Published in final edited form as:

Psychopharmacology (Berl). 2016 March ; 233(5): 773–784. doi:10.1007/s00213-015-4157-x.

Enhanced cocaine-induced locomotor sensitization and intrinsic excitability of NAc medium spiny neurons in adult but not adolescent rats susceptible to diet-induced obesity

Max F. Oginsky, Joel D. Maust, John T. Corthell, and Carrie R. Ferrario

Department of Pharmacology, University of Michigan, Ann Arbor, MI, USA

Abstract

Rationale—Basal and diet-induced differences in mesolimbic function, particularly within the nucleus accumbens (NAc), may contribute to human obesity; these differences may be more pronounced in susceptible populations.

Objectives—We determined whether there are differences in cocaine-induced behavioral plasticity in rats that are susceptible vs. resistant to diet-induced obesity, and basal differences in the striatal neuron function in adult and adolescent obesity-prone and obesity-resistant rats.

Methods—Susceptible and resistant outbred rats were identified based on “junk-food” diet-induced obesity. Then, the induction and expression of cocaine-induced locomotor sensitization, which is mediated by enhanced striatal function and is associated with increased motivation for rewards and reward-paired cues, were evaluated. Basal differences in mesolimbic function were examined in selectively bred obesity-prone and obesity-resistant rats (P70-80 and P30-40) using both cocaine induced locomotion and whole-cell patch clamping approaches in NAc core medium spiny neurons (MSNs).

Results—In rats that became obese after eating “junk-food”, the expression of locomotor sensitization was enhanced compared to non-obese rats, with similarly strong responses to 7.5 and 15 mg/kg cocaine. Without diet manipulation, obesity-prone rats were hyper-responsive to the acute locomotor-activating effects of cocaine, and the intrinsic excitability of NAc core MSNs was enhanced by ~60% at positive and negative potentials. These differences were present in adult, but not adolescent rats. Post-synaptic glutamatergic transmission was similar between groups.

Conclusions—Mesolimbic systems, particularly NAc MSNs, are hyper-responsive in obesity-prone individuals; and interactions between predisposition and experience influence neurobehavioral plasticity in ways that may promote weight gain and hamper weight loss in susceptible rats.

Keywords

obesity; nucleus accumbens; excitability; sensitization; striatum; cocaine

Corresponding Author: Carrie R. Ferrario, University of Michigan, 1150 W. Medical Center Drive, MSRB III 1301, Ann Arbor, MI, 48109; ferrario@umich.edu.

FUNDING AND DISCLOSURES: Cocaine was provided by the NIDA drug supply program. This work was supported by R01DK106188 and by a Brain and Behavior Research Foundation NARSAD Young Investigator Award to CRF. MFO was supported by NIDA T32DA007268.

INTRODUCTION

; Rada et al. 2010; Valenza et al. 2015; Vollbrecht et al. In Press; Vollbrecht et al. 2015) no previous studies have examined basal differences in neurotransmission to MSNs in the NAc core of obesity-prone and obesity-resistant rats.

Sensitization to the locomotor-activating effects of cocaine is associated with alterations in NAc MSN morphology, function, and enhanced cue-triggered reward-seeking behaviors (Ferrario et al. 2005; Robinson and Kolb 2004; Vezina and Leyton 2009; Wolf and Ferrario 2010). Furthermore, locomotor sensitization is also associated with enhanced motivational responses to food cues that can contribute to the pursuit of food rewards (Wyvell and Berridge 2000; 2001). Although several studies have examined the effect of eating sugary, fatty, foods on the acute locomotor-activating effects of amphetamines (Baladi et al. 2012; McGuire et al. 2011), none have examined the subsequent expression of sensitization after a drug-free period or potential differences in the induction vs. expression of sensitization in obesity-susceptible populations. This is important because interactions between the development of obesity and/or ingestion of high fat foods may produce different neurobehavioral alterations in obesity susceptible vs. resistant populations (Jensen and Kirwan 2015; Levin et al. 1997; Murdaugh et al. 2012; Yokum et al. 2011).

Therefore, in the current study, we examined the induction and expression of cocaine-induced locomotor sensitization in rats identified as resistant or susceptible to weight gain when given free access to sugary, fatty, “junk-food”. In addition, we used selectively bred obesity-prone and obesity-resistant rats to examine basal differences in sensitivity to cocaine-induced locomotion, post-synaptic glutamate receptor function, and excitability of MSNs using whole-cell patch clamp approaches in the NAc core. We found that while the induction of locomotor sensitization was similar, the expression of sensitization after a cocaine-free period was enhanced in rats that gained weight when given “junk-food” compared to those that did not. This suggests that consumption of the junk-food and/or alterations accompanying obesity produce more robust changes in mesolimbic function in susceptible individuals. When basal differences were examined in selectively bred rats, we found that obesity-prone rats were more sensitive to the acute locomotor-activating effects of cocaine (i.e., there was a left shift in the dose response function). Furthermore, although basal post-synaptic glutamatergic transmission was similar between groups, intrinsic excitability of MSNs in the NAc core was enhanced in obesity-prone compared to obesity-resistant rats. These neurobehavioral differences were not present during early adolescence, suggesting that differences in NAc function and sensitivity to cocaine in adults are not due to differences occurring earlier in development, but rather may be related to spontaneous weight gain and accompanying physiological changes. These data are discussed in light of enhanced motivational properties of food and food cues in obesity-prone individuals (Berthoud 2012; Demos et al. 2012; Stice et al. 2012; Stice et al. 2011), and potential interactions between pre-disposition and alterations in striatal systems induced by consumption of sugary, fatty, foods.

MATERIALS AND METHODS

Subjects

Rats were maintained on a reverse light-dark schedule (12/12) and all testing was conducted during the dark phase starting ~3–4 hours after dark onset. Adult male Sprague-Dawley rats were purchased from Harlan. Breeding pairs of obesity-prone and obesity-resistant rats were purchased from Taconic and maintained in house by the University of Michigan breeding core. These lines were originally established by Barry Levin (Levin et al. 1997). Rats had free access to food and water throughout. All procedures were approved by The University of Michigan Committee on the Use and Care of Animals.

Drugs & Reagents

Cocaine HCL was provided by the NIDA drug supply program. All other drugs and reagents were obtained from Sigma (St. Louis MO, USA) or Tocris (Minneapolis MN, USA).

Experiment 1: Diet-induced obesity and cocaine sensitization in outbred rats

—The development and expression of cocaine-induced locomotor sensitization were evaluated in outbred male Sprague-Dawley rats given free access to a “junk-food” diet. The “junk-food” diet is a mash composed of: Ruffles original potato chips (40g), Chips Ahoy original chocolate chip cookies (130g), Jiff smooth peanut butter (130g), Nesquik powdered chocolate flavoring (130g), powdered Lab Diet 5001 (200 g; % of calories: 19.6% fat, 14% protein, 58% carbohydrates; 4.5 kcal/g), and water (180 ml). Ingredients were combined in a food processor. Diet composition was based on previous studies establishing individual differences in susceptibility to weight gain (Levin et al. 1997; Robinson et al. 2015; Vollbrecht et al. 2015). K-means clustering based on weight gain after one-month of free access to junk-food was used to identify individuals that are susceptible (Junk-Food Gainer) vs. resistant to diet-induced obesity (Junk-Food Non-Gainer). This statistical method provides an unbiased separation that can be applied uniformly across studies (MacQueen 1967).

