

Responses to drugs of abuse and non-drug rewards in leptin deficient ob/ob mice

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Abstract

Rationale Although leptin receptors are found in hypothalamic nuclei classically associated with homeostatic feeding mechanisms, they are also present in brain regions known to regulate hedonic-based feeding, natural reward processing, and responses to drugs of abuse. The ob/ob mouse is deficient in leptin signaling, and previous work has found altered mesolimbic dopamine signaling and sensitivity to the locomotor activating effects of amphetamine in these mice.

Objectives We directly assessed responses to three drugs of abuse and non-drug rewards in the leptin-deficient ob/ob mouse.

Methods Ob/ob mice were tested in assays of sweet preference, novelty seeking, and drug reward/reinforcement.

Results In assays of novelty seeking, novel open field activity and operant sensation seeking were reduced in ob/ob mice, although novel object interaction and novel environment preference were comparable to wild types. We also found that ob/ob mice had specific phenotypes in regard to cocaine: conditioned place preference for 2.5 mg/kg was increased, while the locomotor response to 10 mg/kg was reduced, and cocaine self-administration was the same as wild types. Ob/ob mice also acquired self-administration of the potent opioid remifentanyl, but breakpoints for the drug were significantly reduced. Finally, we found significant differences in ethanol

drinking in ob/ob mice that correlated negatively with body weight and positively with operant sensation seeking.

Conclusions In conclusion, ob/ob mice displayed task-specific deficits in novelty seeking and dissociable differences in reward/reinforcement associated with cocaine, remifentanyl, and ethanol.

Keywords Alcohol · Cocaine · Leptin · Opioid · Operant sensation seeking · Self-administration · Saccharin · Sucrose

Introduction

Leptin is a peripherally released peptide that powerfully modulates food intake (Friedman and Halaas 1998). The ob/ob mouse is homozygous for a spontaneous mutation in the leptin gene (*lep*), which disrupts leptin signaling, resulting in excessive food intake. This phenotype is largely due to a lack of leptin signaling in neurons of the arcuate nucleus of the hypothalamus (Davis et al. 2011; Friedman 1998; Houseknecht et al. 1998). While the leptin receptors found in this area are classically associated with homeostatic feeding mechanisms, leptin receptors are also present in brain regions known to regulate hedonic-based feeding, natural reward processing, and responses to drugs of abuse (Leinninger 2009; Narayanan et al. 2010). Specifically, the leptin receptor (LepRb) is found in the ventral tegmental area (VTA), nucleus tractus solitarius, and lateral hypothalamus (Caron et al. 2010; Figlewicz et al. 2003; Scott et al. 2009). Consistent with the hypothesis that leptin can modulate non-homeostatic feeding, sucrose self-administration (in non-food restricted animals) is reduced by both i.c.v. and intra-VTA infusions of leptin (Davis et al. 2011; Figlewicz et al. 2006), and VTA knock-down of leptin receptors increases consumption of palatable foods (Hommel et al. 2006).

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There is also evidence that changes in leptin signaling can alter responses to drugs of abuse. Leptin-deficient ob/ob mice have a reduced locomotor response to amphetamine, and administration of leptin increases amphetamine locomotor responses in ob/ob and WT mice (Fulton et al. 2006). A more recent study demonstrated that intra-VTA leptin reduced conditioned place preference for cocaine and cocaine seeking in rats that had previously self-administered cocaine, but had no acute effect on cocaine self-administration (You et al. 2015). Together, these data suggest that leptin signaling may play different roles in locomotor and reward-related responses to psychostimulants, although differences may also be attributable to differences between the chronic leptin deficiency in the ob/ob mouse and acute modulation of leptin signaling in rats. Based on the location of leptin receptors and reported phenotypes of ob/ob mice, we hypothesized that reinforcement of non-calorie-containing rewards would be reduced in these mice. To test this hypothesis and to determine if psychostimulant phenotypes following chronic disruption of leptin signaling would be congruent with data from acute modulation, we examined responses to a variety of drugs of abuse and non-drug rewards in the leptin-deficient ob/ob mouse.

Materials and methods

Animals

Age-matched male ob/ob and wild-type (WT) mice (6–20 weeks old) were bred from heterozygous breeders generated by mating male congenic C57BL/6J ob/ob (Jackson Labs, stock #000632) and female C57BL/6J mice. Mice were housed in MCW animal care facilities on a reverse light cycle (lights off 0800–2000 hours) and experiments were performed between 0900 and 1600 hours. Genotyping was performed according to the PCR method available from Jackson Labs. Food and water was provided ad libitum during all experiments. Mice were housed in groups of two to five with the exception of animals in sweet preference, ethanol drinking, and intravenous drug self-administration experiments, which were single housed prior to experiments. All mice were handled for 3 days prior to experiments as described (Olsen and Winder 2010). All experiments were approved by the Institutional Animal Care and Use Committee at the Medical College of Wisconsin and were performed within the guidelines of the Guide for the Care and Use of Laboratory Animals.

Jugular catheterization

Jugular catheterization was similar to that described (Grueter et al. 2006; Olsen and Winder 2006; Schramm-Sapyta et al. 2006). Mice were anesthetized with isoflurane (3–5 % induction, 1–3 % maintenance) and were implanted with a silicone

catheter (0.2 mm ID, 0.4 mm OD, Access Technologies, Skokie, IL) into the right jugular vein, which exited through the intrascapular region and was connected to a cannula assembly. The cannula assembly consisted of a 26 gauge stainless steel cannula (Plastics One, Roanoke, VA) mounted on a silicone base with nylon mesh (similar to that described in Thomsen and Caine 2007), which was implanted subcutaneously. Rimadyl (carprofen, 5 mg/kg s.c.) was administered immediately following surgery and 24 h later. Mice were allowed to recover ≥ 5 days before experiments.

Apparatus

Novel open field (NOF)/novel object interaction (NOI)

A plastic gray circular chamber (45.5 cm diameter, 32.8 cm height) was placed on a smooth white floor with overhead lighting (150 ± 20 lx). Custom novel objects were made using various Lego pieces. Two variations were used (object 1: $8.9 \times 6.4 \times 6.2$ cm ($l \times w \times h$); object 2: $8.9 \times 3.9 \times 6.6$ cm). Mice were exposed to one object in each of two separate NOI tests (order counterbalanced). Data was recorded by an overhead video camera and analyzed by ANY-maze Video Tracking System (Stoelting, Wood Dale, IL).

