

# Leptin Increases Striatal Dopamine D2 Receptor Binding in Leptin-Deficient Obese (*ob/ob*) Mice

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**KEY WORDS** obesity; food; insulin; autoradiography; reward;  $\beta$ -Imager

**ABSTRACT** Peripheral and central leptin administration have been shown to mediate central dopamine (DA) signaling. Leptin-receptor deficient rodents show decreased DA D2 receptor (D2R) binding in striatum and unique DA profiles compared to controls. Leptin-deficient mice show increased DA activity in reward-related brain regions. The objective of this study was to examine whether basal D2R-binding differences contribute to the phenotypic behaviors of leptin-deficient *ob/ob* mice, and whether D2R binding is altered in response to peripheral leptin treatment in these mice. Leptin decreased body weight, food intake, and plasma insulin concentration in *ob/ob* mice but not in wild-type mice. Basal striatal D2R binding (measured with autoradiography [<sup>3</sup>H] spiperone) did not differ between *ob/ob* and wild-type mice but the response to leptin did. In wild-type mice, leptin decreased striatal D2R binding, whereas, in *ob/ob* mice, leptin increased D2R binding. Our findings provide further evidence that leptin modulates D2R expression in striatum and that these effects are genotype/phenotype dependent. **Synapse 64:503–510, 2010.** © 2010 Wiley-Liss, Inc.

## INTRODUCTION

Obesity has increased dramatically across the world and especially throughout the United States. Obesity can become a self-propagating disease in which there is a breakdown in the regulation of food intake and energy expenditure. This could be the result of the development of tolerance to peptides and/or neurotransmitters that are involved in food-intake regulation. Among these regulatory molecules, leptin and dopamine (DA) are involved in the regulation of both food intake and energy expenditure [for review, see Palmiter (2007)].

Leptin is a 16 kDa peptide that is secreted primarily by adipose tissue and acts on the brain as an adiposity signal (Green et al., 1995). Food intake results in a temporary increase in leptin levels (Baskin et al., 1999), and obese patients and rodents models have been shown to exhibit a resistance to leptin transport and/or action (Sahu, 2003). Leptin is transported across the blood brain barrier by binding to the short form of the leptin receptor, which is primarily located

in specialized cells in cerebral capillaries (Kastin and Pan, 2000). Leptin deficiency leads to a wide variety of symptoms, some of which include reproductive problems, bone formation deficiency, and cardiovascular complications (Sahu, 2003). The *ob/ob* mouse is a naturally occurring spontaneous point mutation in the leptin gene that prevents the peptide from being produced (Maffei et al., 1995). Symptoms in these mice include hyperphagia, hyperglycemia, glucose intolerance, increased plasma insulin, subfertility, impaired wound healing, and obesity (increase in the number and size of adipocytes) (Zhang et al., 1999).

Contract grant sponsor: NIAAA; Contract grant numbers: AA 11034, AA07574, AA07611

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Received 24 June 2009; Accepted 9 October 2009

DOI 10.1002/syn.20755

Published online in Wiley InterScience (www.interscience.wiley.com).

DA in the nucleus accumbens (NAc) is believed to be involved in the motivating properties of food (Baldo and Kelley, 2007; Carelli, 2002; Wise, 2006). In the caudate putamen (CPu) DA plays a role in maintaining food intake and driving the need to eat (Salamone and Correa, 2002). Indeed, imaging studies have shown that severely obese individuals have decreased striatal D2 receptor (D2R) availability (Wang et al., 2001), and leptin-receptor deficient obese rodents also show decreased D2R binding in striatum (Hamdi et al., 1992; Thanos et al., 2008). In contrast, chronic food restriction showed greater striatal D2R binding relative to ad libitum fed rats (Thanos et al., 2008). Genetic studies, although not always consistent, have reported that individuals carrying the Taq 1 A1 allele of the D2R gene, which was associated with decrease in D2R in striatum by some investigators (Thompson et al., 1997), are more vulnerable to addictive behaviors such as compulsive food intake and are more likely to be obese (Noble et al., 1991; Stice et al., 2008). In humans, DA concentration in CSF decreases as leptin increases (Hagan et al., 1999), which could reflect inhibition of DA release by leptin. Indeed, preclinical studies have shown that short-term leptin treatment decreases both DA release and concentration in NAc (Krugel et al., 2003) and hypothalamus in a dose-dependent manner (Brunetti et al., 1999). The D2R agonist, bromocriptine (BC) reduces body fat in laboratory animals (Cincotta and Meier, 1989) and humans (Meier et al., 1992) and decreases food intake while increasing locomotor activity and D2R binding in leptin-receptor deficient obese Zucker rats (Davis et al., 2008). These observations are all consistent with the construct that there is hypoactivity of the DA system including disruption in D2R expression in obesity and obesity-related metabolic disturbances.