After 2 months of free access to the junk-food, the induction and expression of cocaine-induced locomotor sensitization were determined as previously described (Ferrario et al., 2005; Ferrario et al., 2010). Locomotor testing was conducted in standard rat cages (22.86 cm × 44.45 cm × 28 cm). Each chamber has five photobeams; one positioned in the center of the chamber and two on either end positioned 5 cm and 10 cm from the shorter chamber wall. Rearing behavior was not measured. Rats were handled daily for one week, and then habituated to locomotor chambers (40 min) and the injection procedure (saline 1 ml/kg, i.p. 60 min). Next, all rats were given cocaine (15 mg/kg, weight of salt, i.p.) once per day for 8 consecutive days. On each of these pre-treatment days, rats were placed in the locomotor chamber for 40 minutes followed by cocaine injection. This cocaine regimen was chosen because it induces robust locomotor sensitization and striatal plasticity (e.g., (Ferrario et al. 2010). Fourteen days after the last cocaine pre-treatment, rats were brought back to locomotor chambers for a 40 min habituation followed by injection of saline (1 mL/kg, i.p.) and cocaine challenge (7.5 and 15 mg/kg, i.p.). Rats had free access to junk-food throughout all cocaine pre-treatment, 14 day cocaine-deprivation, and cocaine challenge. Food intake

and weight gain were monitored throughout. Total photo-cell beam breaks per 5 min interval were the primary measure of locomotion. In addition, rats were observed for possible stereotypy during testing.

Experiment 2: Acute cocaine-induced locomotion in selectively bred obesity-prone and obesity-resistant rats—The acute locomotor-activating effects of cocaine were evaluated in male adult (P70-80) and adolescent (P30-40) selectively bred obesity-prone and obesity-resistant rats, without any diet manipulation. Rats were habituated to the locomotor chambers and injection procedure as described above. The next day, rats were brought back to the locomotor chambers (40 min), and received an injection of saline (40 min) followed by 3 doses of cocaine: (7.5, 15, and 30 mg/kg, i.p.; as in (Ferrario et al. 2005)). All rats received three doses of cocaine in ascending order; 7.5 and 15 mg/kg doses were given one hour apart and the last injection (30mg/kg) was given 1.5 hrs after the 15 mg/kg dose. As above, total photo-cell beam breaks per 5 min interval were the primary measure of locomotion and rats were observed during testing.

Experiment 3: Electrophysiological Recordings of Medium Spiny Neurons in the NAc core—Whole cell patch clamp recordings of MSNs were made from selectively bred adult (P70-P80) and adolescent (P30-P40) obesity-prone and obesity-resistant rats without diet manipulation or drug exposure using standard procedures (e.g., (Ferrario et al. 2012; Ferrario et al. 2011)). The classification of adult and adolescent age ranges was made based on previous studies (Kasanetz and Manzoni 2009; Spear 2000). Recordings were conducted in the presence of the GABA receptor antagonist picrotoxin (50 μ M). Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), brains were rapidly removed and placed in ice-cold oxygenated (95% O₂-5% CO₂) aCSF containing (in mM): 125 NaCl, 25 NaHCO₃, 12.5 glucose, 1.25 NaH₂PO₄, 3.5 KCl, 1 L-ascorbic acid, 0.5 CaCl₂, 3 MgCl₂, 305 mOsm, pH 7.4. Coronal slices (300 μ m) containing the NAc were made using a Leica VT1200 vibratory microtome (Leica Biosystems, Buffalo Grove, IL, USA) and allowed to rest in oxygenated aCSF for at least 40 min before recording. For the recording aCSF (2 ml/min), CaCl₂ was increased to 2.5 mM and MgCl₂ was decreased to 1 mM. Patch pipettes were pulled from 1.5 mm borosilicate glass capillaries (WPI, Sarasota, FL) to a resistance of 3–7 M Ω with a horizontal puller (Model P97, Sutter Instruments, Novato, CA, USA) and filled with a solution containing (in mM): 130 K gluconate, 10 KCl, 1 EGTA, 2 Mg²⁺-ATP, 0.6 Na⁺-GTP, and 10 HEPES, pH 7.3, 285 mOsm when performing current clamp experiments. When measuring glutamatergic transmission, pipettes were filled with solution containing (in mM): 140 CsCl, 10 HEPES, 2 MgCl₂, 5 Na⁺-ATP, 0.6 Na⁺-ATP, 2 QX314, pH 7.3, 285 mOsm. For current clamp studies, MSNs in the NAc core were identified based on resting membrane potential and action potential firing in response to current injection (–200 to 175–275 pA, 25 pA increments, 500 ms; (Nisenbaum and Wilson 1995; Wilson and Kawaguchi 1996)). The I/V relationship was determined by calculating the difference between the baseline voltage and the voltage at 200 ms after initial current injection. Neuronal excitability was determined by measuring the number of action potentials elicited by each depolarizing current injection. Input resistance was determined by the change in voltage from –50 pA to +50 pA current injections. Rheobase is defined as the minimum amount of current injection to elicit an action potential. The action potential threshold was

determined using the maximum second derivative method (Sekerli et al. 2004). The peak amplitude was defined as the difference between the action potential peak and threshold. The first interspike interval was measured between the peak amplitude of the first two action potentials in a train. The amplitude of the afterhyperpolarization (AHP) was measured from the action potential threshold to the lowest potential of the AHP. Evoked EPSCs (eEPSCs) were elicited by local stimulation (0.05 to 0.30 mA square pulses, 0.3 ms, delivered every 20 s) using a bipolar electrode placed ~300 μ m lateral to recorded neurons. The minimum amount of current needed to elicit a synaptic response with <15% variability in amplitude was used. If > 0.30 mA was required, the neuron was discarded. NMDAR-mediated eEPSCs were recorded at +40 mV whereas AMPAR-mediated eEPSCs were recorded at -70 mV (Scheyer et al. 2014). NMDAR-mediated eEPSCs were determined by the difference in current before and after application of 50 μ M APV at a holding potential of +40 mV.

RESULTS

Experiment 1: The expression of sensitization is enhanced in Junk-Food Gainers vs. Junk-Food Non-Gainers after 14 days of cocaine deprivation

As expected, when given free access to junk-food, only some rats ate significantly more food (Fig. 1a; two-way RM ANOVA; main effect of group $F_{(1,13)} = 4.96$, $p < 0.05$) and gained a substantially more weight (Fig. 1b; two-way RM ANOVA; main effect of group $F_{(1,13)} = 19.95$, $p < 0.001$; Junk-Food Gainers; JF-G, $n=7$) than others given the same free access to junk-food (Junk-Food Non-Gainers; JF-N, $n=8$). In addition, weight gain in Junk-Food Non-Gainers was comparable to that usually seen in rats given standard lab chow (~10–15 g/week; unpublished observations). Although not statistically significant, it is worthwhile to note that JF-G tend to eat slightly more food, even when they are given standard lab chow (Fig 1a, first two data points). This has been noted previously but is not pronounced enough to use as an indication of susceptibility on its own (unpublished observations CRF and personal communication Barry Levin). Weight gain of individual rats in each group after 9 weeks of junk-food consumption and prior to beginning cocaine sensitization are shown in Fig 1c.

