioral Tests

Taste assessment using brief-access tests in the gustometer. Rats were tested individually by using a multibottle gustometer (“Davis Rig” from DiLog Instruments, Tallahassee, FL) as previously described (55). The testing took place during the light phase starting in the morning (~0800) on the third postoperative week and 3 days after the OGTTs. The standard protocol of training and brief-access tests used in our laboratory is described elsewhere (21). 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 ones. To maintain proper hydration during testing aversive taste stimuli, all rats received an additional 120-min access period to water in the home cage following each daily session (1500–1700). Each rat was tested twice for a tantant, in a counterbalanced fashion (i.e., the same stimulus was never presented.
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on 2 consecutive days), but only one stimulus a day was given. In a daily session, five to six concentrations of the same tastant with water were available for 10-s access periods in a randomized order over 20 min. During this period, all concentrations were presented in equal number. 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.

Following a 5-day water training, in the first phase, the rats received water and various concentrations of normally aversive solutions, but only one chemical tested a day: citric acid (1 $\times$ 10$^{-3}$, 3 $\times$ 10$^{-3}$, 6 $\times$ 10$^{-3}$, 0.01, 0.03, 0.1 M), quinine-HCl (1 $\times$ 10$^{-5}$, 3 $\times$ 10$^{-5}$, 1 $\times$ 10$^{-4}$, 3 $\times$ 10$^{-4}$, 1 $\times$ 10$^{-3}$, 3 $\times$ 10$^{-3}$ M), and the nontaste (i.e., trigeminal) stimulus capsaicin (1 $\times$ 10$^{-5}$, 3 $\times$ 10$^{-5}$, 1 $\times$ 10$^{-4}$, 3 $\times$ 10$^{-4}$, 1 $\times$ 10$^{-3}$ M). For testing responsiveness to sweetness and saltiness, rats were first given ad libitum water (for the rest of the experiment) and the following taste stimuli were used: sugars sucrose (disaccharide, 0.01, 0.03, 0.10, 0.3, 1.0, 1.5 M) and fructose (monosaccharide, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 M); nonsugar sweet and umami stimuli sodium saccharin (0.05, 0.1, 0.2, 0.4, 0.8, 1.6% or $\approx$ 2.3, 4.5, 9, 18, 36, 45 mM) and alanine (0.01, 0.03, 0.06, 0.1, 0.3, 1.0 M); and other palatable stimuli NaCl (0.01, 0.03, 0.06, 0.1, 0.3, 1.0 M) and monosodium L-glutamate (MSG: 0.01, 0.03, 0.06, 0.1, 0.3, 1.0 M).

Two-bottle sucrose preference tests. Following the lick-rate tests (on postoperative week 6), preferences for two concentrations of sucrose over water were tested separately in counterbalanced order with 1 day off between the tests (i.e., the rats receiving only water). Two bottles, one filled with 0.3 or 1.0 M sucrose solution and another with water, were used. Placement of the bottles with sucrose and water was randomized across the tests to avoid place preferences. Sucrose and water consumption was measured for the period of 24 h by weighing preweighed bottles at the end of the test. Preference was expressed as ratio of sucrose intake over total fluid intake (i.e., water and sucrose). The sucrose preference test was carried out with food available all the time.

Chemicals used as oral stimuli. All chemicals used in the behavioral experiments 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. Chemicals’ purity was at least 95%. Chemicals were purchased from standard vendors, except for MSG, which was obtained from a local grocery store. Specifically, sucrose, fructose, Na-saccharin (1.2-benzisothiazol-3-2H-thione,1,1-dioxide, sodium salt), citric acid, NaCl, and alanine all were from Fisher Scientific (Fair Lawn, NJ) whereas quinine-HCl and capsaicin were from Sigma (St. Louis, MO).

Statistical analysis. The 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 to calculate the difference score between licks made for a given concentration of chemical and those made for water: lick difference score (chemical $x$) = licks (chemical $x$) − licks (water). The rationale for this was to control for differential water licks between strains and surgical conditions. Repeated-measure analysis of variance (RM-ANOVA; main factors: surgical treatments, strain) with repeated measures (on concentrations) was conducted on the lick difference score for each tastant. Post hoc tests were conducted, when appropriate, by Fisher’s least significant difference tests. Data from OGTt tests were analyzed by two-way ANOVA (strain and surgical conditions as main factors), and the area under the curve (AUC) was calculated for each group and compared between strains at each time point via a Student’s t-test. Body weight was analyzed by two-way ANOVA (strain and surgical conditions as main factors). In all analyses, significance was set at $\alpha$ = 0.05 and Statistica 6.0 software for PC was used (Tulsa, OK).