Here, we assessed the role of leptin on D2R binding in normal and leptin-deficient obese mice. Because leptin has been shown to decrease food intake, weight gain, and DA release and concentrations, we hypothesized that normal and *ob/ob* mice would show changes in D2R binding in response to leptin treatment.

## MATERIALS AND METHODS

### Animals and procedures

Male 8-week-old mice were divided into four groups of eight mice per group: (1) *ob/ob* mice treated with vehicle (*ob-veh*); (2) *ob/ob* mice treated with leptin (*ob-lep*); (3) C57/BL6 wild-type mice treated with vehicle (*wt-veh*), and (4) C57/BL6 wild-type mice treated with leptin (*wt-lep*). Animals were purchased from Jackson Laboratories (Bar Harbor, ME) and individually housed on a reverse 12-h light–dark cycle with lights off at 7 am. All procedures were performed so

as to minimize animal discomfort and in accordance with the National Academy of Sciences Guide for the Care and Use of Laboratory Animals (NAS and NRC, 1996) and Institutional Animal Care and Use Committee (State University of New York, Stony Brook) guidelines.

### Pump implantation

All surgeries were done under sterile conditions. The animals were first anesthetized with isoflurane. The area of implantation was shaved and disinfected. A small incision was then made in between the scapulae, and a small pocket was formed by gently separating the subcutaneous connective tissue. Alzet micro-osmotic pumps (Model 1002, Durect Corp.) were placed inside the pocket with the flow moderator pointing away from the incision. Animals were allowed to completely recover from the anesthesia before being returned to their housing facility and monitored daily for the duration of the experiment. Leptin-treated mice were infused with leptin (Phoenix Peptide, Burlingame, CA) at a rate of 200 ng/h and vehicle-treated mice were infused with saline for a period of 8 days. Mean total food intake and body weight were measured before and at the end of the 8-day treatment period for each mouse.

### D2R autoradiography

On the last day of treatment, mice were fasted overnight. The next morning the animals were anesthetized with isoflurane and then decapitated. The brain was then rapidly removed and frozen in an isopentane and dry ice bath and stored in a  $-80^{\circ}\text{C}$  freezer. The brain was then transferred to a cryostat (Leica CM3050), and sections were cut 14- $\mu\text{m}$  thick at  $-18^{\circ}\text{C}$ . Sections were mounted on glass microscope slides and stored at  $-80^{\circ}\text{C}$  until binding was conducted. Slides were gradually brought back to room temperature and then preincubated at room temperature for 10 min in 50 mM Tris–HCl buffer (pH 7.4). The slides were then incubated in specific binding buffer (50 mM Tris–HCl, 0.4 nM [ $^3\text{H}$ ] spiperone, and 10  $\mu\text{M}$  ketanserin tartrate) at room temperature for 1 h. To determine nonspecific binding, some slides in parallel were incubated for 1 h in specific binding buffer in the presence of 10  $\mu\text{M}$  haloperidol. Next, the slides were washed  $2 \times 10$  min in ice-cold 50 mM Tris–HCl and ice-cold  $\text{dH}_2\text{O}$ . Afterward, 20- $\mu\text{l}$  aliquots of incubation solution were taken and added with 3 ml of Ultima Gold XR (scintillation solution), and the amount of radioactivity was measured with a liquid scintillation counter. Slides were then dried overnight in a dessicator and placed in a glass slide cassette for qualitative and quantitative analysis using a  $\beta$ -Imager (Biospace, Paris, France). Using Betavision+ software (Biospace, Paris, France), region of interests

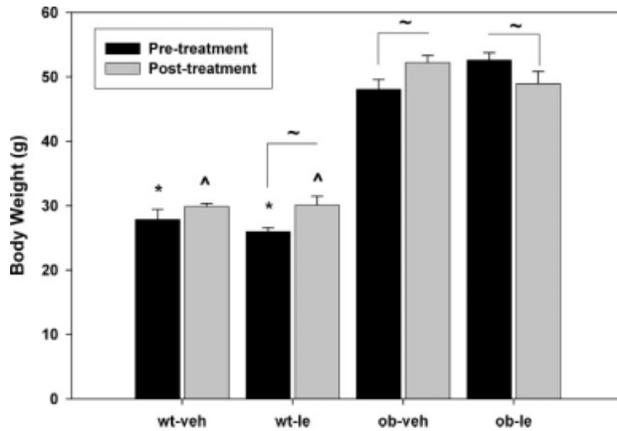


Fig. 1. Mean (+SEM) daily body weight in *ob/ob* and wild-type mice before and after treatment with vehicle or leptin. (\*) wt-veh and wt-le mice weighed significantly less than ob-veh and ob-le mice before treatment. (^) wt-veh and wt-le mice weighed significantly less than ob-veh and ob-le mice after treatment. (~) wt-le and ob-veh mice weighed significantly more posttreatment and ob-le mice weighed significantly less posttreatment.

were drawn on the left and right CPu and NAc and cerebellum (CB) of each brain slice. The data was then calibrated using a tissue homogenate standard. Mean values ( $\mu\text{Ci/g}$ ) of the left and right receptor rich regions (CPu and NAc) for each group were later normalized for receptor poor regions (CB), and the data are reported in respective ratios (receptor rich/receptor poor).

### Plasma leptin and insulin concentrations

At the time of sacrifice, trunk blood was collected using EDTA as an anticoagulant and centrifuged at 3000g for 10 min at 4°C to isolate the plasma. The plasma was used to determine glucose, insulin, and leptin concentrations by using enzyme-linked immunosorbent assays (ELISA). ELISA kits were purchased from Linco Research and completed, in duplicate, following the accompanying protocol. The absorbance ( $\lambda = 450 \text{ nm}$ ) of the immunoplate was measured at the end of the protocol using a microplate reader.

## RESULTS

### Body weight and food intake

A two-way ANOVA showed a significant Group main effect:  $F(3, 42) = 166.609$  in body weight. Pairwise multiple comparisons (Holm–Sidak) showed that ob-lep mice weighed significantly less after leptin treatment ( $t = 2.007$ ;  $P < 0.05$ ). On the other hand, wt-lep mice weighed significantly more after leptin treatment ( $t = 2.067$ ;  $P < 0.05$ ) (Fig. 1). Also, ob-veh mice continued to gain weight as expected ( $t = 2.242$ ;  $P < 0.05$ ) (Fig. 1). Finally, wt-veh and wt-lep mice weighed significantly less before [lep-veh ( $t = 10.187$ ;

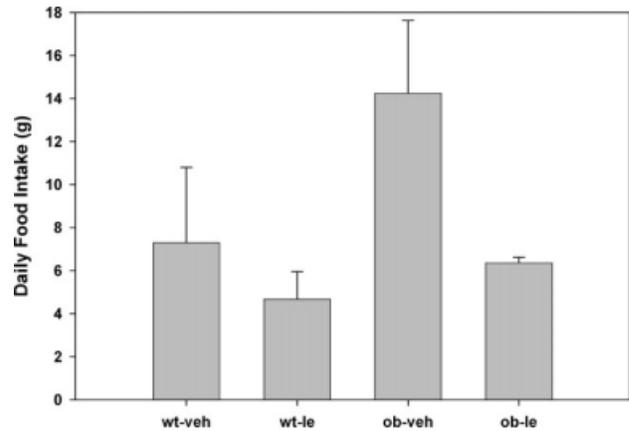


Fig. 2. Mean (+SEM) daily food intake in *ob/ob* and wild-type mice during the treatment period. (\*) ob-le mice showed significantly lower food intake compared to ob-veh mice.