Figure 2 below shows locomotor activity across repeated cocaine injections (15 mg/kg, i.p.). Junk-Food Gainer and Junk-Food Non-Gainer groups showed a similar rate and magnitude of locomotor sensitization across repeated injections (Fig. 2a) and a significantly greater (i.e., sensitized) locomotor response to the last vs. first injection of cocaine (Fig. 2b; total beam breaks/40 min; Two-way RM ANOVA; main effect of injection $F_{(1,12)} = 14.75$, $p < 0.01$; group x time interaction and main effect of group n.s.). However, when re-exposed to cocaine after a 14 day cocaine deprivation period (i.e., cocaine “challenge”), Junk-Food Gainers showed a stronger response to cocaine (7.5 mg/kg) compared to Junk-Food Non-Gainers, indicative of enhanced expression of sensitization in Junk-Food Gainers (Fig. 2c; two-way RM ANOVA; main effect of group $F_{(1,12)} = 12.57$, $p < 0.01$; group x time interaction $F_{(11, 132)} = 3.47$, $p < 0.001$; Fig. 2d: two-way RM ANOVA; group x session $F_{(1,12)} = 4.7$, $p = 0.05$; 7.5 mg/kg JF-G vs. JF-N $t_{12} = 4.2$, $p < 0.01$). Furthermore, in Junk-Food Gainers the response to the 7.5 mg/kg cocaine challenge was as strong as the initial response to 15 mg/kg cocaine (Fig. 2d). In contrast, the response to cocaine challenge

remained low compared to the first cocaine exposure in Junk-Food Non-Gainers (Fig. 2d). Thus, although the initial induction of locomotor sensitization was similar between groups, its expression was enhanced in Junk-Food Gainers after a period of cocaine deprivation. Importantly, locomotor activity during habituation (not shown) or in response to saline did not differ between groups (Fig. 2c).

Experiment 2: Greater sensitivity to cocaine-induced locomotion in selectively bred obesity-prone vs. obesity-resistant rats

Although no differences were found in response to the first injection of cocaine in outbred Junk-Food Gainers vs. Junk-Food Non-Gainers, it is possible that consumption of the junk-food diet (2 months) and accompanying weight gain may have masked potential pre-existing differences and/or that examination of the full dose response curve would have revealed group differences. To examine these possibilities, the acute locomotor-activating effects of cocaine were examined in selectively bred obesity-prone and obesity-resistant rats across multiple doses and without diet manipulation. The time course of cocaine-induced locomotion in adult rats is shown in Fig. 3a ($n=8/\text{group}$). The locomotor response to cocaine was generally greater in obesity-prone vs. obesity-resistant rats across all doses, with the largest differences found at 15 mg/kg (Fig. 3a Two-way RM ANOVA, 15 mg/kg: main effect of group $F_{(1,14)} = 5.57$, $p < 0.04$; 30 mg/kg main effect of group $F_{(1,14)} = 3.34$, $p = 0.08$). In addition, enhanced sensitivity to cocaine-induced locomotion was also evident when total locomotor activity 40 min after injection was examined (Fig. 3b; Two-way RM ANOVA; main effect of group $F_{(1,14)} = 5.29$, $p < 0.04$; group $\times$ dose interaction n.s.). In contrast, the locomotor response to cocaine was similar between groups during adolescence (Fig. 3c; $n=10/\text{group}$). In addition, adult obesity-prone rats were heavier than obesity-resistant rats at the time of testing (Avg. weight $\pm$ SEM: adult OP = 351.9 ± 7.2 g, adult OR = 300.5 ± 6.0 g, $t_{(14)} = 5.4$, $p < .0001$), but weight of adolescent rats did not differ significantly (Avg. weight $\pm$ SEM: adolescent OP = 151.0 ± 7.9 g; adolescent OR = 130.7 ± 9.1 g, $t_{(18)} = 1.6$, $p = .11$). However, this is well inside the range of normal variance for adult outbred Sprague Dawley rats: P70-76 = 274–380g; (McCutcheon and Marinelli 2009). Thus, although heavier, obesity-prone rats at this age are not “obese” (see also (Vollbrecht et al. 2015) for adiposity and metabolic characterization). Importantly, no group differences were seen during habituation (data not shown) or in response to saline injection (Fig. 3a), indicating that our ability to measure locomotor activity accurately in obesity-prone and obesity-resistant rats is not affected by rat size; this is also supported by our data above in outbred rats where Junk-Food Gainers are much fatter than Junk-Food Non-Gainers, but show similar initial locomotor responses.

Experiment 3: Greater intrinsic excitability of NAc core medium spiny neurons in selectively bred obesity-prone vs. obesity-resistant rats

Cocaine-induced locomotion is mediated by dopamine- and glutamate-mediated transmission in the NAc (Karasinska et al. 2005; Pulvirenti et al. 1989), and locomotor sensitization to cocaine is associated with increased glutamate receptor expression and function in MSNs of the NAc core (Boudreau and Wolf 2005; Ferrario et al. 2010; Kourrich et al. 2007). To determine if glutamate receptor function differed between obesity-prone and obesity-resistant rats prior to any diet manipulation, we used whole cell patch clamping

approaches to measure evoked AMPAR- and NMDAR-mediated post-synaptic currents (eEPSC; OP: n=5 cells from 5 rats; OR: n=6 cells from 4 rats). The AMPA/NMDA ratio did not differ between groups, nor did the amplitude of AMPAR- and NMDAR-mediated eEPSCs (peak AMPAR-mediated current amplitude: OP = 187.5 ± 26.6 pA; OR = 179.6 ± 39.7 pA; peak NMDAR-mediated current amplitude: OP = 78.7 ± 36.5 pA; OR = 82.4 ± 44.6 pA; data not shown).

We next examined intrinsic excitability of MSNs in the NAc core of obesity-prone and -resistant rats because there are basal differences in dopamine systems in obesity-prone vs. -resistant rats, dopamine-mediated transmission strongly influences MSN excitability, and cocaine sensitization is associated with alterations in intrinsic excitability, (Geiger et al. 2008; Hu 2007; Kourrich et al. 2015; Kourrich and Thomas 2009; Marinelli et al. 2006; Rada et al. 2010; Valenza et al. 2015; Vollbrecht et al. In Press). Recordings were made from adult and adolescent obesity-prone and obesity-resistant rats without diet manipulation or cocaine exposure (Fig. 4a; OP n=10–11 cells from 7 rats; OR n=10–11 cells from 8 rats). Plotting the current/voltage (I/V) relationship showed a significantly greater change in membrane potential (Vm) in response to current injection in MSNs from adult obesity-prone vs. obesity-resistant rats (Fig. 4b; Two-way RM ANOVA, group x current injection interaction: $F_{(12,130)} = 10.46$, $p < 0.0001$). This effect was seen at both positive and negative current injections. In contrast, there was no difference in the I/V relationship in MSNs from adolescent obesity-prone and obesity-resistant rats (Fig. 4c). This suggests that differences emerge after initial neuronal maturation. Additional analysis of data from adults showed that the number of action potentials elicited by increasing current injection was significantly greater in obesity-prone rats (Fig. 4d; two-way RM ANOVA, group x current injection interaction: $F_{(7,133)} = 17.06$; $p < 0.0001$; Sidak's post test, $p < 0.0001$). Consistent with the shift in the I/V relationship and increased action potential firing, the input resistance was greater in cells from obesity-prone vs. obesity-resistant rats (Fig. 4e; $t_{20} = 2.8$, $p < 0.05$; OP: 193.1 ± 22.4 M Ω vs. OR: 119.1 ± 14.1 M Ω) and the rheobase (*i.e.*, the minimum amount of current injected to elicit an action potential) was lower in the MSNs from obesity-prone rats compared to obesity-resistant (Fig. 4f; $t_{20} = 3.3$; $p < 0.01$; Rheobase OP = 100.0 ± 7.5 pA; OR: = 161.4 ± 17.3 pA). Finally, there were no differences in basal cell parameters including: resting membrane potential, action potential threshold, action potential rise time (10%–90%), action potential amplitude, the duration of 1st interspike interval between groups, or the AHP (Fig. 4g). Because of the large variation in the rheobase between MSNs from obesity-prone and obesity-resistant rats, it was not possible to compare the latency to first spike between groups at the same current injection intensity. However, taken together the shift in the I/V relationship, lower rheobase, and greater firing frequency are consistent with greater excitability of MSNs in adult obesity-prone vs. obesity-resistant rats.

