The Effects of Mu and Kappa Opioid Antagonists on Reinstatement of Sugar Seeking

by

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ABSTRACT

THE EFFECTS OF MU AND KAPPA OPIOID ANTAGONISTS ON REINSTATEMENT OF SUGAR SEEKING

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Excessive consumption of palatable food is related to death caused by cardiovascular disease, fatty liver disease, and diabetes in humans and animals. Resumption of consuming palatable food following abstinence can be thought of as relapse. Reinstatement of sugar seeking in rats serves as animal model for studying relapse to excessive consumption of palatable foods in humans. Given the known role of mu and kappa receptors in hedonic eating, satiety, stress and food seeking, and food-restriction induced drug seeking, it was predicted that Naltrexone (mu-opioid antagonist; doses: 0.3 mg/kg and 3 mg/kg) and JDTic (kappa-opioid antagonist; doses: 3 mg/kg and 10 mg/kg) drugs should prevent reinstatement of sugar seeking caused by food-restriction stress and by priming. Rats were trained to self-administer sugar in an eight arm radial apparatus where tests of seeking and consumption were also tested. It was found that NTX, but not JDTic, blocked reinstatement caused by food-restriction. While these two antagonists had opposite effects on sucrose self-administration during reacquisition, both attenuated the effects of food restriction on reinstatement induced by the sugar prime. Furthermore JDTic appeared to dose dependently enhance nose poking in food restricted rats during the test of prime-induced reinstatement. These data confirm the critical role of opioid systems in sugar consumption and sugar seeking, further demonstrate the clinical relevance of using mu-opioid antagonists to treat problematic eating and warrant further investigation of a role for kappa opioid receptors in feeding related behaviour.

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Table of Contents

The Effects of Mu and Kappa Opioid Antagonists on Reinstatement of Sugar Seeking

Excessive consumption of palatable food

Context

Excessive consumption of palatable food is related to death caused by cardiovascular disease, fatty liver disease, and diabetes in humans and animals (Assy et al., 2008; Castro, et al., 2011; Kanoski & Davidson, 2011; Nseir, Nassar, & Assy, 2010; Ouyang et al., 2008; Pranprawit, et al., 2013; and Stevens et al., 2014;) Excessive eating can occur in obese or non-obese individuals, can be associated with disorders such as bulimia nervosa (BN) or binge-eating disorder (BED) (Hilbert et al., 2014). The onset of binge-eating in BN tended to be predicted by previous fasting (no food consumption for at least 24 hours) as well as various early life stressors, after a 5 year follow up in individuals with BN (Stice, et al., 2008). BED is less well studied, but tends to be also related to early stressors in life, with binging being much more sporadic in BED individuals than BN individuals (Heaner & Walsh, 2013).

Eating for pleasure

Humans and animals alike have been shown to hedonically consume palatable foods such as sugars (i.e. eating for pleasure rather than sustenance) (Peciña & Berridge, 2005). Recent evidence has suggested that while taste is a crucial aspect of hedonic eating, there are post-ingestional consequences associated with hedonic eating independent of taste and satiety (Clouard, et al., 2014). The authors found that pigs given oral as well as intraduodenal infusions of sucrose solution and activity in brain areas associated with hedonic eating and food reward was measured. It was found that intraduodenal infusions alone increased activity in the putamen, ventral anterior cingulate cortex and hippocampus (areas involved in incentive

learning and memory), oral infusions alone increased activity in prefrontal and insular cortices, while both infusions given together resulted in activation only of the right insular cortex. These findings provide neurological evidence that hedonic and motivational value of food is modulated by both taste, and post-ingestional consequences as areas in the brain implicated in incentive learning and hedonic eating are activated by infusions of sucrose solution.

Relapse to excessive consumption of palatable foods

One critical aspect of excessive eating is relapse (Boggiano et al., 2009; Craighead & Agras, 1991; Davis & Carter, 2009; De Jong, et al., 2013; Grilo, Shiffman, & Wing, 1988; Grilo, Shiffman, & Wing, 1989; Sharma, Fernandes, & Fulton, 2013; Sinha & Jastreboff, 2013; Striegel-Moore, et al., 2000; and Willard, 1991). That is, humans attempting cessation of palatable foods may resume overeating when presented with certain environmental stimuli. Grilo et al., 1989 investigated many scenarios that preceded dietary relapse. They found approximately 50% of relapse scenarios occurred following consumption of a meal, or following the presence of a negative event. Furthermore approximately 73% of dietary relapse cases followed the presentation of some food related stimuli. There are many possible reasons or contexts predictive of relapse, but have been broken into three main causes.

Three causes of relapse to excessive consumption of palatable food

The first cause of relapse is exposure to palatable food (Grilo, Shiffman, & Wing, 1989). In this study, it was found that while approximately two-thirds of relapse to problematic eating occurred following a binge eating session, (1000-3400 calories in a single session), approximately one third of the relapses were caused by simple re-exposure to palatable food (i.e apple pie).

The second cause of relapse is exposure to cues related to the food (Asmaro & Liotti, 2014; Grilo et al., 1989; Jansen, Broekmate, & Heymans, 1992). Brain activation was measured in humans viewing cues related to calorically dense food and low calorie foods (Killgore et al., 2003). They found that the medial orbital prefrontal cortex (mPFC), the dorsolateral prefrontal cortex (DLPFC), and the thalamus show greater activation following the presentation of palatable food related cues compared to regular food related cues. Furthermore, activation of the mPFC is greater in fasting participants compared to satiated participants. This provides evidence that palatable food related cues differ from regular food related cues and that individuals attempting to restrict palatable food intake may be more susceptible to these cues than individuals on a standard diet.

The third cause of relapse to overconsumption of palatable food is stress (Byrne, Cooper, & Fairburn, 2003; Fairburn, et al., 2000; Grilo, Shiffman, & Wing, 1988; Grilo, Shiffman, & Wing, 1989; Oliver et al., 2000; Polivy & Herman, 1999; Sinha & Jastreboff, 2013; Striegel-Moore, et al., 2000; Willard, 1991). Participants placed in stress evoking conditions such as forced public speaking, tend to consume more palatable food when given choice between normal diets and palatable food (Oliver et al., 2000). Grilo et al., 1988 found that emotional distress almost always resulted in overeatting, but the ability to cope with stress, both cognitively and behaviourally, was associated with positive outcomes when attempting to reduce overconsumption of palatable food. Additionally low socioeconomic status is correlated with relapse in obese women (Byrne, Cooper, & Fairburn, 2003). Ninety of the women who regained weight in this study admitted to using food as a method for coping with stress.

Stress

Stress is modulated by the hypothalamic-pituitary-adrenal axis (HPA) which is made up of the Hypothalamus, Anterior Pituitary gland, and the Adrenal Cortex. Corticotropic Releasing Hormone (CRH) signals the anterior pituitary gland to secrete adrenocoticotropic hormone (ACTH). This signals the Adrenal Cortex to secrete cortisol, which acts as a negative feedback loop to decrease production of CRH and ACTH. High levels of stress have been shown to augment eating patterns (i.e. binging or restraining intake). Additionally chronic unpredicatable stress has been shown to disregulate the HPA axis, resulting in changes in genes responsible for glucocorticoid production. This increases glucose and insulin levels, as well as increasing preference and intake of palatable foods (Sinha & Jastreboff, 2013).

Food restriction as a stressor

Food restriction (i.e maintaining animals at 85% of their ad-lib body) and deprivation (i.e withholding all food for a period of time) serve as a stressor in laboratory rats (Carr, 1996). Food restriction increases self-administration of cocaine compared to free-fed rats and increases basal plasma cortisol levels. Additionally, plasma and serum corticosterone levels increase in food deprived rats compared to control rats (Beck & Luine, 1999; Dallman et al., 1999; Kiss et al., 1994). Blockade of corticosterone, with the corticosterone synthesis inhibiter metyrapone, attenuates the effects food restriction-induced sensitization to cocaine, suggesting the mechanism through which food restriction modulates drug taking is in part, stress related (Marinelli et al., 1996). Furthermore, like other stressors, food restriction increases immobility in the forced swim test as well as decreasing the time spent in open arms of an elevated plus maze (food restriction is also anxiogenic) (Jahng et al., 2007). Since stress is related to relapse to

palatable food seeking, and food restriction is a stressor, it could be a useful stressor for studying an animal model of relapse.

Using the reinstatement model to measure relapse to palatable foods in animals

In laboratory animals, relapse to food is explored using the reinstatement model (Ghitza, et al., 2006)**.** In the reinstatement model, animals are trained to self-administer food. Responding is then measured under extinction conditions. It has been found that reinstatement of food seeking can be elicited by food related cues, food priming, or by stress (Calu, et al., 2014; Chen, et al., 2014; Ghitza, et al., 2006; Nair, Gray, & Ghitza, 2006; Nair, Golden, & Shaham, 2008; Nair, et al., 2009; and Pickens et al., 2012)**.** For instance, Calu et al., 2013 were able to elicit reinstatement of food seeking by acute pellet priming, a light cue, and with the anxiogenic drug, yohimbine. It appears that dietary history also influences the efficacy of inducing reinstatement in rats. Compared to rats fed regular daily rat chow, rats fed daily palatable food for 7 weeks had reduced cue and prime-induced reinstatement of regular chow pellet seeking. Additionally footshock-induced reinstatement of regular chow pellets was enhanced by a history of palatable food intake (Chen et al., 2014). Furthermore, the type of pellet used to reinstate food seeking also appears to play a role in the effectiveness of the reinstatement model. For instance prime and yohimbine induced reinstatement occurred to a greater degree for rats trained with sugar pellets compared to rats trained with regular rat chow pellets (Nair, Gray, & Ghitza, 2006). These findings suggest that reinstatement of palatable food seeking occurs more readily than reinstatement of less calorically dense foods.

Food Restriction induced reinstatement

Food restriction is an effective stressor in eliciting stress-induced reinstatement of drug cocaine seeking in rats previously trained to respond for cocaine (Carroll, 1985; Shalev, et al., 2003). Food-restriction stress is commonly employed to study neurobiology of stress-induced reinstatement to heroin, cocaine and alcohol seeking (Bongiovanni & See, 2008; Carroll, 1985; Guccione, et al., 2012; Highfield, et al., 2002; Maric, et al., 2012; Sedki, D'Cunha, & Shalev, 2013; Sedki et al., 2014; Shalev, et al., 2000; Shalev, et al., 2003; Shalev, et al., 2006; Shalev, 2012; and Tobin, et al., 2009). Yohimbine has been previously used as a stressor to induce reinstatement of sugar seeking in rats (Nair et al., 2009). Yohimbine is an alpha-2 adrenoceptor antagonist with moderate and weak affinity for various 5HT receptors as well as D2 and D3 receptors (Millan et al., 2000). Due to the complex affinities of systemic injections of yohimbine, it may not be an ideal stressor in inducing reinstatement of sugar seeking, as it is difficult to know how systemic injections of other drugs will be affected by the binding affinities of yohimbine. Since food restriction can elicit reinstatement of seeking for drugs of abuse, it may also be a useful method for eliciting reinstatement of palatable food seeking.

Opioids and food

This study explores the effectiveness of the opioid antagonists, naltrexone (NTX) and JDTic in preventing reinstatement of self-administration of sugar by food-restriction stress and priming. Opioid receptors of particular interest since while they are largely distributed across the central nervous system, they tend to be localized in areas that modulate food intake as well as reward (Cota et al, 2006) Feeding is modulated by opioid receptors in the dorsomedial hypothalamus (DMH), lateral hypothalamus (LH), ventral tegmental area (VTA), nucleus of the solitary tract (NTS), and dorsal hippocampus, (Will et al., 2003). For example, injections of the

mu-opioid agonist DAMGO into these five areas increase ingestion of highly palatable food suggesting these areas are important for feeding behaviour. Additionally, food consumption is stimulated by injections of morphine or other mu-agonists directly into the nucleus accumbens (NAc), suggesting this opioid receptor containing area is also important for the modulation of feeding behaviour through the dopaminergic reward pathway (Bakshi et al, 1994). Furthermore, i.c.v injections of the mu agonist DAMGO, and delta agonist DPDPE increased extracellular dopamine in the NAc while a kappa agonist dynorphin analogue reduced extracellular dopamine in the NAc (Spanagel et al., 1990). These effects were blocked by mu, delta and kappa opioid antagonism. These results further demonstrate a role of opioids and the dopaminergic reward pathway which has been proposed as a mechanism for the modulation of food reward. Finally food deprivation has been shown to increase mRNA expression of mu-opioid receptors in the ventral medial hypothalamus and arcuate nucleus, areas which are also related to food and satiety (Barnes, et al., 2008).

Endogenous opioids

Endogenous opioid ligands are derived from three main precursors: pro-opiomelanocortin, proenkephalin, and prodynorphin (Waldhoer et al., 2004). There are three subtypes of opioid receptors: mu, kappa, and delta. Enkephalin and beta-endorphin ligands bind to mu-opioid receptors. Dynorphins ligands bind to kappa-opioid receptors. Enkephalin ligands also bind to delta-opioid receptors. Endogenous opioids are produced and act throughout the CNS as well as spinal cord and PNS.

Naltrexone and reinstatement

NTX, is a non-selective mu opioid receptor antagonist (Shader, 2003). The plasma half-life for NTX is approximately 4 hours, and has been used in the treatment of opioid and alcohol addiction in humans. No studies have investigated the effects of NTX on food restriction-induced reinstatement of sugar seeking in rats, but one study has investigated the effects of NTX on food deprivation-induced drug seeking in rats (Sedki et al., 2014). Sedki et al., 2014 found that kappa, but not mu-opioid antagonists reduced food deprivation induced heroin seeking in rats. NTX has, however been shown to attenuate reinstatement of alcohol seeking in rats (Burattini, et al., 2006). It is possible that NTX may affect ethanol reinstatement differently than other drugs of abuse due to the consummatory phase associated with alcohol making it similar to a food reinforcer.

Naltrexone and sugar

NTX has been found to reduce perceived craving and the intensity of sweetness of sugar in humans (Bertino, Beauchamp, & Engelman, 1991; Fantino, Hosotte, & Apfelbaum, 1986; Langleben, et al., 2012; Waldhoer, Bartlett, & Whistler, 2004) as well as reducing consumption of food (Melchior et al., 1989). NTX has also been found to reduce consumption of sugar in rhesus monkeys (Williams, et al., 1998)**.** Additionally NTX has been shown to reduce sugar consumption (Avena, et al., 2014; Gosnell et al., 2010; Katsuura & Taha, 2014; Malkusz et al., 2014; Michaels & Holtzman, 2007; Naleid, et al., 2007; Skelly, et al., 2010; Stromberg, et al., 2002; and Wong, Wojnicki, & Corwin, 2009), deprivation-induced food intake (Bodnar, et al., 1995; Islam, et al., 1994; Koch & Bodnar, 1994; Schaefer, Koch, & Bodnar, 1994), positive orofacial responses on the taste reactivity test (Parker et al., 1992) and sugar seeking (Burton, Noble, & Fletcher, 2011) in rats.

JDTic and reinstatement

JDTic is a highly selective long lasting kappa opioid receptor antagonist with a half-life of about 9 days (Munro et al., 2012). JDTic has been found to attenuate stress and cue-induced reinstatement of drug seeking but has not yet been investigated with reinstatement of sugar (Beardsley, et al., 2005; Beardsley, et al., 2010; Deehan, et al., 2012; Schank et al., 2012).

Kappa opioid receptors and sugar

Kappa opioid receptors have been linked to food consumption. For instance, administration of the kappa opioid agonist U50,488H increased consumption of high concentrations of sucrose in rats in addition to increasing the consumption of rat chow (Badiani et al., 2001). The kappa opioid agonists U50,488H and tifluadom also enhance appetite stimulation for palatable food in non-food deprived rats (Jackson & Cooper, 1985). A study using rhesus monkeys also found that kappa opioid agonists U50,488H and ethylketocyclazocine (EKC) increased consumption of food (Mello & Negus, 1998). While there is evidence of kappa opioid receptors modulating food intake and kappa opioid antagonists decreasing reinstatement of drug seeking, there is no literature that directly investigates kappa opioid antagonists and their role on food reinstatement.