Experiment 2: Electrophysiology

Surgery and semichronic preparation. To record taste responses in the PBN, we used a semichronic preparation successfully used by our laboratory in normal (37, 38) as well as in OLETF rats (34, 35). This procedure utilizes a modified chronic recording technique (24) to localize the taste area and sets up an acrylic headpiece with a reclosable opening over the brain target area. This allows repetitive recording with a minimal and noninvasive preparation in a slightly anesthetized condition.

Surgical procedures were carried out under aseptic conditions and general anesthesia using pentobarbital sodium (Nembutal, 50 mg/kg ip, Abbott Laboratories, North Chicago, IL). Rectal temperature was monitored throughout anesthesia and maintained at 37–38°C via a feedback loop connected to a heating pad (FHC, Bowdoinham, ME). The rat was mounted in a stereotaxic apparatus (Stoelting, Wood Dale, IL) by use of blunt ear bars with the skull leveled between bregma and lambda. The skull was exposed with a midline longitudinal incision, cleaned of periosteum. Just caudal to the interparietal suture, a 3 × 5-mm oval area of the bone was drilled away, the exposed dura was excised, and a cap of dental acrylic was anchored to the skull with stainless steel screws (1–72 × 1/8 in., Small Parts, Seattle, WA). Stainless steel wire was soldered onto two screws to serve as a ground. An antibiotic ointment (erythromycin, Bausch & Lomb, Tampa, FL) was placed on the exposed brain and then covered with a nontoxic silicone adhesive (Kwik-Cast, World Precision Instruments, Sarasota, FL). The acrylic was built up on the skull and molded around the conical ends of two sets of stainless steel rods that were attached rigidly to the ear bars. During subsequent recording sessions, these rods were reattached to the ear bars more medially and fitted back into the acrylic impressions, thus painlessly fixing the rat’s head in the stereotaxic plane. After surgery, an antibiotic was administered topically around the head wound (neomycin-polymixin, 0.5%) and systemically (gentamicin sulfate, 6 mg im).

Electrophysiological recordings and rapid stimuli. Following 1 wk of recovery from the first surgery, the rats were reanesthetized with a lower dose of pentobarbital sodium (Nembutal, 35 mg/kg ip, supplemented with 0.1 ml in every hour), mounted by the steel rods in the stereotaxic apparatus, and the silicon cork was removed from the hole on the acrylic headpiece and the underlying brain surface was cleaned with sterile physiological saline. The PBN gustatory area was located electrophysiologically using an electrode tilted 20° off perpendicular (tip anterior) to avoid damage to the transverse sinus. For extracellular single-unit recordings, tungsten microelectrodes were used ($\Omega$ = 3–6 MΩ at 1 kHz; FHC, Bowdoinham, ME). Neuronal activity was recorded online by using Cambridge Electronic Design’s hardware and Spike2 software along with the sapid stimulus onset mark.

For taste stimulation, the tongue was gently pulled out from the mouth and tied down in an $-45°$–45° side-down angle using a 5-0 silk surgical suture in the midline. The anterior 2/3 of the tongue was stimulated using a computer-controlled 16-channel fluid delivery system (Octaflow, ALA Scientific Instruments, Westbury, NY). The taste stimuli were the following: 0.1 M NaCl (Fisher Scientific, Fair Lawn, NJ), 0.01 M citric acid (J. T. Baker, Phillipsburg, NJ), 0.003 M quinine-HCl (Sigma-Aldrich), 0.03 M MSG (Sigma-Aldrich) and 0.03, 0.1, 0.3, 0.6, 0.9, 1.2, and 1.5 M sucrose (Sigma-Aldrich). All solutions were prepared in distilled water, delivered at flow rate of 100 μl/s, and at room temperature (22–25°C). Each taste stimulus was presented for 10 s and was followed by a 10-s water rinse with no pause between, to keep a continuous flow on the tongue avoiding on-off artifacts from mechanical stimulation. Separate stimulation sequences were used for basic taste characterization of a neuron (rinse, water, rinse, 0.3 M sucrose, rinse, NaCl, rinse, citric acid, rinse, quinine-HCl, rinse, MSG, rinse) and for consecutive concentration-response tests for sucrose (seven sucrose concentrations in ascending order, each bracketed with water rinses).

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RESULTS

Experiment 1: Behavioral Studies

Food intake. As expected, preoperative daily food intake was increased approximately twofold in the OLETF vs. LETO rats (36.4 ± 2.5 vs. 20.76 ± 1.5 g; P < 0.01). The mean daily caloric intake normalized to body weight was 27% higher in the OLETF rats (0.28 vs. 0.22 kcal/g body wt). After GBS, we compared food intake from postoperative day 4 to 25. Because the controls were pair fed, the only sensible comparison was between the GBS groups. Whereas absolute daily food intake was similar over time in the LETO-GBS and OLETF-GBS groups (days 4–11: 12.8 ± 1.5 vs. 11.76 ± 1.5 g; days 12–18: 25.2 ± 1.4 vs. 26.8 ± 1.6 g; days 19–25: 23.2 ± 0.6 vs. 25.6 ± 1.4 g), when normalized to body weight the OLETF-GBS overall consumed less calories per body weight than the LETO-GBS animals (0.17 vs. 0.19 kcal/g body wt). However, this consumption represented a smaller (60–65%) reduction relative to the presurgical intake in the OLETF-GBS vs. LETO-GBS rats (85–90%)

Body weight. Figure 1 depicts body weight over time during the 7-wk study period. At the start of experiment 1 OLETF rats weighed 465.6 ± 14.2 g and were significantly heavier than age-matched (21 ± 1.1 wk) LETO (336.5 ± 19.1 g, P < 0.05). 1H-NMR body composition analysis demonstrated increased body fat content (26.54 ± 0.67 vs. 22.33 ± 0.42%) in OLETF compared with LETO rats (P < 0.05). As noted, water restriction is required for testing aversive compounds (postoperative days 28 through 40; solid black line in Fig. 1). Because of the pair-feeding design, all groups lost weight during the study. However, the greatest effect occurred in OLETF-GBS rats, which lost 25–30% of starting weight (OLETF-GBS vs. OLETF-SH: F<sub>1.380</sub> = 0.24, P < 0.001) during the study period. In contrast, weight loss throughout the study was similar in the in LETO-GBS and LETO-SH rats (F<sub>1.387</sub> = 42.19, P = 0.626).

An overall ANOVA on percent change of body weight (not shown) allowed direct comparison of the effects of the surgeries between obese and lean rats with significantly different absolute body weights. The analysis revealed a significant interaction for strain × surgical condition (F<sub>3,110</sub> = 55.05, P < 0.001) and whereas the effect of GBS was significantly different between obese and lean rats (P < 0.01), weight loss in the surgical controls was statistically identical.

Oral glucose tolerance. Data from OGTTs performed in postoperative weeks 2 and 7 are shown in Fig. 2. Fasting blood glucose levels were normal in all groups and did not differ between strains. However, glucose tolerance was significantly impaired in OLETF rats at both time points compared with age-matched LETO controls. The OGTTs showed significantly higher glucose levels in sham-operated OLETF relative to LETO rats at 30 min on postoperative week 2 (30 min: 232 ± 24 vs. 158 ± 15 mg/dl, P < 0.01; open symbols in Fig. 2, A and B). On postoperative week 7, in addition to 30 min, the 60- and 120-min blood glucose readings were also higher in the OLETF rats compared with LETO rats (30 min: 275 ± 29 vs. 167 ± 11 mg/dl, P < 0.01; 60 min: 201 ± 12 vs. 144 ± 17 mg/dl, P < 0.05, 120 min: 141 ± 10 vs. 114 ± 5 mg/dl, P < 0.05; open symbols in Fig. 2).
2, C and D). AUC analysis revealed that OLETF rats expressed 180 and 215% increased blood glucose responses at 2 and 7 wk, respectively ($P < 0.01$ for both comparisons). The OLETF-GBS rats demonstrated significantly improved oral glucose tolerance compared with OLETF-SH at both time points. That is, they displayed lower peak blood glucose values following a gastric load (30 min at 2 wk: $145 \pm 10$ vs. $232 \pm 24$ mg/dl, $P < 0.01$, and 7 wk: $184 \pm 37$ vs. $275 \pm 29$ mg/dl, $P < 0.05$; Fig. 2, B and D), and a lower AUC (2 wk: $-58\%$, and 7 wk: $-35\%$, $P < 0.01$ for both comparisons). In contrast, GBS had no effect on glucose tolerance in lean controls at any time point tested.

**Responses to sweet tastants.** SUCROSE. Concentration-response function generated for sucrose was different between
OLETF and LETO rats (concentration \times strain: F_{5,75} = 5.29, P < 0.001). RM-ANOVA revealed significant strain \times treatment effects (F_{1,15} = 6.71, P < 0.03), and a significant three-way interaction for concentration responses (concentration \times strain \times treatment: F_{5,75} = 3.16, P < 0.02). Post hoc tests showed that, compared with LETO-SH, OLETF-SH rats made more licks for the higher solutions (above 0.1 M, P < 0.01 for all; see Fig. 3A, dashed lines). GBS abolished this difference and reduced sucrose licks in OLETF-GBS rats to the level of the LETO rats (OLETF-GBS vs. OLETF-SH: above 0.1 M, P < 0.05 for all; see Fig. 3A, left). In contrast, in the LETO rats GBS did not alter lick responses to sucrose at any concentrations (Fig. 3A, right).