Novel environment preference (NEP)

A custom two-chamber clear acrylic apparatus ($32.9 \times 21.7 \times 30.4$ cm ($l \times w \times h$)) was housed inside a sound-attenuating chamber (Med Associates, St. Albans, VT) with overhead lighting (105 ± 5 lx) and a fan that provided background noise. The apparatus was divided into two equal chambers (15.3×20.5 cm) by a black plastic divider with an opening at the base to allow access between chambers. A removable black plastic insert was used to block opening. The two chambers differed only in B/W wall pattern (checkerboard vs. diagonal stripes) that was affixed to the outside of the clear walls. Video was acquired from overhead cameras and stored and analyzed by ANY-maze.

Conditioned place preference (CPP) for cocaine

CPP took place in three-chamber apparatuses (Med Associates) in a room with a white noise generator (70 dB). The two main chambers (16.9×12.7 cm) differed in wall color (black or white), floor texture, and illumination (black 14 lx, white 4 lx), and were separated by a center gray compartment (10.0×12.7 cm) with a smooth plastic floor. Animal location and locomotor activity were determined using photobeam detectors within the chambers and data were analyzed by Med-PC IV software. There was no consistent pre-conditioning bias between the main chambers, and assignment of the

cocaine-paired chamber was counterbalanced within each genotype based on pre-conditioning preference test.

Operant conditioning chambers

Operant conditioning studies were performed in self-administration chambers (Med Associates, 21.6 × 17.8 × 12.7 cm) similar to those described (Olsen and Winder 2009), with the exception that the left wall was equipped with two retractable levers and the right wall was equipped with nosepeaks with a liquid receptacle between them. Each manipulandum had a yellow LED mounted above it.

Drugs

Cocaine-HCl (generously provided by the NIDA Drug Supply Program), remifentanyl-HCl (Ultiva, Mylan Pharmaceuticals, Morgantown, WV), and methohexital sodium (Brevital, JHP Pharmaceuticals, Rochester, MI) were diluted in 0.9 % saline (Hospira, Lake Forest, IL). Sucrose and saccharin-sodium (Sigma-Aldrich, St. Louis, MO) and USP-grade ethanol (Decon Labs, King of Prussia, PA) were diluted in drinking water (hyperchlorinated) obtained from the Medical College of Wisconsin vivarium.

Behavioral procedures

Sweet preference

Mice were singly housed with ad libitum food and given access to sweet solutions under a standard two-bottle choice paradigm: one containing water, the other a sweet (saccharin or sucrose) solution. Bottles were available 24 h/day, and mice had access to each concentration for 48 h, with the bottle side switched each 24 h. All concentrations were given in ascending order, and saccharin doses (0.001, 0.003, 0.01, 0.03, 0.1, 0.3 %) were completed before animals were advanced to sucrose (0.03, 0.01, 0.03, 1, 1.8, 3, 10 %).

Novelty seeking assays

Mice ($n = 16$ WT, $n = 23$ ob/ob) underwent a series of novelty seeking assays: novel open field/novel object interaction (NOF/NOI), novel environment preference (NEP), and operant sensation seeking (OSS (Olsen and Winder 2009)). Each novelty test was separated by ≥ 48 h. In the NOF test, mice were placed in the center of the open field and monitored for 60 min and analyzed for distance traveled and center time (center 1/3 of the apparatus). After 60 min, a novel object was placed in the center of the apparatus and interaction was measured for 30 min. Interaction was calculated in ANY-Maze and defined as the head of the mouse being within 2 cm of any edge of the object. Mice were then tested for

NEP using similar methods to those described for rats (Belin et al. 2011; Meyer et al. 2010). Mice were habituated to one of the two chambers (counterbalanced within genotype) for 30 min, then allowed access to both chambers for the 20-min test. The preference ratio was calculated as *time in the novel chamber/test time*. Next, mice were tested for OSS in 2-h sessions as described (Olsen and Winder 2009), with the exception that each light flash was mirrored on the opposite wall, resulting in either two front or two rear lights being simultaneously illuminated. FR-1 sessions continued until criteria were met (three consecutive sessions of ≥ 30 reinforcers and $\geq 2:1$ ratio of active to inactive lever presses, minimum 8 sessions) or 12 sessions had been completed. After completion of OSS, mice underwent a second NOF/NOI test. To avoid potential confounding effects of the novel object being located in the center, a second NOI test was conducted where the novel object was placed in the perimeter of the open field. Mice were placed into the NOF apparatus for 1 h, then a novel object (different than the one previously used) was placed in the perimeter of the apparatus and NOI was measured for 30 min. Data from one subject in the NOI and NEP were lost due to a software error.

Cocaine conditioned place preference (CPP)

Mice ($n = 47$ WT, $n = 44$ ob/ob) were tested for cocaine CPP similar to described methods (Vialou et al. 2012). Day 1 consisted of a pre-conditioning test where mice were placed into the center chamber and had access to all three chambers for 15 min. Mice were then assigned one of the two main chambers to be paired with cocaine (counterbalanced, the alternate main chamber was assigned to saline), and over the next 4 days, mice underwent one conditioning session per day. During conditioning, mice received either saline (10 ml/kg) or cocaine (2.5, 5, or 10 mg/kg, i.p.) immediately prior to being confined to their assigned conditioning chamber for 20 min. Cocaine and saline conditioning sessions alternated daily, resulting in two conditioning sessions per treatment. The order of cocaine/saline sessions was counterbalanced within genotype. On the final day, a post-conditioning test was performed under the same conditions as the pre-conditioning test. Data were analyzed by calculating the time spent on the cocaine-paired chamber in the pre-test to the post-test.

Cocaine self-administration

Mice ($n = 11$ WT, $n = 17$ ob/ob) were implanted with jugular catheters and underwent cocaine self-administration without prior training or food restriction. Sessions began with turning on the fan (house light remained off), extension of the two levers, and a single non-contingent dose of cocaine (0.5 mg/kg, 40 μ l infusion over 2.3 s) with presentation of the cue light above the active lever (5 s). Each mouse was

assigned either the left or right lever as the active lever (counterbalanced within genotype). Mice first underwent fixed ratio-1 (FR-1) sessions, where a single press on the active lever resulted in delivery of cocaine and cue light, followed by a 10-s timeout. An additional non-contingent dose of cocaine (with cue light) was delivered if no active lever presses occurred within 30 min of the session start. FR-1 sessions were 3 h in length, but terminated early if 64 infusions were earned. FR-1 sessions continued until criteria were met (three consecutive sessions of ≥ 15 infusions and $\geq 2:1$ ratio of active to inactive lever presses, minimum 7 sessions) or 12 sessions had been completed. Next, the reinforcement schedule was advanced to a progressive ratio (PR). PR sessions were 4 h in length unless no reinforcer was earned within a 1-h period, in which case the session terminated early. During PR sessions, only the active lever was extended and each ratio was calculated based on the formula described by (Richardson and Roberts 1996), using $j=0.18$. PR sessions were conducted for 3 days using 0.5 mg/kg cocaine, then for 3 days using 1.0 mg/kg cocaine. Catheter patency was determined at the end of FR-1 and PR sessions using Brevital (9 mg/kg, i.v.) and any mouse not meeting criteria for patency (sedation within 5 s) was removed from the study. Four WT and five ob/ob mice were removed due to non-patent catheters, resulting in $n=7$ WT and $n=12$ ob/ob mice in FR-1 analysis. In some cases, catheter patency was verified after the FR-1, but not the PR stage and thus only FR-1 data from those animals was included in analysis.

