DIABETES

Striatal dopamine regulates systemic glucose metabolism in humans and mice

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The brain is emerging as an important regulator of systemic glucose metabolism. Accumulating data from animal and observational human studies suggest that striatal dopamine signaling plays a role in glucose regulation, but direct evidence in humans is currently lacking. We present a series of experiments supporting the regulation of peripheral glucose metabolism by striatal dopamine signaling. First, we present the case of a diabetes patient who displayed strongly reduced insulin requirements after treatment with bilateral deep brain stimulation (DBS) targeting the anterior limb of the internal capsule. Next, we show that DBS in this striatal area, which induced dopamine release, increased hepatic and peripheral insulin sensitivity in 14 nondiabetic patients with obsessive-compulsive disorder. Conversely, systemic dopamine depletion reduced peripheral insulin sensitivity in healthy subjects. Supporting these human data, we demonstrate that optogenetic activation of dopamine D_1 receptor–expressing neurons in the nucleus accumbens increased glucose tolerance and insulin sensitivity in mice. Together, these findings support the hypothesis that striatal neuronal activity regulates systemic glucose metabolism.

INTRODUCTION

Insulin resistance is the pathophysiological hallmark of obesity, nonalcoholic fatty liver disease, and type 2 diabetes (*1*, *2*). Whereas genetics, lifestyle, and cellular defects in adipocytes, hepatocytes, and skeletal myocytes have been the focus of study for many years (*3*), the brain has also emerged as an important regulator of systemic glucose and energy metabolism (*4*, *5*). The role of the hypothalamus in maintaining glucose homeostasis and energy balance is now well established (*6*, *7*), and other brain areas may be involved as well (*8*, *9*). In this regard, the ventral striatum, an area involved in motivated behavior and in human obesity (*10*), is of particular interest and may play a role in obesity-related changes in glucose control. However, precisely how this region is involved in systemic glucose control is unknown.

The nucleus accumbens (NAc), a region in the ventral striatum with dense projections to the hypothalamus (*11*), is involved in motivation, reward, and addiction (*12*–*16*). There is also increasing evidence that striatal dopamine plays a role in the regulation of energy homeostasis. We, and others, have shown that striatal dopamine $D_{2/3}$ receptor ($D_{2/3}R$) availability is reduced in obese insulin-resistant humans (*17*, *18*) and increases after long-term bariatric surgery–induced weight loss (*19*). Neurons in the NAc can be activated or inhibited by glucose (*20*), and sucrose administration to rodents stimulates dopamine release within the NAc (*21*, *22*). Striatal dopamine activity may also be involved in systemic glucose control. Intracerebroventricular administration of bromocriptine, a potent dopamine D_2 receptor (D_2R) agonist, to obese hamsters improves glucose tolerance and insulin sensitivity (*23*), and systemic bromocriptine administration to humans improves glucose metabolism in some (*24*, *25*), but not in all, trials (*26*); however, the sites and mechanisms of action are unknown. Moreover, observational data on possible associations between striatal dopamine receptor availability, measures of glucose metabolism, and insulin action in humans have been inconsistent (*27*–*30*), and there is a need for more direct evidence from translational studies.

The introduction of deep brain stimulation (DBS) in humans has enabled specific modulation of one or more brain areas in vivo (*31*), thereby providing a powerful tool for translational neuroscience. In recent years, DBS has emerged as a safe and effective treatment option for pharmacoresistant obsessive-compulsive disorder (OCD) (*32*, *33*), with the most effective stimulation area located at the border of the NAc core and ventral anterior limb of the internal capsule (vALIC) (*34*). We have recently shown that vALIC-DBS acutely reduces striatal $D_{2/3}R$ binding potential (BP) and chronically increases plasma homovanillic acid concentrations in OCD patients (*34*), indicating that it increases occupancy of the $D_{2/3}R$ by enhancing endogenous dopamine release. Therefore, these patients offer a unique in vivo model to study whether striatal dopamine release affects glucose metabolism in human subjects.

Here, we present the case of an obese patient with type 2 diabetes who displayed strongly reduced insulin requirements after treatment with bilateral vALIC-DBS for his concomitant OCD. We confirm that vALIC-DBS, which induces striatal dopamine release (*34*), increased insulin sensitivity in this patient and in nondiabetic patients. Conversely, systemic dopamine depletion, which effectively reduces striatal dopamine concentrations (*35*), reduced insulin sensitivity in healthy subjects. In rodents, optogenetic activation of dopamine D_1 receptor (D_1R)–expressing cells in the NAc increased glucose tolerance and insulin sensitivity. Together, these data show that striatal neuronal activity is involved in whole-body glucose homeostasis.

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RESULTS

Insulin sensitivity is increased in a diabetes patient after vALIC-DBS

A 55-year-old man with severe OCD symptoms since childhood was treated with bilateral DBS at 53 years of age. He was diagnosed with insulin-dependent type 2 diabetes 5 years before DBS placement. Before DBS treatment, his diabetes was managed with metformin and insulin (total daily dose of 226 IU), whereas his total daily insulin dose was reduced by 46 IU after the start of DBS treatment. This led to the intriguing hypothesis that vALIC-DBS improves insulin sensitivity. The patient did not lose weight after DBS treatment but did stop taking quetiapine, an atypical antipsychotic that is known to be associated with increased diabetes risk (*36*). To test whether DBS directly affected glucose metabolism in this patient, we determined basal and insulin-mediated glucose fluxes (assessed by a two-step hyperinsulinemic-euglycemic clamp using [6,6-$^2H_2$]glucose as tracer) on two occasions: once while the DBS was switched off for 17 hours (unstimulated state) and once while the DBS was switched on for 17 hours (stimulated state). The patient was instructed to stop his usual medication, including insulin, 1 day before each of the two study days, which were scheduled 1 month apart. His clinical characteristics and clamp results are presented in Table 1. The fasting plasma glucose concentration and basal rate of endogenous glucose production (EGP) did not differ between unstimulated and stimulated conditions. In contrast, fasting plasma insulin was lower during DBS, suggesting higher insulin sensitivity (*37*). Accordingly, DBS was associated with increased suppression of EGP, suppression of free fatty acids (FFAs), and glucose disposal [rate of disappearance (Rd)], indicating that, in this patient, electric stimulation along the vALIC is associated with increased hepatic, adipose tissue, and muscle insulin sensitivity, respectively. Steady-state plasma concentrations of glucose, insulin, and glucagon were similar between both clamp days. Overall, this case suggested that striatal DBS may improve insulin action on multiple target tissues.