DISCUSSION

Individual differences in mesolimbic function

Both pre-existing and diet/obesity-induced differences in the function of mesolimbic circuits, particularly within the NAc, are thought to contribute to obesity in people (Dagher 2009; Stice et al. 2012; Tomasi and Volkow 2013). Consistent with the idea of pre-existing

differences, we recently found that prior to any diet manipulation, outbred rats subsequently identified as susceptible to obesity were hyper-responsive to the motivational properties of a food cue (Robinson et al., 2015), and that obesity-prone rats show robust Pavlovian to instrumental transfer, a classic measure of incentive motivation, whereas obesity-resistant rats do not (CRF unpublished observations). These behavioral responses are mediated in part by dopamine- and glutamate-mediated transmission in MSNs of the NAc core (Balleine 2005; Cardinal et al. 2002; Crombag et al. 2008a; Crombag et al. 2008b; Kelley 2004; Setlow et al. 2002). In addition, consumption of sugary, fatty foods and obesity are associated with changes in mesolimbic function (Baladi et al. 2012; Hryhorczuk et al. 2015; McGuire et al. 2011; Rada et al. 2010). Here, we first examined the induction and expression of cocaine-induced locomotor sensitization in outbred rats identified as susceptible or resistant to weight gain when given free access to junk-food. We next used selectively bred rats to examine basal differences in the acute response to cocaine and the function of MSNs in the NAc core, without diet manipulation. We found that the expression of sensitization after a cocaine-free period was enhanced in Junk-Food Gainers vs. Junk-Food Non-Gainers. In addition, prior to any diet manipulation obesity-prone rats were hyper-responsive to the acute locomotor-activating effects of cocaine. Furthermore, intrinsic excitability of MSNs in the NAc core was enhanced in obesity-prone compared to obesity-resistant rats. Together, these data suggest that there are both basal and experience-induced differences in striatal function in individuals that are susceptible to obesity that manifest in part as enhanced responsivity of mesolimbic reward circuits. We propose that this hypersensitivity may contribute to greater cue-triggered motivation in obesity-susceptible individuals.

Junk-food effects on induction and expression of locomotor sensitization to cocaine

Although the effect of eating sugary, fatty, foods on the acute locomotor-activating effects of amphetamines has been examined (Baladi et al. 2012; McGuire et al. 2011), potential differences in the induction and expression of sensitization in obesity-susceptible populations has not. It is well established that only a sub-set of rats will become obese when given free access to a moderately fatty diet (Levin et al. 1997; Robinson et al. 2015). Thus, we began by using a junk-food diet to identify obesity susceptible and resistant outbred rats and then evaluated cocaine-induced sensitization. As expected, junk-food produced substantial weight gain in a sub-set of susceptible individuals, Junk-Food Gainers, that was attributable at least in part to greater food intake in (Fig. 2). While rats still had access to junk-food, cocaine-induced locomotion was examined. The acute response to the first cocaine injection was similar between Junk-Food Gainers and Junk-Food Non-Gainers (Fig. 2a). This is consistent with our previous study showing a similar magnitude of amphetamine-induced locomotion in Junk-Food Gainers and Junk-Food Non-Gainers (Robinson et al. 2015). Because the focus here is on differences between obesity-susceptible and -resistant populations, comparisons to chow-fed controls were not included. However, previous studies have found both increased and decreased locomotor responses to amphetamine after high-fat diet exposure (e.g., (Hryhorczuk et al. 2015; Robinson et al. 2015). Interestingly, the study by Hryhorczuk et al. (2015) suggests that the source of dietary fat substantially impacts the nature of diet-induced changes in mesolimbic function. Of course, data here do not rule out the possibilities that lower doses of cocaine may be

needed to reveal differences between groups, or that junk-food exposure may have masked potential basal differences between susceptible and resistant populations (see also below).

When cocaine was given repeatedly, the development of locomotor sensitization was similar in Junk-Food Gainers and Junk-Food Non-Gainers (Fig. 2a). This is consistent with a recent report showing that exposure to either unlimited or restricted high-fat food (35% fat) did not alter the induction of cocaine-induced locomotor sensitization (Baladi et al. 2015). However, these data are in contrast to cross-sensitization between high-fat diets and amphetamines where methamphetamine-induced sensitization is enhanced during high-fat diet exposure (McGuire et al. 2011; Robinson et al. 2015). Although cocaine and amphetamines both increase extracellular dopamine levels via actions at the dopamine transporter (DAT), they do so through different mechanisms. Specifically, cocaine blocks the DAT, preventing the removal of dopamine that is released through normal membrane depolarization; whereas amphetamine causes reverse transport of dopamine through the DAT, regardless of normal dopamine release. High-fat diets, insulin and leptin (which are increased by obesity) can all alter DAT function (Baladi et al. 2015; Daws et al. 2011; Figlewicz et al. 1994; Perry et al. 2010). Thus, enhanced induction of locomotor sensitization in response to amphetamine, but not cocaine, after diet-induced obesity may be due to the effects of diet manipulation on DAT function and the enhancement of amphetamine's ability to reverse transport dopamine through the DAT, but do not alter cocaine's effects on DAT blockade. This possibility deserves further investigation.

After repeated cocaine treatment, junk-food fed rats were given a 14 day drug-free period and were then re-exposed to cocaine (i.e., cocaine challenge). The expression of locomotor sensitization after this cocaine deprivation was more robust in Junk-Food Gainers. Specifically, the locomotor response to 7.5 mg/kg cocaine was increased in Junk-Food Gainers vs. Junk-Food Non-Gainers (Fig. 2c), and produced locomotor activity in Junk-Food Gainers that was comparable to that induced by the first injection of 15 mg/kg (Fig. 2d). To our knowledge, no other studies have examined interactions between diet manipulation and subsequent expression of sensitization after drug deprivation.