Remifentanil self-administration

Remifentanil self-administration was conducted in the same manner as cocaine self-administration, with the exception of drug and dose. Mice ($n=10$ WT, $n=13$ ob/ob) self-administered 0.003 mg/kg remifentanil under an FR-1 schedule of reinforcement. Mice were next tested under PR conditions (as described for cocaine) for 0.003 mg/kg, then 0.01 mg/kg remifentanil. Catheter patency was also verified after FR-1 and PR stages of self-administration as described for cocaine. Two WT and three ob/ob mice were removed due to non-patent catheters, resulting in $n=8$ WT and $n=10$ ob/ob mice in FR-1 analysis.

Ethanol two-bottle choice

In order to determine if any of the novelty seeking measures correlated with ethanol drinking (Manzo et al. 2014; Parkitna et al. 2013), mice from the first two (of four) cohorts that underwent the novelty seeking battery ($n=8$ WT, $n=9$ ob/ob) were singly housed, then exposed to ethanol in a continuous access two-bottle choice paradigm as described (Lee et al. 2013). Mice had access to food at all times and were exposed to each concentration of ethanol (3, 6, 10, 14, and

20 %) in ascending order for 4 days each. Every 2 days, bottles and animals were weighed and the positions of the two bottles were switched to prevent any side bias. To determine consumption, the weight of 1 ml of solution was determined for each concentration of ethanol using the same balance as that used throughout the experiment. Additionally, fluid loss (estimated by placing bottles into empty cages along the same schedule) was subtracted from measurements.

Data analysis

Data were analyzed by two-tailed Student's *t* test, Fisher's exact test, and ANOVA (repeated measures when appropriate) followed by Holm-Sidak multiple comparisons using Prism 6.0 (*t* tests), SPSS 21 (three-way repeated measures ANOVA), or SigmaPlot 11.0 (other tests). In the case of unequal variances in ANOVA, statistics were performed on ranked values and denoted with asterisk (*). In the case of unequal variances in *t* tests, Welch's correction was applied, and adjusted degrees of freedom are reported and denoted with asterisk (*). Significance was set at $p=0.05$.

Results

Sweet preference

Despite a previous report of enhanced sweet preference in db/db mice (mice that lack a functional leptin receptor) (Ninomiya et al. 1995), and the ability of leptin to directly regulate signaling in sweet-responsive taste bud cells (Yoshida et al. 2013), we found no group differences between WT and ob/ob mice in preference for either saccharin or sucrose under continuous access conditions (Fig. 1a, b). Although the preference was comparable, ob/ob mice had significantly higher fluid intake during saccharin ($F(1, 45)^* = 52.8$, $p < 0.001$) and sucrose ($F(1, 45)^* = 34.5$, $p < 0.001$) preference tests (Fig. 1c, d).

Novelty seeking assays

To examine novelty seeking, we tested mice in a battery of novelty seeking assays that included tests of forced exposure, free choice, and self-administration of novelty (Bardo et al. 1996; Olsen and Winder 2009). Activity in the novel open field was significantly reduced in ob/ob mice ($t(37) = 11.2$, $p < 0.0001$; Fig. 2a), as previously described (Pelleymounter et al. 1995), but center time was not significantly different between genotypes (Fig. 2c). Our pilot data indicated a possible difference between WT and ob/ob mice in center time, so NOI was analyzed using side placement of the novel object. There were no significant group differences in NOI (Fig. 2d) or novel environment preference (Fig. 2f). Contrary to results from the

Fig. 1 Sweet preference and fluid intake using continuous access two-bottle choice procedure. Mice had continuous access to chow during preference tests for saccharin (a) and sucrose (b). Total fluid intake during preference tests for saccharin (c) and sucrose (d). Symbols represent mean ± SEM. *n* = 5–6/group. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 compared to lowest concentration