Table 1. Metabolic parameters of a 55-year-old diabetes patient with vALIC-DBS. Indicators of improved insulin sensitivity are highlighted in bold.

	Unstimulated (DBS off)	Stimulated (DBS on)
Height (cm)	171	
Weight (kg)	123	
BMI (kg/m^2)	42	
Body fat content (%)	41	
Glucose (mM)*	6.9	6.9
Insulin (pM)*	265	**206**
FFAs (mM)*	0.47	0.38
EGP (μmol kg^{-1} min^{-1})*	9.84	9.96
Glucose (mM)†	5.3	5.0
Insulin (pM)†	340	378
Glucagon (ng/liter)†	96	99
Suppression of EGP (%)†	37	**47**
Suppression of FFAs (%)†	11	**18**
Glucose Rd (μmol kg^{-1} min^{-1})†	6.8	**7.3**
Glucose (mM)‡	4.8	5.0
Insulin (pM)‡	540	529
Glucagon (ng/liter)‡	90	90
Suppression of EGP (%)‡	68	**75**
Suppression of FFAs (%)‡	55	**66**
Glucose Rd (μmol kg^{-1} min^{-1})‡	9.1	**10.9**
Resting energy expenditure (kcal/day)	2258	2418

*In the basal state, after an overnight fast.
†During step 1 of the clamp (insulin infusion dose, 20 mIU m^{-2} min^{-1}).
‡During step 2 of the clamp (insulin infusion dose, 40 mIU m^{-2} min^{-1}).

Targeting the striatum with vALIC-DBS increases hepatic and muscle insulin sensitivity

To test whether DBS treatment directly affects glucose metabolism, we recruited 14 OCD patients with bilateral vALIC-DBS (Table 2). We determined basal and insulin-mediated glucose fluxes on two occasions: once while the DBS was switched off for 17 hours (unstimulated state) and once while the DBS was switched on for 17 hours (stimulated state).

vALIC-DBS lowered fasting plasma insulin concentrations (table S1) without affecting basal EGP (Fig. 1A). As a consequence, the product of fasting insulin and basal EGP, an index of hepatic insulin resistance (*38*), was reduced by vALIC-DBS [964 ± 206 pM (μmol kgFFM^{-1} min^{-1}) versus 555 ± 141 pM (μmol kgFFM^{-1} min^{-1}); $P = 0.016$], suggesting that basal hepatic insulin sensitivity was increased by DBS. During a two-step hyperinsulinemic-euglycemic clamp protocol (see Materials and Methods), insulin-mediated suppression of EGP during low-dose insulin infusion (step 1) increased upon stimulation (Fig. 1B). During high-dose insulin infusion (step 2), the insulin-stimulated Rd of glucose was also higher when the DBS was switched on (Fig. 1C). In addition, DBS induced a reduction in plasma FFA concentrations (table S1), suggesting an increase in adipose tissue insulin sensitivity (*39*), and a shift from fat to carbohydrate oxidation during hyperinsulinemic conditions, consistent with increased glucose disposal. Stimulation also decreased plasma glucagon concentrations during hyperinsulinemia, whereas plasma concentrations of insulin, cortisol, epinephrine, and norepinephrine were not modulated. These results support the hypothesis that vALIC-DBS increases hepatic, adipose tissue, and muscle insulin sensitivity in nondiabetic OCD patients.

Psychiatric evaluation showed that DBS decreased signs of anxiety, depression, obsessions, and compulsions (table S2); however, we did not observe any correlation between insulin sensitivity and psychiatric responses to DBS treatment.

Long-term obesity is associated with changes in the striatal dopamine system (*17*, *18*). Therefore, we assessed whether the effects of vALIC-DBS on glucose metabolism are dependent on body weight status. Seven of 14 patients were lean [body mass index (BMI) <25 kg/m^2], and 7 patients were overweight/obese (BMI ≥25 kg/m^2) (Table 2). DBS reduced striatal $D_{2/3}R$ availability in lean, but not in overweight/obese, subjects (Fig. 1D), suggesting that the treatment induced significantly more striatal dopamine release in lean subjects ($P = 0.018$ for Δ $D_{2/3}R$ BP in lean versus overweight/obese subjects). In agreement, DBS exerted a more pronounced effect on peripheral insulin sensitivity in lean

Table 2. Characteristics of OCD patients with vALIC-DBS. Data are count, means ± SD, or median (interquartile range), depending on type and distribution. Overweight/obesity was defined as BMI ≥25 kg/m^2. HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

