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Cocaine and desipramine elicit distinct striatal noradrenergic and behavioral responses in selectively bred obesity-resistant and obesity-prone rats

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Abstract

Previous studies have demonstrated a role for norepinephrine (NE) in energy regulation and feeding, and basal differences have been observed in hypothalamic NE systems in obesity-prone vs. obesity-resistant rats. Differences in the function of brain reward circuits, including in the nucleus accumbens (NAc), have been shown in obesity-prone vs. obesity-resistant populations, leading many researchers to explore the role of striatal dopamine in obesity. However, alterations in NE transmission also affect NAc mediated behaviors. Therefore, here we examined differences in striatal NE and the response to norepinephrine transporter blockers in obesity-prone and obesity-resistant rats. We found that striatal NE levels increase following systemic cocaine administration in obesity-prone, but not obesity-resistant rats. This could result from either blockade of striatal norepinephrine transporters (NET) by cocaine leading to reduced NE reuptake, or circuit-based responses following cocaine administration resulting in increased NE release. Retrodialysis of the NET inhibitor, desipramine, into the ventral striatum did not cause selective increases in striatal NE levels in obesity-prone rats, suggesting that circuit-based mechanisms underlie NE increases following systemic cocaine administration. Consistent with this, systemic desipramine treatment decreased locomotor activity in obesity-prone, but not obesity-resistant rats. Furthermore, obesity-prone rats were also more sensitive to desipramine-induced reductions in food intake compared to obesity-resistant rats. Taken together, these data expand our understanding of differences in NE systems of obesity-prone vs. resistant rats, and provide new insights into basal differences in striatal systems that may influence feeding behavior.

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Keywords

norepinephrine; microdialysis; diet induced obesity; norepinephrine transporter; striatum; food intake

1. Introduction

Norepinephrine (NE) systems play a role in energy regulation, including modulation of feeding behaviors [1–6]. For example, peripheral administration of the norepinephrine transporter (NET) inhibitor nisoxetine decreases food intake after high-fat diet-induced obesity [7]. In addition, NE delivered directly to the lateral hypothalamus increases feeding in sated rats [8,9], whereas NE administration into the ventral medial hypothalamus reduces food intake even in fasted rats [9]. Furthermore, administration of NE into the globus pallidus initiates food intake in sated rats [9].

Previous studies using selectively bred rats have found evidence for differences in hypothalamic NE systems in obesity-prone vs. obesity-resistant rats, both before and after diet-induced obesity [10–13]. For example, prior to obesity, obesity-prone rats exhibit decreased NE turnover in the ventromedial and lateral hypothalamus [10], whereas after obesity onset, hypothalamic NE levels are increased and NET binding is reduced in obesity-prone rats compared to their obesity-resistant counterparts [11,14]. However, potential differences in NE in other brain regions involved in non-homeostatic feeding and motivation, such as the ventral striatum, have not been as closely examined.

Recent work in both humans and rodents indicates a role for altered striatal function in over-eating that in turn drives obesity [15–18]. For example, neural activations in response to images of food are greater in the ventral striatum of obese people, while activations in the dorsal striatum in response to consumption of food itself are reduced [19,20]. The striatum plays key roles in reward and motivation and is tightly linked to both normal and atypical motivation for food [17,21–27]. There are direct and indirect connections between hypothalamic, striatal, and pallidal regions that influence feeding [28–31]. Thus, interactions between homeostatic and motivational circuits may affect feeding behaviors, particularly feeding that goes beyond homeostatic needs, and may do so differently in obesity-prone vs. obesity-resistant populations [30,32].

We recently found that the magnitude of cocaine-induced locomotor activity is stronger in obesity-prone than in obesity-resistant rats; this was not associated with differences in cocaine-induced increases in striatal dopamine [33–35]. In addition, obesity-prone rats are more sensitive to the D₂ agonist quinpirole than their obesity-resistant counterparts [34]. While cocaine-induced locomotor activity is often linked to dopamine transmission within the ventral striatum, alterations in NE transmission also affect psychostimulant induced locomotion (For review see [36]). For example, global knockout of the norepinephrine transporter in mice leads to supersensitivity to cocaine-induced locomotion, and causes an enhanced D_{2/3} receptor response [37]. Therefore, we hypothesized that systemic administration of cocaine would lead to significant increases in extracellular NE levels in obesity-prone rats. In addition, if these increases were a direct result of NET blockade by

cocaine, then local administration of the NET inhibitor, desipramine, should also cause significant increases in extracellular NE levels. Furthermore, we hypothesized that systemic administration of desipramine would have stronger effects on locomotor activity, body temperature, and food intake in obesity-prone compared to obesity-resistant rats, based on basal differences in hypothalamic NE in obesity-prone rats and the role of NE activation in maintaining core body temperature [38–41], augmenting locomotor activity [42,43] and reducing food intake [7].

2. Methods

2.1 Subjects

Obesity-prone (OP) and obesity-resistant (OR) rats, originally developed by Barry Levin [44] and obtained from Taconic, were bred in-house. Animals were 70–90 days old at the start of experiments unless otherwise specified and were pair-housed on a reverse 12 h light/dark cycle (lights off at 7 am). All experimental measures were performed during the dark cycle unless otherwise specified. Rats had free access to food and water throughout and all measures were made in age-matched male rats. All rats were treated as approved by the University of Michigan Unit for Laboratory Animal Medicine (ULAM) and in accordance with the National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals. Cocaine-induced locomotor activity, and striatal dopamine levels from these same rats were previously published [33], but effects of cocaine on striatal NE were not previously reported (OP N = 6, OR N = 6). Studies of the effects of local desipramine (OP N = 4, OR N = 4) and systemic desipramine (OP N = 6 OR N = 6) were conducted in separate sets of rats.

2.2 Chemicals and Reagents

All chemicals, drugs, and reagents were purchased from Sigma Aldrich (St. Louis, MO) unless otherwise noted. Water and acetonitrile for reagents and mobile phases were Burdick & Jackson HPLC grade (VWR, Radnor, PA). Artificial cerebrospinal fluid (aCSF) consisting of 145 mM NaCl, 2.68 mM KCl, 1.01 mM MgSO₄, 1.40 mM CaCl₂, 0.45 mM NaH₂PO₄, and 1.55 mM Na₂HPO₄, adjusted pH to 7.4 with 0.1 M NaOH, was used to dilute standards and as the perfusions fluid for microdialysis. (+/–)-Norepinephrine-2,5,6,α,β-d₆ HCl (d6-NE) was purchased from C/D/N isotopes, (Pointe-Claire, Canada).

2.3 Microdialysis Surgery and Dialysate Collection

Microdialysis probes (CMA 12 Elite 2mm, CMA/Microdialysis AB Stockholm, Sweden) were implanted unilaterally, with probe tips located in the NAc of obesity-prone and obesity-resistant rats 20–24 h before microdialysis sampling. Probes were implanted using the following coordinates from bregma: anterior-posterior (AP) +1.4, medial-lateral (ML) ±2.5, dorsal-ventral (DV) –7.8 [45] under isoflurane anesthesia (1–5%). Probes were secured to the skull using skull screws (Fine Science Tools Cat#19010-10) and acrylic dental cement (Cat #'s 0921392 & 0921395; Bosworth Co., Skokie, IL). Animals were awake and freely moving during experiments. Microdialysis probes were initially flushed at 2 µL/min with aCSF for 1 h using a syringe pump. The rate of perfusion was reduced to 1 µL/min for another h before dialysate collection. Samples were collected every 3 min (3 µL).

2.4 Microdialysis Probe Placements

For all subjects, brains were removed and frozen at -80°C . Coronal brain sections ($50\text{ }\mu\text{m}$) were cut through the forebrain using a Leica CM1850 cryostat (Leica Microsystems, Buffalo Grove, IL), mounted onto slides and stained with cresyl violet to verify probe placements.

2.5 Systemic Cocaine Exposure

As previously described [33], rats were placed in locomotor chambers for a 1 h habituation period prior to receiving an injection of saline (1 mL/kg , i.p.) followed 45 min later by a cocaine injection (15 mg/kg , i.p.). Animals were returned to their home cage 1 h after cocaine injection. Locomotor activity was recorded throughout all testing and time locked with dialysate collection in 3 min bins.

2.6 Local Desipramine

The effects of intra-striatal desipramine on neurotransmitter levels were determined in a separate set of animals. Microdialysis samples were collected for 30 min to establish a baseline. For quantitative microdialysis of NE, d₆-NE was dissolved in aCSF (100 nM) and then perfused through the microdialysis probe beginning at 30 min and persisted through the remainder of the experiment. As shown previously, inclusion of d₆-NE allows for in vivo calibration by measuring the concentration of d₆-NE lost from the probe to calculate the fraction (i.e. the extraction fraction) of NE that is recovered by the probe [46,47]. This method accounts for any differences in probe function and tissue sampling environment. NE concentrations were corrected for in vivo probe recovery and reported as C_{app} . Desipramine dissolved in aCSF was administrated into the ventral striatum by retrodialysis at $100\text{ }\mu\text{M}$ ($t=60\text{ min}-90\text{ min}$), and $500\text{ }\mu\text{M}$ ($t=90\text{ min}-120\text{ min}$) to measure the effects of local NET inhibition.

2.7 HPLC-MS Analysis

All microdialysis samples and standards were derivatized by adding carbonate buffer (100 mM), benzoyl chloride (2% in acetonitrile v/v) and an internal standard solution in a 2:1:1 ratio, as previously described [48]. Internal standard stocks were comprised of $500\text{ }\mu\text{M}$ Glutamate, Taurine, $50\text{ }\mu\text{M}$ GABA, glutathione, and $5\text{ }\mu\text{M}$ 5-HT, NE, DA, derivatized with $^{13}\text{C}_6$ benzoyl chloride. Internal standards were stored at -80°C and then diluted 500x into the final sample.

A binary HPLC pump and autosampler (Thermo Accela, Waltham, MA) with a Phenomenex (Torrance, CA) Kinetex biphenyl reverse-phase column ($2.1\text{ mm} \times 100\text{ mm}$, $1.7\text{ }\mu\text{m}$, $100\text{ }\text{\AA}$ pore size) was used for separation. Mobile phase A was 10 mM ammonium formate and 0.15% (v/v) formic acid in water. Mobile phase B was acetonitrile. For the cocaine study, the mobile phase gradient was as follows: initial, 5% B; 0.1 min, 15% B; 2 min, 40% B; 2.1 min, 100% B; 3 min, 100% B; 3.1 min, 5% B; 3.5 min, 5% B. In the desipramine study, the following gradient was utilized: initial, 5% B; 0.1 min, 19% B; 1 min, 26% B; 1.5 min, 75% B; 2.5 min, 100% B; 3.0 min, 100% B; 3.1 min, 5% B, 3.5 min, 5% B. The flow rate was $450\text{ }\mu\text{L/min}$, and sample injection volume was $5\text{ }\mu\text{L}$ in "no waste" injection mode and total cycle times were 4.5 min. The autosampler and HPLC column were kept at ambient temperature. A Thermo TSQ Quantum Ultra triple quadrupole mass spectrometer operating

in positive SRM mode with a heated electrospray ionization (HESI) probe (at 350 °C) was used for detection. The limit of detection for this instrument is 0.04 nM. Thermo XCalibur software (version 3.0) was used to integrate peaks for each analyte and quantification was determined by dividing analyte peak area by internal standard peak area. Peaks were visually inspected to verify correct integration.

2.8 Locomotor Activity, Body Temperature, and Feeding Following Desipramine Treatment

For these studies, rats were singly housed in a standard 12 h light/dark cycle. Locomotor activity was measured via radio transmitter telemetry devices (model ER-4000 E-Mitter, Mini Mitter Co., Bend, OR) surgically implanted within the abdominal cavity. Rats were anesthetized with ketamine (90 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), the abdomen was cleaned with betadine and alcohol, and then a 1–2 cm rostral-caudal incision was made in the skin and underlying musculature to expose the peritoneal cavity. The telemeter was placed inside the peritoneal cavity and the incision closed with absorbable Ethicon Vicryl 5-0 coated suture (muscle) and non-absorbable Ethicon Nylon 5-0 suture (skin). Rats were allowed to recover for 6–8 days. The telemeter transmitted activity and temperature data to a receiver (model ER-4000 Receiver, Mini Mitter Co.) placed directly under the home cage of each rat. Cages were placed on the receiver pad 5 h prior to injection (i.p.) with either vehicle (veh: sterile water) or desipramine (0, 3.2 and 10 mg/kg). Injections were given in a counterbalanced design with one week between each injection. Locomotor activity and body temperature data were collected every 5 min for 5 h prior to and 24 h after each injection using Vital View software (Mini Mitter Co.). Food was weighed at the start and end of each 24 h session.

2.9 Statistical Analysis

For comparisons between two groups, two-tailed t-tests, one or two-way repeated measure (RM) ANOVAs were used unless otherwise noted. When appropriate, Sidak's post-tests were utilized. Statistical analyses were conducted in Prism 6 (GraphPad, San Diego, CA).

3. Results

3.1 Effects of Systemic Cocaine on Striatal NE Levels

Obesity-prone rats were significantly heavier at the time of testing than obesity-resistant rats (OP = 446.2 ± 14.19g, OR = 382.3 ± 10.44g; $t_{10} = 3.623$, $p < 0.005$ [34]) and insulin levels were higher, although not statistically significant (OP = 42.12 ± 9.49 µU/mL, OR = 23.94 ± 5.96 µU/mL, $p < 0.1434$, [34]). Obesity-prone rats showed a significantly stronger locomotor response to cocaine (15 mg/kg, i.p.) compared to obesity-resistant rats (Fig 1A; Two-way RM ANOVA, strain x condition interaction: $F_{(2,20)} = 5.16$, $p < 0.02$). Importantly, brain concentrations of cocaine were similar between groups (OP = 835.6 nM ± 197.8; OR = 668.6 nM ± 106.5; [34]). Acute cocaine administration increased extracellular NE levels in the ventral striatum of obesity-prone but not obesity-resistant rats (Fig. 1B; Two-way RM ANOVA, strain x time interaction: $F_{(29, 290)} = 1.877$, $p < 0.01$; Fig. 1B Inset: area under curve $t_{10} = 3.821$, $p < 0.01$) No basal differences in NE were found between groups.

3.2 Effect of Desipramine Retrodialysis on NE Levels

We next used retrodialysis to determine whether local NET blockade within the ventral striatum is sufficient to selectively increase NE levels in obesity-prone rats. Again, obesity-prone rats were significantly heavier than obesity-resistant rats at the time of testing in this cohort ($OP = 424 \pm 14.58\text{g}$, $OR = 357 \pm 5.56\text{g}$; $t_6=4.294$, $p < 0.01$) and no basal differences in NE levels were observed (Fig 2A inset; see Table 1 for baseline concentrations of all analytes). Retrodialysis of 100 μM desipramine did not produce significant changes in NE, dopamine or serotonin (5-HT) in either group (Fig 2). Administration of 500 μM desipramine resulted in a significant increase in extracellular NE in obesity-resistant rats only (Figure 2A; Two-way RM ANOVA, dose x strain interaction: $F_{(39, 234)} = 2.666$; $p < 0.001$; Post-hoc: $p < 0.05$ at time 96–102 min). However, this concentration of desipramine also caused significant off-target effects, producing increases in extracellular dopamine (Fig 2B; Two-way RM ANOVA, main effect of dose: $F_{(39, 234)} = 6.902$; $p < 0.001$) and serotonin that were similar between groups (Fig 2C; Two-way RM ANOVA, main effect of dose: $F_{(39, 234)} = 6.442$; $p < 0.0001$).

3.3 Microdialysis Probe Placements

Probe placements were all between +1.25 and +1.65 mm from bregma. The ML and DV locations show that probe tips were located in the ventral striatum. There was no systematic difference in probe location (AP/ML) between obesity-prone (black) and obesity-resistant (gray) rats (Fig 1C, cocaine administration; Fig 2D, desipramine retrodialysis).

3.4 Effects of Systemic Desipramine on Locomotion, Body Temperature, and Food Intake

We next examined the effect of systemic desipramine on food intake, activity levels, and body temperature in obesity-prone and obesity-resistant rats. Home cage locomotor behavior was similar between obesity-prone and obesity-resistant rats after vehicle treatment (Figure 3A, B). Following administration of desipramine (3.2 mg/kg or 10 mg/kg i.p.), a significant decrease in locomotor activity in obesity-prone rats was observed (Fig 3B; Two-way RM ANOVA significant dose x time interaction: $F_{(74, 518)} = 1.733$, $p < 0.001$; Post-hoc: $p < 0.05$ veh vs. 3.2 mg/kg 30, 60 and 90 min post injection; veh vs. 10 mg/kg 15–25, 40–50, 60–70, and 90 min post injection). Comparisons of locomotor activity in obesity-resistant rats following desipramine revealed no interactions between dose and time, and no main effect of either variable (Figure 3A). Desipramine produced a significant decrease in body temperature in obesity-prone rats (Fig 3D; Two-way RM ANOVA significant dose x time interaction: $F_{(74, 518)} = 5.668$, $p < 0.001$; Post-hoc: $p < 0.05$, veh vs. 3.2 mg/kg: 50–90 min post injection; veh vs. 10.2 mg/kg: 10–90 min post injection; Main effect of dose: $F_{(2, 15)} = 39.68$; $p < 0.001$) and in obesity-resistant rats (Fig 3C; Two-way RM ANOVA significant dose x time interaction: $F_{(74, 407)} = 1.976$, $p < 0.0001$; four trials were removed due to equipment errors). However, the magnitude of reductions in body temperature following desipramine were similar between obesity-prone and obesity-resistant rats (Fig 3D inset; Two-way ANOVA no significant dose x strain interaction, main effect of dose: $F_{(2, 25)} = 10.11$, $p < 0.001$; Main effect of strain: $F_{(2, 25)} = 0.8972$, $p = 0.35$).

As expected, food intake over a 24 h period was significantly greater in obesity-prone vs. obesity-resistant rats (Two-way ANOVA: main effect of strain: $F_{(1, 30)} = 4.606$, $p < 0.05$). In

addition, desipramine decreased food intake in both groups (Two-way ANOVA: dose x strain interaction $F_{(2,30)}=3.33$, $p<0.05$; main effect of dose: $F_{(2, 30)} = 126$, $p<0.0001$), however, post hoc analyses revealed that food intake was significantly reduced following 3.2 and 10 mg/kg in obesity-prone rats, whereas decreases in obesity-resistant rats only occurred following a 10 mg/kg dose (Fig 4).

4. Discussion and Conclusion

Previous studies have separately demonstrated the involvement of the reward pathway and NE circuits in obesity and feeding behaviors [2,33–35,49]. In addition, both peripheral and hypothalamic NE systems differ in selectively bred obesity-prone vs. obesity-resistant rats [11,14,44]. However, differences in striatal NE or behavioral response to desipramine have not been examined in obesity-prone vs. obesity-resistant groups. Importantly, the ventral noradrenergic bundle contributes to NE release in the hypothalamus, but also projects to the ventral striatum [6,50], and lesion of the ventral noradrenergic bundle leads to hyperphagia and obesity specifically mediated by NE [3], supporting the idea that interactions between hypothalamic and striatal NE play important roles in feeding.

Here, we found that systemic cocaine administration enhanced striatal NE levels in obesity-prone but not obesity-resistant rats (Fig 1B). Cocaine blocks all monoamine transporters including the NET [51]. Thus, differential effects of cocaine found here could be due to direct blockade of NET within the ventral striatum, or due to indirect circuit-mediated effects [52–56]. In order to determine if effects were due to NET blockade in the striatum alone, desipramine (a more selective NET blocker than cocaine), was infused directly into the ventral striatum of obesity-prone and obesity-resistant rats via retrodialysis while simultaneously measuring NE levels by microdialysis. Our data show that 100 μ M desipramine (a dose which selectively produces NET blockade) delivered directly into the ventral striatum was insufficient to increase NE concentrations of obesity-prone rats (Fig 2A). Thus, increases in striatal NE following systemic cocaine administration are not likely a result of local NET blockade, but rather may be the result of broader circuit-based responses. In addition, retrodialysis of 500 μ M desipramine resulted in an increase in extracellular levels of NE in obesity-resistant, but not obesity-prone rats (Figure 2A). Furthermore, as shown in Table 1, 500 μ M desipramine also produced increases in extracellular dopamine and serotonin in both groups. This is consistent with effects of desipramine at the dopamine and serotonin transporters at higher concentrations, and confirms that desipramine did in fact reach the target tissue and is able to elicit increases in NE.

The absence of any increase in NE in obesity-prone rats following intra-striatal desipramine suggests that NET expression or function may be attenuated in the ventral striatum of obesity-prone vs. obesity-resistant rats. While protein expression of NET in the brain has been difficult to characterize fully, Schroeter and colleagues demonstrated that NET is present in the caudal portion of the nucleus accumbens shell [57], and functional NE transporters have been found in the ventral striatum [58]. Interestingly, global knockout of the norepinephrine transporter in mice leads to supersensitivity to cocaine as measured by locomotor activity, and causes an enhanced $D_{2/3}$ receptor response [37]. Consistent with

potential reductions in NET, cocaine-induced locomotor activity is enhanced in obesity-prone rats, and they are more sensitive to the D₂ agonist quinpirole, than obesity resistant rats [33–35]. Given that NET binding in hypothalamic regions is reduced in obesity-prone rats compared to obesity-resistant rats [10,14], the absence of desipramine-induced increases in locomotor activity in the current study could suggest that a similar reduction may also exist in the ventral striatum, although further studies are necessary to verify this possibility. None-the-less, it's clear from our current results that increases in NE following systemic cocaine are not likely due to blockade of the NET within the striatum itself, but rather arise from broader circuit effects of cocaine.

Systemic desipramine treatment was used to further characterize differences in the NE systems of obesity-prone and obesity-resistant rats. Consistent with the hypothermic effects of elevations in hypothalamic NE [38,40,59, See also 60 for review], intraperitoneal injection of desipramine caused decreases in body temperature that were similar in obesity-prone and obesity-resistant groups (Figure 3C, D, D inset). Furthermore, previous studies have demonstrated that acute desipramine decreases locomotor activity in a novel environment, but not a familiar environment [61–63]. Here we found that acute desipramine treatment in the home cages decreased locomotor activity in obesity-prone, but not obesity-resistant rats. This again is consistent with an enhanced sensitivity to modulation of NE systems in obesity-prone animals (see also above for discussion of role of NE in locomotion), however, similar decreases in body temperature found in both strains following desipramine treatment suggest that there is some specificity to the differential response to NET blockade. One caveat to note is that differences in body composition could lead to differences in metabolism of systemic desipramine. Although we cannot rule this possibility out, the fact that we observe differences in some measures without differences in others, suggests that animals are being equivalently dosed.

When effects of desipramine on food intake were examined, we found that a lower dose of desipramine was able to reduce food intake in obesity-prone vs. obesity-resistant rats (Figure 4). Specifically, both 3.2 and 10 mg/kg desipramine (i.p.) reduced food intake in obesity-prone animals, while significant reductions in food intake were observed in obesity-resistant rats only following a 10 mg/kg dose (Figure 4). These data are consistent with human studies demonstrating effectiveness of the NET inhibitor atomoxetine in reducing body weight in individuals with obesity and binge eating behaviors [64,65] and with reductions in food intake and weight in response to a general monoamine reuptake inhibitor, tesofensine, or the NET inhibitor nisoxetine following diet-induced obesity in rats [7,66]. No diet manipulation was employed here and while obesity-prone rats were heavier than obesity-resistant rats, no significant differences were observed in fasted insulin levels. This suggests that even modest to moderate weight gain, independent of obesity or metabolic syndrome, may enhance sensitivity to the anorectic effects of NET inhibitors. Furthermore, differences in responsivity between obesity-prone and obesity-resistant groups suggest that individual susceptibility to obesity is also a factor in sensitivity to NET inhibitors.

In summary, our data demonstrate that striatal NE responses and sensitivity to effects of NET blockade differ in obesity-prone and obesity-resistant rats. Cocaine induced increases in extracellular striatal NE are likely due to circuit level effects of the drug, as direct

administration of desipramine within the ventral striatum does not alter NE levels in obesity-prone rats. We also demonstrate that systemic administration of the NET inhibitor desipramine causes significant decreases in body temperature and locomotor activity in obesity-prone rats, with little effect on locomotor activity in obesity-resistant rats. Finally, systemic desipramine is more effective at reducing food intake in obesity-prone rats than in obesity-resistant rats, with a relatively low dose of desipramine decreasing 24 h food intake only in obesity-prone rats. Overall, these data lend further support to the idea that NE systems are disrupted in obesity-prone models, and identify novel differences in NE systems that may influence feeding behavior.

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Highlights

- Systemic cocaine administration increases striatal norepinephrine in obesity-prone rats
- Striatal blockade of norepinephrine transporter via desipramine retrodialysis does not increase striatal norepinephrine levels in obesity-prone rats
- Systemic desipramine treatment leads to decreases in locomotor activity in obesity-prone rats when compared to obesity-resistant rats
- Desipramine decreases food intake at lower doses in obesity-prone rats than in obesity-resistant rats.

Figure 1.

Cocaine administration increases locomotor behavior and extracellular norepinephrine in the ventral striatum. A) OP animals exhibited an enhanced locomotor response to cocaine when compared to OR animals * indicates $p < 0.05$ by Sidak's post-test. B) Norepinephrine was significantly increased in OP rats following acute cocaine administration (Cocaine injection shown by dotted line). Inset compares the respective area under the curve for OP and OR rats. C) Microdialysis probes were localized to the ventral striatum of OP and OR rats during cocaine administration experiments. * indicates $p < 0.05$ by Sidak's post-test (A, B) or by unpaired students t-test (inset of B).

Figure 2.

Desipramine retrodialysis increases monoamine levels with differential effects on norepinephrine levels. A) Basal (white) and 100 μ M desipramine (light gray) had no significant effect on NE in either OP or OR rats, while 500 μ M desipramine (dark gray) led to significant increases in NE only in OR rats. Inset shows basal NE concentrations do not differ between OP and OR rats. B) 100 μ M desipramine had no significant effect on dopamine, while 500 μ M (dark gray) lead to a significant increase in dopamine levels in both OP and OR rats. C) 500 μ M desipramine (dark gray) led to increases in serotonin concentrations in both OP and OR rats. D) Microdialysis probes were localized to the ventral striatum of OP and OR rats during local desipramine microdialysis experiments. * indicates $p < 0.05$ by Sidak's post-test.

Figure 3.

Desipramine differentially effects locomotor activity and body temperature in obesity-prone and obesity-resistant rats. A) No significant effects on locomotion were observed in obesity-resistant rats following desipramine treatment. B) A significant decrease in locomotor behavior was observed in obesity-prone animals following desipramine treatment. C) Body temperature was reduced in obesity-resistant rats following desipramine treatment. D) A significant decrease in core body-temperature was observed in obesity-prone rats following desipramine treatment. Inset shows average temperature over last 30 minutes for both OP and OR animals, demonstrating that no differences exist in the magnitude of change between the two strains following any dose of desipramine. Sidak's Post-hoc analysis: * = significant difference between vehicle and 10 mg/kg, # = significant difference between 3.2 mg/kg dose and vehicle, Ψ = significant difference between 3.2 mg/kg and 10 mg/kg.

Figure 4.

Desipramine is more effective in reducing 24-hour food intake in OP rats. Systemic desipramine administration significantly reduced food intake in both OP and OR rats at a dose of 10 mg/kg. At a dose of 3.2 mg/kg a significant effect of desipramine on food intake was only observed in OP animals. A main effect of strain was observed indicating that OP rats consumed greater amounts of food than obesity-resistant rats. * indicates $p < 0.05$ by Sidak's post-test.

Table 1

In vivo dialysate concentrations (nM) at baseline and following retrodialysis of desipramine (500 μ M).

<u>Analyte</u>	<u>Obesity-Resistant</u>		<u>Obesity-Prone</u>	
	<u>Baseline</u>	<u>500 μM Desipramine</u>	<u>Baseline</u>	<u>500 μM Desipramine</u>
Norepinephrine	0.20 $\pm$ 0.07	2.86 $\pm$ 0.55	0.18 $\pm$ 0.06	1.19 $\pm$ 0.64
Dopamine	3.00 $\pm$ 0.34	17.3 $\pm$ 2.74	4.11 $\pm$ 0.74	23.3 $\pm$ 4.4
Serotonin	0.06 $\pm$ 0.02	0.74 $\pm$ 0.10	0.04 $\pm$ 0.02	0.45 $\pm$ 0.14

All values given are means $\pm$ SEM. OP: N=4 rats, OR: N=4 rats