**Fructose.** As with sucrose, the concentration-response function for fructose differed between groups with respect to strain and treatment (F_{5,75} = 2.82, P < 0.03). Compared with LETO-SH, OLETF-SH showed increased responses to the highest concentrations (0.8 and 1.6 M, P < 0.05 for both

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**Fig. 3.** Responses to sweet tastants. Mean number of licks made for increasing concentrations of sweet chemicals minus water licks (“0” on the abscissa) by OLETF (left) and LETO rats (right). Solid symbols: groups received GBS; open symbols: sham-operated controls. **A:** responses to sucrose. **B:** responses to fructose. **C:** responses to Na-saccharin. **D:** responses to alanine. Statistical symbols represent post hoc comparisons between surgical conditions: #P < 0.05, *P < 0.01. For more details and statistics, see RESULTS.
concentrations, see Fig. 3B). Again, GBS significantly allevi-
ated these increased lick responses in the OLETF rats ($P < 0.05$, see Fig. 3B, left), while having no effect on fructose licking in lean rats (Fig. 3B, right).

**Saccharin.** Unlike carbohydrates, an RM-ANOVA re-
vealed a nonsignificant (NS) strain × treatment effect for the noncaloric sweetener Na-saccharin ($F_{1,13} = 0.23, P = 0.68, NS$; Fig. 3C). Nevertheless, post hoc analysis showed a decreased response at 0.1 and 0.2% Na-saccharin in OLETF rats that received GBS compared with pair-fed surgical controls (OLETF-SH; $P < 0.01, P < 0.05$, respectively, see Fig. 3C).

**alanine.** Similar to preferred carbohydrates, the concentra-
tion-response function for the sweet amino acid alanine dif-
fered between groups ($F_{4,56} = 3.22, P < 0.05$). Whereas the LETO groups showed a flat concentration function for alanine, and no effect by GBS (Fig. 3D, right), OLETF-SH rats had an increased response to the higher concentrations of alanine solutions (0.3, and 1.0 M, $P < 0.05$ for both, see Fig. 3D, left) and a significant reduction following GBS ($P < 0.05$).

**Responses to nonsweet tasting compounds.** **NaCl.** The strain × treatment effect was not significant for NaCl ($F_{1,15} = 0.14, P = 0.72, NS$; Fig. 4A). Whereas OLETF rats exhibited a nonsignificant trend of increased lick responses to the most preferred NaCl concentrations (0.03–0.1 M; see Fig. 4A, left), there was no reliable effect by surgery on concentration-response function ($F_{4,60} = 1.16, P = 0.37, NS$; Fig. 4A).

**MSG.** The concentration-response function for MSG was overfl at with a trend for increased responsivity to higher concentrations by the OLETF rats compared with LETO. However, GBS had no effect in either strain ($F_{1,15} = 0.81, P = 0.38$, NS; Fig. 4B) or concentration between strains ($F_{4,60} = 0.84, P = 0.51$, NS; Fig. 4B).

**CITRIC ACID.** Concentration-response function generated for citric acid was not different between groups (Fig. 4C). Both strains preferred only the lower concentrations (0.01, 0.03 M) while showing an indifference-aversiveness response to higher concentrations. Again, GBS had no effect on either strain in either strain ($F_{4,60} = 1.60, P = 0.18, NS$; Fig. 4C).

**Quinine-HCl.** Whereas RM-ANOVA revealed no overall dif-
ference in concentration responses to quinine-HCl between strains as a function of surgery ($F_{5,75} = 1.07, P = 0.38$, NS; Fig. 4D), OLETF-GBS rats appeared to be less sensitive to the lowest concentration (0.03 mM/l) compared with any other groups (Fig. 4D, left).

**Capsaicin.** The trigeminal stimulus, the irritant capsaicin was used to test nontaste orosensory effects of GBS (not shown). The concentration × strain × treatment interaction was not significant when assessing lick difference score ($F_{4,53} = 1.12, P = 0.36, NS$).

**Two-bottle sucrose preference.** To eliminate the potentially confounding impact of sucrose’s postingestive effects, the 24-h two-bottle sucrose preference tests were performed after the gustometer studies on postoperative week 6. Both lean and obese pair-fed controls drank significantly more of the 0.3 M vs. the 1.0 M sucrose solution (LETO-SH: 126.5 ± 2.4 vs. 40.5 ± 3.6 g; OLETF-SH: 124.8 ± 14.5 vs. 38.5 ± 9.5 g). The GBS procedure reduced sucrose intake in both strains with an accentuated effect in OLETF rats (0.3 M: −59 vs. −44%; 1.0 M: −38 vs. −26%; OLETF-GBS vs. LETO-GBS). Water intake from the second bottle concurrently with sucrose was negligible (0–4 g) in all rats except the OLETF-GBS rats. This resulted in significantly reduced preference score for OLETF-GBS compared with LETO-GBS (0.3 M over water: 0.65 ± 0.01 vs. 0.96 ± 0.08, $P < 0.01$; 1.0 M over water: 0.58 ± 0.09 vs. 0.89 ± 0.08, $P < 0.01$; Fig. 5).

**Experiment 2: Electrophysiology**

**Body weight and oral glucose tolerance.** The rats obtained from Tokushima Research Institute for experiment 2 had somewhat higher body weights compared with the subjects of experiment 1. Table 1 includes the initial and final body weight for rats used in experiment 2. At this point, OLETF-SH rats were significantly heavier than OLETF-GBS (+27%, $P < 0.01$). In experiment 2, a single OGTT was performed in each individual rat at the end of the study before the animal was euthanized (14–16 wk following the surgeries). In these older animals (38.8 ± 1.9 wk) as diabetes progressed in the ad libitum fed OLETF-SH, an effect of GBS on improving glu-
cose tolerance was even more pronounced than in experiment 1. Compared with OLETF-SH, OLETF-GBS rats had lower fasting blood glucose (95.2 ± 7.7 vs. 110.8 ± 5.3 mg/dl, $P < 0.05$) and improved responses to glucose load (30 min: 245.2 ± 18.4 vs. 304.4 ± 15.4 mg/dl, $P < 0.05$; 120 min: 172.2 ± 13.1 vs. 192.4 ± 28.1 mg/dl, NS).

**Histology and recording sites.** A total of 138 penetrations were made into the pons in 16 rats, and in all rats both the left and right sides were explored. Single or multiunit electrophysiological responses to sapid stimuli occurred in 101 of these penetrations. Taste testing of neurons resulted in significant taste responses in a total of 170 distinct single neuron units.

On the basis of the stereotaxic coordinates of the penetra-
tions and the residual track marks from the histology, the taste-responsive neurons were located in the caudalmedial quadran of the PBN extending from near the dorsal surface of the pons through the brachium conjunctivum and into the compact layer of cells between the brachium conjunctivum and the mesencephalic trigeminal nucleus. This approximate location is consistent with previous samples from chronic and semichronic studies (24, 37, 38) and with acute experiments in which localization was an objective (48). Since the penetrations were made over the course of a month, and assuming an accuracy of at best 100 μm for such measurement in chronic studies, more precise localization of individual recording sites is impractical.

**Basic characteristics of taste neurons.** We recorded and analyzed taste responses elicited by 170 PBN neurons in eight obese OLETF and eight lean LETO rats. On the basis of the largest response to the standard stimuli, the neurons were classified in subcategories. These categories and the distribution of the recorded neurons are summarized in Table 1.

Whereas the average spontaneous firing rate (10.08 ± 0.64 Hz) did not differ between strains and surgical groups, a detailed analysis revealed lower baseline firing rate in the sucrose-best units in LETO-GBS compared with LETO-SH rats (4.76 ± 1.46 Hz vs. 8.39 ± 1.98 Hz, $P < 0.02$). In contrast, GBS did not alter spontaneous activity of sucrose-best neurons in OLETF rats (6.18 ± 0.84 Hz vs. 6.04 ± 1.48 Hz).

**Overall response profiles.** An analysis of normalized re-
sponse magnitudes (spikes/s) revealed no significant difference in neural responses to NaCl, citric acid, quinine, or MSG.
between the four experimental groups. In contrast, we found significant differences in the sucrose responses with respect to surgical conditions and the applied concentration (Fig. 6). Overall ANOVA showed differences between groups above 0.3 M sucrose that became larger with increasing concentrations (0.3 M: $F_{4.5} = 49.005, P < 0.001, n = 107$; 0.6 M: $F_{4.3} = 26.163, P < 0.01, n = 116$; 0.9 M: $F_{4.5} = 81.37, P < 0.001, n = 106$; 1.2 M: $F_{4.5} = 41.435, P < 0.001, n = 98$; 1.5 M: $F_{4.3} = 100.401, P < 0.002, n = 98$). Post hoc tests revealed that these differences were due to the following effects: 1) GBS reduced sucrose responses in OLETF rats to concentrations above 0.9 M compared with all other groups (0.9 M: $P < 0.01, P < 0.03, P < 0.001$; 1.2 M: $P < 0.001, P < 0.01, P < 0.03$; 1.5 M: $P < 0.01, P < 0.01, P < 0.001$, compared with OLET-F-GBS, LETO-SH, and LETO-GBS, respectively); and 2) GBS reduced responsiveness to sucrose in LETO-GBS compared with LETO-SH at lower concentrations (0.3 and 0.6 M: $P < 0.02$ and $P < 0.05$, respectively).

Fig. 4. Responses to nonsweet compounds. Mean number of licks made for increasing concentrations of sweet chemicals minus water licks ("0" on the abscissa) by OLETF (left) and LETO rats (right). Solid symbols: groups received GBS; open symbols: sham-operated controls. A: responses to NaCl. B: responses to monosodium L-glutamate (MSG). C: responses to citric acid. D: responses to quinine-HCl. For more details and statistics, see RESULTS.
Sucrose concentration-response function. A detailed analysis revealed significant differences in specific aspects of neural concentration responses to oral sucrose. These effects are summarized in Fig. 7. Multivariate ANOVA showed strain × treatment interaction effects for the response threshold, or the first significant response concentrations ($F_{3,139} = 2.921, P < 0.05$) and maximum response concentrations to sucrose ($F_{3,139} = 8.589, P < 0.001$). In contrast, there was no statistical difference in the maximum effective concentrations. Taste-responsive neurons in obese OLETF-SH rats showed a shift in their concentration-response functions toward higher concentrations of sucrose compared with lean LETO-SH controls. Specifically, in the OLETF-SH the first significant response concentration and maximum response concentration were elevated by 65.7 ($P < 0.05$) and 72.6% ($P < 0.001$), respectively. The major observation was that GBS reversed this shift back to control levels. Compared with obese OLETF-SH, OLETF-GBS rats showed lower first significant response concentration ($P < 0.02$) and lower maximum response concentration ($P < 0.001$). In fact, all concentration responses were statistically identical between the OLETF-GBS and LETO-SH rats. In addition, GBS did not affect the sucrose-concentration responses in lean LETO rats (LETO-SH vs. LETO-GBS).

An analysis of the maximal neuronal responses to sucrose as a function of the most effective sucrose concentration (Fig. 8) revealed similar differences between experimental groups for the maximal responses ($F_{3,116} = 6.260, P < 0.001$) and the respective sucrose concentrations ($F_{3,116} = 3.350, P < 0.05$). Whereas there was no significant difference in the magnitude of maximal responses, the largest responses appeared at 0.46 M higher sucrose concentrations in OLETF-SH compared with LETO-SH rats ($P < 0.01$). This difference vanished after GBS, and there was no significant difference between LETO-SH and OLETF-GBS with respect to the applied concentrations. An additional effect of GBS was a decrease in the maximal response magnitudes in both strains (OLETF-GBS vs. OLETF-SH, $P < 0.01$ and LETO-GBS vs. LETO-SH, $P < 0.02$).

Table 1. Summary of experiment 2

<table>
<thead>
<tr>
<th></th>
<th>OLETF-SH</th>
<th>OLETF-GBS</th>
<th>LETO-SH</th>
<th>LETO-GBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats, $n$</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Number of neurons, $n$</td>
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<td>47</td>
<td>40</td>
<td>47</td>
</tr>
<tr>
<td>Body weight, g ± SE</td>
<td>670.5 ± 24.2</td>
<td>662.2 ± 17.3</td>
<td>480.5 ± 14.6</td>
<td>472.3 ± 20.3</td>
</tr>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>N-best</td>
<td>6 (16.7)</td>
<td>16 (34.0)</td>
<td>12 (30.0)</td>
<td>18 (38.3)</td>
</tr>
<tr>
<td>S-best</td>
<td>19 (52.7)</td>
<td>24 (51.1)</td>
<td>26 (65.0)</td>
<td>24 (51.1)</td>
</tr>
<tr>
<td>C-best</td>
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<td>4 (8.5)</td>
<td>2 (5.0)</td>
<td>3 (6.4)</td>
</tr>
<tr>
<td>Q-best</td>
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<td>1 (2.1)</td>
<td>0 (0)</td>
<td>1 (2.1)</td>
</tr>
<tr>
<td>M-best</td>
<td>2 (5.6)</td>
<td>2 (4.2)</td>
<td>0 (0)</td>
<td>1 (2.1)</td>
</tr>
</tbody>
</table>

Groups and classification of recorded neurons based on their best responses to oral stimulation with different tastants. OLETF, Otsuka Long-Evans Tokushima Fatty rats; LETO, Long-Evans Tokushima Otsuka rats; GBS, gastric bypass surgery; SH, sham operation; N-best, NaCl best response; S-best, sucrose best response; C-best, citric acid best response; Q-best, Quinine-HCl best response; M-best, monosodium glutamate best response units. Body weights indicate initial and final body weight. Note that controls animals, OLETF-SH and LETO-SH, were not pair fed as in experiment 1.
whereas the two GBS groups did not differ from each other in either variable.

DISCUSSION

The present study demonstrates decreased taste preference for palatable sucrose and other sweet-tasting compounds following Roux-en-Y gastric bypass surgery in the obese OLETF rat model. We also report the first electrophysiological evidence that central taste processing for sweet is altered by GBS. The use of the CCK-1 receptor-deficient OLETF rats, which develop obesity due to impaired meal-size control, is helpful to delineate contribution of CCK signaling to the beneficial effects of GBS (e.g., glucose control, behavior, and neural functions). The following discussion reviews specific findings, speculates on potential underlying mechanisms and identifies targets for future research.

Effects of GBS on Body Weight and Glucose Control

The present study is the first to report on weight loss and glucose tolerance in the obese OLETF rat following a bariatric surgery. In this model, we found that weight loss and improvements in blood glucose control are similar to that observed in GBS patients. The observed weight loss (26%) is similar to the 30% reduction commonly observed in patients and was maintained over a long postsurgical period also in our rat model. These findings support the notion that CCK signaling is not critical to weight management and improved appetite control following GBS (58). Improvements in oral glucose tolerance were apparent shortly after GBS (2 wk), were maintained over time, and appear to be related to the effects of GBS on gastrointestinal anatomy rather than food restriction alone. These observations reinforce previous studies indicating that restricted caloric intake following GBS is not sufficient to...
explain postoperative improvements in glucose homeostasis. The use of rats provided us with the unique opportunity to study effects of GBS in a lean subject. Surprisingly, in lean rats GBS caused no additional weight loss compared with caloric restriction from pair feeding in experiment 1. This finding supports the notion that restricted intake following GBS is not the most critical factor contributing to improved weight management.

Behavioral Effects of GBS on Sucrose Preferences

Altered food preferences in GBS patients have long been considered to contribute to its beneficial effects (4, 6). For example, Sclafani (54) described altered carbohydrate preferences following various gastrointestinal surgeries in rats. In contrast, the present study used a gustometer to evaluate taste functions, which minimizes the effects of postigestive feedback (11). We identified increased responsiveness to sweet-tasting compounds in OLETF rats (13, 21) and also determined the effect of GBS on taste sensitivity (concentration responses) to various chemicals. The major finding of the present study is that GBS alleviates increased taste preference in OLETF rats in a specific manner, affecting only responsiveness to sweet tastants while concentration functions remain unaltered to other taste qualities. Moreover, the taste effects of GBS and the corresponding reduced intake of sucrose in long-term choice tests were observed in obese but not lean rats. This observation supports our hypothesis that taste preferences in OLETF are altered in ways that are specific to mechanisms that code for sweet and the effect of GBS, at least in part, to reverse these deficits. As with body weight and glucose control, CCK signaling appears to have a negligible effect in behavioral responses to sweet taste following GBS.

Electrophysiological Effects of GBS on PBN Taste Coding

Using single neuron recordings in the PBN of OLETF rats, our laboratory recently demonstrated decreased neural responses to lower concentrations of sucrose and exaggerated responses to higher concentrations (35). The present study extends this observation to characterize the effects of GBS on taste coding. Whereas the findings in the surgical controls (OLETF-SH vs. LETO-SH) basically reproduced the strain differences observed in our previous study, the effects of GBS were novel and surprising. Following GBS, OLETF rats demonstrate neural concentration-response functions to oral sucrose stimulation that are indistinguishable from those of lean rats with or without GBS. Specifically, GBS attenuated the increased neural responses to higher concentrations of sucrose in the OLETF rats to the level of lean controls. Following GBS, the taste-responsive PBN neurons in the OLETF rats expressed concentration sensitivity (i.e., the maximum responses occurring at lower concentrations) similar to lean rats. Thus taste code in the PBN not only reflects chronically increased appetite in this model of obesity but also appears to be influenced by postsurgical changes in gastrointestinal anatomy. These findings support the notion that PBN taste activity, at least in part, carries affective information that probably includes the actual hedonic value of the stimulus, commonly described as palatability. The presence of a corresponding behavioral change (i.e., reduced preference and intake of palatable meals) following GBS further supports this claim.

Potential Mechanisms

Taste functions encompass primary sensory and affective processes. Consequently, the GBS procedure may impact either or both processes. Sensory processing is initiated by taste cells located on the tongue and in the oral cavity. Hormones that regulate feeding status and metabolic states may alter taste signaling acting on receptors located on taste cells or on the afferent nerve terminals to the brain. For example, taste cells express receptors for insulin (59), leptin (29), CCK, and also GLP-1 (27). Taste cells may also release GLP-1 (14). In the gut, a similar relationship exists: intestinal neuroendocrine cells producing GLP-1 or expressing CCK-1 receptors also have the molecular machinery identical to those the oral taste cells to detect sweet-tasting intraluminal nutrients (33, 51). On this basis, one may suppose that the observed effects in the OLETF rat were due to the loss of CCK-1 receptor functions. However, there is evidence arguing against this supposition. First, it has been shown that peripheral CCK-1 receptors are not required for acquisition of conditioned taste preferences or aversions (12, 32). Second, it has been generally considered that CCK is not involved in the mechanisms of GBS (31, 58). Lastly, our preliminary data (20) and recent replication by others (64) show similar alterations in behavioral taste functions following GBS in dietary obese rats with intact CCK signaling.

The reward produced by ingesting food, the so-called hedonic responses to taste, modulates homeostatic controls such as the meal size and, in turn, the overall regulation of body weight. Thus it is also feasible that GBS alters integration of peripheral and central signals that are critical to food reward. Such an effect might occur in forebrain reward areas, such as the mesolimbic dopamine system, or in the sensory nuclei themselves, or both. For taste, there is evidence for both (46). In addition to research showing that the hindbrain taste relays are uniquely positioned to integrate systems controlling energy balance and sensory-motivational aspects of taste-guided behaviors (see Refs. 7, 43, 137 for reviews), we recently demonstrated that the gustatory PBN is critical to sucrose’s oral effects to stimulate the mesoaccumbens reward system (22, 45, 47).

There are multiple signals and mechanisms that can modulate neural processing of afferent information within the PBN. In addition to expressing insulin and leptin receptors (17), PBN neurons also receive descending anorexigenic (melanin-concentrating hormone) and orexigenic (neuropeptide Y) projections from the hypothalamus (1). Unlike in the hypothalamus, the exact role of the insulin and leptin receptors expressed in the hindbrain remains unknown. However, recent studies suggest that in addition to energy homeostasis, reward processes are also influenced by insulin and leptin (15). Therefore, it is possible that improvements in insulin and leptin signaling following GBS contribute to the observed changes in taste reward functions.

Whatever the cause for altered PBN taste processing after GBS (i.e., modulation of the taste afferents or top-down influence from reward or homeostatic regulating circuitries), the coding scheme observed in the present study is consistent with the effect of gastric distension, an inhibitory feedback signal that enhances satiation and diminishes taste reactivity (7, 18). Gastric distension has been shown to reduce sucrose-evoked
neuronal activity in the PBN whereas NaCl responses remain unaltered (2). This suggests that the PBN may take part in neural processes underlying hedonic evaluation of gustatory information and may contribute to the regulation of meal size by increasing or reducing the orosensory positive feedback. The present data reinforce previous findings showing that pontine taste code is altered in obese rats and demonstrates for the first time that GBS reverses these deficits. It remains to be determined, however, whether these observed changes in gustatory neuronal responses lead or follow the changes in appetite and food preferences.

Finally, the lack of effect of GBS on either behavioral or neural taste functions in lean rats strongly suggests that GBS resets obesity-related alterations in taste and ingestive behavior. A commonly posited mechanism to explain decreased sweet preferences after GBS is an acquired aversion to carbohydrate-rich meals that may result in an intestinal discomfort and diarrhea, collectively called dumping syndrome (62). However, the present study found no evidence for development of taste aversion to sucrose as a consequence of GBS. The CCK-1 receptor-deficient phenotype of the OLETF rat cannot explain this, because we found no taste aversion in the control LETO rats that received GBS. However, in the present study rats had only limited exposure to sucrose unlike patients with substantial intake, at least initially, as they make their transition from eating high-carbohydrate diets to healthier diets. As with all feeding studies in rats, there must be a consideration of the lack of cognitive factors and dietary awareness, all of which are important components of postsurgical dietary management of the GBS patients. Therefore, further studies are required to delineate different aspects of taste functions in GBS patients.

**Perspectives**

This study demonstrates effects of GBS on basic variables of both the sensory input and behavioral output branches of the neural systems mediating sucrose preference. We do not suggest, however, that the findings reported in this paper are the only neural consequences of GBS related to altered postsurgical food preferences. Further research focusing on the afferent signals (e.g., gastrointestinal hormones), as well as the mechanisms upstream to the steps mediating activation of the food reward circuits (e.g., dopamine, opioid, cannabinoid modulation), are still critically important. Understanding the underlying mechanisms by which GBS affects the hedonic processing of taste information may help in identifying therapeutic targets that mimic the beneficial effects of GBS on appetite control and food choices without the risks and complications of an invasive surgical procedure.

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