	Overall (n = 14)	Lean (n = 7)	Overweight/obese (n = 7)	P*
Men/women	8/6	4/3	4/3	1.000
Age (years)	45 (40–51)	43 (38–49)	45 (41–53)	0.535
Length (cm)	175 ± 10	175 ± 8	175 ± 13	1.000
Weight (kg)	82 ± 17	73 (67–78)	93 (82–102)	0.011
BMI (kg/m^2)	27.0 ± 5.4	24.0 (22.3–24.7)	30.4 (26.7–30.6)	0.001†
Body fat content (%)	30.8 ± 9.1	25.0 (21.5–28.3)	33.7 (32.7–35.8)	0.004
Glucose (mM)‡	4.9 ± 0.7	4.7 ± 0.7	5.2 ± 0.7	0.150
Insulin (pM)‡	59 (31–86)	45 (8–81)	79 (44–144)	0.209
Triglycerides (mM)‡	1.2 (0.6–2.2)	0.9 (0.5–1.3)	2.2 (1.1–2.4)	0.053
Cholesterol (mM)‡	4.6 ± 1.1	5.0 ± 1.1	4.3 ± 1.1	0.271
HDL (mM)‡	1.2 ± 0.4	1.4 ± 0.4	1.0 ± 0.2	0.054
LDL (mM)‡	2.8 ± 1.1	3.2 ± 1.2	2.5 ± 0.9	0.275
AST (U/liter)	24 ± 8	25 ± 7	23 ± 10	0.687
ALT (U/liter)	23 (13–33)	24 (18–39)	18 (13–28)	0.456

*Lean versus overweight/obese. †By study design. ‡In the basal state, after an overnight fast.

Table 3. Characteristics of healthy men in dopamine depletion experiments (n = 10). Data are means ± SD.

Age (years)	23 ± 3
Length (cm)	183 ± 5
Weight (kg)	74 ± 8
BMI (kg/m^2)	22.0 ± 1.9
Body fat content (%)	15.4 ± 4.3

subjects (Fig. 1E). We did not observe differential effects of DBS on hepatic insulin sensitivity (Fig. 1F), suggesting that central control of EGP may involve additional factors independent from dopamine release.

Dopamine depletion reduces insulin sensitivity

If striatal dopamine release increases hepatic and muscle insulin sensitivity, we would expect that reducing striatal dopamine concentration reduces insulin sensitivity. To test this hypothesis, we recruited 10 healthy lean men (Table 3) and determined basal and insulin-mediated glucose fluxes on two occasions in a randomized controlled crossover design: once during conditions of systemic dopamine depletion and once during control conditions. Acute systemic dopamine depletion can be achieved by pharmacological inhibition of tyrosine hydroxylase, the rate-limiting enzyme in endogenous dopamine synthesis (*40*), using the inhibitor α-methyl-para-tyrosine (AMPT). This method has been shown to effectively increase striatal $D_{2/3}$R availability and thus reduce striatal dopamine concentrations in humans (*35*). Treatment with AMPT reduced plasma homovanillic acid (Fig. 2A) and increased plasma prolactin concentrations throughout the AMPT study day (Fig. 2B), indicating that dopamine is successfully reduced (*41*).

AMPT did not affect basal EGP (Fig. 2C) or hepatic insulin sensitivity (Fig. 2D) but decreased the insulin-stimulated Rd of glucose (Fig. 2E). The results indicate that acute systemic dopamine reduction decreases peripheral insulin–mediated glucose uptake, which primarily reflects muscle insulin sensitivity (*42*), in healthy subjects. The data are also consistent with the notion that central control of EGP may involve additional factors.

Analysis of metabolic parameters showed that AMPT administration increased plasma insulin and decreased plasma catecholamines during low-dose insulin infusion (table S3). Blood pressure, heart rate, low-frequency (LF) variability, high-frequency (HF) variability, or LF/HF ratio in either the supine or upright position was not modulated by AMPT (table S4).

Optogenetic activation of NAc D_1R^+ neurons improves glucose tolerance in mice

The present human studies suggest that there is a link between striatal dopamine and glycemic control. To investigate the mechanism mediating this effect, we evaluated whether direct optogenetic activation of D_1R-expressing neurons affects glucose tolerance and insulin sensitivity in mice. To this end, we expressed the excitatory opsin channelrhodopsin-2 (hChR2) in D_1R-expressing neurons in the NAc in vivo in adult mice (n = 9) to allow for specific light-mediated activation of D_1R^+ neurons (fig. S1, A to C). We chose to target the NAc because DBS of this ventral striatal nucleus previously modulated glucose metabolism in rodents (*43*, *44*).

Activation of D_1R^+ neurons in the NAc enhanced glucose clearance during an intraperitoneal glucose tolerance test (IPGTT) (Fig. 3A). All mice were tested under light-activated (laser on) and control (laser off) conditions in a controlled crossover design (see Materials and Methods). Fasting glucose concentrations did not differ between these conditions (9.2 ± 0.7 mM versus 10.0 ± 0.7 mM; P = 0.471). After glucose administration, all mice displayed a rapid rise in blood glucose at 15 min. At 30 and 60 min, light-induced stimulation of D_1R-expressing neurons reduced plasma glucose concentrations, indicating that