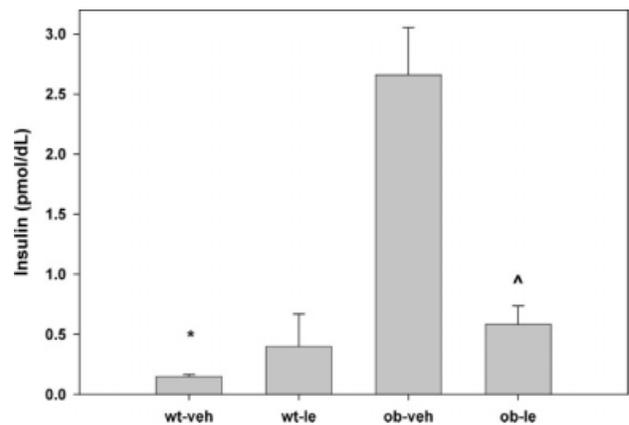


Fig. 3. Mean (+SEM) plasma leptin concentrations in *ob/ob* and wild-type mice after treatment with vehicle or leptin. (\*) wt-le mice had significantly greater plasma leptin levels than wt-veh mice. (^) ob-le mice had significantly greater plasma leptin levels than ob-veh mice.

$P < 0.05$ ); wt-lep ( $t = 14.748$ ;  $P < 0.05$ )] and after treatment [lep-veh ( $t = 10.522$ ;  $P < 0.05$ ); lep-lep ( $t = 9.417$ ;  $P < 0.05$ )] compared to ob-veh and ob-lep mice (Fig. 1). The only significant difference, we observed in food intake, was between ob-lep and ob-veh mice. A  $t$ -test revealed significantly lower food intake in ob-le mice ( $t = 8.439$ ;  $P < 0.05$ ; Fig. 2).

### Insulin and leptin

A two-way ANOVA showed significant main effects in strain [ $F(1, 14) = 24.332$ ;  $P < 0.001$ ], treatment [ $F(1, 14) = 11.183$ ;  $P = 0.007$ ], and their interaction [ $F(1, 14) = 18.114$ ;  $P = 0.001$ ]. Multiple pairwise comparisons showed significantly higher insulin levels in ob-veh compared to ob-lep mice ( $t = 5.594$ ;  $P < 0.05$ ) and wt-veh mice ( $t = 6.261$ ;  $P < 0.05$ ; Fig. 3).

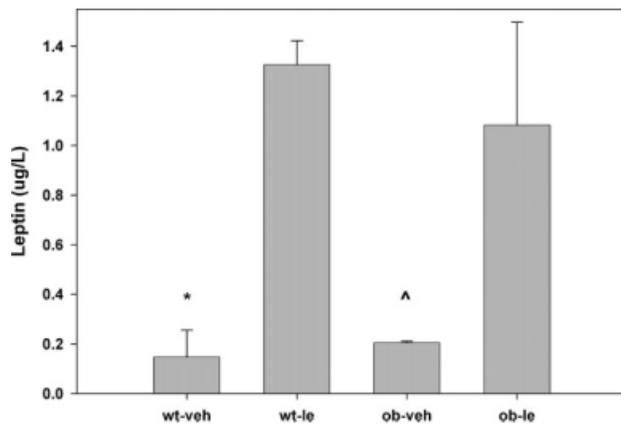


Fig. 4. Mean (+SEM) plasma insulin concentrations in *ob/ob* and wild-type mice after treatment with vehicle or leptin. (\*) wt-veh mice had significantly lower plasma insulin levels than ob-veh mice (^) ob-le mice had significantly lower plasma insulin levels than ob-veh mice.

A two-way ANOVA showed a significant main effect in treatment [ $F(1, 11) = 20.407$ ;  $P = 0.002$ ]. Multiple pair-wise comparisons showed significantly higher leptin levels in wt-lep compared to wt-veh ( $t = 3.560$ ;  $P < 0.05$ ) and ob-lep compared to ob-veh ( $t = 2.809$ ;  $P < 0.05$ ) mice (Fig. 4). Although wt-lep mice showed greater leptin levels compared to ob-lep mice, due to variability within the ob-lep group, this difference was not significant.

#### D2R BINDING Caudate putamen

A two-way ANOVA showed a significant main effect in strain [ $F(1, 25) = 7.104$ ;  $P < 0.01$ ] and the interaction between strain and treatment [ $F(1, 25) = 14.715$ ;  $P < 0.001$ ]. Multiple pairwise comparisons (Holm-Sidak) showed significantly greater D2R binding in ob-lep compared to ob-veh ( $t = 2.358$ ;  $P < 0.05$ ) and wt-lep ( $t = 4.451$ ;  $P < 0.05$ ) mice (Fig. 5). Also, wt-veh mice showed greater D2R binding compared to wt-lep ( $t = 3.047$ ;  $P < 0.05$ ) mice (Fig. 5).

#### Nucleus accumbens

A two-way ANOVA showed a significant main effect in strain [ $F(1, 25) = 6.988$ ;  $P < 0.01$ ] and the interaction between strain and treatment [ $F(1, 25) = 13.383$ ;  $P < 0.001$ ]. Multiple pairwise comparisons (Holm-Sidak) showed significantly lower D2R binding in wt-lep compared to wt-veh ( $t = 3.310$ ;  $P < 0.05$ ) and ob-lep ( $t = 4.315$ ;  $P < 0.05$ ) mice (Fig. 5).

A one-way ANOVA showed significantly greater binding in the NAc compared to the CPu in all treatment groups [ $F(7, 51) = 29.313$ ;  $P < 0.001$ ]. Multiple pairwise comparisons (Holm-Sidak) showed significantly greater binding in the NAc compared to the CPu in wt-veh ( $t = 6.809$ ;  $P < 0.05$ ) and wt-lep ( $t =$

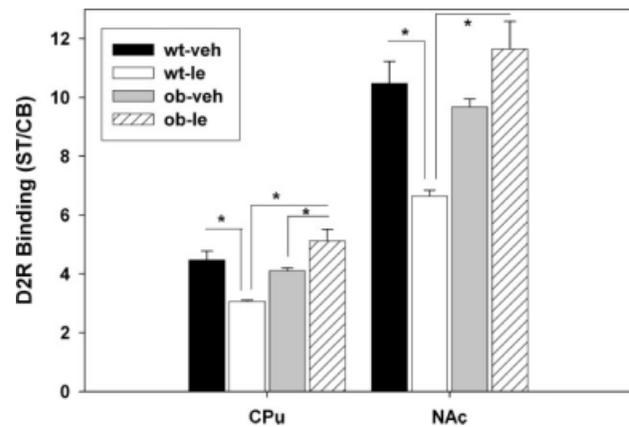


Fig. 5. Mean (+SEM) [ $^3\text{H}$ ] spiperone D2R binding in *ob/ob* and wild-type mice in the CPu and NAc after treatment with vehicle or leptin. Statistically significant differences are denoted by the (\*) symbol and connector lines.

4.058;  $P < 0.05$ ), ob-veh ( $t = 7.294$ ;  $P < 0.05$ ), and ob-lep ( $t = 7.392$ ;  $P < 0.05$ ).

