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Striatal Dopamine Homeostasis is Altered in Mice Following Roux-en-Y Gastric Bypass Surgery

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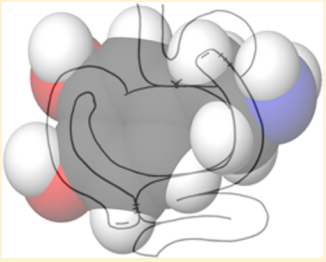
Supporting Information

ABSTRACT: Roux-en-Y gastric bypass (RYGB) is an effective treatment for obesity. Importantly, weight loss following RYGB is thought to result in part from changes in brain-mediated regulation of appetite and food intake. Dopamine (DA) within the dorsal striatum plays an important role in feeding behavior; we therefore hypothesized that RYGB alters DA homeostasis in this subcortical region. In the current study, obese RYGB-operated mice consumed significantly less of a high-fat diet, weighed less by the end of the study, and exhibited lower adiposity than obese sham-operated mice. Interestingly, both RYGB and caloric restriction (pair feeding) resulted in elevated DA and reduced norepinephrine (NE) tissue levels compared with ad libitum fed sham animals. Consequently, the ratio of NE to DA, a measure of DA turnover, was significantly reduced in both of these groups. The RYGB mice additionally exhibited a significant increase in phosphorylation of tyrosine hydroxylase at position Ser31, a key regulatory site of DA synthesis. This increase was associated with augmented expression of extracellular-signal-regulated kinases ERK1/2, the kinase targeting Ser31. Additionally, RYGB has been shown in animal models and humans to improve insulin sensitivity and glycemic control. Curiously, we noted a significant increase in the expression of insulin receptor-β in RYGB animals in striatum (a glucosensing brain region) compared to sham ad libitum fed mice. These data demonstrate that RYGB surgery is associated with altered monoamine homeostasis at the level of the dorsal striatum, thus providing a critical foundation for future studies exploring central mechanisms of weight loss in RYGB.



KEYWORDS: *RYGB, bypass, dopamine, obesity, brain, striatum, insulin*

Obesity is a growing health epidemic in both youth and adult populations. Over 15% of American children and over 30% of adults are obese.[1] The associated comorbidities of obesity, including type 2 diabetes and heart disease, are a major source of mortality.[2] Notably, bariatric surgery has been an effective means of weight loss for obese individuals who are able to undergo surgery.[3] Roux-en-Y gastric bypass (RYGB) is the most widely used bariatric surgical procedure performed in the United States, which results in sustained weight loss and improved metabolic parameters.[4,5] The procedure reroutes the upper stomach to a more distal portion of the small intestine (proximal jejunum), thus bypassing the major portion (~90%) of the distal stomach, the duodenum, and the proximal jejunum.[6]

A number of nonmutually exclusive mechanisms have been suggested to account for weight loss following RYGB. Ochner et al. (2011) estimated that restrictive effects of limiting stomach size and malabsorptive effects of bypassing a portion of the proximal gut account for approximately 55−80% of observed weight loss, while the additional weight loss must be explained by alternative mechanisms.[7] One of the more striking consequences of RYGB is a sustained loss of appetite associated with a reduction in food intake, likely mediated by adaptations within the central nervous system to postoperative changes in levels of circulating hormones, including insulin.[8−12] The brain plays an important role in regulating appetite and feeding, which requires the integration of information about nutritional requirements, energy stores, the availability and desirability of foods, and the motivation to work for palatable foods.[13−15] The latter may be particularly relevant to the etiology of the contemporary obesity epidemic in which the evolutionarily adaptive drive to consume energy-dense foods becomes maladaptive in the setting of abundance.

Functional brain imaging studies in humans have demonstrated significantly blunted MRI responses to the consumption of palatable food in obese individuals compared with healthy weight controls,[16] suggesting a model by which deficits in the experience of foods promotes compensatory overconsumption. Under this model, weight loss in RYGB might be explained, in

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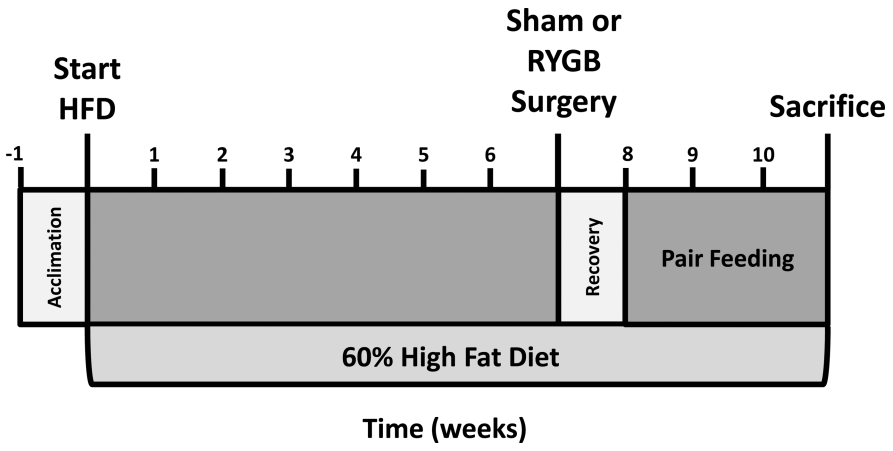