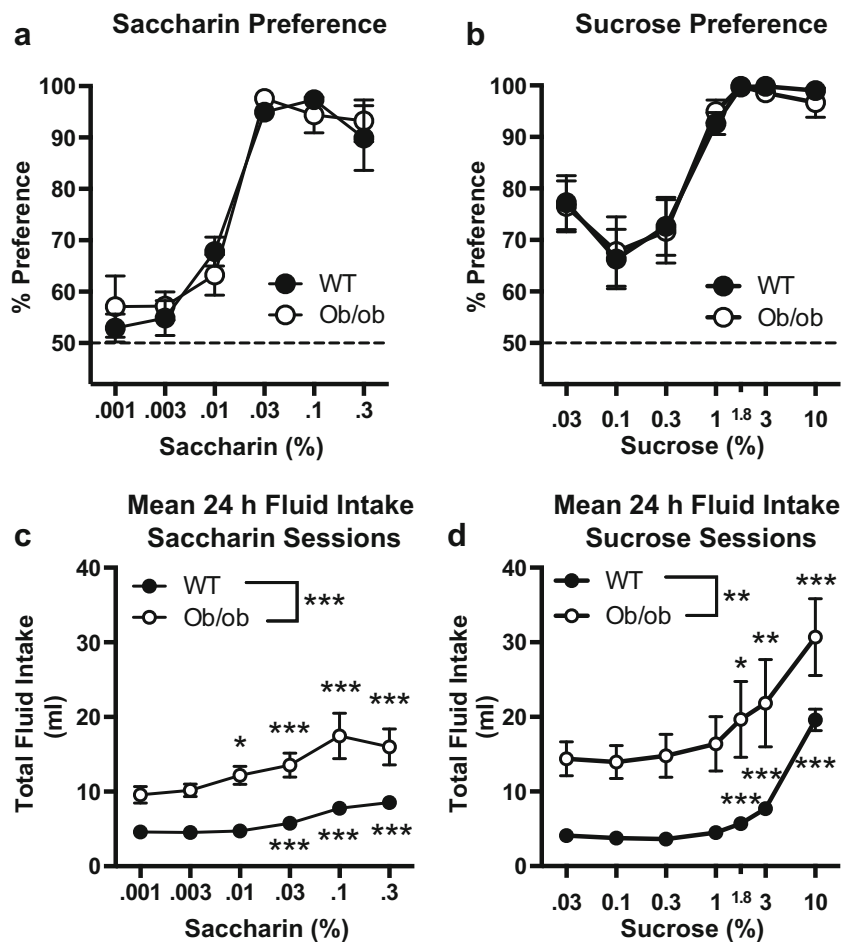
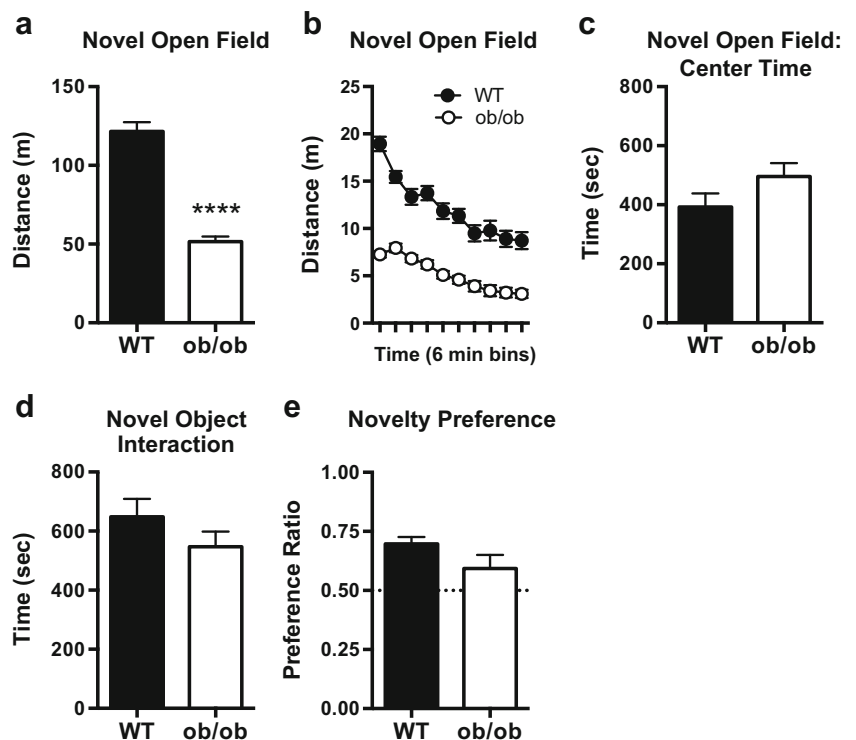


Fig. 2 Novelty seeking assessed by novel open field (a, b), novel object interaction (d), and novel environment preference (e) tests. Testing was performed >48 h apart. Bars and symbols represent mean ± SEM. *n* = 16–23/group. *****p* < 0.0001



first three tests, ob/ob mice had significantly reduced novelty seeking in the OSS task. In the initial 8 days of OSS, there were main effects of genotype ($F(1,74)^* = 95.4, p < 0.001$), lever ($F(1,74)^* = 32.7, p < 0.001$), and session ($F(7, 518)^* = 10.7, p < 0.001$) (Fig. 3a). There was also a significant interaction of session and lever ($F(7,518)^* = 7.9, p < 0.001$), and genotype and lever ($F(1,74)^* = 4.1, p < 0.05$), but not session and genotype or three-way interaction. When the final 3 days of OSS were aligned for animals that met criteria (Fig. 3b), there were significant effects of session ($F(2,100)^* = 4.1, p < 0.05$), genotype ($F(1,50)^* = 16.9, p < 0.001$), and lever ($F(1,50)^* = 234.9, p < 0.001$), but no significant interactions. However, there were no effects of genotype or session on the number of reinforcers earned during these sessions (Fig. 3c). Despite the similar OSS performance among mice that met criteria, a significantly smaller proportion of ob/ob mice met criteria than WT (52 vs. 94 %, $p < 0.05$, Fisher's exact test, Fig. 3d), and among mice that met OSS criteria, ob/ob required significantly more sessions ($t(14.7)^* = 3.7, p < 0.01$; Fig. 3e).

Cocaine conditioned place preference (CPP)

Comparison of WT and ob/ob mice in cocaine CPP revealed a significant main effect of dose ($F(1,83)^* = 7.1, p < 0.001$), but not genotype or interaction (Fig. 4a). Both genotypes had a significant CPP for the 10 mg/kg dose, and ob/ob had a significantly greater difference score than WT at the 2.5 mg/kg dose. Locomotor responding during conditioning sessions for the

highest dose of cocaine was also different between WT and ob/ob mice. There was a significant effect of genotype ($F(1, 19) = 30.0, p < 0.001$) and session ($F(2,38) = 8.2, p < 0.01$) on locomotor activity during the first saline and the two cocaine conditioning sessions, and when locomotor activity during the first saline conditioning session was compared to the activity during cocaine conditioning, only WT mice showed elevated cocaine-associated locomotor activity ($p < 0.01$; Fig. 4b).

Cocaine self-administration

Both WT and ob/ob mice had significant cocaine CPP, although we observed a reduced locomotor effect of the drug as has been previously reported for amphetamine (Fulton et al. 2006). There is substantial evidence for distinctive neural mechanisms mediating Pavlovian and instrumental conditioning. Therefore, we also tested self-administration of cocaine in WT and ob/ob mice. Comparison of the initial 7 days of cocaine self-administration revealed significant main effects of lever ($F(1, 32)^* = 19.8, p < 0.001$; Fig. 5a) and session ($F(6,192)^* = 12.1, p < 0.001$), but not genotype. Analysis of lever pressing (Fig. 5b) and reinforcers earned (Fig. 5c) during the last 3 days of FR-1 sessions revealed no significant differences in genotype. Furthermore, there was no significant difference between WT and ob/ob mice in the number of days or the proportion of mice to meet acquisition criteria (Fig. 5d, e). Under a progressive ratio, a main effect of dose ($F(1,9) = 7.1, p < 0.05$) was observed, but not genotype or interaction (Fig. 5f).

Fig. 3 Operant sensation seeking. **a** FR-1 responding during the first eight sessions in all mice. **b** Nosepokes and **c** reinforcers earned during the final three sessions in mice that acquired. **d** Proportion of mice that met acquisition criteria. **e** Number of days taken to reach criteria for mice that acquired OSS. $n = 16\text{--}23/\text{group}$ * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

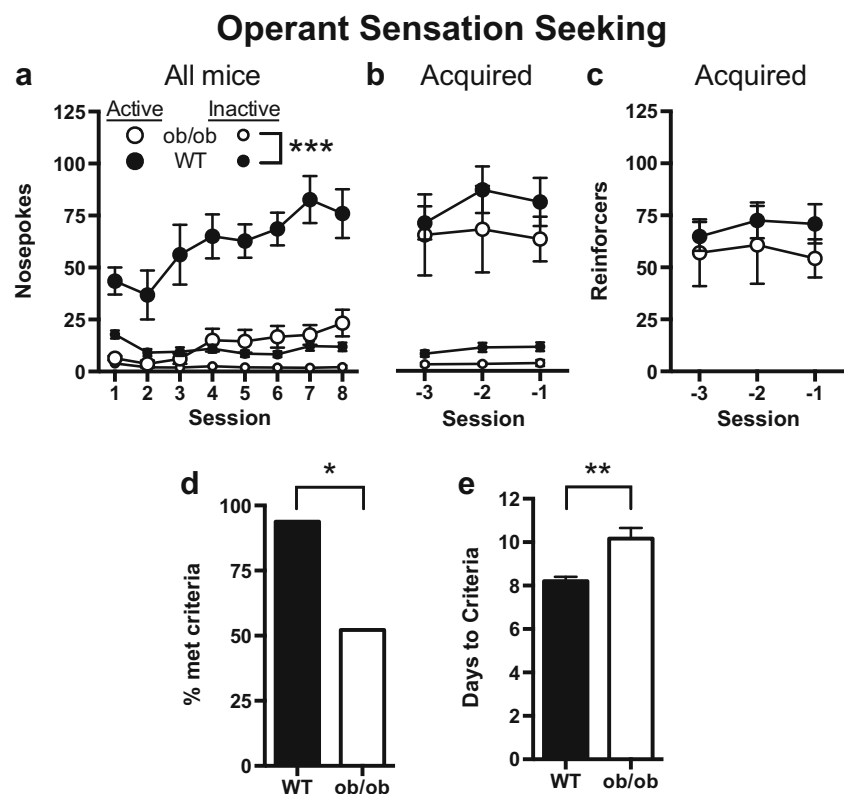
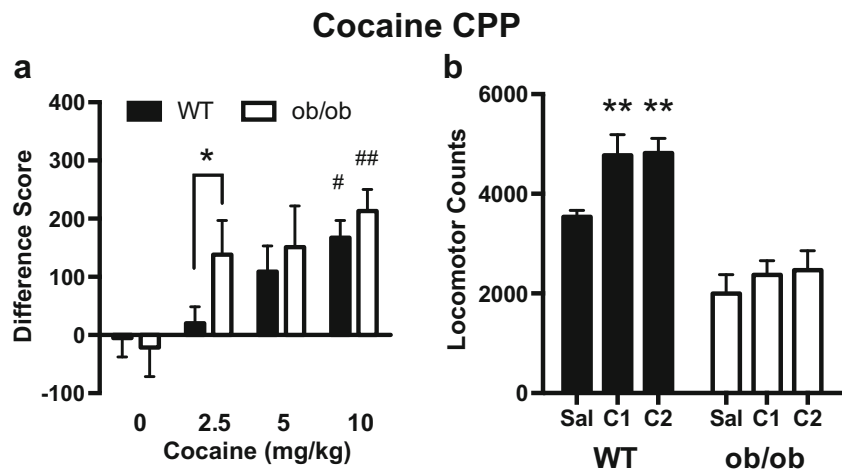


Fig. 4 Conditioned place preference for cocaine. **a** Difference score (post-conditioning minus pre-conditioning time in cocaine-paired side), $n = 7-15/\text{group}$. **b** Locomotor counts during saline (Sal) and the 2 cocaine (C1, C2) conditioning sessions ($n = 10-11/\text{group}$). Bars represent mean + SEM. # $p < 0.05$, ## $p < 0.01$ compared to 0 mg/kg, * $p < 0.05$, ** $p < 0.01$



Remifentanil self-administration

To determine if opioid reinforcement is altered in ob/ob mice, we performed intravenous remifentanil self-administration. Our initial studies using 0.01 mg/kg remifentanil yielded variable results during acquisition, so the training dose was reduced to 0.003 mg/kg. Thus, FR-1 acquisition includes animals ($n = 1$ WT, $n = 3$ ob/ob) that had up to 5 days of previous self-administration for 0.01 mg/kg, although this did not have a significant impact on the number of days to reach criteria. Comparison of the initial 7 days of remifentanil self-administration (Fig. 6a) revealed significant main effects of

genotype ($F(1,32)^* = 5.6, p < 0.05$), session ($F(6,192)^* = 12.3, p < 0.001$), and lever ($F(1, 32)^* = 21.2, p < 0.001$), with only the interaction of session and lever being significant ($F(6,192)^* = 4.4, p < 0.001$). Analysis of lever pressing (Fig. 6b) and reinforcers earned (Fig. 6c) during the last 3 days of FR-1 sessions revealed a significant genotype difference only in reinforcers earned ($p < 0.05$)*. There was no significant difference between WT and ob/ob mice in the number of days or the proportion of mice to meet acquisition criteria (Fig. 6d, e). Under progressive ratio remifentanil self-administration, there was a significant main effect of genotype ($F(1,10)^* = 8.7, p < 0.05$), where ob/ob mice earned significantly fewer infusions at both doses (Fig. 6f).

Fig. 5 Intravenous cocaine self-administration. **a** FR-1 responding during the first seven sessions in all mice for cocaine (0.5 mg/kg/infusion). **b** Lever presses and **c** reinforcers earned and corresponding cocaine intake during the final three sessions in mice that acquired. **d** Proportion of mice that met acquisition criteria. **e** Number of days taken to reach criteria for mice that acquired cocaine self-administration. **f** Number of infusions earned and corresponding breakpoint during progressive ratio responding for 0.5 and 1.0 mg/kg/infusion cocaine. Bars represent mean + SEM. $n = 8-12/\text{group}$ (FR-1), $n = 4-7/\text{group}$ (PR)

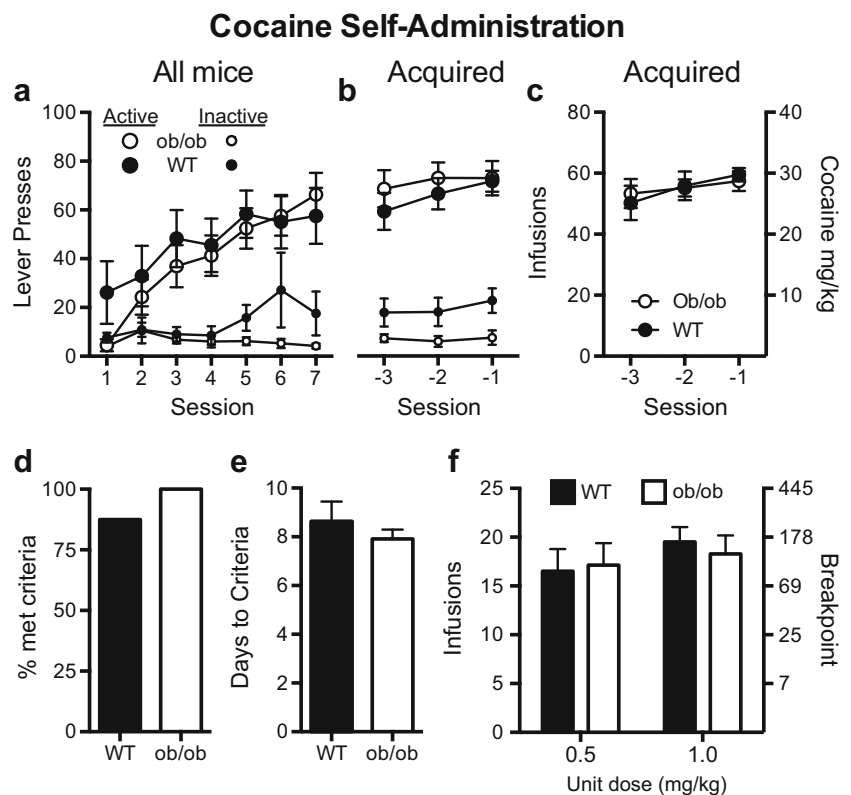
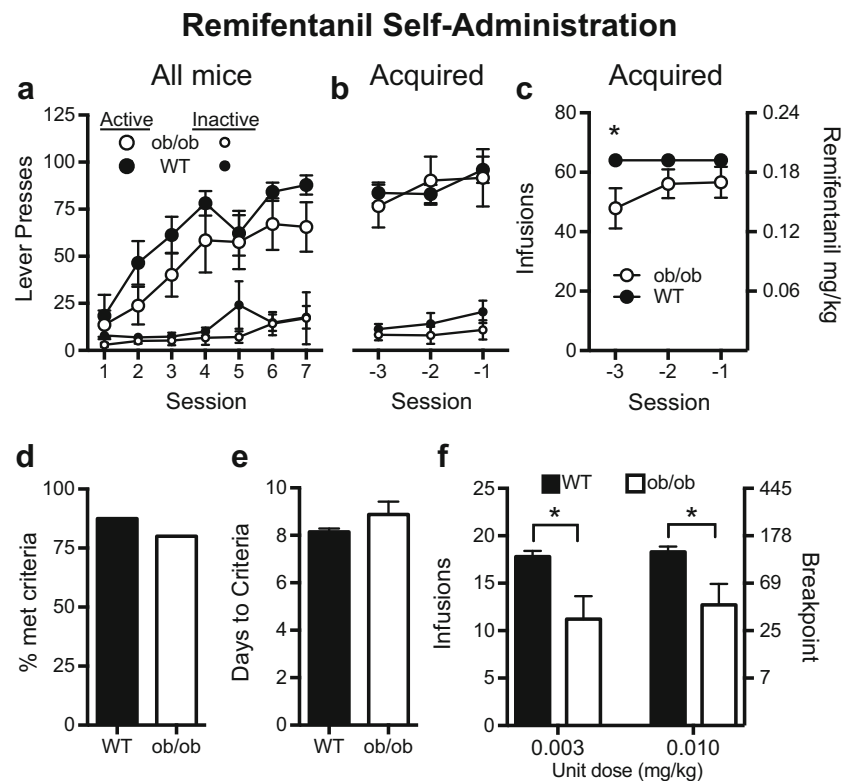


Fig. 6 Intravenous Remifentanyl self-administration. **a** FR-1 responding during the first seven sessions in all mice for remifentanyl (0.003 mg/kg/infusion). **b** Lever presses and **c** reinforcers earned and corresponding remifentanyl intake during the final three sessions in mice that acquired. **d** Proportion of mice that met acquisition criteria. **e** Number of days taken to reach criteria for mice that acquired remifentanyl self-administration. **f** Number of infusions earned and corresponding breakpoint during progressive ratio responding for 0.003 and 0.01 mg/kg/infusion remifentanyl. Bars represent mean + SEM. $n = 8\text{--}10/\text{group}$ (FR-1), $n = 5\text{--}7/\text{group}$ (PR). * $p < 0.05$ WT compared to ob/ob mice



Ethanol two-bottle choice

Alcohol intake was compared under continuous access two-bottle choice conditions for 4 days at each concentration as described (Lee et al. 2013). Analysis of ethanol consumption revealed a main effect of concentration ($F(4,76) = 18.6$, $p < 0.001$) and interaction ($F(4,84) = 6.9$, $p < 0.001$), but no significant main effect of genotype (Fig. 7a). Differences between WT and ob/ob mice became larger with higher concentrations of ethanol, and ob/ob mice had significantly lower intake of 20 % ethanol than WT ($p < 0.05$).

Analysis of ethanol preferences also revealed a main effect of concentration without an effect of genotype or interaction (Fig. 7b). When total intake across all concentrations was analyzed, ob/ob mice had greater variability compared to WT ($F(8,7) = 11.8$, $p < 0.01$), and further analysis revealed a significant inverse correlation between body weight and ethanol intake in ob/ob ($F(1,7) = 15.3$, $p < 0.01$, $r^2 = 0.69$), but not WT mice (Fig. 7c). This is not explained by age differences, as there was no significant correlation between age and ethanol intake in either ob/ob mice or WT mice (Fig. 7d).

To determine if this relationship extended to the other measures, and to examine relationships between the novelty seeking measures and ethanol intake, we performed correlations between body weight and each of the behavioral outcomes (Table 1). In ob/ob mice, significant inverse relationships were found between body weight and novel open field distance ($r^2 = 0.20$, $p < 0.05$) and novel environment preference

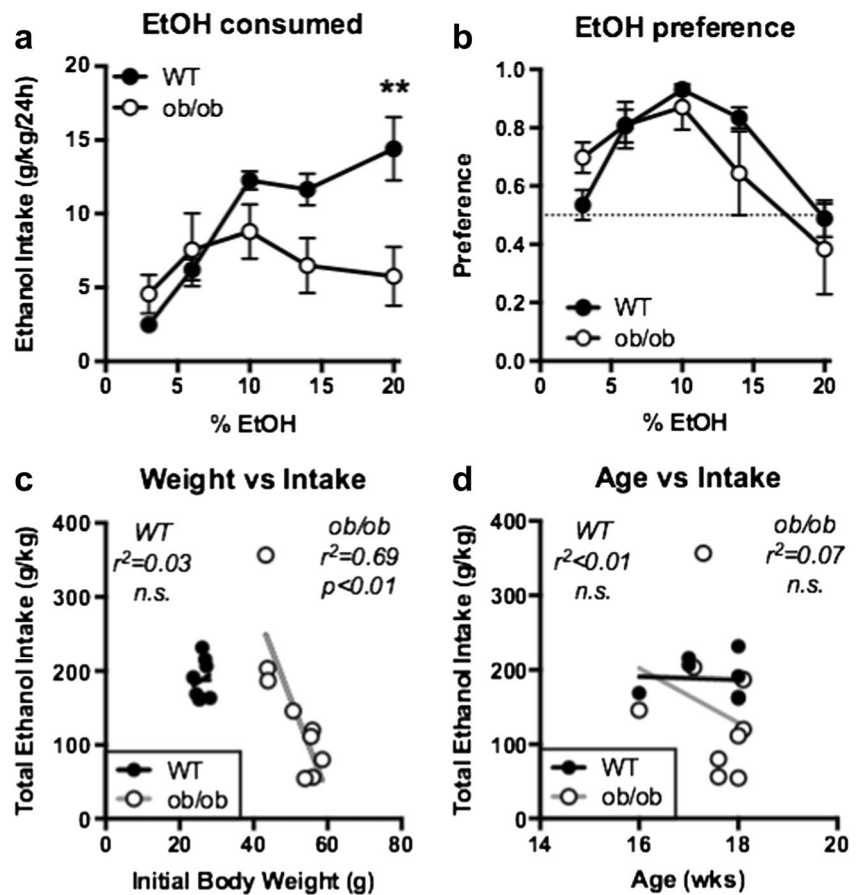
($r^2 = 0.22$, $p < 0.05$), although age was also negatively correlated these measures ($r^2 = 0.24$, $p < 0.05$ and $r^2 = 0.30$, $p < 0.01$, respectively). In WT mice, we observed a significant correlation between weight and novel object interaction ($r^2 = 0.34$, $p < 0.05$), which also generalized to age ($r^2 = 0.27$, $p < 0.05$). Among the novelty seeking measures, OSS was most highly correlated with ethanol intake in ob/ob mice ($r^2 = 0.49$, $p < 0.05$), but this relationship was not significant in WT mice. Thus, while correlations were observed between weight and several measures in ob/ob mice, only the relationship with ethanol intake was unique to weight. Weight differences did not explain the variance observed in remifentanyl self-administration, as correlations between weight and self-administration were not significant on either the FR-1 or PR schedule.

Discussion

To gain insight into how leptin deficiency may alter behavioral responses to natural and drug rewards, we screened ob/ob mice in a variety of assays. Surprisingly, we found no significant differences between ob/ob and WT mice in many of the assays performed, despite expected differences in locomotor activity. Differences were, however revealed in cocaine CPP, OSS, and opioid self-administration.

In our screen of sweet preference, we found no difference between genotypes for either a non-caloric (saccharin) or a calorie-containing (sucrose) sweetener, although overall fluid

Fig. 7 Ethanol drinking under continuous access two-bottle choice conditions. **a** Average amount consumed per day and **b** corresponding ethanol preference for each of the ethanol concentrations. **c** Correlation of body weight and total ethanol intake during the 20-day experiment. **d** Correlation of age and total ethanol intake during the 20-day experiment. $n = 8-9/$ group. $**p < 0.01$ between genotypes



consumption in ob/ob mice was approximately double that of WT throughout the experiment. A previous study found enhanced sucrose preference in the leptin receptor-deficient db/db mouse (Ninomiya et al. 1995). One potential reason for this difference in findings is that there may have been a ceiling effect in our study, where WT mice in Ninomiya et al. (1995) did not reach a maximum preference level for any concentrations tested. Another potential reason is that the lowest

concentration of sucrose used in the current study produced greater than 50 % preference in both genotypes, which could have masked an increase in preference for lower sucrose concentrations in ob/ob mice.

We also performed a series of novelty seeking assays. Significant evidence suggests that novelty seeking is associated with mesolimbic dopamine transmission (Gjedde et al. 2010; Olsen and Winder 2009; Rebec et al. 1997). Leptin

Table 1 Correlative analysis of novelty seeking and ethanol intake

WT	ob/ob	Weight	NOF	NOI	NEP	OSS	Ethanol Intake
			0.18	0.56*	-0.10	0.15	0.27
		Weight	-0.42*	0.14	-0.46*	-0.45	-0.78**
		Novel Open Field (NOF)		0.29	0.19	0.37	0.39
		Novel Object Interaction (NOI)			0.13	-0.05	-0.57
		Novel Environment Preference (NEP)				-0.30	0.06
		Operant Sensation Seeking (OSS)				0.15	0.51
		Ethanol Intake					0.46
							0.70*

Table represents Pearson’s r among novelty seeking and ethanol intake measures. Within each cell, r values for WT (top left) and ob/ob (bottom right) are listed

* $p < 0.05$; ** $p < 0.01$

receptors are found within dopaminergic and non-dopaminergic neurons of the VTA, and leptin can regulate activity of VTA dopamine neurons both directly and indirectly (Hommel et al. 2006; Leininger 2009; Thompson and Borgland 2013). Ob/ob mice appear to have reduced mesolimbic dopamine signaling, as tyrosine hydroxylase (the rate limiting enzyme in catecholamine synthesis) and evoked dopamine release are reduced in these mice (Fulton et al. 2006). Although we observed the expected reduction in novel open field activity (Sakkou et al. 2007) in ob/ob mice, we observed no differences in novel object interaction or novel environment preference. This is in contrast to reports of novelty-associated anxiety-like phenotypes in ob/ob mice (Asakawa et al. 2003; Finger et al. 2010). Reasons for this discrepancy are unclear, although it should be noted that mice in the current study were tested during the dark phase of their light cycle and handled prior to testing. Ob/ob mice in the present study did not show decreased center time in the novel open field, suggesting that under the experimental conditions employed here, novelty did not provoke anxiogenic responses.

In contrast to these free choice novelty seeking assays (NOI and NEP), we found that only 52 % of ob/ob mice acquired OSS. This was not a result of impaired learning, as ob/ob mice readily acquired self-administration of cocaine and remifentanyl. Thus, although novelty promoted exploration in the NOI and NEP tests in ob/ob mice, the OSS stimuli (which novelty represents a significant component of (Olsen and Winder 2012)) failed to reinforce instrumental responding in nearly half of these mice.