It is possible that differences in pharmacokinetics could contribute to differences observed during cocaine challenge. However, we do not think this is likely because Junk-Food Gainers were already substantially heavier than Junk-Food Non-Gainers at the time of the very first cocaine exposure (Fig 2A). The expression of sensitization is mediated in large part by alterations in striatal function and organization, particularly within the NAc (Vanderschuren and Pierce 2010; Vezina 2004). Furthermore, the neuroadaptations accompanying locomotor sensitization contribute to enhanced incentive-motivational properties of reward-paired cues (Flagel et al. 2008; Robinson and Berridge 1993; Vanderschuren and Pierce 2010; Vezina and Leyton 2009; Wolf and Ferrario 2010). Thus, expression of greater sensitization in Junk-Food Gainers found here is consistent with the idea that interactions between predisposition and consumption of fatty, sugary, foods may produce alterations in mesolimbic function that enhance the incentive motivational properties of food and food-paired stimuli in susceptible individuals (Jansen et al. 2008; Stice et al. 2012). This is also consistent with our recent observation that eating junk-food enhances cue-triggered approach (CRF unpublished observations). Interestingly, fMRI

studies in people find that NAc activation in response to stimuli associated with food (food cues) is enhanced in obese individuals, whereas dorsal striatal activations in response to the consumption of food itself is reduced after obesity develops (Burger and Stice 2014; Cosgrove et al. 2015; Dagher 2009; Demos et al. 2012; Stice et al. 2008; Stice et al. 2010; Stoeckel et al. 2008; Tetley et al. 2009; Vainik et al. 2013). Thus, the enhanced expression of cocaine-induced sensitization in susceptible rats found here, does not rule out the possibility of opposing effects of fatty, sugary, foods on different striatal sub regions that underlie the pursuit of food vs. responses to its consumption (e.g., (Geiger et al. 2009; Rada et al. 2010); see also (Castro et al. 2015)).

Cocaine-induced locomotion in obesity-prone and obesity-resistant rats

As mentioned above, the initial cocaine-response was similar in outbred Junk-Food Gainers vs. Junk-Food Non-Gainers. However, consumption of the junk-food diet itself may produce neuroadaptations that affect cocaine-induced locomotion. Therefore, we next used selectively bred obesity-prone and obesity-resistant rats to examine neurobehavioral differences without any diet manipulation. Here, sensitivity to cocaine-induced locomotion was enhanced in obesity-prone vs. obesity-resistant rats in response to the first cocaine exposure across multiple doses (Fig. 3). This is consistent with our earlier study using a single dose of cocaine (Vollbrecht et al. In Press). Thus, obesity-prone rats are sensitized compared to their obesity-resistant counterparts. It is worthwhile to note that the weight of obesity-prone and obesity-resistant rats at the time of testing is well inside the range of normal variance for adult outbred Sprague Dawley rats: P70-76 = 274–380g (McCutcheon and Marinelli 2009). Thus, although obesity-prone rats are heavier, they were not “obese” at the time of testing. In addition, we have found that adiposity and fasted insulin levels do not differ substantially between obesity-prone and obesity-resistant rats during early adulthood (P70-80), although we have observed trends for increased insulin levels (Vollbrecht et al. 2015).

The acute locomotor activating effects of cocaine rely in large part on the activation of MSNs in the NAc. Interestingly, although lower basal dopamine levels have been found in the NAc of obesity-prone rats (Geiger et al. 2008; Rada et al. 2010), the magnitude of amphetamine induced increases in NAc dopamine were greater in obese rats (Geiger et al. 2009). These data suggest that although basal levels may be lower in some striatal regions of obesity-resistant rats, the responsivity of these circuits to stimulants may nonetheless be enhanced. In addition to dopamine, this activation is also influenced by glutamatergic transmission and intrinsic properties of MSNs (Kourrich et al. 2015; Vanderschuren and Pierce 2010; Wolf 2010; Wolf and Ferrario 2010). However, No differences in post-synaptic glutamatergic transmission were found between groups.

Enhanced MSN excitability in adult, but not adolescent, obesity-prone rats

Here, we found that excitability of MSNs was enhanced in obesity-prone rats. Excitability is determined predominantly by inwardly-rectifying K⁺ currents at negative membrane potentials (<−90 mV) and by A-type K⁺ currents at positive membrane potentials (>−40 mV; (Nisenbaum and Wilson 1995; Perez et al. 2006). In addition, A-type K⁺ currents also influence repetitive action potential firing (Perez et al. 2006). Here, we found a shift in the

I/V relationship at both positive and negative current injections (Fig. 4b), suggesting that there is a reduction in both inwardly-rectifying K^+ currents and A-type K^+ currents in MSNs from adult obesity-prone rats. In addition, evoked action potential firing was greater in MSNs from obesity-prone rats (Fig. 4d), also consistent with a reduction in A-type K^+ currents. Given these changes in excitability and action potential frequency, a reduced spike latency would be expected. However, due to the large difference in current intensities required to elicit an action potential between obesity-prone and obesity-resistant cells, comparable measures of spike latency could not be calculated. However, the lower rheobase in obesity-prone rats is also indicative of enhanced cellular excitability (Fig. 4f) and is consistent with smaller A-type K^+ currents. For example, blocking the A-type K^+ current reduces the amount of outward rectification in these neurons allowing the cell to more strongly depolarize with lower current injections, thus reducing the rheobase (Nisenbaum and Wilson 1995). Although voltage-gated Na^+ or Ca^{2+} currents could also contribute to reductions in the rheobase, this seems unlikely given that the threshold for action potential firing and action potential rise time (mediated by Na^+ currents) and the AHP (influenced by Ca^{2+} currents) are similar between groups. We speculate that enhanced excitability may reduce the threshold for, and increase transitions into “up-state” membrane potentials (i.e., where neurons remain close to the firing threshold), ultimately increasing neural firing and enhancing network activity (Wilson and Kawaguchi 1996) in obesity-prone rats. However, future studies are needed to directly measure differences in K^+ channel properties and alterations in the transition between “up-” and “down-states” in this model.

The differences in excitability described above were found in MSNs from adult, but not MSNs from adolescent obesity-prone vs obesity-resistant rats. Specifically, measures of excitability, spike number, rheobase, and input resistance found in adolescent MSNs of both selectively bred lines were similar to those previously reported in MSNs from outbred adolescent Sprague Dawley rats (Kasanetz and Manzoni 2009). This suggests that the initial maturation of MSN excitability is normal in these selectively bred rats. In addition, our data from MSNs in adult obesity-resistant rats were similar to those previously reported in adult MSNs from outbred rats. However, somewhat surprisingly, the profile of intrinsic properties of MSNs in obesity-prone rats more closely resembles that of MSNs from juvenile (P14-P21) rats (Kasanetz and Manzoni 2009), with greater spike number and input resistance, enhanced excitability, and reduced rheobase compared to adults. Thus, while intrinsic excitability of MSNs in obesity-prone rats is normal during adolescence (P30-40), it appears to revert to a more juvenile-like state as they age. However, it is worth noting that the magnitude of inward rectification in MSNs from adult obesity-prone rats is less strong than that previously observed in juveniles. Thus, different mechanisms are likely involved.

Although by definition obesity-prone rats are “sensitized” to cocaine compared to obesity-resistant rats (Fig. 3), the alterations in NAc core MSN function found in obesity-prone rats are not the same as those seen after sensitization induced by repeated cocaine exposure. Specifically, locomotor sensitization induced by *repeated* systemic cocaine treatment has been associated with *reduced* excitability in the NAc and *increased* post-synaptic AMPAR expression and function (see (Wolf 2010; Wolf and Ferrario 2010) for reviews, and (Kourrich et al. 2015) for discussion of opposing effects in NAc core vs. shell. In contrast,

the enhanced sensitivity to cocaine in obesity-prone rats found here was associated with *increased* excitability in the absence of differences in post-synaptic AMPAR transmission (Fig. 4). While caution must be used when comparing basal differences in cocaine sensitivity to effects induced by repeated cocaine exposure, our data suggest that the neural mechanisms underlying enhanced sensitivity to acute cocaine-induced locomotion in obesity-prone rats may be distinct from those found in outbred rats sensitized by repeated cocaine exposure.