## DISCUSSION

### Effects of leptin deficiency and treatment on food intake and body weight

Leptin deficiency was associated with higher food intake (although not significant due to high-sample variability) and body weight. Ob-veh mice consumed 47% more food and weighed 40% more than wt-veh-treated mice during the treatment period. Leptin treatment was associated with decreases in food intake of 66% in ob-lep (significant) and of 37% in wt-lep mice (nonsignificant). The effects of leptin on body weight differed in the two groups; leptin decreased weight only in the *ob/ob* mice (7% decrease), whereas it did not affect weight in the wild-type mice. Indeed, the wt-lep showed a 15% weight increase similar to that observed in the wt-veh mice. These findings support the lack of a universal effect of leptin treatment to decrease weight in humans (Jequier, 2002). It should be noted that the food-intake values, we present in Figure 2, are high compared to what others have previously reported. Specifically, previous studies have shown that similarly aged wild-type and *ob/ob* mice, respectively, consume around 4 and 6 g/day (Szczyepka et al., 2000). Our findings show corresponding food intake levels at around 7 and 14 g/day for vehicle-treated wild-type and *ob/ob* mice, respectively. We attribute this discrepancy to methodological differences in food measurements. We measured food only at the beginning and end of the 8-day treatment period, and we did not account for any food that was hoarded in the bedding, which probably lead to inflated food intake values.

### Effects of leptin deficiency and treatment on plasma leptin and insulin

Ob-veh and wt-veh mice showed similar plasma leptin concentrations. This is an unexpected finding, because *ob/ob* mice are characterized by leptin deficiency. Because, fasting decreases leptin, the low-leptin concentrations observed in wt-veh mice are likely to reflect their fasting status at the time of the measurements (O'Doherty and Nguyen, 2004). Both groups of leptin-treated animals showed increased plasma leptin levels (up to  $\sim 1.4$  ng/ml), whereas the untreated animals were approximately between 0.15 and 0.2 ng/ml or about a sixfold increase. Previously, it has been shown that baseline unfasted *ob/ob* and control mice had leptin levels of 1.5 and 1.6 ng/ml, respectively, and that leptin treatment increased plasma leptin levels about three to fourfold; (Harris et al., 1998). Therefore, leptin treatment was associated with a significant increase in plasma leptin concentrations in wt-lep and ob-lep mice, respectively. Leptin decreased the plasma hyperinsulinemia in the ob mice (plasma insulin was decreased 160%) resulting in insulin concentrations similar to those in wt mice (Fig. 4). This is consistent with the prior studies showing that leptin inhibits insulin secretion and insulin gene expression in pancreatic  $\beta$ -cells of *ob/ob* mice (Seufert et al., 1999). Because, in our study, the reduction in insulin occurred even when the weight of the ob-lep was still significantly greater than that of the wt mice, it supports the notion that leptin deficiency may underlie insulin resistance in obesity (Seufert, 2004).

### Effects of leptin deficiency and treatment on D2R binding

We did not detect any differences in D2R binding between the vehicle-treated *ob/ob* and wild-type mice groups. Because pre and postsynaptic D2R levels, respectively, modulate and are in part modulated by extracellular DA signaling (Geiger et al., 2008), this finding may suggest that *ob/ob* and normal wild-type mice are characterized by similar basal DA levels in the CPU and NAc. Studies assessing DA levels in ventral striatum have been inconsistent with one in vivo microdialysis study showing that *ob/ob* and wild-type mice did not differ in basal and cocaine stimulated DA release in ventral striatum (Roseberry et al., 2007), whereas a study in brain slices reported that basal and electrically evoked DA release in ventral striatum was significantly reduced in *ob/ob* mice compared to wild-type (Fulton et al., 2006). These discrepancies could reflect technical issues such as animal age and stimulus and techniques used to measure DA release (Roseberry et al., 2007). Therefore, the DA profile of *ob/ob* mice remains unclear, and further studies are required to clarify this issue. Our

findings are more compatible with a lack of difference in basal extracellular DA levels in these two strains. This view raises implications for the hyperphagia that the *ob/ob* mice exhibit and for the involvement of mesolimbic DA in this behavior. This is relevant because DA has been shown to be required for the hyperphagia that *ob/ob* mice exhibit (mutant *ob/ob* mice lacking both leptin and DA are not hyperphagic) (Szczycka et al., 2000). However, it is unclear if DA's involvement in the hyperphagia of *ob/ob* is mediated by varying extracellular DA levels, DA neuronal firing, or DA receptor concentration and function. Our interpretation is limited by the fact that we only have measures of D2R binding but not of DA release. Nonetheless, the fact that leptin differentially decreased hyperphagia and increased D2R in *ob/ob*, but not in wild-type controls, suggests that D2R are involved in the response to leptin in *ob/ob* mice. DA concentrations in the CPU are thought to be related to eating for survival while in the NAc DA's role is thought to be related to eating for reward [for review, see Palmiter (2007)]. In both cases, increased DA signaling promotes feeding, and the magnitude of this increase is thought to underlie motivational aspects of feeding to promote satiety [for review, see Phillips et al. (2007)]. Therefore, lower DA release in response to food in *ob/ob* mice may predispose these mice to eat more to reach satiety, eventually contributing to hyperphagia. In this respect, an upregulation of postsynaptic D2R with leptin treatment could serve to amplify the weak DA signals in *ob/ob* mice decreasing the hyperphagic behavior.

In the vehicle-treated animals, we did not see a difference in D2R between *ob/ob* and wild-type mice, which is different from findings in leptin-receptor impaired obese rats that show decreases in D2R binding (Hamdi et al., 1992; Thanos et al., 2008) compared to lean littermates. Leptin receptors are in fact expressed on DA neurons (Finglewicz, 2003; Hommel et al., 2006) and because leptin-deficient mice do not differ in D2R binding from controls, while leptin receptor-deficient rats do, our findings point to unique D2R and leptin receptor interactions on DA neurons, so that the lower D2R levels in Zucker rats may reflect the deficiency of leptin receptors.

Leptin-treated wild-type animals showed a decrease in D2R binding, whereas *ob/ob* mice treated with leptin showed an increase in D2R binding. This is the first study to document changes in D2R in striatum of both *ob/ob* as well as normal mice in response to peripheral leptin administration. To our knowledge, the only previous study to examine changes in markers of central DA function in response to peripheral leptin treatment (Roseberry et al., 2007) showed that a very similar leptin-treatment procedure as ours restored cocaine-induced increases in the D2R-mediated somatodendritic synaptic current to similar

levels as observed in wild-type mice. Our findings taken together with those from prior studies (Fulton et al., 2006; Krugel et al., 2003) provide further evidence that peripheral leptin concentrations affect central DA mechanisms. However, the nature of this interaction is still far from clear (Palmiter, 2007). For example, central and peripheral leptin administration has been linked to the increases in DA production in VTA (Fulton et al., 2006; Roseberry et al., 2007) but decreases in DA release in NAc (Krugel et al., 2003). These findings seem to point to a differential effect of leptin on DA activity in different brain regions. It has been speculated that the decreases in DA release in NAc produced by leptin treatment may be related more to the anorectic effects of leptin than to direct effects of changes in leptin signaling in the brain (Krugel et al., 2003). Indeed, we previously showed that food restriction leads to greater D2R binding in normal and leptin-receptor deficient obese rodents, when compared with ad libitum fed rats (Thanos et al., 2008), and therefore the increases in D2R binding, we observe in *ob/ob* mice in the CPu and NAc, may be related more to secondary effects of leptin treatment via decreases in food intake than to direct effects of leptin on DA. Future studies aimed at pair feeding *ob/ob* mice to food intake levels of leptin-treated counterparts may help in establishing causality. On the other hand, decreases in D2R binding in normal weight rats may serve as a "safety" mechanism to protect from negative energy balance states such as starvation, because decreases in D2R would promote increases in feeding. This view is particularly interesting, because it suggests that apart from motivation to procure and consume food, striatal DA function may also be involved in energy balance as well.