We found that cocaine established CPP in ob/ob mice, despite a lack of significant locomotor activating effects of the psychostimulant in this assay. Furthermore, we found that ob/ob mice had a greater preference score for 2.5 mg/kg cocaine than WT mice. Our finding that CPP for the lowest dose of cocaine tested was surprising, but is consistent with a recent study showing a reduction in cocaine CPP following intra-VTA leptin (You et al. 2015). This is unlikely to be related to the increase in food intake/body weight, as diet-induced obese mice were found to have reduced cocaine CPP (Morales et al. 2012). Similarly, increased food intake/body weight likely does not explain the lack of locomotor effects of cocaine, as cocaine-induced locomotor activity is increased in mice fed high fat and/or high sugar diets (Collins et al. 2015; Olsen 2011).

As a complementary approach, we performed an experiment to determine if ob/ob mice would self-administer intravenous cocaine. We found that neither acquisition of cocaine self-administration nor motivation to obtain it (as measured by progressive ratio responding) was altered in ob/ob mice. While this may seem inconsistent, CPP and self-administration do not measure the same constructs (conditioned reward and reinforcement, respectively (Bardo and Bevins 2000; Mackintosh 1974; Tzschentke 2007)). Considering reports that ob/ob mice have reductions in basal locomotor activity (Goulding et al.

2008), psychostimulant-induced locomotor activity (Fulton et al. 2006), and mesolimbic dopamine levels (Fulton et al. 2006) (although see Roseberry et al. 2007), our finding that ob/ob mice achieved similar breakpoints for cocaine is especially noteworthy, as dopamine signaling is critically important for effort based responding in operant conditioning tasks (Salamone and Correa 2012).

Similar to cocaine, we found that ob/ob mice acquired intravenous self-administration of remifentanyl without prior training. Despite similar levels of intake under FR-1 conditions, PR responding was significantly reduced in ob/ob mice. This is consistent with remifentanyl not being as effective as a reinforcer in ob/ob mice (Richardson and Roberts 1996). Cocaine was able to support high levels of PR responding in ob/ob mice, indicating that task performance is not compromised in these mice. Instead, these differences may reflect a reported reduction of brain mu opioid receptors (MORs) in ob/ob mice (Khawaja et al. 1989).

Finally, we found that ob/ob mice had significant differences in ethanol consumption that were associated with body weight. A possibility is that the aforementioned reduction in mu opioid receptors (Khawaja et al. 1989) reduced drinking in ob/ob mice. Ethanol intake is significantly reduced by knock-out or blockade of the mu opioid receptor (Hall et al. 2001; Le et al. 1993). Another possibility is that heavier ob/ob mice may have a greater severity of metabolic disturbances than those with lower body weight. Blednov et al. found that the pharmacokinetic profile of ethanol is markedly different in ob/ob mice (Blednov et al. 2004). A possible consequence of this is excessive accumulation of acetaldehyde (an intermediary of ethanol metabolism), resulting in dysphoria and subsequent ethanol avoidance. Consistent with this idea, Blednov et al. (2004) also reported decreased ethanol consumption in ob/ob mice.

It is difficult to identify a specific mechanism for chronic leptin deficiency that explains our pattern of findings, although several lines of evidence suggest that altered opioid signaling may be involved. Ob/ob mice have approximately 40 % fewer MOR binding sites than wild types (Khawaja et al. 1989). Furthermore, genetic deletion of MORs results in strikingly similar phenotypes to those reported here. Similar to our results from ob/ob mice, MOR knockout mice have reduced novelty seeking (Yoo et al. 2004), opioid self-administration (Becker et al. 2000), and alcohol intake (Hall et al. 2001; Roberts et al. 2000). The similarities also extend to reward-related behaviors that are not altered by MOR deletion: MOR knockout mice do not have significant differences in cocaine self-administration (Gutierrez-Cuesta et al. 2014) or sucrose consumption when measured under the conditions used in the present study (Kas et al. 2004; Ostlund et al. 2013). Alterations in MOR signaling may also explain the negative association between body weight and alcohol intake we observed. Diet-induced obesity has been shown to reduce MOR mRNA in the VTA and nucleus accumbens (Vucetic et al.

2011), and diet-induced obesity results in a reduction in alcohol consumption (Thanos et al. 2012). Despite these similarities, there are also distinct differences between some of the observed phenotypes in ob/ob and MOR knockout mice. MOR knockout mice do not have altered novel open field activity (Hall et al. 2004), and the locomotor response to cocaine has been reported to either be elevated (Hall et al. 2004; Hummel et al. 2004), unchanged (Lesscher et al. 2005), or reduced (Chefer et al. 2004). Cocaine CPP is also reduced in MOR knockout mice (Hall et al. 2004), contrary to our finding of elevated CPP at low doses of cocaine.

The putative nature of these hypotheses and the fact that some of our findings are consistent with results from acute leptin inhibition (e.g., cocaine CPP), while others are consistent with a reduction in MOR signaling highlight the fact that the ob/ob mouse is a chronic model of leptin deficiency. Thus, as with other chronic models of gene disruption, phenotypes observed in ob/ob mice may be a direct result of leptin deficiency or secondary to chronic deficiency. In particular, chronic deficiency could influence the development of circuitry that is involved in reward-related behaviors. Ob/ob mice have reduced brain weight and myelination (Bereiter and Jeanrenaud 1979; Hashimoto et al. 2013), and basal and evoked dopamine in the nucleus accumbens are reduced in these mice (Fulton et al. 2006). This reduction is contrary to what would be predicted by experiments demonstrating that acute leptin administration depresses mesolimbic dopamine function (Hommel et al. 2006; Thompson and Borgland 2013; You et al. 2015).

It should also be noted that all of the current studies were performed without food restriction. Food restriction increases performance on food and drug-associated behaviors (Baldo et al. 2013; Carr 2007), and the influence of leptin signaling on reward-related behaviors is modulated by the macronutrient content and availability of food (Figlewicz et al. 2006; Fulton et al. 2000), even in a chronic model of disrupted leptin signaling (Fulton et al. 2004).

Our data indicate that ob/ob mice do not have overarching differences in behavioral responses to rewards, and differences may be most apparent under conditions that require effort. For example, normal performance in the novel open field requires sustained activity, and OSS requires instrumental responding to obtain novel audiovisual stimuli. On the contrary, novelty seeking in the NOI and NEP tasks does not require additional activity or effort. Similarly, remifentanyl self-administration was only reduced in ob/ob mice under a progressive ratio schedule—a schedule that requires increasing effort to maintain drug intake.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to report.

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