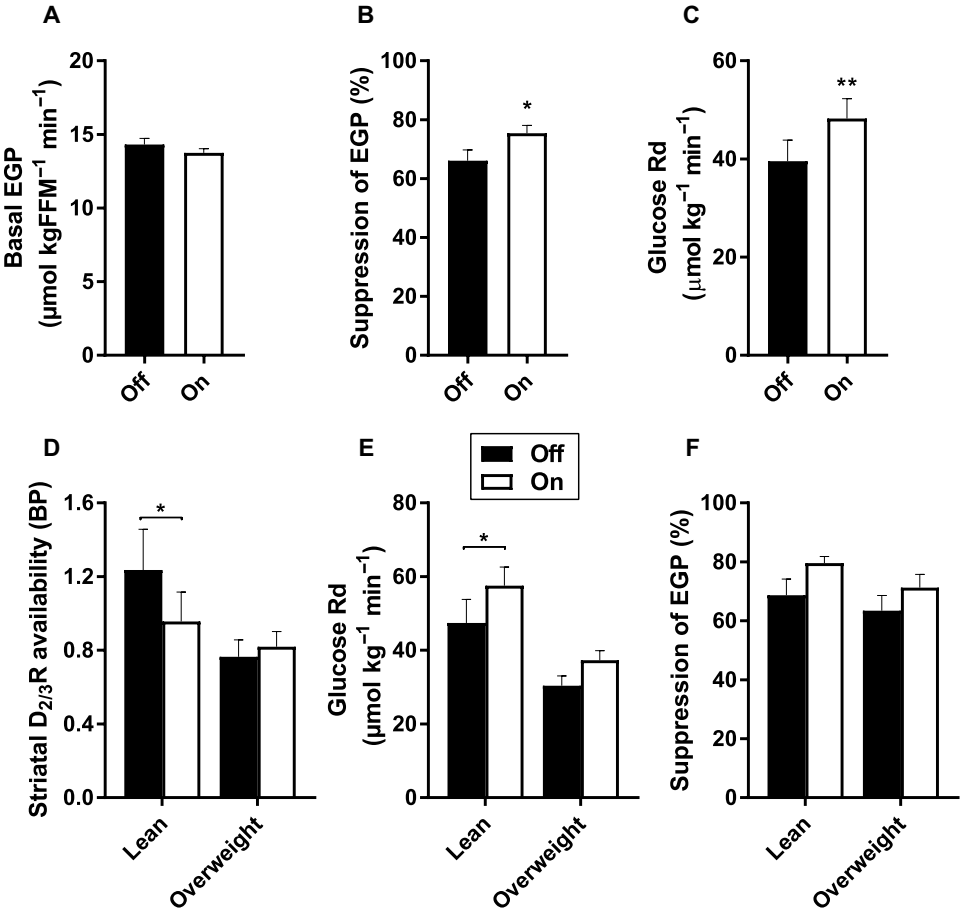

Fig. 1. Effect of vALIC-DBS on insulin sensitivity in nondiabetic OCD patients. (**A** to **F**) Bar graphs showing the effect of vALIC-DBS on basal rate of EGP assessed after an overnight fast (A), insulin-mediated suppression of basal EGP (B), insulin-stimulated Rd of glucose (C), striatal dopamine release in lean and overweight/obese subjects (P = 0.018 for Δ $D_{2/3}$R BP in lean versus overweight/obese subjects) (D), peripheral insulin sensitivity in lean and obese subjects (E), and hepatic insulin sensitivity in lean and obese subjects (F). n = 13 to 14. Data are means ± SEM. *P < 0.05 by two-sided paired t tests (A to C) or Wilcoxon signed-rank test (D and E); **P < 0.01 by two-sided paired t test.

activation of NAc D_1R^+ neurons increased glucose tolerance. The effects of laser stimulation on blood glucose were observed only in the context of glucose tolerance testing, because laser stimulation did not lower blood glucose concentrations in saline-injected animals (n = 4) (Fig. 3A). Some mice were retested again under control conditions to assess the potential contribution of the ordering of experimentation, and no differences were observed.

Plasma insulin concentrations at 30 min after glucose administration did not differ between stimulated and unstimulated conditions (Fig. 3B), suggesting that enhanced glucose tolerance was likely due to higher insulin sensitivity rather than increased insulin secretion. This experiment suggests that activation of NAc D_1R^+ neurons acutely increases glucose clearance by enhancing insulin sensitivity, further supporting the link between striatal dopamine signaling and glycemic control.

DISCUSSION

In this series of human and rodent experiments, we show that modulating striatal and systemic dopamine affects whole-body glucose metabolism. Using a unique human cohort to study acute striatal dopamine release in vivo (*34*), we show that hepatic and peripheral insulin sensitivity are acutely regulated by vALIC-DBS in nondiabetic OCD patients, suggesting that striatal dopamine release enhances whole-body insulin sensitivity. Consistent with this finding, we also show that AMPT-mediated dopamine reduction, which effectively reduces intrasynaptic dopamine concentrations in the striatum (*35*, *45*), produced the opposite response, reducing peripheral insulin sensitivity in healthy subjects. This effect was likely not mediated by peripheral AMPT effects on glucoregulatory hormones or by effects on the cardiovascular system as reflected by the lack of changes in spectral heart rate variability (*46*, *47*). Finally, in support of our human data, we observed increased glucose clearance when we activated D_1R-expressing neurons in the rodent NAc, suggesting that activating this specific brain region is sufficient to modulate whole-body glucose control. Overall, these translational observations support a mechanism by which striatal dopamine signaling may physiologically regulate systemic glucose metabolism.

Relationships between the central dopamine system, food intake, obesity, and insulin sensitivity in humans have been proposed before, but it has been difficult to establish the underlying mechanisms as well as their directionality. Although obesity has been suggested to be a hypodopaminergic state, where decreased dopamine-mediated reward may drive excessive food intake (*17*), human association studies do not imply causality. Moreover, studies on the correlation between BMI and striatal $D_{2/3}$R or dopamine transporter availability produced mixed results, likely due to the presence of confounding factors, including differences in obesity-related insulin sensitivity (*27*–*29*). Here, we used three different interventional settings to demonstrate how the modulation of striatal or dopaminergic systems affects glucose metabolism, independent of effects of obesity on dopamine and independent of striatal/dopaminergic effects on the regulation of food intake and body weight.

The central nervous system, in general, and dopamine, in particular, have been shown to mediate brain output to control peripheral glucose metabolism in animals. Interventions that directly target the central dopamine system, including DBS of the NAc shell (*43*, *44*), infusion of the selective dopamine reuptake inhibitor vanoxerine into the NAc shell (*48*), and central bromocriptine administration (*23*), modulate peripheral metabolism in rodent models. Our human data provide additional evidence in support of the central regulation of glucose metabolism.

The observation that activation of D_1R^+ neurons in the rodent NAc was sufficient to improve glucose tolerance suggests a key role

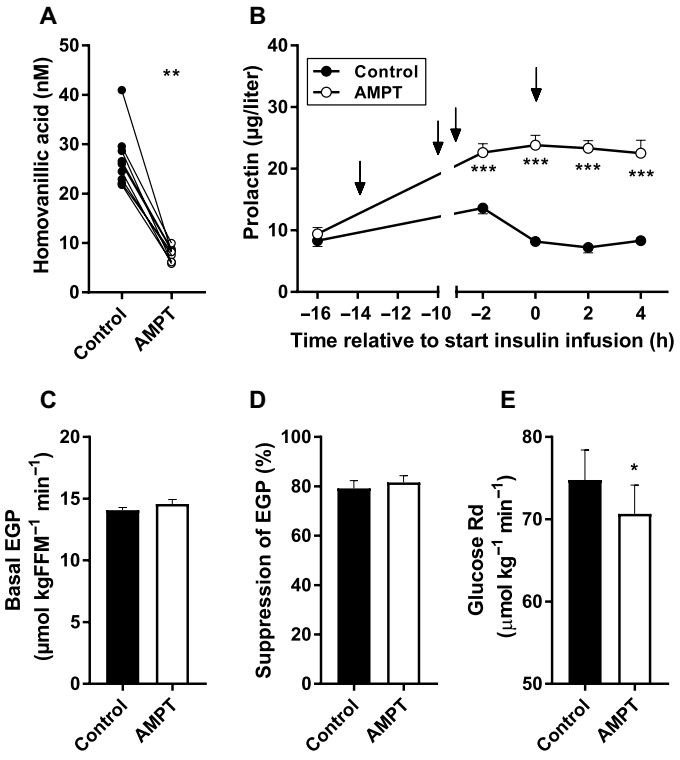

Fig. 2. Effect of dopamine reduction on insulin sensitivity in healthy subjects. (**A**) Effect of AMPT administration on plasma homovanillic acid. (**B**) Effect of AMPT administration on plasma prolactin. The arrows indicate the times of AMPT administration. (**C** to **E**) Bar graphs showing basal EGP (C), insulin-mediated suppression of basal EGP (D), and insulin-stimulated Rd of glucose (E). $n = 10$. Data are means ± SEM. *$P < 0.05$ by two-sided paired t test; **$P < 0.01$ by Wilcoxon signed-rank test; ***$P < 0.001$ by two-sided paired t tests.

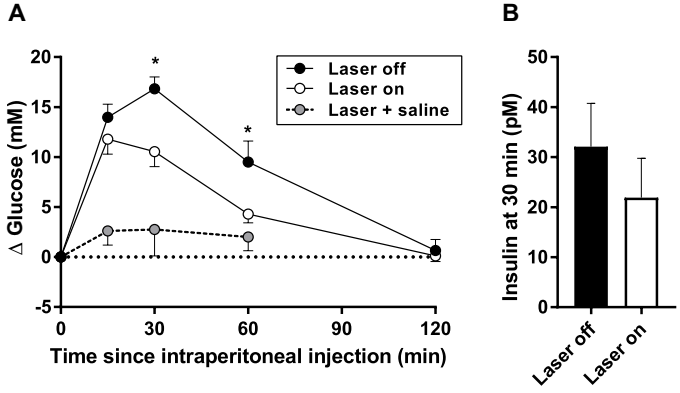

Fig. 3. Effect of optogenetic activation of NAc D_1R^+ neurons on glucose tolerance in transgenic mice. (**A**) Effect of optical stimulation of NAc D_1R^+ neurons on glucose concentrations during IPGTT. (**B**) Plasma insulin concentrations during IPGTT. $n = 9$. Data are means ± SEM. *$P < 0.01$ for laser on versus laser off by two-way analysis of variance (ANOVA) (stimulation-time interaction $F = 4.31$, $P = 0.004$; main effect stimulation $F = 14.42$, $P = 0.002$).

for striatal neuronal activity in the central regulation of metabolism. Nevertheless, the extent to which the NAc and/or its surrounding regions mediate such effects in humans remains to be elucidated. We highlight that DBS and AMPT are relatively crude translational interventions, and their effects on insulin sensitivity may involve the direct modulation of NAc dopamine neurons as well as the activation or inhibition of brain regions that receive neuronal projections from the NAc. In this regard, the hypothalamus is a likely candidate that might be involved in these effects, because (i) it receives dense projections from the NAc (*11*); (ii) we have previously shown that DBS of NAc shell increases c-Fos, a marker for neuronal activity, in the lateral hypothalamus in rats (*43*); and (iii) it has been shown to control glucose metabolism via autonomic nervous projections to peripheral tissues, including the liver, pancreas, adipose tissue, and muscle (*49*–*54*). Together, our results support previous findings suggesting key roles for the striatum and hypothalamus in integrating metabolic input (nutrient, hormone, and energy sensing) and output (central regulation of systemic metabolism) (*5*).

We observed that vALIC-DBS improved peripheral insulin sensitivity in lean OCD patients, in association with striatal dopamine release, whereas DBS did not significantly alter striatal $D_{2/3}R$ availability or insulin sensitivity in the overweight/obese subgroup. Although these data may suggest that there is no effect of DBS on striatal dopamine release in obese subjects, we acknowledge that single-photon emission computed tomography (SPECT) may not be sensitive enough to detect smaller changes in dopamine release and that, in overweight/obese subjects, DBS may have induced a slight increase in extracellular dopamine below this threshold; however, obese individuals also display reduced striatal dopamine release in response to amphetamine (*55*) or food-related stimuli (*56*, *57*). These observations support the hypodopaminergic hypothesis of obesity development, where decreased dopaminergic activity may contribute to compensatory overconsumption (*17*). Our data suggest that decreased central dopamine signaling may also contribute, in part, to obesity-related insulin resistance. A better understanding of the central glucose–controlling mechanisms may open up new avenues for the prevention and treatment of insulin resistance and type 2 diabetes.