In summary, our data show that while the induction of locomotor sensitization was similar, the subsequent expression of sensitization was enhanced in Junk-Food Gainer vs. Junk-Food Non-Gainer groups. In addition, prior to diet manipulation the function of mesolimbic circuits was enhanced in obesity-prone individuals. This was accompanied by an increase in excitability of MSNs in the core of the NAc. Although direct tests are needed, it is possible that enhanced NAc excitability in susceptible rats may promote subsequent plasticity induced by repeated activation of mesocorticolimbic circuits in response to the consumption of fatty, sugary foods, ultimately resulting in sensitization of striatal systems and enhancing cue-triggered motivation for food (Robinson et al. 2015). This possibility, as well as the relationship between pre-existing differences in NAc function and enhanced motivation triggered by food cues should be investigated in future.

Acknowledgments

Cocaine was provided by the NIDA drug supply program. This work was supported by NIDDK R01DK106188 and by a Brain and Behavior Research Foundation NARSAD Young Investigator Award to CRF. MFO was supported by NIDA T32DA007268. Technical assistance was provided by Cameron W. Nobile, and helpful comments during the preparation of this manuscript were provided by Drs. Peggy Gnegy and Paul Meyer.

References

- Baladi MG, Horton RE, Owens WA, Daws LC, France CP. Eating high fat chow decreases dopamine clearance in adolescent and adult male rats but selectively enhances the locomotor stimulating effects of cocaine in adolescents. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*. 2015;18.
- Baladi MG, Koek W, Aumann M, Velasco F, France CP. Eating high fat chow enhances the locomotor-stimulating effects of cocaine in adolescent and adult female rats. *Psychopharmacology*. 2012; 222:447–57. [PubMed: 22418731]
- Balleine BW. Neural bases of food-seeking: affect, arousal and reward in corticostriatolimbic circuits. *Physiology & behavior*. 2005; 86:717–30. [PubMed: 16257019]
- Berthoud HR. The neurobiology of food intake in an obesogenic environment. *The Proceedings of the Nutrition Society*. 2012; 71:478–87. [PubMed: 22800810]
- Boudreau AC, Wolf ME. Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2005; 25:9144–51. [PubMed: 16207873]
- Burger KS, Stice E. Greater striatopallidal adaptive coding during cue-reward learning and food reward habituation predict future weight gain. *NeuroImage*. 2014; 99:122–8. [PubMed: 24893320]
- Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience and biobehavioral reviews*. 2002; 26:321–52. [PubMed: 12034134]
- Castro DC, Cole SL, Berridge KC. Lateral hypothalamus, nucleus accumbens, and ventral pallidum roles in eating and hunger: interactions between homeostatic and reward circuitry. *Frontiers in systems neuroscience*. 2015; 9:90. [PubMed: 26124708]

- Cosgrove KP, Veldhuizen MG, Sandiego CM, Morris ED, Small DM. Opposing relationships of BMI with BOLD and dopamine D2/3 receptor binding potential in the dorsal striatum. *Synapse* (New York, NY). 2015; 69:195–202.
- Crombag HS, Sutton JM, Takamiya K, Holland PC, Gallagher M, Huguier RL. A role for alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid GluR1 phosphorylation in the modulatory effects of appetitive reward cues on goal-directed behavior. *The European journal of neuroscience*. 2008a; 27:3284–91. [PubMed: 18598267]
- Crombag HS, Sutton JM, Takamiya K, Lee HK, Holland PC, Gallagher M, Huguier RL. A necessary role for GluR1 serine 831 phosphorylation in appetitive incentive learning. *Behavioural brain research*. 2008b; 191:178–83. [PubMed: 18455244]
- Dagher A. The neurobiology of appetite: hunger as addiction. *International journal of obesity*. 2009; 33(Suppl 2):S30–3. [PubMed: 19528977]
- Daws LC, Avison MJ, Robertson SD, Niswender KD, Galli A, Saunders C. Insulin signaling and addiction. *Neuropharmacology*. 2011; 61:1123–8. [PubMed: 21420985]
- Demos KE, Heatherton TF, Kelley WM. Individual differences in nucleus accumbens activity to food and sexual images predict weight gain and sexual behavior. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2012; 32:5549–52. [PubMed: 22514316]
- Everitt BJ, Wolf ME. Psychomotor stimulant addiction: a neural systems perspective. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2002; 22:3312–20. [PubMed: 11978805]
- Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE. Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. *Biological psychiatry*. 2005; 58:751–9. [PubMed: 16098484]
- Ferrario CR, Goussakov I, Stutzmann GE, Wolf ME. Withdrawal from cocaine self-administration alters NMDA receptor-mediated Ca²⁺ entry in nucleus accumbens dendritic spines. *PloS one*. 2012; 7:e40898. [PubMed: 22870207]
- Ferrario CR, Li X, Wang X, Reimers JM, Uejima JL, Wolf ME. The role of glutamate receptor redistribution in locomotor sensitization to cocaine. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2010; 35:818–33. [PubMed: 19924109]
- Ferrario CR, Loweth JA, Milovanovic M, Ford KA, Galinanes GL, Heng LJ, Tseng KY, Wolf ME. Alterations in AMPA receptor subunits and TARPs in the rat nucleus accumbens related to the formation of Ca(2+)-permeable AMPA receptors during the incubation of cocaine craving. *Neuropharmacology*. 2011; 61:1141–51. [PubMed: 21276808]
- Figlewicz DP, Szot P, Chavez M, Woods SC, Veith RC. Intraventricular insulin increases dopamine transporter mRNA in rat VTA/substantia nigra. *Brain research*. 1994; 644:331–4. [PubMed: 8050044]
- Flagel SB, Watson SJ, Akil H, Robinson TE. Individual differences in the attribution of incentive salience to a reward-related cue: influence on cocaine sensitization. *Behavioural brain research*. 2008; 186:48–56. [PubMed: 17719099]
- Geiger BM, Behr GG, Frank LE, Caldera-Siu AD, Beinfeld MC, Kokkotou EG, Pothos EN. Evidence for defective mesolimbic dopamine exocytosis in obesity-prone rats. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2008; 22:2740–6. [PubMed: 18477764]
- Geiger BM, Haburcak M, Avena NM, Moyer MC, Hoebel BG, Pothos EN. Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. *Neuroscience*. 2009; 159:1193–9. [PubMed: 19409204]
- Hryhorczuk C, Florea M, Rodaros D, Poirier I, Daneault C, Des Rosiers C, Arvanitogiannis A, Alquier T, Fulton S. Dampened Mesolimbic Dopamine Function and Signaling by Saturated but not Monounsaturated Dietary Lipids. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2015
- Hu XT. Cocaine withdrawal and neuro-adaptations in ion channel function. *Mol Neurobiol*. 2007; 35:95–112. [PubMed: 17519508]