Figure 1. Study design. Mice were acclimated to the mouse metabolic phenotyping core facility for 1 week. They were then placed on a 60% high-fat diet for 7 weeks. At week 7, the mice underwent surgery, either RYGB or sham. They were given 1 week for recovery, after which they were singly housed and the sham group was split into pair-fed animals and ad-libitum-fed animals. At week 11 after starting the high fat diet, all mice were sacrificed and striatal tissue was collected for analysis (n = 6−8 per group).

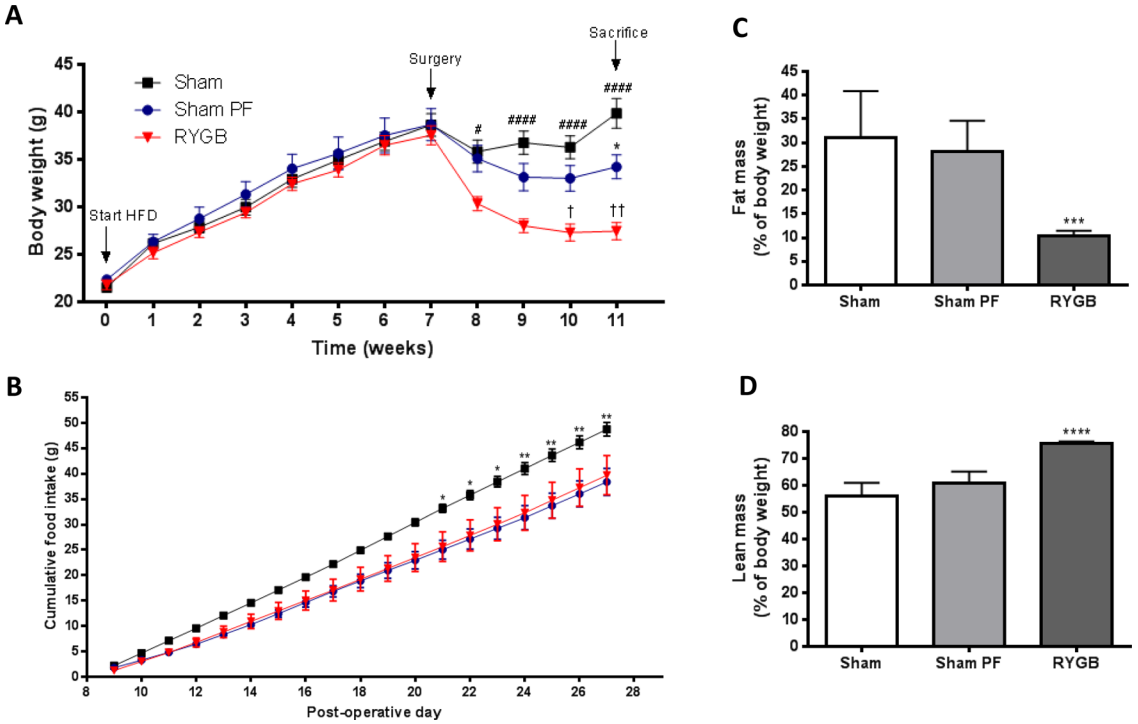

Figure 2. RYGB decreases body weight, food intake, and fat mass. (A) Body weights of sham, sham pair-fed, and RYGB mice over the course of the study. There were no significant differences in body weight prior to surgery. #Significant differences between sham and RYGB groups. *Significant differences between sham and sham pair-fed groups. †Significant differences between RYGB and sham pair-fed groups (n = 6−8). (B) Cumulative intake of a 60% high-fat diet was measured starting at day 9 (start of pair-feeding) through day 27 postsurgery. *Significant difference between the sham-operated ad-libitum-fed group and the RYGB-operated group (n = 5−8). (C) Fat mass and (D) lean mass in each of the surgical/feeding groups quantified as percent of total body weight at week 11 (n = 5−8). Together, fat mass and lean mass compose the entirety of body weight with the exception of free fluid mass. Significant differences were noted between the sham and RYGB groups for both fat (***p < 0.001) and lean mass (****p < 0.0001). Data are represented as mean ± SEM.

part, by amelioration of central dysfunction. The aim of the current study was to begin to define the central neurochemical and molecular candidates by which RYGB may regulate feeding behavior in the setting of obesity.

In this study, we focused on the nigrostriatal dopamine (DA) axis. This pathway has been strongly implicated in the motivation to seek food.[17,18] Importantly, impairments in striatal DA signaling have been repeatedly associated with obesity.[19−21] In this context, it is important to point out that circulating hormones which are altered in states of obesity and diabetes, such as insulin and glucagon-like peptide 1 (GLP-1), promote changes in DA homeostasis within the striatum.[12,22] Further, it has been previously shown that appropriate insulin signaling at the level of the striatum prevents excessive high fat feeding.[22,23] These findings provide a theoretical framework in which dopaminergic tone is impaired under the state of altered hormonal signaling in obesity and, importantly, that RYGB

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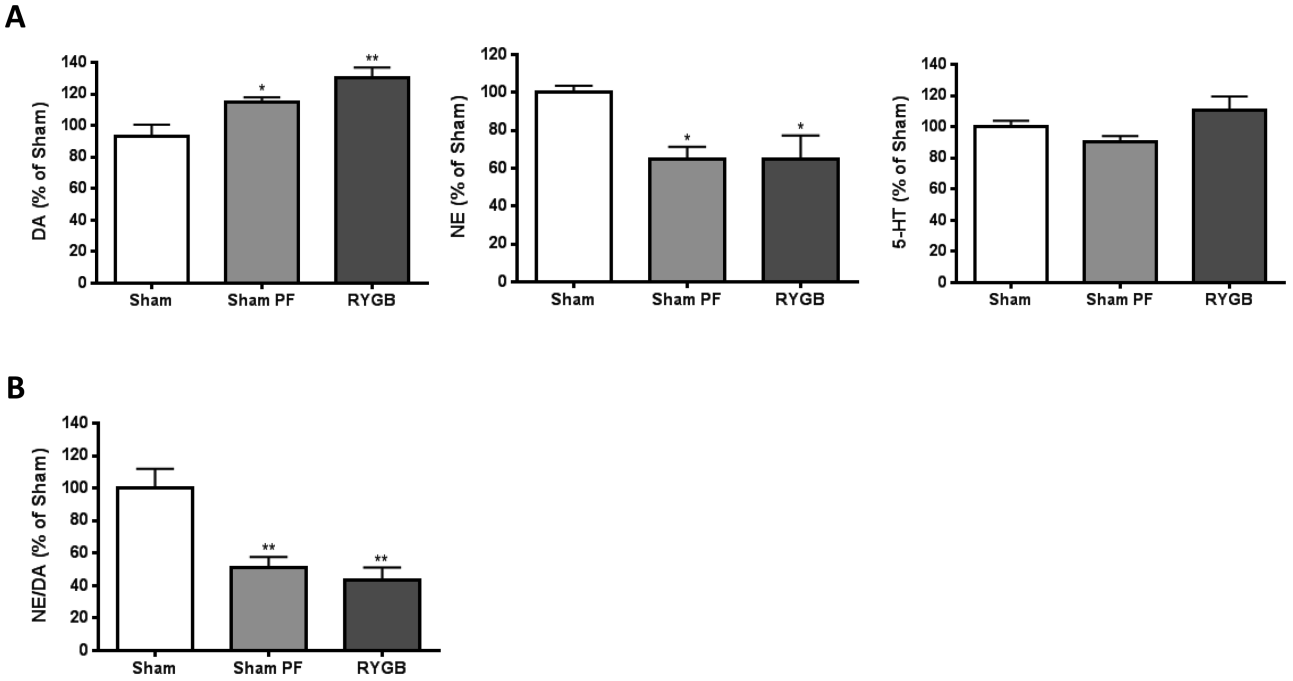

Figure 3. DA levels are elevated in the dorsal striatum of sham pair-fed and RYGB-operated mice. (A) Striatal DA measured by HPLC was significantly elevated in both sham pair-fed ($*p < 0.05$) and RYGB mice ($**p < 0.01$) compared to sham-operated animals (left; $n = 5–8$). In contrast, NE was significantly decreased in both sham pair-fed and RYGB mice compared to sham-operated animals (center; $*p < 0.05$; $n = 5–7$) while 5-HT was not significantly different between groups (right; $n = 6–7$). (B) Conversion of DA to NE was significantly impaired in the pair-fed and RYGB mice, as noted by the ratio of NE to DA ($**p < 0.01$; $n = 5–6$). Data are represented as mean ± SEM.

would enhance striatal DA neurotransmission in the setting of obesity.

RESULTS AND DISCUSSION

RYGB Reduces Body Weight, Food Intake, and Adiposity in Mice. After 7 weeks on a high fat diet, mice were randomly assigned to receive sham or RYGB surgery (Figure 1). All mice were allowed to feed on a high-fat diet ad libitum for 1 week following surgery, after which the sham group was randomly assigned either to continue ad libitum feeding or to be pair-fed to the RYGB group (see Methods for details). Body weights for animals in each of these three groups were monitored for the duration of the study ($n = 6–8$ per group). Following the initial 7 weeks on the high-fat diet, the average weight of the animals was 38.4 ± 0.8 g with no significant differences among animals ($p > 0.05$). At the end of the study (4 weeks after surgery), the RYGB group weighed significantly less than the sham animals (27.5 ± 0.9 g vs 40.0 ± 1.7 g; $^{\#\#\#\#}p < 0.0001$ by two-way ANOVA and Bonferroni's multiple comparison test), with an average weight loss of 26.9 ± 1.4% from peak weight immediately before surgery to 4 weeks postsurgery. In contrast, the sham pair-fed group only lost 11.1 ± 2.4% of their peak body weight, which was significantly different from the ad libitum sham group at week 11 ($*p < 0.05$). Importantly, the RYGB group also weighed significantly less than the pair-fed group ($^{\dagger\dagger}p < 0.01$).

The RYGB animals consumed significantly less food by weight compared to the shams (Figure 2B; $**p < 0.01$ in last 4 days of intake measurement by two-way ANOVA and Bonferroni's multiple comparison test). At 4 weeks postsurgery, the body fat in the RYGB group was 10.25 ± 0.55%, and 31.05 ± 3.5% in the sham group (Figure 2C; $***p < 0.001$). In contrast, the adiposity of the pair-fed group (28.08 ± 2.3%) was no different from that of ad-libitum-fed shams ($p > 0.05$). RYGB also resulted in greater lean mass composition by the end of the study with respect to sham ad libitum mice, our control group for the remainder of the study (Figure 2D; $****p < 0.0001$).

Mice That Have Undergone RYGB or Caloric Restriction Exhibit Higher Levels of DA and Reduced Levels of NE in the Striatum. Four weeks postsurgery, all groups were sacrificed and dorsal striatum tissue was collected. Levels of monoamines and their metabolites were determined by HPLC. DA levels were significantly elevated in both the RYGB and sham pair-fed groups with respect to shams fed ad libitum (Figure 3A; $**p < 0.01$ and $*p < 0.05$ for DA in RYGB and sham PF animals, respectively; mean HPLC values were 120.9 ± 9.7, 148.9 ± 4.1, and 168.8 ± 8.9 ng/mg protein for sham, sham PF, and RYGB groups, respectively; $n = 5–8$ per group). On the other hand, NE levels were significantly reduced in the RYGB and sham PF mice (Figure 3B; $*p < 0.05$ in RYGB and sham PF mice; mean HPLC values were 3.1 ± 0.1, 2.0 ± 0.2, and 2.0 ± 0.4 ng/mg protein, respectively; $n = 5–7$ per group), while there were no significant differences in the levels of the noncatecholaminergic monoamine, serotonin (5-HT) ($p > 0.05$).

DA levels within terminals are highly regulated by a number of homeostatic mechanisms, including metabolism, synthesis, and synaptic reuptake.[24] In relation to DA metabolism, there were no significant differences between groups in terms of levels of DA metabolites (Supporting Information Figure S1). However, a small proportion of DA is also converted to NE in the striatum by the enzyme DA β-hydroxylase.[25] Thus, it was important to determine whether the conversion of DA to NE was affected by RYGB by measuring changes in the ratio of NE to DA. This ratio was significantly reduced both in the pair-fed and RYGB animals relative to sham ad libitum-fed animals

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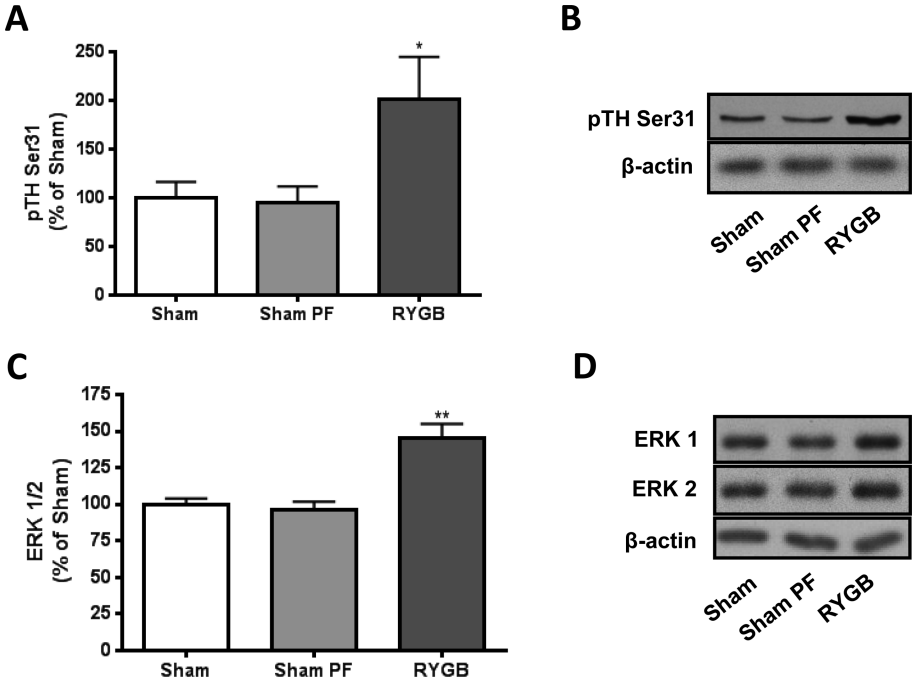

Figure 4. TH phosphorylation at Ser31 and expression of ERK 1/2 are elevated in RYGB-operated mice. (A) Phosphorylation of tyrosine hydroxylase at residue Ser31 (pTH-Ser31) was significantly elevated in the RYGB group compared to the sham ad libitum fed animals (*$p < 0.05$; $n = 6–8$). Data were normalized to β-actin. (B) Representative immunoblots. (C) Expression of ERK 1/2 was elevated in the RYGB mice compared to shams (**$p < 0.01$; $n = 5–6$). Data were normalized to β-actin and (D) a representative immunoblot is shown. Data are represented as mean ± SEM.

(Figure 3B; **$p < 0.01$). These findings demonstrate that, in the setting of high-fat feeding, both food restriction and RYGB act to increase striatal DA content and that this effect is potentially mediated in small part by reduced conversion of DA to NE.

RYGB but Not Caloric Restriction Increases TH Phosphorylation at Residue Ser31. In addition to DA metabolism, DA levels in neurons are also homeostatically regulated by reuptake and synthesis. Given that altered conversion of DA to NE can only explain partially the increase in DA in the RYGB animals, we thus asked whether these other components of DA homeostasis were altered by either RYGB or caloric restriction (pair-feeding). First, we determined whether expression of the DA transporter (DAT) is altered by either intervention. The DAT acts to clear DA from the synapse by active uptake into the terminals. Total expression of DAT was not significantly different across the three different experimental groups (100.0 ± 9.1%, 115.2 ± 9.4%, and 140.5 ± 35.4% in sham, sham PF, and RYGB, respectively; $n = 6$). Similarly, total expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, was unchanged across groups (100 ± 24.7%, 104.9 ± 10.7%, and 124.9 ± 32.8% in sham, sham PF, and RYGB, respectively; $n = 4–6$).

The activity of TH in relation to DA synthesis is regulated, in part, by phosphorylation of the residues Ser31 and Ser40. Phosphorylation at either of these residues results in increased activity of TH and increased DA synthesis.[26] Due to the low stoichiometric presence of Ser40 in striatum,[27] we focused on measuring striatal changes in phosphorylation of TH at Ser31. Interestingly, RYGB animals showed increased expression of phospho-TH Ser31 compared with sham animals (Figure 4A and B; *$p < 0.05$), while there was no significant difference between sham pair-fed animals and shams fed ad libitum.

The phosphorylation of TH at Ser31 is known to be regulated by extracellular signal-regulated kinases 1 and 2 (ERK1/2).[26,28] Although we did not observe an increase in ERK1/2 phosphorylation (100 ± 18.7%, 127.7 ± 30.5%, and 98.6 ± 14.1% in sham, sham PF, and RYGB, respectively; $n = 6–8$), a significant increase in ERK1/2 expression in the RYGB animals was noted, which may account for the observed increase in phospho-TH Ser31 (Figure 4C and D; **$p < 0.01$). Individually, both ERK 1 and ERK 2 were significantly increased in the RYGB group (100 ± 12.5%, 91.3 ± 9.9%, and 167.8 ± 9.9% for ERK1 in sham, sham PF, and RYGB, respectively; $p < 0.001$; 100 ± 10.5%, 125.7 ± 11.1%, and 177.3 ± 25.1% for ERK2 in sham, sham PF, and RYGB, respectively; $p < 0.01$; $n = 6–8$ per group).

RYGB Promotes Expression of the Insulin Receptor β Subunit in Striatum. It is well documented that levels of circulating hormones are altered by RYGB surgery.[7] The brain receives constant information about the body's nutritional status via these circulating hormones including, but not limited to, insulin, leptin, GLP-1, orexin, and ghrelin. Receptors for each of these hormones have been found in brain regions involved in energy homeostasis and feeding behavior, including striatum.[29−31] These enteroendocrine signaling factors have been shown to regulate feeding and body weight through central mechanisms.[32] In particular, within the central nervous system, insulin signaling has previously been linked to changes in DA homeostasis and food reward.[33−35] Given that circulating insulin levels are proportional to adiposity,[36] that RYGB improves insulin sensitivity,[37] and that in our study the RYGB group exhibited lower levels of adiposity than the pair-fed animals, possible changes in striatal insulin signaling were determined in our experimental groups. Here, expression of the β subunit of the insulin receptor was elevated in the striatum of

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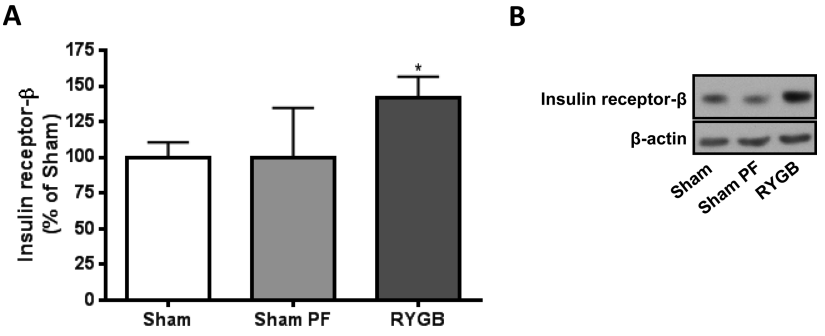

Figure 5. Expression of the insulin receptor β subunit is elevated in RYGB-operated mice. (A) Expression of the insulin receptor β was higher in the RYGB mice compared to shams ($*p < 0.05$; $n = 6–7$). Data were normalized to β-actin and (B) a representative immunoblot is shown. Data are represented as mean ± SEM.

animals that underwent RYGB, but not in the pair-fed group (Figure 5; $*p < 0.05$).

After uncovering a number of molecular changes associated with RYGB, we were interested to know whether any of these changes correlated with reductions in body weight. Such an analysis might provide insight into the contribution of body weight and associated metabolic changes toward adaptations in the brain dopaminergic system. Incorporating all animals from all groups, we measured the correlation between body weight and striatal DA, DAT, pERK1/2, ERK1/2, TH, pTH-Ser31, and the insulin receptor β subunit (Table 1). Significant inverse correlations were found between body weight and DA, ERK1/2 expression, pTH-Ser31 expression, and expression of the insulin receptor β subunit. Given that the RYGB group lost more weight than the pair-fed group, it is difficult from this study to determine whether the changes were caused primarily by a reduction in body weight/adiposity or through a more surgery-dependent, weight-independent mechanism.

Table 1. Correlation between Body Weight and Studied Molecular Markers of DA Homeostasis[a]

	R^2	df	P value
DA	0.182	20	**0.048**
DAT	0.091	19	0.183
pERK1/2	0.001	19	0.921
ERK1/2	0.454	17	**0.002**
pTH-Ser31	0.204	20	**0.035**
TH	0.003	17	0.812
IR-β	0.265	18	**0.020**

[a]Body weight was significantly inversely correlated with DA levels, ERK1/2 expression, phosphorylation of TH at residue Ser31, and IR-β expression. R^2 = coefficient of determination; df = degrees of freedom.

Both human and rodent studies indicate that RYGB alters the rewarding properties of palatable foods.[9,38−41] People who undergo bariatric surgery self-report less desire to consume palatable foods than before surgery[9,38−40] and reduce their intake.[42] Shin et al. (2010) performed RYGB in rats and found that, compared with sham animals, they exhibited more positive orofacial responses to a low concentration sucrose solution and a lower rate of licking of a high concentration sucrose solution.[43] Importantly, RYGB resulted in reduced consumption of a high-fat diet. This same group also noted that RYGB rescued food motivation to the level of lean controls in both an incentive runway paradigm and a progressive ratio operant paradigm.[43,44] These results promote a model by which RYGB reduces the need to consume large quantities of palatable food by restoring appropriate levels of hedonic stimulation. These behavioral correlates implicate adaptations within brain reward circuitry; yet the neural mechanisms driving changes in feeding behavior in bariatric surgery are unclear.

Dysregulated DA signaling within the striatum has been strongly associated with high-fat feeding, obesity, and reward.[15,19,21,23,45,46] Positron emission tomography (PET) studies point to altered DA signaling in the dorsal striatum of obese individuals.[21,47] Similarly, rodent models of obesity, including diet-induced obesity models[19,23,45,48] and obesity-prone genetic lines,[49] have all exhibited deficits in striatal DA homeostasis. Importantly, viral-mediated knockdown of the striatal D2 receptor suggest that impaired DA signaling may be a causal factor in the etiology of obesity.[19] Of note, amphetamine as well as other DA-targeting drugs possess potent anorectic properties,[50,51] supporting our hypothesis that RYGB reduces intake of obesogenic food by enhancing DA neurotransmission. Of course, these drugs possess addictive properties; thus, defining other targets of regulation within this system through the study of RYGB has the potential to reveal novel and safer pharmacological targets for the treatment of obesity.

Although the literature makes strong reference to differences in levels of appetite-regulating gut hormones following bariatric surgery,[7,10−12,52] few attempts have been made to define the neurobiological adaptations that result from this altered neurohormonal milieu.[47,53−55] The aim of the current study was to define the neurochemical and molecular phenotype of RYGB within the striatum in a well-controlled preclinical mouse model. Furthermore, we sought to determine which of these phenotypes resulted from a simple reduction in caloric intake versus an effect of the surgical procedure. We focused our study on monoamine signaling within the dorsal striatum, as DA within this region plays a critical role in the consummatory drive for food.[17,18] We observed altered catecholamine (DA and NE) levels in the dorsal striatum of both RYGB and pair-fed (chronically food restricted) animals, but not the noncatecholaminergic neurotransmitter 5-HT. This finding is consistent with the idea that DA signaling within the dorsal striatum supports the rewarding properties of palatable food,[56] and that food is more rewarding under conditions of restriction.[57] A potential explanation for a small component of this effect may lie in alterations in DA conversion to NE, as observed in the current study. As this process occurs within vesicles and without changes to DA metabolism, we would expect a reduction in DA processing to NE to result in an

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increase in DA accumulation in vesicles with enhanced DA release in both chronically food-restricted and RYGB mice. In fact, it has previously been reported that electrically evoked DA release in the dorsal striatum and nucleus accumbens is attenuated in slices taken from rats fed a cafeteria diet for 15 weeks versus rats on a regular chow diet.[48] Future studies will determine whether evoked DA release is corrected in the RYGB model.

To further explain this increase in striatal DA content, we next focused on determining possible changes in the function of key regulators of the DA synthetic pathway. We determined that animals which underwent RYGB had significantly greater levels of TH phosphorylation at Ser31. This phenotype was not observed in either the sham or the sham pair-fed groups, suggesting that the increased DA levels in the sham pair-fed group stems from a mechanism independent of changes in TH function. Consistent with the increase in phospho-TH, the expression of ERK1/2 was elevated in the RYGB mice. Since phosphorylation of Ser31 is targeted by ERK1/2, the increase in expression of ERK1/2 may suggest a possible mechanism by which TH activity is upregulated.[28] Finally, given the extensive literature on altered neuro-hormonal levels following RYGB, we explored markers of differential hormonal signaling in the RYGB group which could connect changes in gut anatomy with the changes DA homeostasis observed in the dorsal striatum. Our group and others have previously found that insulin signaling in the dorsal striatum acts to regulate DA homeostasis,[33,34] and that this signaling is dysregulated in rodents fed a high-fat diet.[23] Importantly, insulin resistance and type 2 diabetes often develop in the setting of obesity and are corrected by RYGB.[37,58] We found that expression of the insulin receptor β subunit was significantly upregulated in mice that underwent RYGB surgery, but not in either of the sham groups. The relevance of these data is enhanced considering that the striatum has been described as a glucosensing brain region[59,60] and that increased brain glucose availability (i.e., hyperglycemia) and glucose oxidation disrupt both nigrostriatal neurotransmission and striatal DA turnover.[61,62]

Here, we report for the first time neurochemical changes at the level of the striatum in a preclinical model of RYGB. These changes include an elevation of DA levels with reduced conversion to NE, increased phosphorylation of TH, increased expression of the regulatory kinase ERK1/2, and increased insulin receptor-β expression. RYGB, while generally effective as a treatment for obesity, is not equally effective for all individuals.[63] Here, we have speculated that neural mechanisms may be an important factor mediating weight loss in RYGB. Understanding these neural contributions to weight loss may allow for the development of pharmacotherapeutic interventions to improve clinical outcomes in patients undergoing bariatric surgery. Of course, pharmacological weight loss approaches, or so-called "knifeless surgery", if sufficiently effective would be beneficial as a means to avoid surgery altogether and bring relief to a larger patient population.[64,65]

METHODS

Mice. Male C57BL/6J mice arrived from Jackson Laboratory (Bar Harbor, ME) at 6 weeks of age and were initially group-housed at the Vanderbilt Mouse Metabolic Phenotyping Center (Nashville, TN) with a 12 h light/dark cycle (lights on at 6:00 a.m.). Mice were given 1 week to acclimate to the facility before being switched from a standard chow diet to a 60% kcal fat diet (Research Diets, Inc., New Brunswick, NJ) for 7 weeks prior to surgery to establish diet-induced obesity (Figure 1). Mice were kept on this diet for the remainder of the study. Animals which had undergone sham surgery were split into pair-fed and ad-libitum-fed groups. On day 7 following surgery, all animals were moved from group housing to single housing. Feeding from this point on was measured every 2−4 days (grams of food given minus grams of food remaining). Mice which were noticeably crumbling food were excluded from food intake analysis (n = 1). Pair feeding was started on day 9 postsurgery; this consisted of providing animals with a measured amount of food each morning and evening, which matched food consumed per half day in the RYGB animals from the same cohort in the prior 2−4 days. Body weight throughout the study was measured on a weekly basis. All experiments, procedures, and surgeries involving mice were performed in compliance with and were approved by the Institutional Animal Care and Use Committee of Vanderbilt University.

RYGB Surgical Preparations. RYGB and sham surgeries were performed under inhaled 3−5% isoflurane anesthesia as previously described ("RYGB" procedure).[66] Mice which did not achieve a body weight of at least 33 g on the day before surgery were excluded from the study. Prior to surgery, mice were fasted for approximately 12 h. On the morning of surgery, the mice received 0.03 mg of buprenorphine analgesic (Patterson Veterinary via Hospira Inc., Nashville, TN) and 0.1 mL of 0.9% saline (intraperitoneal). Animals were administered 0.017 mg of ketoprofen (Cayman Chemical, Ann Arbor, MI) once/day as needed for 1−2 days following surgery and weekly iron dextran injections (10 mg/kg; Durvet, Inc., Blue Springs, MO) to prevent anemia.

Whole Body Composition. Body composition was measured in conscious mice using the mq10 NMR analyzer (Bruker Optics Inc., Billerica, MA) at the Vanderbilt University Mouse Metabolic Phenotyping Center as described previously.[66] Measurements were made 1 week prior to surgery (±1 day) and each week thereafter.

Monoamine Content. Mice were sacrificed 4 weeks following surgery under inhaled isoflurane anesthesia. A section of brain including the striatum was blocked. A small portion of the dorsal striatum was taken by punch microdissection for determination of monoamine content, while the rest of the striatum was dissected and saved for Western blotting. Tissue saved for both monoamine determination and Western blotting was immediately placed in tubes on dry ice. Tissue punches were analyzed at the Vanderbilt University Neurochemistry Core via high performance liquid chromatography (HPLC) with amperometric detection as described previously.[67]

Tissue Preparation and Immunoblotting. Tissue punches from dorsal striatum were collected and homogenized on ice in buffer containing 22 mM HEPES, 133 nM NaCl, 1% triton, 0.1% each of leupeptin, pepstatin, and aprotinin, 1% phosphatase inhibitor cocktail 3 (Sigma-Aldrich, St. Louis, MO), and 0.5 mM PMSF, and then spun at 13 000g for 30 min at 4 °C. The supernatant was taken and combined with pulldown buffer containing 24 mM HEPES, 146 nM NaCl, 0,1% triton, 0.1% each of leupeptin, pepstatin, and aprotinin, 1% phosphatase inhibitor cocktail 3 (Sigma-Aldrich, St. Louis, MO), and 0.5 mM PMSF. The protein content was then assessed, compensated so that each sample contained the same amount of total protein, and analysis performed. Protein was eluted with 2× sample buffer for 5 min at 95 °C, cooled, and separated by 10% SDS-PAGE. Resolved proteins were then transferred to polyvinylidene difluoride (PVDF) membrane and blocked in either 5% milk or 2.5% BSA in 0.1% Tween 20 in Tris-buffered saline. Blots were then incubated in primary antibody rocking either at room temperature for 1 h or overnight at 4 °C. The primary antibodies used in this study included tyrosine hydroxylase (1:1000; Cell Signaling Technology; Danvers, MA), phospho-tyrosine hydroxylase serine 31 (1:500; Cell Signaling Technology; Danvers, MA), dopamine transporter (1:10 000; Dr. Roxanne Vaughan, University of North Dakota School of Medicine), ERK 1/2 (1:1000; Promega; Madison, WI), phospho-ERK 1/2 (3:2000; Promega; Madison, WI), and insulin receptor-β (1:300; Santa Cruz Biotechnology, Santa Cruz, CA). All proteins were detected using HRP conjugated secondary antibodies (1:5000; Santa Cruz Biotechnology, Santa Cruz, CA). After chemiluminescent visualization (Amersham ECL-Plus; Piscataway, NJ) on Hyblot CL

film (Denville Scientific, South Plainfield, NJ), protein band densities were quantified using ImageJ software (ImageJ, National Institutes of Health, Bethesda, MD). All measures of protein were normalized to measures of β-actin (1:2000; Sigma-Aldrich; St. Louis, MO) from the same samples and expressed as a percentage of the average optical density of the sham control group.

Statistics. Results are presented as mean ± standard error of the mean (SEM). All statistical analyses were performed using GraphPad Prism version 6.00 for Windows (San Diego, CA). Comparisons between sham, sham pair-fed, and RYGB groups were made by one-way ANOVA with Dunnett's post test against the sham group unless otherwise noted. For all measures, data points which fell greater than or equal to an interquartile range outside the first and third quartiles for the surgical/feeding group were excluded from analysis as outliers. Correlation analyses were performed by linear regression in GraphPad Prism. Significance was defined as a p value < 0.05.

■ ASSOCIATED CONTENT

Supporting Information

Analysis of striatal dopamine metabolites. Striatal content of dopamine metabolites is not altered by RYGB or pair-feeding. Levels of DA metabolites (A) DOPAC (n = 5−8), (B) 3-MT (n = 5−8), and (C) HVA (n = 6−8) normalized to total DA were the same across surgical and feeding groups (p > 0.05). Data are represented as mean ± SEM. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Author Contributions

I.A.R. collected and analyzed the data. I.A.R. and A.G. wrote the paper. I.A.R., A.G., N.N.A., and D.H.W. participated in study design. A.H.H., J.E.A., N.N.A., and D.H.W. provided critical revisions of the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

RYGB, Roux-en-Y gastric bypass; PF, pair-fed; DA, dopamine; NE, norepinephrine; 5-HT, serotonin; TH, tyrosine hydroxylase; DAT, dopamine transporter; ERK, extracellular signal-regulated kinase; PET, positron emission tomography; HPLC, high performance liquid chromatography

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