Some nuances and limitations with respect to our findings should be discussed. First, the downstream neuronal circuits underlying the observed effects remain to be elucidated, and other neurotransmitters that may be affected by striatal DBS should be considered. In animals, stimulation of the NAc shell increases concentrations of both dopamine and serotonin (*58*, *59*). It is currently unknown whether DBS in this region induces serotonin release in humans, but evidence from multiple species supports a role for serotonin signaling in the control of glucose metabolism (*60*–*63*). Other neurotransmitters, including glutamate and GABA (γ-aminobutyric acid) (*64*–*66*), are also affected by DBS of the NAc area and may be involved in its glucoregulatory effects. In light of this, facilitation of glutamatergic NMDA (*N*-methyl-D-aspartate) receptor–mediated neurotransmission by pharmacological increase of extracellular glycine, an essential co-agonist, has recently been shown to improve glucose tolerance and energy homeostasis (*9*). Here, (i) striatal dopamine release and peripheral insulin sensitivity were differentially regulated by DBS in lean and obese patients, whereas the DBS effect on hepatic insulin sensitivity was similar in lean and obese patients; (ii) pharmacological dopamine reduction with AMPT acutely reduced peripheral, but not hepatic, insulin sensitivity; and (iii) D_1R^+ neuronal activation in mice enhanced glucose clearance, but did not lower fasting blood glucose, which is primarily determined by EGP. These findings suggest that central control of EGP may involve additional factors.

A second limitation involves the debated effects of DBS at circuit level. The vALIC contains descending glutamatergic fibers from the ventromedial prefrontal cortex and the medial orbitofrontal cortex (*67*, *68*). Dorsal fibers within this ventral part of the internal capsule

travel to the thalamus, whereas the ventral fibers descend to the brainstem. Finally, the medial forebrain bundle traverses the vALIC and connects to the NAc, whereas the inferomedial branch reaches the lateral hypothalamus (*69*), both structures known to be involved in central control of metabolism. Animal and three-dimensional simulation models show that DBS targets in OCD hit fibers descending to the thalamus and brainstem (*68*). Previous observations also indicate that DBS may excite efferent axons, thereby providing stimulation to a target nucleus (*70*). Thus, one possible mechanism of vALIC-DBS may be stimulation of dopaminergic axons to the NAc. It should, however, be clear that the exact mechanism of action of DBS, as well as the underlying neural circuitry, remains to be determined. In addition, electric stimulation may affect brain regions other than those adjacent to the electrodes. We have previously shown that bilateral DBS of the subthalamic nucleus, in contrast to the present stimulated area, does not affect basal glucose metabolism or insulin sensitivity in humans (*71*), but we cannot rule out that other nuclei were activated or inhibited by vALIC-DBS and contributed to the observed effects. However, the lack of significantly increased insulin sensitivity, concomitant with lower or absent striatal dopamine release, upon DBS in the overweight/obese subgroup and the direct effect of NAc D_1R^+ neuronal activation on glucose tolerance suggest that striatal dopamine signaling is a major contributor to central glucose regulation. Future studies should address aspects of timing (acute versus chronic) and magnitude (physiological versus supraphysiological) to determine whether dopamine-mediated regulation of metabolism is clinically relevant for chronic human diseases such as obesity and type 2 diabetes.

Finally, we acknowledge that the OCD patients who participated in the DBS study were not randomly assigned to treatment order. In our current nonrandomized controlled crossover design, we were able to study the acute effects of DBS on glucose metabolism in humans, yet minimizing the amount of time that these symptomatic patients were exposed to unstimulated (untreated) conditions. Thus, we cannot rule out that our DBS study findings were influenced by independent order effects. For instance, stress levels may have changed during the course of the study because of the study procedures or psychiatric symptom severity. Although we did not observe differences in cortisol concentrations during hyperinsulinemia, we cannot rule out a stress effect on insulin sensitivity.

To conclude, our results demonstrate that DBS targeting the vALIC at the border of the NAc, which results in striatal dopamine release (*34*), reduces insulin requirements in a diabetic patient and increases hepatic and peripheral insulin sensitivity in OCD patients. We also show that pharmacological inhibition of dopamine synthesis, which reduces striatal dopamine concentrations (*35*), decreases peripheral insulin sensitivity in healthy subjects. Supporting these human data, we demonstrate that direct activation of D_1R^+ neurons in the rodent NAc improves glucose tolerance and insulin sensitivity. We acknowledge that human models of striatal dopamine modulation (vALIC-DBS and AMPT) have methodological limitations, but all of our results point in the same direction, thereby supporting the hypothesis that striatal neuronal activity regulates systemic glucose metabolism.

MATERIALS AND METHODS

Study design

We aimed to determine whether modulation of the striatal dopamine system affects glucose metabolism in humans and mice.

DBS study

The study was prospectively registered in the Netherlands Trial Registry (www.trialregister.nl; NTR2000). We determined outcomes of glucose metabolism and insulin sensitivity on two occasions: once while the DBS was switched off (unstimulated state) and once while the DBS was switched on (stimulated state). To this end, the DBS was switched off at 3:00 p.m. on the day before the first study day. It then remained off for 1 week and was switched back on at 3:00 p.m. on the day before the second study day.

AMPT study

The study was prospectively registered (www.trialregister.nl; NTR1999). We determined outcomes of glucose metabolism and insulin sensitivity on two occasions in a randomized controlled crossover design: once during conditions of systemic dopamine depletion and once during control conditions. Acute dopamine depletion was induced by oral administration of AMPT, which temporarily inhibits tyrosine hydroxylase, the rate-limiting enzyme in endogenous dopamine synthesis (*40*). The AMPT dose (40 mg/kg, up to 4 g) was selected to achieve effective inhibition of tyrosine hydroxylase activity with minimal side effects (*35*) and administered in four equal doses: at 6:00 p.m. and 10:00 p.m. on the day before the clamp and at 7:00 a.m. and 10:00 a.m. on the day of the clamp. Subjects were instructed to drink plenty of water to prevent the formation of AMPT crystals in the urine.

Participants

All procedures were approved by the Academic Medical Center medical ethics committee, and all subjects provided written informed consent in accordance with the Declaration of Helsinki.

DBS study

Patients were recruited from the psychiatric outpatient clinic of the Academic Medical Center. All patients were diagnosed with pharmacoresistant primary OCD on the basis of DSM-IV (Diagnostic and Statistical Manual of Mental Disorders-IV) criteria. Between 2005 and 2008, bilateral quadripolar DBS electrodes were implanted in the vALIC during stereotactic surgery as described (*32*). The patients were treated with monopolar stimulation of the two dorsal contacts, thereby providing most effective stimulation at the border of the vALIC and the NAc core. Patients were stimulated with an average of 4.8 V (range, 3.5 to 6.2); a frequency of 130 Hz ($n = 11$) or 185 Hz ($n = 4$); and a pulse width of 90 μs ($n = 12$), 130 μs ($n = 2$), or 150 μs ($n = 1$). They were instructed to stop psychoactive drugs and/or other drugs that may influence dopamine or glucose metabolism before the start of the study ($n = 13$), or, if discontinuation was not possible, take the drug at the same time during both study days ($n = 1$). The same patients participated in a concurrent study that was designed to determine the effect of DBS on striatal dopamine release (*34*).

AMPT study

Subjects were recruited from the general population through local advertisements. They were in good health, which was confirmed by medical history, physical examination, and routine blood tests. Eligibility criteria included age 18 to 35 years, BMI of 20 to 25 kg/m^2, Caucasian ethnicity, and normal glucose tolerance in accordance with American Diabetes Association criteria (*72*). Exclusion criteria were a history of psychiatric disorders, a family history of diabetes, intense physical activity (three or more times per week), and the use of any medication.

Metabolic study protocol

Glucose kinetics and tissue-specific parameters of insulin sensitivity were assessed during a two-step hyperinsulinemic-euglycemic clamp

study as described (*37*). Briefly, subjects were admitted to the clinical research unit after an overnight fast. At 8:00 a.m., a primed continuous infusion of the stable isotope-labeled glucose tracer [6,6-$^2H_2$]glucose (prime, 11 μmol/kg; continuous, 0.11 μmol kg^{-1} min^{-1}; Cambridge Isotope Laboratories) was started and continued until the end of the experiment. At 10:00 a.m., infusion of insulin (Actrapid; Novo Nordisk Farma) was started at a rate of 20 mU [m^2 body surface area]$^{-1}$ min^{-1} for step 1 of the clamp. During insulin infusion, plasma glucose was measured every 10 min, and exogenous glucose (enriched with [6,6-$^2H_2$]glucose to approximate plasma enrichment) was infused at a variable rate to maintain plasma glucose at 5.0 mM. At 12:10 p.m., insulin infusion was increased to 60 mU m^{-2} min^{-1} for step 2 of the clamp and continued for another 2 hours and 10 min. Plasma glucose enrichment, glucoregulatory hormones, and FFAs were determined at 9:50 a.m. to 10:00 a.m., 11:50 a.m. to 12:10 p.m., and 2:00 p.m. to 2:20 p.m. for the calculation of basal, step 1, and step 2 glucose fluxes, respectively.

Glucose, insulin, glucagon, cortisol, FFAs, and [6,6$^2H_2$]glucose enrichment (tracer-to-tracee ratio) were determined as described (*37*, *73*, *74*). Epinephrine and norepinephrine were determined by high-performance liquid chromatography with intra-assay variation of 9 and 2%, respectively, and inter-assay variation of 18 and 10%, respectively. Prolactin and homovanillic acid were determined as described (*34*, *35*). Body composition was determined by bioelectrical impedance analysis (BF906; Maltron International). Indirect calorimetry was performed using a ventilated hood system (Vmax Encore 29n; CareFusion). Cardiovascular tone was estimated from heart rate variability (*46*), which was determined by power spectral analysis of continuous blood pressure and heart rate measurements (Nexfin; BMEYE) as described (*75*).

Glucose fluxes (EGP and Rd) were calculated using modified versions of the Steele equations for the steady state (basal) or non–steady state (during insulin infusion) (*76*, *77*). Hepatic insulin sensitivity was expressed as insulin-mediated suppression of EGP during step 1 of the clamp. Adipose tissue insulin sensitivity was expressed as insulin-mediated suppression of circulating FFAs during step 1 of the clamp (*39*). The insulin-stimulated Rd of glucose, assessed during step 2 of the clamp, primarily reflects glucose disposal into muscle, but also into adipose tissue (*42*). Resting energy expenditure, glucose oxidation, and fat oxidation were calculated as described (*78*). The experimental protocol for the patient presented in the case report was as described above, except that the duration of step 1 (low-dose insulin) was 1 hour and 10 min and the rate of insulin infusion during step 2 was 40 mU m^{-2} min^{-1}.

Striatal $D_{2/3}R$ availability

DBS study—To determine the effect of DBS on acute striatal dopamine release, striatal $D_{2/3}R$ availability was determined 1 week after DBS discontinuation and 1 hour after switching it on, using SPECT as described (*34*). Briefly, a primed continuous infusion of the radiotracer [^{123}I]iodobenzamide [prime, 80 megabecquerel (MBq); continuous, 20 MBq/hour] was started and continued until the end of the experiment. Sustained binding equilibrium is achieved after 2 hours of radiotracer infusion (*35*, *79*). Baseline SPECT images of the brain, as well as SPECT images after the DBS was switched on, were acquired using a brain-dedicated SPECT scanner as described (*35*, *79*). Data were reconstructed and analyzed by an experienced, blinded investigator. Striatal $D_{2/3}R$ availability was expressed as BP and calculated as (striatal radiotracer binding – occipital binding)/occipital binding. The occipital cortex does not express $D_{2/3}R$ and was used as reference tissue. Endogenous dopamine release in the striatum was determined as described (*35*).