- Jansen A, Vanreyten A, van Balveren T, Roefs A, Nederkoorn C, Havermans R. Negative affect and cue-induced overeating in non-eating disordered obesity. *Appetite*. 2008; 51:556–62. [PubMed: 18495294]
- Jensen CD, Kirwan CB. Functional brain response to food images in successful adolescent weight losers compared with normal-weight and overweight controls. *Obesity (Silver Spring, Md)*. 2015; 23:630–6.
- Karasinska JM, George SR, Cheng R, O'Dowd BF. Deletion of dopamine D1 and D3 receptors differentially affects spontaneous behaviour and cocaine-induced locomotor activity, reward and CREB phosphorylation. *The European journal of neuroscience*. 2005; 22:1741–50. [PubMed: 16197514]
- Kasanetz F, Manzoni OJ. Maturation of excitatory synaptic transmission of the rat nucleus accumbens from juvenile to adult. *Journal of neurophysiology*. 2009; 101:2516–27. [PubMed: 19244354]
- Kelley AE. Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neuroscience and biobehavioral reviews*. 2004; 27:765–76. [PubMed: 15019426]
- Kourrich S, Calu DJ, Bonci A. Intrinsic plasticity: an emerging player in addiction. *Nature reviews Neuroscience*. 2015; 16:173–84. [PubMed: 25697160]
- Kourrich S, Rothwell PE, Klug JR, Thomas MJ. Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2007; 27:7921–8. [PubMed: 17652583]
- Kourrich S, Thomas MJ. Similar neurons, opposite adaptations: psychostimulant experience differentially alters firing properties in accumbens core versus shell. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2009; 29:12275–83. [PubMed: 19793986]
- Levin BE, Dunn-Meynell AA, Balkan B, Keesey RE. Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. *The American journal of physiology*. 1997; 273:R725–30. [PubMed: 9277561]
- MacQueen, JB. *Proceedings of 5th Berkeley Symposium on Mathematical Statistics and Probability*. University of California Press; 1967. Some Methods for classification and Analysis of Multivariate Observations; p. 281-297.
- Marinelli M, Rudick CN, Hu XT, White FJ. Excitability of dopamine neurons: modulation and physiological consequences. *CNS & neurological disorders drug targets*. 2006; 5:79–97. [PubMed: 16613555]
- McCutcheon JE, Marinelli M. Age matters. *The European journal of neuroscience*. 2009; 29:997–1014. [PubMed: 19291226]
- McGuire BA, Baladi MG, France CP. Eating high-fat chow enhances sensitization to the effects of methamphetamine on locomotion in rats. *European journal of pharmacology*. 2011; 658:156–9. [PubMed: 21371470]
- Murdaugh DL, Cox JE, Cook EW 3rd, Weller RE. fMRI reactivity to high-calorie food pictures predicts short- and long-term outcome in a weight-loss program. *NeuroImage*. 2012; 59:2709–21. [PubMed: 22332246]
- Nisenbaum ES, Wilson CJ. Potassium currents responsible for inward and outward rectification in rat neostriatal spiny projection neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1995; 15:4449–63. [PubMed: 7790919]
- Perez MF, White FJ, Hu XT. Dopamine D(2) receptor modulation of K(+) channel activity regulates excitability of nucleus accumbens neurons at different membrane potentials. *Journal of neurophysiology*. 2006; 96:2217–28. [PubMed: 16885524]
- Perry ML, Leininger GM, Chen R, Luderman KD, Yang H, Gnegy ME, Myers MG Jr, Kennedy RT. Leptin promotes dopamine transporter and tyrosine hydroxylase activity in the nucleus accumbens of Sprague-Dawley rats. *Journal of neurochemistry*. 2010; 114:666–74. [PubMed: 20412389]
- Pulvirenti L, Swerdlow NR, Koob GF. Microinjection of a glutamate antagonist into the nucleus accumbens reduces psychostimulant locomotion in rats. *Neuroscience letters*. 1989; 103:213–8. [PubMed: 2771183]
- Rada P, Bocarsly ME, Barson JR, Hoebel BG, Leibowitz SF. Reduced accumbens dopamine in Sprague-Dawley rats prone to overeating a fat-rich diet. *Physiology & behavior*. 2010; 101:394–400. [PubMed: 20643155]

- Robinson MJ, Burghardt PR, Patterson CM, Nobile CW, Akil H, Watson SJ, Berridge KC, Ferrario CR. Individual Differences in Cue-Induced Motivation and Striatal Systems in Rats Susceptible to Diet-Induced Obesity. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2015
- Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain research Brain research reviews*. 1993; 18:247–91. [PubMed: 8401595]
- Robinson TE, Kolb B. Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology*. 2004; 47(Suppl 1):33–46. [PubMed: 15464124]
- Scheyer AF, Wolf ME, Tseng KY. A protein synthesis-dependent mechanism sustains calcium-permeable AMPA receptor transmission in nucleus accumbens synapses during withdrawal from cocaine self-administration. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2014; 34:3095–100. [PubMed: 24553949]
- Sekerli M, Del Negro CA, Lee RH, Butera RJ. Estimating action potential thresholds from neuronal time-series: new metrics and evaluation of methodologies. *IEEE Trans Biomed Eng*. 2004; 51:1665–72. [PubMed: 15376515]
- Setlow B, Holland PC, Gallagher M. Disconnection of the basolateral amygdala complex and nucleus accumbens impairs appetitive pavlovian second-order conditioned responses. *Behavioral neuroscience*. 2002; 116:267–75. [PubMed: 11996312]
- Spear LP. The adolescent brain and age-related behavioral manifestations. *Neuroscience and biobehavioral reviews*. 2000; 24:417–63. [PubMed: 10817843]
- Stice E, Dagher A. Genetic variation in dopaminergic reward in humans. *Forum of nutrition*. 2010; 63:176–85. [PubMed: 19955785]
- Stice E, Figlewicz DP, Gosnell BA, Levine AS, Pratt WE. The contribution of brain reward circuits to the obesity epidemic. *Neuroscience and biobehavioral reviews*. 2012
- Stice E, Spoor S, Bohon C, Veldhuizen MG, Small DM. Relation of reward from food intake and anticipated food intake to obesity: a functional magnetic resonance imaging study. *Journal of abnormal psychology*. 2008; 117:924–35. [PubMed: 19025237]
- Stice E, Yokum S, Bohon C, Marti N, Smolen A. Reward circuitry responsivity to food predicts future increases in body mass: moderating effects of DRD2 and DRD4. *NeuroImage*. 2010; 50:1618–25. [PubMed: 20116437]
- Stice E, Yokum S, Zald D, Dagher A. Dopamine-based reward circuitry responsivity, genetics, and overeating. *Current topics in behavioral neurosciences*. 2011; 6:81–93. [PubMed: 21243471]
- Stoeckel LE, Weller RE, Cook EW 3rd, Twieg DB, Knowlton RC, Cox JE. Widespread reward-system activation in obese women in response to pictures of high-calorie foods. *NeuroImage*. 2008; 41:636–47. [PubMed: 18413289]
- Tetley A, Brunstrom J, Griffiths P. Individual differences in food-cue reactivity. The role of BMI and everyday portion-size selections. *Appetite*. 2009; 52:614–20. [PubMed: 19501758]
- Tomasi D, Volkow ND. Striatocortical pathway dysfunction in addiction and obesity: differences and similarities. *Critical reviews in biochemistry and molecular biology*. 2013; 48:1–19. [PubMed: 23173916]
- Vainik U, Dagher A, Dube L, Fellows LK. Neurobehavioural correlates of body mass index and eating behaviours in adults: a systematic review. *Neuroscience and biobehavioral reviews*. 2013; 37:279–99. [PubMed: 23261403]
- Valenza M, Steardo L, Cottone P, Sabino V. Diet-induced obesity and diet-resistant rats: differences in the rewarding and anorectic effects of D-amphetamine. *Psychopharmacology*. 2015
- Vanderschuren LJ, Pierce RC. Sensitization processes in drug addiction. *Current topics in behavioral neurosciences*. 2010; 3:179–95. [PubMed: 21161753]
- Vezina P. Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. *Neuroscience and biobehavioral reviews*. 2004; 27:827–39. [PubMed: 15019432]
- Vezina P, Leyton M. Conditioned cues and the expression of stimulant sensitization in animals and humans. *Neuropharmacology*. 2009; 56(Suppl 1):160–8. [PubMed: 18657553]