Leptin impairment has been hypothesized to increase the "threshold" for DA's effects on food intake (Fetissov et al., 2002). According to this view, rats and mice with impaired leptin signaling (*ob/ob* mice and obese Zucker rats) need to consume more food than normal mice and rats to reach the same DA level at which the normal mice experience satiety and stop consuming food. Our findings of increased D2R binding in *ob/ob* mice following leptin treatment further support this view, because increased D2R could translate to a lower DA "threshold."

Leptin treatment in the *ob/ob* mice decreased plasma-insulin concentrations. Like leptin, DA, and, specifically, the D2-like receptor family, have both been implicated in mediating inhibition of insulin secretion in pancreatic  $\beta$ -cells (Rubi et al., 2005). Furthermore, a recent study has shown that insulin may be involved in regulating striatal DA release via its actions on the DA transporter (DAT) (Williams et al., 2007). Specifically, this study showed that insulin depletion was associated with decreases in

striatal DAT cell-surface expression and amphetamine-induced DA release. These findings suggest that insulin may have an indirect effect on striatal D2R via its actions on the DAT and DA signaling. Therefore, it is possible that the decrease in peripheral insulin in leptin-treated *ob/ob* mice in our study may be related to the increases in D2R binding, we observed, as decreases in DA signaling would lead to D2R upregulation. This interpretation implicates a regulatory role for central DA and specifically the D2R in peripheral energy metabolism and merits further investigation. Indeed, one recent study reported increases in DA release in the NAc in response to purely postingestive factors (calorie load) and not to taste (de Araujo et al., 2008). In humans, reports of changes in peripheral glucose and insulin concentrations after administration of D2R agonists (Scranton et al., 2007) and antagonists (Pezzarossa et al., 1986) further support this view. In particular, the D2R agonist BC currently holds promise as an effective diabetes medication (Durant et al., 2007; Scranton et al., 2007).

The decreased D2R binding, we observed in leptin-treated wild-type mice, does not support the idea that reduction in food intake results in increases in D2R. A potential explanation for this may be that the decreases in food intake, we observed, were not robust enough or over a long enough time period to cause an increase in D2R binding. Another explanation may be that the decreased D2R binding in leptin-treated wild-type mice may reflect changes in presynaptic D2R (D2 autoreceptors). DA release is mediated by presynaptic D2R (Farnebo and Hamberger, 1971) and peripherally administered leptin decreases DA release in the NAc (Krugel et al., 2003). The decreases in D2R binding, we observed in leptin-treated wild-type mice, support this interpretation and suggest that a potential reason for decreased DA release in the NAc due to either direct (leptin itself) or indirect (anorectic effects of leptin) effects on decreasing presynaptic D2R. This interpretation is purely speculative, because our D2R measures with spiperone do not allow us to differentiate between pre versus postsynaptic D2R. Similarly, we cannot determine if the increases in D2R binding in *ob/ob* mice are due to changes in D2R at pre or postsynaptic sites.

In this study, we did not monitor locomotor activity and thus we cannot determine the extent to which differences in locomotor activity with and without leptin treatment contribute to the changes in body weight and D2R.

A limitation for this study was that our measurements were limited to dorsal striatum and NAc but not to other regions such as hypothalamus and amygdala, where leptin receptors are abundant and which are also involved in modulating feeding behavior.

## CONCLUSION

Vehicle-treated *ob/ob* mice continued to gain weight, consumed more food, and showed greater peripheral insulin levels compared to wild-type mice, whereas D2R binding was the same in both groups. Leptin-treated *ob/ob* mice lost weight, their fasting insulin levels were lowered to an equivalent level to wild-type mice, and food intake decreased and D2R binding increased in CPU and NAc. In contrast, leptin-treated wild-type mice did not lose weight, plasma insulin did not change, and food intake decreased (albeit not significantly) while D2R binding decreased. The basal D2R binding did not differ between *ob/ob* and lean wild-type, which suggests that striatal D2R do not contribute to the phenotype of *ob/ob* mice. However, leptin administration was found to induce significant and differential changes in striatal D2R in these two strains, and therefore the effect of leptin treatment in *ob/ob* mice on DA metabolism merits further investigation.

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