Psychiatric symptoms

DBS study—Psychiatric symptom scores were assessed using visual analog scales to evaluate the effect of vALIC-DBS (stimulated versus unstimulated) on anxiety, restlessness, depression, obsession, compulsion, and avoidance symptoms.

Animals and surgical procedures

Adult *Drd1a-cre*$^+$ mice (GENSAT strain EY262) were used in this study. Male (n = 6) and female (n = 3) mice underwent intracranial surgery targeting the NAc [anterior-posterior, 1.2 mm (from bregma); mediolateral, ±0.6 mm (from midline); dorsoventral, −4.5 mm (from skull)]. Mice were anesthetized (ketamine, 10 mg/kg; xylazine, 1 mg/kg) and placed in a stereotactic frame (Stoelting). After craniotomy, 0.5 μl of AAV-DIO-Ef1a-hChR2(H134R)-EYFP (UNC Vector Core) was delivered bilaterally over 5 min by a 32-gauge Hamilton syringe. Transduction efficiency of this commercially available adeno-associated virus (AAV) construct has been demonstrated thoroughly (*80*). Immediately after infusion of the construct, fiber-optic cannulas (DFC_200/240-0.22_5mm_GS1.2_FLT; Doric Lenses) were implanted and secured to the skull using adhesive cement (C&B-METABOND S-380; Parkell). Mice were single-housed under 12-hour light-dark cycles, provided with standard chow and water ad libitum, and allowed to fully recover for more than 2 weeks before subsequent testing. Cannula placement, tissue integrity, and enhanced yellow fluorescent protein (EYFP) expression were confirmed at the time of sacrifice (fig. S1, A to C). All procedures were conducted in accordance with the Institutional Animal Care and Use Committee.

Animal IPGTT protocol

After overnight fasting, mice were transported to a testing room and individually tethered to an overhead fiber-optic cable via their indwelling optic fibers. They were allowed to move freely. Mice were tested under laser-stimulated or control (tethered, but no laser activation) conditions. Experiments were scheduled several days apart. At t = 0:00 hours, a glucose dose of 2 g/kg was injected intraperitoneally. Immediately upon injection, the laser was turned on (a repeating profile of 3 min on, 3 min off at 10 Hz) for the duration of the test. Power output at the fiber-optic cable was maintained between 20 and 22 mW in all mice. Tail vein blood was collected at t = 0:00, t = 0:15, t = 0:30, t = 1:00, and t = 2:00 hours. Glucose was determined at all time points using a OneTouch Verio IQ glucose meter (LifeScan). Plasma insulin was determined at t = 0:30 hours by radioimmunoassay performed by the Yale Diabetes Research Core. After testing, mice were returned to the animal facility, provided with chow and water ad libitum, and allowed to recover for several days before additional experiments. Male and female mice showed similar responses and were grouped together in analyses.

Fiber-optic cannula targeting sites and ChR2-EYFP expression

Mice were anesthetized and perfused with cold phosphate-buffered saline (PBS) for 1 min, followed by cold 10% formalin for >5 min. Cannulas were carefully removed to preserve targeting sites, and brains were postfixed in 10% formalin overnight at 4°C. Brains were then cryopreserved in 30% sucrose/PBS at 4°C until they sank. Coronal sections (35 μm) were stored in 0.01% sodium azide/PBS until

further evaluation. Sections were rinsed, blocked with donkey serum, and incubated with the primary antibody (1:500; chicken anti–green fluorescent protein) overnight at room temperature. Sections were then rinsed, incubated with the secondary antibody (1:500; Alexa Fluor 488–conjugated donkey anti-chicken), rinsed again, and dried overnight. The following day, slides were rehydrated in PBS, followed by dehydration in increasing solutions of ethanol (50, 75, 90, 100, and 100%) and CitriSolv (2×, 3 min). Finally, slides were coverslipped using DPX mounting media.

Statistical analyses

Normally distributed parameters were evaluated by two-sided paired *t* tests (within-subject differences) or independent sample *t* tests (between-subject differences). Non-normally distributed parameters were evaluated by Wilcoxon signed-rank tests (within-subject differences) or Mann-Whitney *U* tests (between-subject differences). Time series data (animal experiments) were evaluated by two-way ANOVA and Bonferroni's post hoc tests. Findings were considered significant if $P < 0.05$. Analyses were performed using IBM SPSS Statistics v23 and GraphPad Prism v7.

SUPPLEMENTARY MATERIALS

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Fig. S1. Summary of fiber-optic cannula targeting sites and ChR2-EYFP expression.
Table S1. Metabolic parameters in OCD patients with vALIC-DBS.
Table S2. Psychiatric symptom severity in OCD patients with vALIC-DBS.
Table S3. Metabolic parameters during control conditions or dopamine depletion.
Table S4. Blood pressure and heart rate variability during control conditions or dopamine depletion.
Data file S1. Raw data for Fig.1.
Data file S2. Raw data for Fig.2.
Data file S3. Raw data for Fig.3.

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Striatal dopamine regulates systemic glucose metabolism in humans and mice

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A stimulating therapy for diabetes

Blood glucose concentration is controlled by the hormone insulin. In patients with type 2 diabetes, insulin resistance leads to elevated blood glucose concentration and increased risk of developing cardiovascular disorders. The brain has been shown to participate in glucose metabolism; however, whether and how modulation of brain activity affects systemic blood concentrations of glucose is poorly understood. ter Horst *et al*. show that in diabetic and nondiabetic patients, striatal dopamine release induced by deep brain electrical stimulation of the ventral anterior limb of the internal capsule improved insulin sensitivity. Conversely, pharmacological systemic dopamine depletion reduced the insulin-mediated blood glucose uptake. The findings open up a potential avenue for treating pharmacoresistant type 2 diabetes.