- Vollbrecht PJ, Mabrouk OS, Nelson AD, Kennedy RT, Ferrario CR. Interactions between pre-existing differences and junk-food induced alterations in striatal dopamine systems of obesity-prone rats. *Obesity* (Silver Spring, Md). In Press.
- Vollbrecht PJ, Nobile CW, Chadderdon AM, Jutkiewicz EM, Ferrario CR. Pre-existing differences in motivation for food and sensitivity to cocaine-induced locomotion in obesity-prone rats. *Physiology & behavior*. 2015; 152:151–160. [PubMed: 26423787]
- Wilson CJ, Kawaguchi Y. The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1996; 16:2397–410. [PubMed: 8601819]
- Wolf ME. The Bermuda Triangle of cocaine-induced neuroadaptations. *Trends in neurosciences*. 2010; 33:391–8. [PubMed: 20655604]
- Wolf ME, Ferrario CR. AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine. *Neuroscience and biobehavioral reviews*. 2010; 35:185–211. [PubMed: 20109488]
- Wyvell CL, Berridge KC. Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward “wanting” without enhanced “liking” or response reinforcement. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2000; 20:8122–30. [PubMed: 11050134]
- Wyvell CL, Berridge KC. Incentive sensitization by previous amphetamine exposure: increased cue-triggered “wanting” for sucrose reward. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2001; 21:7831–40. [PubMed: 11567074]
- Yokum S, Ng J, Stice E. Attentional bias to food images associated with elevated weight and future weight gain: an fMRI study. *Obesity* (Silver Spring, Md). 2011; 19:1775–83.

Fig. 1.

Food intake and weight gain in outbred rats. **a** Average weekly food intake ($\pm$ SEM) in Junk-Food Gainers (JF-G) vs. Junk-Food Non-Gainers (JF-N). **b** Average ($\pm$ SEM) weekly weight gain in Junk-Food Gainers vs. Junk-Food Non-Gainers. **c** Weight gain of individual rats ($\pm$ SEM) after 9 weeks of junk-food. When given access to junk-food, some outbred rats (JF-G, $n=7$) eat significantly more food (a) and gain more weight (b) than others given the same free access to junk-food (JF-N, $n=8$). * = $p < 0.05$

Fig. 2.

The induction of locomotor sensitization was similar, but the expression of sensitization after cocaine deprivation was enhanced in Junk-Food Gainers. **a** Average locomotor response ($\pm$ SEM) per 5 min after each cocaine injection. **b** Total locomotion ($\pm$ SEM) during 40 min after the first and last cocaine injection. **c** Average locomotor response ($\pm$ SEM) per 5 min to 7.5 mg/kg cocaine after 14 days of cocaine deprivation (i.e., cocaine “challenge”). **d** Total locomotion ($\pm$ SEM) during 40 min after cocaine challenge (7.5 mg/kg) and in response to the first cocaine injection (15 mg/kg). While still on the junk-food diet, Junk-Food Gainers (JF-G, $n=7$) and Junk-Food Non-Gainers (JF-N, $n=7$; one Non-Gainer was removed from the study because it developed a urinary tract infection and the treatment required could compromise cocaine metabolism) the induction of locomotor sensitization was similar between groups (a, b). However, Junk-Food Gainers show more robust sensitization than Junk-Food Non-Gainers after 14 days of cocaine deprivation and cocaine challenge (c). In addition, the locomotor response to 7.5 mg/kg cocaine during the challenge was as large as the response to the first injection of 15 mg/kg cocaine in Junk-Food Gainers only. Together, these data show that repeated cocaine exposure produces more robust locomotor sensitization in rats that are susceptible to diet-induced obesity compared to those that are obesity resistant. * = $p < 0.05$

Fig. 3.

Adult obesity-prone rats were more sensitive to the locomotor-activating effects of cocaine than obesity-resistant rats. **a** Average locomotor response ($\pm$ SEM) to increasing concentrations of cocaine in adult obesity-prone (OP) and obesity-resistant rats (OR; $n=8$ /group). **b** Total locomotion ($\pm$ SEM) during 40 min after each dose of cocaine in adult rats (P70-P80). **c** Total locomotion ($\pm$ SEM) during 40 min after each dose of cocaine in adolescent rats (P30-P40; $n=10$ /group). Adult obesity-prone rats showed a stronger locomotor response to cocaine than obesity-resistant rats (i.e., OPs are sensitized compared to ORs) (a,b), consistent with enhance responsivity of mesolimbic circuits. In contrast, cocaine-induced locomotion was similar between groups tested during adolescence. * = $p < 0.05$

Fig. 4.

Intrinsic excitability of MSNs in the NAc core is enhanced in obesity-prone vs. obesity resistant adult, but not adolescent, rats. All data shown are average $\pm$ SEM. **a** Example traces from current-clamp recordings of MSNs from obesity-prone (OP; $n=11$ cells from 7 rats) and obesity-resistant rats (OR; $n=11$ cells from 8 rats). **b** The change in membrane potential at each current injection in MSNs from adult rats (-200 pA to 100 pA). Rectification is reduced in MSNs from adult obesity-prone rats, consistent with increased excitability. **c** The change in membrane potential at each current injection in MSNs from adolescent rats (-200 pA to 100 pA). Rectification was similar in MSNs from adolescent obesity-prone and obesity-resistant rats, suggesting that differences in adulthood emerged after initial development. **d** The number of action potentials elicited by each current injection (0 to 175 pA). The same current injection elicited more action potentials in MSNs from obesity-prone vs. obesity-resistant rats, consistent with enhanced excitability. **e** The input resistance was determined by the change in voltage from -50 to $+50$ pA. Input resistance is greater in MSNs from obesity-prone vs. obesity-resistant rats. **f** The minimum amount of current injection needed to elicit an action potential (rheobase). The rheobase was lower in MSNs from obesity-prone rats. **g** Table of basic membrane properties from adult MSNs. Measurements were taken from the first action potential elicited by the minimum current injection. Action potential threshold was determined by the maximum second derivative method. Action potential amplitude is the difference between the action potential threshold and peak. The 1st interspike interval (ISI) is the difference in time between the first two action potentials. The amplitude of the AHP is the difference between the firing threshold

and the lowest point of the hyperpolarizing potential of the AHP; * = $p < 0.05$, ** = $p < 0.01$.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript