Roux-en-Y Gastric Bypass in Rats Increases Sucrose Taste-Related Motivated Behavior
Independent of Pharmacological GLP-1-Receptor Modulation.

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ABSTRACT

Roux-en-Y gastric bypass surgery (RYGB) has been shown to decrease consummatory responsiveness of rats to high sucrose concentrations, and genetic deletion of glucagon-like-1-peptide-receptors (GLP-1R) has been shown to decrease consummatory responsiveness of mice to low sucrose concentrations. Here we assessed the effects of RYGB and pharmacological GLP-1R modulation on sucrose licking by chow-fed rats in a brief access test that assessed consummatory and appetitive behaviors. Rats were tested while fasted presurgically and postsurgically, and while nondeprived postsurgically and 5h after intraperitoneal injections with the GLP-1R antagonist exendin-3(9-39) (30µg/kg), agonist exendin-4 (1µg/kg), and vehicle in 30-min sessions during which a sucrose concentration series (0.01-1.0M) was presented in 10-s trials. Other rats were tested postsurgically or 15min after peptide or vehicle injection while fasted and while nondeprived. Independent of food-deprivation state, sucrose experience, or GLP-1R modulation, RYGB rats took 1.5-3x as many trials as sham-operated rats, indicating increased appetitive behavior. Under nondeprived conditions, RYGB rats with presurgical sucrose experience licked more to sucrose relative to water compared with sham-operated rats. Exendin-4 and exendin-3(9-39) impacted 0.3M sucrose intake in a 1-bottle test, but never interacted with surgical group to affect brief access responding. Unlike prior reports in both clearly obese and relatively leaner rats given RYGB and GLP-1R knockout mice, we found that neither RYGB nor GLP-1R blockade decreased consummatory responsiveness to sucrose in our less obese chow-fed rats. Collectively, these results highlight the fact that changes in taste-driven motivated behavior to sucrose after RYGB and/or GLP-1R modulation are very model- and measure-dependent.

Keywords

Gustatory, bariatric surgery, sugar, Type 2 diabetes mellitus, anorexigenic peptides
INTRODUCTION

Roux-en-Y gastric bypass surgery (RYGB) is currently considered a successful long-term treatment option for morbid obesity and can cause remission of Type 2 diabetes (38, 49, 59). In this procedure, the majority of the stomach and the proximal intestine are surgically isolated from contact with food, and the remaining small gastric pouch is anastamosed to the jejunum (Figure 1). The residual stomach and proximal small intestine are reattached to the jejunum distal to its junction with the gastric pouch. The weight loss and relatively swift improvement of glycemic status that follow RYGB are thought to be due in large part to behavioral and neuroendocrine changes.

That RYGB decreases caloric intake in patients has been consistently shown across many studies (7, 9, 26, 29, 58) and has been reported to continue up to 15 years after the operation (48, 49). It is not clear, however, if patients only eat less in general or if they also choose different types of foods to eat after RYGB. Some RYGB patients have reported, via food diaries and food-frequency questionnaires, that they eat fewer sweet or fatty foods (7, 26, 58), but the interpretation as to the extent to which the avoidance is due to learned aversions, decreased motivation for food, or to changes in the sense of taste differs across studies. For example, some patients after RYGB claim that sweet foods taste sweeter and they accordingly change their food selections (14). In contrast, other patients hedonically rate a series of sucrose concentrations similarly before and after the operation (13). Still other patients indicate that they continue to regard high-calorie foods as delicious, but avoid these foods because of tolerability and/or lack of interest (22).

Some investigators have used animal models to explore the effect of RYGB on sucrose responsiveness by using high-fat diet-fed obese (as determined by body composition analysis and leptin levels) rats (46) or rats with genetic manipulations that result in increased adiposity.
Shin et al. (46) reported that high-fat diet-fed obese rats licked at a lower rate to very low sucrose concentrations (those near detection threshold; see 8, 54, 60) and at a higher rate to high sucrose concentrations during 10-s trials in a brief access test compared to age-, but not body-weight-matched, chow-fed rats with significantly lower adiposity and leptin levels. The concentration-response function seen in the leaner chow-fed rats was rescued in high-fat diet-fed obese rats when they were tested after RYGB. Similar effects at high, but not low, sucrose concentrations were seen in cholecystokinin (CCK)-1 receptor-deficient Otsuka Long-Evans Tokushima Fatty (OLETF) rats after RYGB when they were compared with sham-operated OLETF rats that were pair-fed based on the intake of RYGB rats (21).

Similar to Hajnal et al. (21), Tichansky et al. (62) found that chow-fed rats with no genetic manipulation that received RYGB licked at lower rates to high but similar rates to low sucrose concentrations in a brief access test compared with sham-operated rats (though note that these sham-operated rats were not pair-fed to the intake of RYGB rats as in 21; also, adiposity and leptin levels were not reported in this study). Also using a previously established model in chow-fed rats (10-13, 27, 31, 32), Bueter et al. (13) reported that RYGB rats showed less preference for sucrose across a range of concentrations in a 24-h 2-bottle test, but this effect was partially attenuated by presurgical exposure to sucrose. Although these studies all used different models and methods, they all suggest that the affective component of sucrose taste may be blunted, at least at higher concentrations, along with the body weight loss that accompanies RYGB surgery; however, the mechanisms underlying such changes in taste-guided behavior remain to be elucidated.

The changes in responsiveness to and preference for sucrose seen after RYGB in rats, and, potentially, food selection in humans, may stem from how the surgery alters the hormonal milieu. RYGB increases postprandial release of peptide-YY (PYY) and glucagon-like-peptide-1 (GLP-1), hormones released by the gut in the presence of nutrients (6, 30, 31, 33; for reviews...
see 39, 41, 67); this has also been seen in a rat model of RYGB (e.g., 11, 13, 32). Systemic administration of the GLP-1 receptor agonist exendin-4 (Ex4) decreases intake of food and sucrose solution (3, 5, 6, 35, 68, 72, 74), and this effect is blocked by the GLP-1 receptor antagonist exendin-3(9-39) (Ex9) (3, 68, 73, 74), which, under certain conditions, independently increases food intake (73, 74; although see 37, 43). GLP-1 is also expressed in taste receptor cells on the tongue and its receptors are present on afferent gustatory nerve fibers innervating taste buds (47). Mice with genetic deletion of the GLP-1 receptor show decreased responsiveness to low, but not high, concentrations of sucrose and low concentrations of the non-nutritive sweetener, sucralose, compared with wild type mice in a brief access taste test (47). This suggests that alterations in GLP-1 levels and/or GLP-1 receptor activity could be responsible for any observed effect of RYGB on affective taste processing. Accordingly, sucrose responsiveness in rats could be altered by pharmacological modulation of GLP-1 receptors independent of RYGB surgery and/or exaggerated or nullified after RYGB.

Here, we sought to recapitulate the blunted behavioral responsiveness to high concentrations of sucrose seen after RYGB in other rat models in a brief access test designed to assess both the appetitive and consummatory aspects of taste-guided affective behavior. We also explored the independent and possible interactive effects of pharmacological GLP-1 receptor modulation on affective responsiveness to sucrose in rats with and without RYGB. In our version of the brief access test, water and each sucrose concentration were presented in randomized blocks of 10-s trials and a given trial began only when contact was made with the stimulus. Therefore licking during each trial was measured only in instances in which the stimulus contacted taste receptors; as such, licks served as a measure of consummatory responsiveness. The total number of trials initiated across each session served as a complementary measure of taste-motivated behavior indicative of overall appetitive
responsiveness (i.e., approach) associated with the opportunity to engage the reinforcing stimulus.

MATERIALS AND METHODS

General methods.

Subjects.

Male Sprague-Dawley rats (Charles River) were used in all of the experiments. All rats were housed individually in conventional polycarbonate tub cages in vivarium rooms where temperature, humidity, and lighting (12-h lights on; 12 h lights off) were controlled automatically. The rats did not receive high fat diets at any time and, except when otherwise noted, rats received ad libitum access to standard maintenance chow (PMI 5001, LabDiets, St. Louis, MO) and reverse osmosis deionized water. Body weight was measured daily but we performed no direct measures of adiposity. All procedures were approved by the Animal Care and Use Committee of Florida State University.

Test stimuli.

Test stimuli consisted of reverse-osmosis deionized water and sucrose solutions (BDH Chemicals, West Chester, PA). All solutions used were prepared daily and presented at room temperature.

Peptides.

The GLP-1 receptor antagonist exendin-3(9-39) (Ex9) and agonist exendin-4 (Ex4) (California Peptide Research, Inc., Napa, CA) were dissolved in phosphate buffered saline. Solutions of the necessary concentrations were prepared en masse and frozen in aliquots; the
amount needed for injection each day was thawed that morning and injected at room
temperature. A washout period of at least 24 h occurred between each peptide administration.

Because there is some evidence that central administration of GLP-1 in rats can serve
as an unconditioned stimulus in taste aversion learning (e.g., 27, 45, 61) and patients receiving
peripheral injections of the GLP-1 analog exenatide (Byetta) have reported nausea and malaise
as side effects (e.g., 2, 75), we chose the highest doses, particularly of Ex4 (1 µg/kg; Ex9: 30
µg/kg), that appear to produce consistent effects on feeding without the threat of nonspecific
effects on behavior that could interfere with our testing protocol.

Brief access apparatus and procedure.

The rats were trained and tested in a lickometer known as a Davis rig (Davis MS-160,
DiLog Instruments; see 50). We used a brief access procedure (see 51) in which a rat was
placed in the test chamber and allowed access to solutions during a 30-min session. In a series
of sessions, water was presented from a stationary spout, water from different tubes was
presented in 10-s trials, or a concentration series of sucrose was presented individually in 10-s
trials. Access to taste stimuli was available to the rat via spouts attached to small glass tubes
that held solutions. The tubes and spouts were seated in a horizontal array on a computer-
controlled motorized rack and were positioned individually behind a slot, covered by a shutter, in
the front panel of the test chamber. During sessions in which the stimuli were presented in
successive trials, the shutter moved to uncover the access slot and a trial was initiated with the
first lick, after which the solution was available for 10 s. Upon completion of the trial, the shutter
was closed over the access slot, the rack was moved, and a different tube was placed into
position. After an inter-trial interval of 7.5 s, the shutter was again opened and the rat was given
the opportunity to initiate another trial. If the rat did not sample a presented stimulus, the rack
would not advance and the unsampled stimulus would stay in place for the remainder of the
session. Presentation order of the stimuli was randomized without replacement in blocks of trials, which means that once the rat had sampled all the concentrations in one block of seven trials, another block would begin and trials would cycle once again through all of the concentrations, which were presented in random order within each block.

In general, to train the animals to sample in the Davis rigs, water bottles were removed from the rats’ cages ~23 h prior to a session in which water was presented from one spout that remained stationary. A session with these parameters was repeated the following day. On the next two days, sessions were conducted once daily in which water was presented in multiple bottles across successive trials. On the 5th day during which the rats were water restricted, water and the sucrose concentration series (0.01, 0.03, 0.06, 0.1, 0.3, 1.0 M) were presented in trials. Water was returned to the rats ~30 min after this final training session. While water-restricted, the body weights of the rats were monitored daily with a contingency in place that any rat falling below 85% of its pre-restriction body weight would receive 10 ml supplemental water 30 min after completion of its session. After training, the rats were tested once daily in sessions in which the sucrose concentration series was presented in trials, either after they had been ~23 h fasted or had been provided with ad libitum access to food (i.e., nondeprived).

Data analysis.

Our measures for each phase of the studies in which brief access performance was measured consisted of: a) the number of licks of water taken and interlick interval (ILI) during the second day of testing during which water was presented from a stationary spout; b) the Lick Score, defined as the mean number of licks taken per trial of each sucrose concentration less the mean number of licks taken per trial of water during testing with the sucrose concentration series; and c) the number of trials initiated by the rats during each session. The individual ILIs, which, under constant test conditions, are relatively invariant in rodents (15, 64, 69, 71) and,
ultimately, place a limit on the maximal lick rate, were filtered to include those between 50 and 250 msec in duration because values less than 50 msec were considered double licks and those values greater than 250 msec were considered pauses between licking bursts (17); the individual ILIs meeting the criterion were averaged across session. Only rats that had initiated at least 2 trials of each concentration across the days that they were tested in each phase were included in the statistical analysis of Lick Score. This was done to increase the veracity of our estimate of concentration-dependent responsiveness and minimize the influence of potential spurious values. However, all rats were included in the analysis of trials initiated because even if an animal did not engage a sufficient number of trials to form a reliable estimate of consummatory responsiveness across the concentration range, the number of trials initiated is still an accurate reflection of the animal’s general appetitive tone.

The average Lick Score of each surgical group in each condition was used to fit the three-parameter logistic function:

$$f(x) = \frac{a}{1 + 10^{\left(\frac{\log_{10} x - c}{b}\right)}}$$  \hspace{1cm} (1)

in which \(x\) = sucrose concentration; \(a\) = asymptotic lick response adjusted for water; \(b\) = slope; and \(c\) = \(\log_{10}\) concentration at the inflection point (EC\(_{50}\)).

Analysis of Variance (ANOVA) was used to compare data from sham-operated and RYGB-operated groups, which were between-subject factors, and, during certain phases, injection conditions and sucrose concentration, which were within-subject factors. When two-way ANOVA revealed significant effects, t-tests and/or 1-way ANOVA were conducted to compare the appropriate conditions; the resulting p-values were Bonferroni-corrected. An alpha of 0.05 was used throughout the experiments as the statistical rejection criterion.
Experiment 1: Effects of RYGB and pharmacological GLP-1 receptor manipulations on brief access responding to sucrose and water.

Age and body weight of subjects.

In this experiment, the rats weighed, on average, 340 g and were ~2.5 months old upon arrival and 540 g and ~4 months old at the time of surgery (Figure 2).

Peptide doses and time course.

During portions of testing in which the effects of pharmacological manipulations of GLP-1 receptors were assessed, all of the rats were injected with Ex9 (30 µg/kg ip), Ex4 (1 µg/kg ip), and vehicle (1 ml/kg ip) 5 h prior to testing. The doses and time course of the peptides were chosen based on our unpublished pilot work that showed a 220% increase and 17% decrease in chow intake of rats (n=6 per peptide group) 5 h after injection of Ex9 and Ex4, respectively, and the published work of others that showed similar effects (72-74).

Presurgical brief access training and testing.

During training (Table 1), provision of supplemental water was necessary for two rats. The rats were fasted and tested with sucrose every-other day for one week (Table 1).

Surgery.

Rats were assigned to either RYGB or sham-operated (SHAM) surgery groups such that the following parameters were matched: licks of water taken on the second day of training when water was presented continuously, licks and trials of sucrose taken during presurgical testing, and body weight.
Food was removed from the rats overnight prior to surgery. Anesthesia was induced in a chamber filled with 5% isoflurane in oxygen (1 l/min). Each rat was shaved from sternum to pelvis and the area disinfected with a betadine scrub. The rat was then placed in supine position on an isothermal heating pad and positioned in a nosecone that maintained anesthesia (2-4% isoflurane in 1 l/min oxygen) for the duration of the surgery. All surgeries were conducted by one surgeon (M.B.) as previously described (10-13, 32). Briefly, for the RYGB procedure (Figure 1), which lasted approximately 70 min, the small bowel was transected 20 cm distal to the pylorus of the stomach. The portion of the intestine caudal to the transection was anastomosed to a small section of gastric mucosa (~4-5 mm), which was separated from the stomach close to the gastro-esophageal junction. The portion of the intestine anterior to the transection, continuous with the remaining portion of the stomach, was anastomosed to the ileum approximately 25-30 cm from the cecum. For SHAM operations, which took approximately 30 minutes, an anterior gastrotomy and a jejunotomy with subsequent closures were performed. The abdominal wall and the skin were closed in layers after both operations.

Immediately following surgery, each rat was injected subcutaneously with an antibiotic (gentamycin; 8 mg/kg), an analgesic (ketoralac; 2 mg/kg), and 5 ml of saline and was placed on an isothermal pad in a polycarbonate cage until fully recovered from anesthesia, at which time the animal was returned to its home cage. The rats were allowed at least 10 days to recover prior to further testing; body weight was monitored daily during this time. For the first 3 days after surgery, each rat was injected with analgesic and antibiotic and was given wet mash (1 part powdered chow and 2 parts water), which was presented fresh daily. Normal chow pellets were returned ~24 h after surgery.

Across 7 days, RYGB surgery was performed on 23 rats and SHAM surgery on 15 rats. Postsurgical complications that we observed after RYGB included excessive salivation, difficult respiration, lethargy, accumulation of fluid under the incision site, and soft feces. Of the 23
RYGB rats, 1 died during surgery, 5 died or were euthanized 1-4 days after surgery, and 2 were euthanized 10-12 days after surgery. No complications or deaths were seen after SHAM surgery. Of the 15 surviving RYGB rats, 7 were used in this study along with 7 of the 15 SHAM rats; the remaining rats were used in a study not presented here.

*Postsurgical brief access testing.*

To assess the effect of RYGB and SHAM operations on intake during sessions in which water was presented continuously and to refamiliarize the rats with the Davis rigs, the training procedure used presurgically was repeated (Table 1). During this time we also assessed effects on the mean ILI. Rats were allowed access to water for 48 h prior to the next phase of testing, during which time they fully gained back the body weight they had lost while on the postsurgical water-restriction schedule.

To examine if RYGB and SHAM surgery impacts trial initiation and licking responses to sucrose, the testing procedure that was performed presurgically was repeated postsurgically. However, a 72-h period of refeeding was allowed between fasts, during which time the body weights of the RYGB rats rose to prefast levels. Thus, only two test sessions, one on Monday and the other on Friday, were conducted. Rats were also tested with sucrose while nondeprived. These testing sessions under nondeprived conditions took place on Tuesday and Saturday to remain consistent with the procedure used postsurgically to test the rats while they were fasted (Table 1).

*Postsurgical brief access testing with pharmacological GLP-1 receptor manipulations.*

The rats were tested once a day for a total of two sessions each after injection of the peptides active at the GLP-1 receptor, starting with the antagonist Ex9. Two vehicle test sessions were conducted both prior to and after the test series during which Ex4 and Ex9 were injected (Table 1). Food was removed from the rats at the time of injection, which was 5 h prior
to the tests, and returned 30 min after completion of the sessions. Because of the delay between injection and testing, the rats were divided into two squads, with one tested on Mondays and Wednesdays and the other on Tuesdays and Thursdays for a total of 4 weeks. SHAM and RYGB rats were represented equally in both of the squads.

After testing with sucrose, the effects of pharmacological GLP-1 receptor manipulation on the intake and ILI of water-restricted rats licking water from a stationary spout were assessed. Water bottles were removed from the rats ~23 h prior to each test session and returned 30 min after the completion of each session. The peptides and vehicle were administered as described above for the sucrose tests, except that only one test session was conducted for each peptide and for vehicle, the latter of which was performed prior to testing with the peptides (Table 1). The rats were tested in two squads, with one tested on Monday, Wednesday, and Friday and the other on Tuesday, Thursday, and Saturday across 1 week.

Data analysis.

Differences in average licks and ILI during water testing between SHAM and RYGB were analyzed separately for the presurgical, postsurgical, and injection conditions. Differences in average sucrose Lick Score and number of sucrose trials initiated between SHAM and RYGB were analyzed separately for the presurgical fasted, postsurgical fasted, postsurgical nondeprived conditions, and injection conditions. Due to computer error, total lick data of one of the RYGB rats postsurgically were lost, and so presurgical values from that rat were also not included in analysis. One SHAM rat did not take enough trials to meet our inclusion criterion and so its Lick Score data were excluded from all conditions.

Experiment 2: Effect of RYGB on brief access responding to sucrose and water in rats without presurgical sucrose testing experience.
**Age and body weight of subjects.**

Twenty male Sprague-Dawley rats that weighed, on average, 335 g and were ~2.5 months old at receipt and 467 g and ~3.5 months old at the time of surgery were used in this experiment (Figure 5).

**Surgery.**

Across 7 days, the RYGB operation described in Experiment 1 was performed on 10 rats and the sham operation was performed on 9 rats. The groups were established such that average body weight did not statistically differ between them. Two RYGB rats were euthanized prior to the start of testing (one 11 days after surgery and one 23 days after surgery), both due to continual weight loss and marked lethargy. No postoperative complications were seen in the SHAM group.

**Postsurgical brief access training and testing.**

After at least 10 days of recovery from surgery, the rats were trained as described (Table 1). Following training, two sucrose test sessions separated by 6 days were conducted while the rats were fasted (Table 1). Two RYGB rats were euthanized due to marked lethargy prior to the second day of testing while fasted, and so their data were removed from the entire study, reducing the overall sample size of the RYGB group to 6. Approximately 3 weeks later, we also performed two sessions of testing, which were separated by 48 h, while the rats were nondeprived (Table 1). Two RYGB rats were not tested during this phase because they did not completely recover the body weight they had lost during the fasted testing conditions, thus the subject size during tests in which food had not be removed from the rats was reduced to 4.

**Data analysis.**
Differences in the average licks of water taken and ILI during water testing between SHAM and RYGB were analyzed postsurgically, and sucrose Lick Score and number of sucrose trials initiated were analyzed separately for the postsurgical fasted and postsurgical nondeprived conditions. Due to machine failure, 2 SHAM rats were not tested on the second day of nondeprived testing, but their performance on the single session was sufficient to meet the criterion for inclusion. A different SHAM rat did not take enough trials across the sessions to be included in Lick Score analysis.

Experiment 3: Assessment of shorter time-course of pharmacological GLP-1 receptor manipulations on brief access responding to and intake of sucrose in unoperated rats.

Subjects.

Sixteen male Sprague-Dawley rats that weighed, on average, 250 g and were ~2 months of age at the beginning of testing were used.

Peptide dose and time course.

The doses of Ex4 and Ex9 listed in Experiment 1 were used, but they were injected 15 min prior to presentation of water and/or sucrose solution.

Brief access training and testing.

The rats were trained to lick water as described, with the exception that between the first set of training sessions in which water was presented continuously and the second set in which water was presented in trials, we measured the effect of Ex4 or Ex9 (and vehicle) on licks and ILI. After training in which water was presented in trials, we measured the effect of the peptides
on number of trials initiated and licks taken during water trials. The details of these tests are presented in Table 2.

To assess the effect of Ex4 and Ex9 on concentration-dependent licking to sucrose, the rats were tested every other day for a total of two sessions after injection with their assigned peptide. A total of four vehicle test sessions were conducted: two prior to and two after the peptide tests (Table 2). This testing series was performed first while the rats were fasted and then repeated when the rats were nondeprived.

*Home-cage intake of 0.3 M sucrose.*

Food was removed from the rats ~23 h prior to injection with Ex9 or Ex4, which was administered according to their assigned peptide group; water was removed at the time of injection. Fifteen minutes after injection, a 100-ml bottle with a curved sipper spout that was filled with 0.3 M sucrose solution was presented in the position normally occupied by the water bottle. Sucrose intake was measured at 0.5 and 1 h after presentation; the bottle was removed at the last time point and the maintenance water bottle replaced. Food was returned to the rats ~1 h after removal of the sucrose. Two test days with the peptides and two test days with vehicle, both before and after peptide testing, were performed (Table 2), with a day off in between each test that served as a period of refeeding for the rats and washout of the peptide.

We also measured the extent to which the dose of Ex9 that we selected was able to block the effect of our chosen Ex4 concentration. We used only those rats that had previous experience with Ex4. Food was removed from the rats ~23 h prior to injection with Ex9; water was removed at the time of this injection. Fifteen minutes after Ex9 injection, the rats were injected with Ex4. Fifteen minutes after that, a bottle of 0.3 M sucrose solution was presented. Measures were taken at 0.25, 0.5, and 1 h after presentation. Food and water was returned to the rats as described above. Two test days with the antagonist challenge of the agonist, and
two test days with two vehicle injections (one injection for each peptide), both before and after peptide testing, were performed, with a day off in between each test (Table 2).

Data analysis.

Differences in the average number of licks of water taken, ILI, and the number of trials initiated during water testing between each peptide group were analyzed with injection condition (peptide versus vehicle) as the within-subject factor. Sucrose Lick Score and number of sucrose trials initiated under vehicle and peptide conditions for each group were analyzed separately for the postsurgical fasted and nondeprived conditions. All rats in both groups met the criterion for Lick Score inclusion when tested while fasted, but, when tested while ad libitum-fed, only 5 rats in the Ex4 group and 4 in the Ex9 group took enough trials in both injection conditions to be included in Lick Score analysis.

An average total intake of 0.3 M sucrose at each time point was calculated for each rat for the two peptide sessions and four vehicle sessions, and also for the two antagonist challenge sessions and their corresponding four vehicle sessions. The average intake of each peptide group was compared to their own intake during vehicle conditions at each time point by one-way ANOVA.

RESULTS

Experiment 1: Effect of RYGB and pharmacological GLP-1 receptor manipulations on brief access responding to sucrose and water.

Body weight change after RYGB.
After RYGB, the rats lost an average of 67 g during the 10-20 day recovery period prior to the start of postsurgical experimental procedures, whereas sham-operated rats gained an average of 33 g (Figure 2). By the end of the experiment, SHAM rats weighed ~220 g more than RYGB rats. No other measures of adiposity were obtained.

*Brief access responding to water and sucrose before and after RYGB.*

Prior to surgery, no differences between the rats identified for RYGB or SHAM operations were seen in licks of water taken during sessions in which water was presented continuously or licks of sucrose taken relative to water in sessions during which the sucrose concentration series was presented in 10-s trials (i.e., Lick Scores; Figure 3a). There were also no differences postsurgically when the rats were tested while water-deprived (Table 3) or while fasted (Table 4, Figure 3c). However, when tested while nondeprived, rats with RYGB had overall higher Lick Scores than SHAM rats (Table 4, Figure 3e). RYGB rats also initiated over 3 times as many trials as SHAM rats when tested nondeprived (F(1,12)=40.752, p<0.001; Figure 3f) and 2.5 times as many when tested while fasted (F(1,12)=29.04, p<0.001; Figure 3d). This suggests that in conditions during which taste would be the main factor motivating licking, our model of RYGB increased consummatory behavior to sucrose during brief access tests in rats. In addition, the difference in trials initiated between the groups implies that, independent of food-deprivation state, our model of RYGB increased appetitive behavior toward sucrose in a brief access test in rats.

*Brief access responding to water and sucrose after RYGB and GLP-1 receptor peptides.*

There was no effect of surgery on licks of water taken (F(1,11)=0.009, p=0.928; Table 5) during water testing while the RYGB and SHAM rats were water-deprived and injected with Ex9, Ex4, and vehicle, nor was there a surgery x injection interaction (F(2,22)=2.489, p=0.106). There was, however, a significant main effect of the pharmacological GLP-1 receptor
manipulations on licks of water taken ($F(2,22)=37.234$, $p<0.001$). Post hoc testing revealed an overall effect of Ex4 relative to vehicle on licks of water taken ($F(1,12)=47.817$, $p<0.001$), but no overall effect of Ex9 ($F(1,12)=1.305$, $p=0.552$). Similarly, no effect of surgery was seen on the average ILI ($F(1,11)=0.557$, $p=0.471$; Table 5), nor was there a surgery x injection interaction ($F(2,22)=0.259$, $p=0.774$). There was, however, a significant main effect of the pharmacological GLP-1 receptor manipulations on ILI ($F(2,22)=4.652$, $p=0.021$), but no overall effect of Ex4 ($F(1,12)=5.306$, $p=0.08$) or Ex9 ($F(1,12)=0.487$, $p=0.998$) was revealed when each peptide was compared individually with vehicle. Thus, while administration of a potent GLP-1 receptor agonist, at a dose that has been shown to decrease food intake, decreased water intake, this decrease was not seen in RYGB rats whose endogenous levels of GLP-1 presumably increased after surgery; likewise, the dose used of the GLP-1 receptor antagonist Ex9 had no effect on water-licking by RYGB rats, which performed similarly to SHAM rats.

There was no significant effect of surgery on sucrose Lick Scores after pharmacological GLP-1 receptor manipulations ($F(1,11)=0.783$, $p=0.395$; Figure 4a), nor did surgery interact with injection condition ($F(2,22)=0.444$, $p=0.647$), concentration ($F(5,55)=0.292$, $p=0.915$), or both ($F(10,110)=1.385$, $p=0.197$). There was, however, a main effect of injection condition ($F(2,22)=3.964$, $p=0.034$), but post hoc tests that compared each peptide to vehicle revealed no overall effect of either peptide (Ex4: $F(1,12)=4.413$, $p=0.114$; Ex9: $F(1,12)=0.063$, $p\geq0.99$) or significant interaction with concentration (Ex4: $F(5,60)=2.553$, $p=0.074$; Ex9: $F(5,60)=1.748$, $p=0.276$). As seen previously, RYGB rats initiated more trials to sucrose than did SHAM rats ($F(1,11)=41.621$, $p<0.001$), but neither a significant independent ($F(2,24)=3.191$, $p=0.059$) nor interactive effect ($F(2,24)=2.705$, $p=0.087$) of the peptides was revealed in our study (Figure 4b). These findings suggest that additional GLP-1-receptor activity via pharmacological agonism with this dose of Ex4 does not significantly further increase consummatory licking or appetitive trial initiation in RYGB rats, nor does it recapitulate in SHAM rats the increase in
approach or lick responsiveness toward sucrose seen in RYGB rats when tested nondeprived. Likewise, the findings also suggest that pharmacological antagonism of GLP-1 receptors in rats after RYGB was not sufficient to reduce the number of trials initiated to the level seen in SHAM rats. Because the increased consummatory behavior that was seen when RYGB rats were tested when nondeprived was not observed under vehicle conditions, it could not be tested if Ex9 would block this effect. It should be noted, though, that because food had been removed at the time of injection 5 h prior to testing, the rats were not in a completely nondeprived state during the tests. Although we expect that the impact of this short period of lack of food access was minimal because it occurred during the light phase, which is when food intake is normally lower compared with the dark phase, this difference from the original nondeprived testing protocol, along with potential stress caused by the injection, does not allow direct comparison of these two tests.

Experiment 2: Effect of RYGB on brief access responding to sucrose and water in rats without presurgical sucrose experience.

Body weight change.

Rats after RYGB lost an average of 111 g during the 10-20 day recovery period prior to the start of postsurgical experimental procedures, whereas sham-operated rats gained, on average, 24 g during recovery (Figure 5). By the end of the experiment, SHAM rats weighed ~190 g more than RYGB rats. No other measures of adiposity were obtained.

Brief access responding to water and sucrose after RYGB.

After RYGB, rats that had no presurgical brief access or sucrose experience did not differ in a statistically significant way from SHAM rats in terms of licks of water taken or ILI
during training while rats were water-deprived (Table 3), nor were any significant effects of surgery seen on Lick Scores to sucrose either when rats were tested while fasted or while nondeprived (Table 4; Figures 6a, 6c). Similar to Experiment 1, however, in either deprivation state, RYGB rats took significantly more trials than SHAM rats (fasted: $F(1,13)=17.693$, $p=0.001$; nondeprived: $F(1,9)=55.269$, $p<0.001$; Figure 6b, 6d). Thus, as in Experiment 1, the rats did not decrease their lick responsiveness to sucrose even though they had no presurgical experience with the taste stimulus. We did not, however, replicate the increased lick responsiveness after RYGB observed in Experiment 1 when rats were tested while nondeprived, so this phenomenon could either be contingent on prior sucrose exposure or may not be particularly robust. We did replicate, however, in both states, that RYGB increased appetitive taste-guided behavior in that RYGB rats with no presurgical sucrose experience took about three times as many trials as SHAM rats when tested while nondeprived and almost twice as many trials when tested while fasted.

**Experiment 3: Assessment of a shorter time-course of pharmacological GLP-1 receptor manipulations on brief access responding to and intake of sucrose in unoperated rats.**

*Brief access responding to sucrose 15 min after injection of Ex4 or Ex9.*

Ex4 decreased the number of licks of water taken by water-restricted rats when water was presented continuously, which is similar to its effect when injected 5 h prior to the test (Table 6). Further, Ex4 decreased the number of trials initiated and the average number of licks per trial taken by water-restricted rats when water was presented in trials (Table 6). The peptide also increased ILI (Table 6), suggesting that increasing GLP-1 receptor activity may directly or indirectly influence activity in the putative central-pattern generator (see 64, 71) in addition to decreasing water intake. Ex9 did not affect licks of water taken, ILI, or average licks taken per
trial. It did, however, significantly decrease the number of water trials taken by water-restricted rats, but the effect was relatively minor. This suggests that decreasing GLP-1 receptor activity via pharmacological antagonism has little effect on water intake under our test conditions.

Neither Ex4 nor Ex9, at the doses used, had an effect on concentration-dependent responding to sucrose (Table 7, Figures 7 and 8) when the rats were tested while fasted. Although Ex9 significantly increased overall responsiveness to sucrose while rats were nondeprived, the magnitude of this effect was clearly small (Figure 7c). Ex4 had no effect on sucrose licking when the rats were tested while either fasted or nondeprived (Figure 8a and 8c), but the peptide did decrease trials taken when the rats were tested while nondeprived (F(1,7)=9.299, p=0.019; Figure 8d) but not when they were tested while fasted (F(1,7)=0.478, p=0.512; Figure 8b). Ex9 had no significant effect on trials initiated when rats were tested either fasted (F(1,7)=0.062, p=0.811, Figure 7b) or nondeprived (F(1,7)=4.355, p=0.075; Figure 7d).

Although the peptides had some effects on lick responsiveness and trial initiation to sucrose when injected 15 min rather than 5 h prior to testing, they did not mimic the effects seen by RYGB, especially with respect to trial initiation.

*Intake of 0.3 M sucrose after GLP-1 receptor peptides.*

Ex4 significantly decreased intake of 0.3 M sucrose in fasted rats 30 min and 1 h after presentation (Table 8). Ex9 had no effect on sucrose intake at either time point (Table 8). Ex9 when injected 15 min prior to Ex4 did not completely block the hypophagic effect of Ex4 (Table 8), but the percent reduction in sucrose intake by rats when injected with Ex4 alone compared to vehicle was cut roughly by half by the antagonist challenge compared to vehicle at both 30 min (F(1,7)=8.942, p=0.02) and 1 h (F(1,7)=8.912, p=0.02) (Figure 9). Thus, we were able to demonstrate that our preparation of the peptides affected sucrose intake during the same time frame in which brief access testing occurred. Furthermore, we showed that injection of Ex9,
prior to Ex4 administration, decreased the agonist’s hypophagic effect, indicating that the impact of Ex4 on sucrose intake was mediated at least in part by a GLP-1 receptor-specific action.

DISCUSSION

Effects of RYGB on responsiveness to sucrose in a brief access test.

To our surprise and in contrast to three reports in the literature, which used a variety of rat models of RYGB (21, 46, 62), in two experiments using our model of RYGB in rats that did not have high fat diets at any time, we did not see an attenuation of licking responses to a concentration series of sucrose solutions in a brief access taste test. If anything, in our hands, RYGB increased sucrose licking overall across concentrations during trials when the rats that had been tested with sucrose presurgically were tested postsurgically while nondeprived. Collectively these data suggest that our model of RYGB in rats that were chow fed promotes little change in consummatory responses of rats for sucrose, at least in our version of the short-term brief access taste test. This type of test is designed to limit the ability of an animal to associate postingestive events arising from the mixing of ingested fluids with a specific concentration during a trial because of the very limited duration of access, which is on the order of seconds, and the randomized nature of stimulus presentation. Consummatory behavior is defined as the final act of the appetitive chain that is typically stimulated by contact of the goal object with receptors. In the brief access test, upon initiation of the trial with the first lick, the taste stimulus comes into direct contact with orosensory receptors, triggering further oromotor responses. Accordingly, once a very brief trial is engaged, the lick rate (e.g., licks produced in the 10 s trial) can be considered to be largely driven by consummatory processes and reflects the relative affective value of the solution. In general, the concentration-response function was unaltered by RYGB, with the exception of a 0.28 log_{10} leftward shift relative to sham-operated
controls in Experiment 1 when animals were tested in a nondeprived state. Thus, in our hands, when the surgery had an effect on consummatory responsiveness to sucrose, it was an increase, and the expression of this change was not consistently observed throughout all testing conditions.

This is not to say that there was only a minor effect of the RYGB surgery on motivated behavior toward sucrose in the brief access test. We observed, on average, a 3-fold increase in the number of trials initiated after RYGB independent of the deprivation state in which the rats were tested or whether the rats were given presurgical sucrose experience. An increase in the total number of trials initiated is indicative of an RYGB-induced facilitation of overall appetitive behavior, which is defined as behavior that brings the animal to the goal object (e.g., foraging, approaching a drinking spout). The increase in the number of trials initiated coupled with either a lack of change or even an increase in concentration-dependent licking by rats after RYGB means that these rats actually ingested more sucrose than the SHAM controls under these test conditions. For example, when the rats in Experiment 1 were tested while nondeprived, we estimated from lick data that the RYGB rats drank, on average, ~13 ml across all concentrations including water, amounting to ~6 kcal, while SHAM rats drank ~3 ml or ~2 kcal.

Our results differ with those of several recent studies (21, 46, 61), which, while employing a variety of models of RYGB, all showed that, after RYGB, rats licked less to high concentrations of sucrose. A close comparison of our work with these other reports suggests that differences in the diet used before and after surgery, the level of adiposity and metabolic state of the animals, the exact nature of the surgical intervention, and/or the behavioral methodology employed may be at the root of the disparity in the results. Our rats received normal chow at all times and were not exposed to high fat chow. Moreover the surgical procedure for producing RYGB differs from those used in all of the other studies in how much stomach remains contiguous with the jejunum in the alimentary limb. In our rat model of RYGB,
a very small gastric pouch, consisting of <5% of the original stomach volume, is fashioned (Figure 1); in other models, 10-20% of the stomach remains. Thus, in our preparation, food moves directly into the jejunum rather than being diluted in the pouch by other foods and fluids and then slowly transported into the jejunum (e.g., 57). These differences in the restrictive nature of the stomach remnant have in some cases been shown to affect weight loss in humans (42, 44; but see 11, 40, 63), and the transit time to the intestine may impact ingestive bouts of sucrose by rats. It should be noted, though, that the type of gastric tissue (e.g., muscular, glandular) that remains in contact with food in the surgical preparations differs anatomically between the rat and human stomach (24), and the effect of these differences on food intake has not, to our knowledge, been explored. Finally, while less interference with the stomach would presumably cause less damage to the vagus nerve, the extent to which vagal afferents are damaged by these various procedures remains to be explicitly tested (11).

Although differences in the geometry of the gut remodeling could be contributing to the lack of consistency between our results and those of others, an equally possible source is some fundamental variations in the design of the brief access test itself. For example, in Experiment 1, our animals had three presurgical test sessions allowing us to measure baseline performance and counterbalance the groups while familiarizing the rats with the sucrose stimuli and their availability from the test apparatus. Although in all of the other studies the animals had presurgical exposure to solid or liquid diets that consisted of a higher percentage of sugar than chow, in none of these studies were the rats tested presurgically, and thus they were not explicitly exposed to the procedure and sucrose solutions before postsurgical testing. Because there is evidence that presurgical 2-bottle preference testing with a series of sucrose concentrations can significantly attenuate the effectiveness of RYGB to decrease sucrose preference (13), we tested rats after RYGB that were not presurgically exposed to sucrose (Experiment 2). Once again we saw no evidence of decreased consummatory responsiveness
to sucrose after RYGB and we replicated the RYGB-induced increase in appetitive behavior seen previously in the form of increased trials.

The procedural parameters of our brief access test differed from those used by others (21, 46, 62) in that animals could initiate as many trials as possible, presented in randomized blocks, during the 30 min session. We chose to do this because: a) it increased the reliability of our estimate of consummatory responding, b) it provided an assessment of the effect of RYGB on appetitive behavior, and c) this procedure has been shown to be sensitive to manipulations of the gustatory system (47, 52-54, 64). Tichansky et al. (62) also allowed rats to initiate as many trials as possible in 30 min, while Shin et al. (46) presented two series of ascending concentrations and Hajnal et al. (21) limited presentations to 5 or 6 at each concentration. Nevertheless, the fact that in our study licking increased monotonically as concentration was raised suggests that, unlike short-term single-bottle intake tests which generally produce an inverted-U concentration-response function, satiation did not influence relative responding to the various concentrations.

Our brief access procedure also required that the rats sample the taste stimulus before another one was presented; as such, a measure of consummatory responsiveness was made every time a stimulus was presented. In the Hajnal et al. (21) and Shin et al. (46) studies, each concentration was present for 10 sec, during which the animals could lick or not, and then the next concentration was moved into place. Although this standardizes the amount of access time that each rat has to each stimulus, it also means that a rat could have trials in which the stimulus was never sampled or sampled late in the access period, which could have had a disproportionate effect on the nature of the curves, especially considering the limited number of trials during which each concentration was presented and that the animals were receiving pure sucrose solution for the first time ever. Tichansky et al. (62) also used a limited hold at which point a new concentration was presented, although it was set at 120 sec.
The final major procedural consideration deals with the relative body weight and adiposity, as well as the resulting metabolic state, between groups of animals at the time of surgery and testing. Interestingly, Hajnal et al. (21) found that RYGB-operated chow-fed OLETF rats licked high concentrations of sucrose significantly less than sham-operated OLETF rats, but that there was no such effect of RYGB observed in Long Evans Tokashima Otsuka (LETO) rats compared with their sham-operated controls, which are leaner than OLETF rats. Such an outcome supports the possibility that body composition at the time of surgery might be a critical factor in whether RYGB affects consummatory responsiveness to sucrose. Because we did not measure body composition or metabolic parameters such as leptin levels or glucose tolerance, we cannot conclude that our animals had adiposity levels similar to those of Hajnal et al. (21), Shin et al., (46), or Tichansky et al. (62), and so the role of this factor in RYGB-induced effects on taste-guided behavior in our model remains to be established. Until recently, RYGB has only been carried out in humans with a body mass index (BMI) greater than or equal to 35 (38), but new initiatives have been taken to perform the surgery in individuals who are not categorized as obese (e.g., BMI=27; BMI>30 is considered obese) with the specific intent to treat Type 2 diabetes (25). It would be interesting to see if differences in taste perceptions would differ after surgery between these groups of bariatric candidates.

Whether some or all of the parametric differences in the designs of these experiments involving the use of the brief access test led to the differences between our results and those of these other studies remains to be resolved. Nevertheless, it must be emphasized that RYGB surgery in rats clearly does not lead unequivocally to a decrease in motivated licking of high sucrose concentrations. Indeed, under our conditions, appetitive responses in the form of trial initiation were remarkably augmented. This latter result receives some support from a linear runway experiment conducted by Shin et al. (46) in which, during the initial testing trials, high-fat diet-fed obese rats that received RYGB and chow-fed leaner sham-operated controls reached the goal box significantly sooner than high-fat diet-fed obese sham-operated rats when access
to a small amount (2 g) of Fruit Loops (a sweet tasting cereal to humans) was used as the reinforcer. Although this result could in part be explained by the large body weight difference between the high-fat diet-fed obese sham-operated rats and the leaner RYGB and chow-fed sham-operated groups creating differences in the work demand of the task, the finding that an appetitive response toward a putatively sweet-tasting stimulus appears to be enhanced by RYGB is nonetheless in agreement with the RYGB-induced increase in the number of sucrose trials initiated in our study. Therefore, it appears that the ingestive response to sucrose, and perhaps sweet-tasting stimuli in general, is not universally decreased by RYGB, but, rather, may vary as a function of the parameters of the surgical manipulation itself, the environmental context (i.e., test conditions) in which the sucrose is offered, and/or the level of adiposity and metabolic state of the animal at the time of surgery and testing.

The effects of GLP-1 receptor modulation on responsiveness to sucrose in the brief access test

Because postprandial GLP-1 levels are elevated in humans after RYGB (e.g., 6, 30, 32, 33) and rats show increased baseline and postprandial levels of the peptide after similar surgery (e.g., 10, 11, 31), we reasoned that SHAM rats injected with the GLP-1 receptor agonist Ex4 might take as many trials of sucrose as RYGB rats, and that RYGB rats injected with Ex9 might reduce the number of trials that they initiate to the level of SHAM controls. However, injection of Ex4 and Ex9, at doses and time courses that have been shown to affect food and sucrose intake (72-74, unpublished data), did not affect sucrose trial initiation of RYGB or SHAM rats in our version of the brief access test to the degree that we saw with the surgical manipulations alone. We also hypothesized that injection of Ex4 might increase Lick Scores in SHAM rats and that Ex9 injection might decrease it in both groups, similar to that seen in GLP-1 receptor
knockout mice (47), but we saw no significant effect relative to vehicle of either peptide when injected 5 h prior to tests.

Although the 5-h delay between injection and test was chosen on the basis of the effectiveness of these peptides to influence food intake (i.e., 73, 74, unpublished data), it is possible that their time course of action on gustatory processes is more rapid. Accordingly, in intact rats, we examined the effects of these peptides on responding to sucrose in the brief access test when they were injected 15 min prior to the start of the test during both fasted and nondeprived states. As when injecting RYGB- and sham-operated rats with Ex4 and Ex9, we hypothesized that Ex4 would increase trials and possibly Lick Score, and that Ex9 would decrease Lick Score and possibly decrease trials. In contrast to our expectations, under our testing conditions, Ex9 resulted in a slight, albeit statistically significant, increase in lick rate for sucrose when the rats were tested while nondeprived with no effect on trials. This is different from findings using GLP-1 receptor knock-out mice (47), but it should be noted that a lower concentration series of sucrose was presented to the knockout mice than what was used in our study (0.001-0.1 M compared to 0.01-1.0 M), and, at 0.4 M sucrose, lick responses of GLP-1 receptor knockout mice were not significantly different from those observed in wild-type mice. Ex4 caused a significant drop in the number of trials initiated, but only when the rats were tested while nondeprived, and had no effect on Lick Score. To functionally confirm the potency of the peptides, we injected rats with Ex4 15 min prior to a 1-bottle 0.3 M sucrose intake test and showed that it decreased their intake by more than 40%, and that this effect was partially blocked by administration of Ex9 15 min prior (similar to 67). These results suggest that exogenous modulation of GLP-1 receptor signaling via the peptide doses and time courses used here has little effect on brief access responding for sucrose, and that GLP-1 receptor activity is not sufficient to explain the effects of RYGB during our tests.
One of the most interesting effects of pharmacological GLP-1 receptor modulation that we observed was that Ex4 injected 15 min prior to testing increased the time between lick onsets (i.e., increased ILI). Although the change, approximately 20 ms, would only account for a difference of 7 licks from 62 in a 10-s trial in which the rat was licking without pause, it could have a greater impact over a longer time scale of ingestion. The effect of a variety of age, environmental, and surgical manipulations on ILI is typically mild (e.g., 23, 36, 53, 56, 70); changes seen occasionally in this parameter are rarely of the magnitude seen here and typically occur with drugs that produce overt motor disturbances (e.g., 19, 20, 34), which were not observed in this study with Ex4. That the ILI was affected to such a notable degree by Ex4 suggests that GLP-1 receptor activation might directly or indirectly act on premotor or motor neurons in the brainstem that control oromotor function (see 64, 71). This interpretation is also consistent with a study by Aja et al. (1) in which the ILI-increasing effect of central administration of cocaine-and-amphetamine-related transcript (CART) was blocked by central administration of Ex9.

**Perspectives**

In these studies, rats that were only exposed to normal chow diets and given our model of RYGB increased appetitive behavior and, under some conditions, increased consummatory behavior toward a concentration series of sucrose solutions. Our results in rats seem to be at odds with reports of decreased preference for sweet-tasting foods and beverages in human bypass patients. As noted in the literature, however, there are reasons other than potential changes in the sensory nature or unconditioned hedonic value of sweets as to why patients would avoid some such foods and fluids (e.g., learning from postingestive consequences, nutritional counseling). Moreover, although patients eat fewer total calories after RYGB, their
selection from the macronutrient categories either remains stable or returns to preoperative levels within 9 months (4, 7, 9, 16, 26, 29, 66). Because in virtually all studies intake is assessed through dietary recall in RYGB patients, it remains to be directly established if the macronutrient intake or actual food selections within macronutrient categories are altered after surgery. It would be useful to complement the measures currently used to assess food preference and intake in RYGB patients with others that quantify actual food choices and ingestive behavior itself to provide a full characterization of the nature of surgically-induced changes in feeding (e.g., 18, 28), as well as provide an objective bridge to behavioral findings from parallel experiments in animal models.
Acknowledgments

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References


Figure captions

Figure 1. Schematic illustration of the small bowel anatomy before (A) and after (B) RYGB operation.

Figure 2. Mean (+ SE) absolute body weight of SHAM and RYGB rats (n=7 per surgical group) at receipt and through acclimation (RECEIPT-ACCLIM), during presurgical training and testing (PRE TEST), the first 10 days after surgery (RECOV), and throughout postsurgical testing (POST TEST). Slight changes in weight during testing can be attributed to food and water manipulations, which are noted on Table 1.

Figure 3. Mean (+ SE) Lick Scores and number of trials initiated to sucrose during brief access tests of rats when tested presurgically while fasted (A, B), when tested postsurgically while fasted (C, D), and when tested postsurgically while nondeprived (E, F). Prior to surgery, rats assigned to SHAM and RYGB groups (n=7 per group) did not significantly differ in Lick Scores or trials initiated. After surgery and when tested while fasted, RYGB rats took significantly more trials than SHAM rats, but RYGB rats did not have Lick Scores different than that of SHAM rats (n=6/7). When tested while nondeprived, RYGB rats had higher overall Lick Scores to sucrose than SHAM rats (n=6/7), as well as taking more trials.

Figure 4. Mean (+ SE) Lick Scores of and number of trials initiated to sucrose by rats given RYGB or SHAM surgery (n=7 per group) and injected ip on separate brief access testing series with the GLP-1 receptor antagonist exendin-3(9=39) (Ex9; 30 µg/kg), agonist exendin-4 (Ex4; 1
µg/kg), and vehicle (Veh; 1 ml/kg) 5 h prior to the test session. Food was removed from the rats at the time of injection. There were no differences in Lick Scores between SHAM rats (n=6/7) and RYGB rats, no significant effect of either peptide relative to vehicle, and no significant interaction of the peptides with surgical condition overall or at any concentration (A). RYGB rats initiated more trials than SHAM rats, and the peptides had no independent or interactive impact on this effect (B).

Figure 5. Mean (+ SE) absolute body weight of SHAM rats (n=9) and RYGB rats (n=4-6) at receipt and during acclimation, for the first 10 days after surgery (RECOVERY), and throughout postsurgical testing. Slight changes in weight during testing can be attributed to food and water manipulations, which are noted on Table 1.

Figure 6. Mean (+ SE) Lick Scores and number of trials initiated to sucrose during brief access tests of rats when tested postsurgically while fasted (A, B) and when tested postsurgically while nondeprived (C, D). After surgery and when tested while fasted, RYGB rats (n=6) took significantly more trials than SHAM rats (n=9), but RYGB rats did not have Lick Scores different from that of SHAM rats. When tested while nondeprived, RYGB rats (n=4) took more trials than SHAM rats (n=8/9), but did not have statistically different Lick Scores.

Figure 7. Mean (+ SE) Lick Scores and number of trials initiated to sucrose of unoperated rats injected ip with 30 µg/kg Ex9 and vehicle 15 min prior to separate brief access sessions while either fasted (n=8; A, B) or nondeprived (n=4/8; C, D). Ex9 slightly but significantly increased overall sucrose Lick Scores of nondeprived rats.
Figure 8. Mean (+ SE) Lick Scores and number of trials initiated to sucrose of unoperated rats injected ip with 1 µg/kg Ex4 and vehicle 15 min prior to separate brief access sessions while either fasted (n=8; A, B) or nondeprived (n=5/8; C, D). Ex4 decreased trials initiated to sucrose by nondeprived rats.

Figure 9. Mean (+ SE) difference in 0.3 M sucrose intake expressed as a percent from vehicle (raw data presented in Table 8) by fasted rats (n=8 per group) injected ip with either Ex9 or Ex4 15 min prior to access during a 1-bottle home-cage test; the rats injected with Ex4 were also on a separate occasion injected with Ex9, 15 min later injected with Ex4, and then 15 min later given 0.3 M sucrose access. Ex4 alone significantly decreased 0.3 M sucrose intake at 0.5 and 1 h after sucrose presentation. Ex9 when injected 15 min prior to Ex4 significantly decreased this effect.
Table 1: Training and testing protocol for experiments 1 and 2.

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<td>GLP-1 receptor manipulations</td>
<td></td>
<td>Sucrose</td>
<td>2</td>
<td>Ex9</td>
<td>35, 37 or 36, 38</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sucrose</td>
<td>2</td>
<td>Ex4</td>
<td>39, 41 or 40, 42</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sucrose</td>
<td>2</td>
<td>Vehicle</td>
<td>43, 45 or 44, 46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water-restricted 6</td>
<td>Water: stationary</td>
<td>1</td>
<td>Vehicle</td>
<td>49 or 50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water: stationary</td>
<td>1</td>
<td>Ex9</td>
<td>51 or 52</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water: stationary</td>
<td>1</td>
<td>Ex4</td>
<td>53 or 54</td>
<td></td>
</tr>
</tbody>
</table>

1 Presented in 30-min sessions in a Davis rig lickometer.
2 Injections were performed 5 h prior to session.
3 Due to different postoperative recovery periods for each rat, Day 0 reflects the start of postsurgical testing and lower numbers are counted backward from Day 10 of recovery after surgery (See Figures 2 and 5).
4 Only performed in Experiment 1.
5 Food or water removed from cage ~23 h prior to session.
6 Food was removed at time of injection.
Table 2: Experiment 3 protocol for training and testing.

<table>
<thead>
<tr>
<th>Deprivation state</th>
<th>Test procedure</th>
<th>Stimuli</th>
<th>Injection</th>
<th># of test sessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-restricted</td>
<td>1-bottle intake in Davis rig</td>
<td>Water</td>
<td>None</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vehicle</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exendin</td>
<td>1</td>
</tr>
<tr>
<td>Brief access trials in Davis rig</td>
<td>Water</td>
<td>None</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vehicle</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exendin</td>
<td>1</td>
</tr>
<tr>
<td>Sucrose array</td>
<td>None</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted</td>
<td>Brief access trials in Davis rig</td>
<td>Sucrose array</td>
<td>Vehicle</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exendin</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vehicle</td>
<td>2</td>
</tr>
<tr>
<td>Nondeprived</td>
<td>Brief access trials in Davis rig</td>
<td>Sucrose array</td>
<td>Vehicle</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exendin</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vehicle</td>
<td>2</td>
</tr>
<tr>
<td>Fasted</td>
<td>1-bottle intake in home cage</td>
<td>0.3 M Sucrose</td>
<td>Vehicle</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exendin</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vehicle</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Veh + Veh</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ex9 + Ex4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Veh + Veh</td>
<td>2</td>
</tr>
</tbody>
</table>

1 One group (n=8) received only Ex4 and the other (n=8) Ex9, and both groups were injected with vehicle; injections were administered 15 min prior to the start of the session.
2 Food or water removed from cage ~23 h prior to session.
3 Vehicle or Ex9 was injected 15 min prior to vehicle or Ex4 injection. Only the group of rats that were previously injected with Ex4 was used in this phase.
Table 3: Mean ± SE and statistical comparison of licks and interlick interval (ILI) of RYGB and SHAM rats to water during postsurgical testing in experiments 1 and 2.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Measure</th>
<th>RYGB Mean (± SE)</th>
<th>SHAM Mean (± SE)</th>
<th>1-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Licks</td>
<td>2926.2 ± 436.3</td>
<td>3370.4 ± 188.5</td>
<td>F(1,11)=0.974, p=0.345</td>
</tr>
<tr>
<td></td>
<td>ILI</td>
<td>160.8 ± 5.4</td>
<td>163.4 ± 5.5</td>
<td>F(1,11)=0.120, p=0.736</td>
</tr>
<tr>
<td>2</td>
<td>Licks</td>
<td>2742.4 ± 169.3</td>
<td>3026.8 ± 202.1</td>
<td>F(1,15)=1.131, p=0.304</td>
</tr>
<tr>
<td></td>
<td>ILI</td>
<td>163.3 ± 4.9</td>
<td>158.5 ± 4.2</td>
<td>F(1,15)=0.573, p=0.461</td>
</tr>
</tbody>
</table>
Table 4. Two-way repeated measures ANOVA values of sucrose Lick Scores comparisons in experiments 1 and 2.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Condition</th>
<th>Surgery</th>
<th>Concentration</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Presurgery:</td>
<td>F(1,11)=0.208,</td>
<td>F(5,55)=158.721,</td>
<td>F(5,55)=0.459,</td>
</tr>
<tr>
<td></td>
<td>fasted</td>
<td>p=0.657</td>
<td>p&lt;0.001</td>
<td>p=0.805</td>
</tr>
<tr>
<td></td>
<td>Postsurgery:</td>
<td>F(1,11)=0.251,</td>
<td>F(5,55)=102.829,</td>
<td>F(5,55)=1.393,</td>
</tr>
<tr>
<td></td>
<td>fasted</td>
<td>p=0.627</td>
<td>p&lt;0.001</td>
<td>p=0.241</td>
</tr>
<tr>
<td></td>
<td>Postsurgery:</td>
<td>F(1,11)=5.462,</td>
<td>F(5,55)=81.178,</td>
<td>F(5,55)=1.367,</td>
</tr>
<tr>
<td></td>
<td>nondeprived</td>
<td>p=0.039</td>
<td>p&lt;0.001</td>
<td>p=0.251</td>
</tr>
<tr>
<td>2</td>
<td>Postsurgery:</td>
<td>F(1,13)=0.021,</td>
<td>F(5,65)=95.859,</td>
<td>F(5,65)=0.832,</td>
</tr>
<tr>
<td></td>
<td>fasted</td>
<td>p=0.887</td>
<td>p&lt;0.001</td>
<td>p=0.532</td>
</tr>
<tr>
<td></td>
<td>Postsurgery:</td>
<td>F(1,7)=0.06,</td>
<td>F(5,35)=37.673,</td>
<td>F(5,35)=1.234,</td>
</tr>
<tr>
<td></td>
<td>nondeprived</td>
<td>p=0.813</td>
<td>p&lt;0.001</td>
<td>p=0.314</td>
</tr>
</tbody>
</table>
Table 5. Mean ± SE licks and interlick interval (ILI) of RYGB and SHAM rats to water during stationary water testing following pharmacological GLP-1 receptor manipulations.

<table>
<thead>
<tr>
<th>Surgery</th>
<th>Injection condition</th>
<th>ILI</th>
<th>Total Licks</th>
</tr>
</thead>
<tbody>
<tr>
<td>RYGB</td>
<td>Vehicle</td>
<td>166.4 ± 5.3</td>
<td>2309.3 ± 200.9</td>
</tr>
<tr>
<td></td>
<td>Exendin-3(9-39)</td>
<td>166.5 ± 7.8</td>
<td>2294.0 ± 338.0</td>
</tr>
<tr>
<td></td>
<td>Exendin-4</td>
<td>177.5 ± 6.7</td>
<td>656.1 ± 261.1</td>
</tr>
<tr>
<td>SHAM</td>
<td>Vehicle</td>
<td>159.2 ± 2.5</td>
<td>2621.2 ± 379.6</td>
</tr>
<tr>
<td></td>
<td>Exendin-3(9-39)</td>
<td>161.6 ± 3.2</td>
<td>2552.8 ± 456.6</td>
</tr>
<tr>
<td></td>
<td>Exendin-4</td>
<td>165.5 ± 3.6</td>
<td>1308.5 ± 523.9</td>
</tr>
</tbody>
</table>
Table 6. Licks, interlick interval (ILI), and trials taken to water by unoperated rats after pharmacological GLP-1 receptor manipulations (Ex4=exendin-4; Ex9=exendin-3(9-39)).

<table>
<thead>
<tr>
<th>Group</th>
<th>Measure</th>
<th>Total Licks</th>
<th>ILI</th>
<th>Number of trials</th>
<th>Licks per trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex4</td>
<td>Veh: Mean + SE</td>
<td>3201.4 ± 253.5</td>
<td>159.7 ± 3.5</td>
<td>52.8 ± 3.7</td>
<td>59.0 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Ex4: Mean + SE</td>
<td>2114.6 ± 308.1</td>
<td>178.7 ± 8.0</td>
<td>33.6 ± 4.5</td>
<td>49.1 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>ANOVA</td>
<td>F(1,7)=12.629, p=0.009</td>
<td>F(1,7)=7.678, p=0.028</td>
<td>F(1,7)=10.393, p=0.015</td>
<td>F(1,7)=8.887, p=0.020</td>
</tr>
<tr>
<td>Ex9</td>
<td>Veh: Mean + SE</td>
<td>3220.5 ± 174.3</td>
<td>159.3 ± 3.4</td>
<td>53.0 ± 2.4</td>
<td>59.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Ex9: Mean + SE</td>
<td>3137.4 ± 135.4</td>
<td>159.6 ± 3.8</td>
<td>44.9 ± 1.7</td>
<td>59.7 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>ANOVA</td>
<td>F(1,7)=0.147, p=0.712</td>
<td>F(1,7)=0.053, p=0.825</td>
<td>F(1,7)=6.342, p=0.040</td>
<td>F(1,7)=0.371, p=0.561</td>
</tr>
</tbody>
</table>
Table 7. Comparison of Lick Scores during brief access testing with sucrose of rats 15 min after pharmacological GLP-1 receptor modulation (Ex4=exendin-4; Ex9=exendin-3(9-39)).

<table>
<thead>
<tr>
<th>Deprivation State</th>
<th>Group</th>
<th>Injection</th>
<th>Concentration</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted</td>
<td>Ex4</td>
<td>F(1,7)=0.402, p=0.546</td>
<td>F(5,35)=239.941, p&lt;0.001</td>
<td>F(5,35)=0.428, p=0.826</td>
</tr>
<tr>
<td></td>
<td>Ex9</td>
<td>F(1,7)=0.502, p=0.501</td>
<td>F(5,35)=138.051, p&lt;0.001</td>
<td>F(5,35)=0.311, p=0.903</td>
</tr>
<tr>
<td>Nondeprived</td>
<td>Ex4</td>
<td>F(1,4)=1.337, p=0.312</td>
<td>F(5,20)=59.736, p&lt;0.001</td>
<td>F(5,20)=1.350, p=0.284</td>
</tr>
<tr>
<td></td>
<td>Ex9</td>
<td>F(1,3)=20.977, p=0.020</td>
<td>F(5,15)=65.251, p&lt;0.001</td>
<td>F(5,15)=0.946, p=0.480</td>
</tr>
</tbody>
</table>
Table 8. Analysis of home-cage 0.3 M sucrose intake of unoperated rats after administration of the GLP-1 receptor antagonist Exendin-3(9-39) (Ex9) or agonist Exendin-4 (Ex4) alone, or after Ex9 challenge of Ex4.

<table>
<thead>
<tr>
<th>Group</th>
<th>Measure</th>
<th>0.25 h</th>
<th>0.5 h</th>
<th>1 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Veh: Mean ± SE</td>
<td>NA</td>
<td>23.2 ± 1.7</td>
<td>26.3 ± 1.9</td>
</tr>
<tr>
<td>Ex4</td>
<td>Ex4: Mean ± SE</td>
<td>12.5 ± 2.1</td>
<td>16.1 ± 2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ANOVA</td>
<td></td>
<td>F(1,7)=49.206, p&lt;0.001</td>
<td>F(1,7)=49.560, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Veh: Mean ± SE</td>
<td>21.0 ± 1.5</td>
<td>21.6 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Ex9</td>
<td>Ex9: Mean ± SE</td>
<td>24.6 ± 1.2</td>
<td>27.2 ± 1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ANOVA</td>
<td></td>
<td>F(1,7)=0.107, p=0.753</td>
<td>F(1,7)=4.068, p=0.084</td>
</tr>
<tr>
<td></td>
<td>Veh + Veh: Mean ± SE</td>
<td>23.6 ± 1.6</td>
<td>29.2 ± 2.4</td>
<td>31.5 ± 2.6</td>
</tr>
<tr>
<td>Ex9 + Ex4</td>
<td>Ex9 + Ex4: Mean ± SE</td>
<td>18.1 ± 2.3</td>
<td>21.3 ± 3.3</td>
<td>25.1 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>ANOVA</td>
<td></td>
<td>F(1,7)=17.488, p=0.004</td>
<td>F(1,7)=44.842, p&lt;0.001</td>
</tr>
</tbody>
</table>
POSTSURGERY (NO PRESURGICAL EXPERIENCE): FASTED

A

LICK SCORE

RYGB
SHAM

SUCROSE CONCENTRATION (M)

0.01 0.1 1

B

TRIALS INITIATED

RYGB SHAM


POSTSURGERY (NO PRESURGICAL EXPERIENCE): NONDEPRIVED

C

LICK SCORE

RYGB
SHAM

SUCROSE CONCENTRATION (M)

0.01 0.1 1

D

TRIALS

RYGB SHAM