Rationale

Given the known role of mu and kappa receptors in hedonic eating, satiety, stress and food seeking, it was predicted that both drugs should prevent reinstatement caused by food-restriction stress and by priming. It was hypothesized that kappa and mu-opioid receptors modulate sweet food seeking and consumption. The following predictions were made: 1) Since food restriction elicits reinstatement of drug seeking, the same effect is expected to be

observed for sugar (Epstein et al., 2006); 2) Since JDTic attenuates stress but not prime induced reinstatement of drug seeking, the same should be observed for food restriction and prime induced reinstatement of drug seeking (Beardsley et al., 2005); 3) Since naltrexone reduces food restriction induced intake of sugar, and cue-induced reinstatement of sugar seeking, it is expected that naltrexone will reduce food-restriction and prime-induced reinstatement of sugar seeking (Bodnar, et al., 1995; Burton, et al., 2011); 4) Since reinstatement of sugar induced by priming and yohimbine is reduced in free fed animals compared to food-restricted animals, the same should be observed in all tests of seeking and consumption in the current study (Nair et al., 2009).

While stress-induced reinstatement of sugar seeking has been induced by the pharmacological stressor yohimbine, and food-restriction has been used to reinstate seeking of drugs of abuse, food-restriction has not been used to induce reinstatement of sugar seeking. Food-restriction has been shown to increase palatability, hedonic value and incentive salience of palatable food in humans and animals (Epstein, et al., 2003; Stice, Burger, & Yokum, 2013; Wassum, et al., 2009) in addition to reinstating seeking of drugs of abuse such as heroin, cocaine and alcohol (Bongiovanni & See, 2008; Carroll, 1985; Guccione, et al., 2012; Highfield, et al., 2002; Maric, et al., 2012; Sedki, D'Cunha, & Shalev, 2013; Sedki et al., 2014; Shalev, et al., 2000; Shalev, et al., 2003; Shalev, et al., 2006; Shalev, 2012; and Tobin, et al., 2009). Food-restriction may be an ideal stressor to reinstate seeking of sugar due to the parallels between food-restriction in rats, and dieting in humans. Because of their long duration of action, NTX and JDTic may be ideal for treating relapse to palatable foods.

Methods

Subjects

One hundred twenty Male Sprague-Dawley rats (Charles River, St-Constant, QC) weighing 225-250 g at the beginning of the experiment were individually housed and maintained on 12 hour reverse light cycle (7:00am dark, 7:00pm light). Rats were given ad libitum access to food and water unless otherwise specified. All experiments were approved by the Animal Care Committee of the University of Guelph, and were carried out in accordance with the recommendations of the Canadian Council on Animal Care.

Apparatus

Behavioural testing was conducted in an automated radial apparatus (Med Associates, St. Albans, VT) with 8 arms radiating from a central octagonal hub, and made of opaque, white polycarbonate. The central hub was equipped with eight automatically operated guillotine doors. A pellet receptacle was located at the end of each arm, and a photo beam situated inside detected nose pokes. A pellet dispenser was positioned behind each receptacle. Additional photo beams were positioned in each arm to record arm entries and proximity to the pellet receptacles. A computer running MED-PC for Windows (Version 1.15) controlled experimental events with a 10-msec resolution. Pellets contained: dextrose, fructose, cellulose, and magnesium stearate. The nutritional profile of the pellets was: 0% fat, 0% protein, 89.5% carbohydrate (609 g/kg monosaccharaides, 285 g/kg disaccharides), <10% moisture, and 3.8% fibre. The percent of monosaccharaides used was 55% fructose and 45% glucose. The pellets were manufactured and purchased from Bio-Serv (45mg Dustless Precision Pellets, Frenchtown, NJ).

Procedure

Behavioural testing involved 6 phases of testing.

Phase 1: Acquisition

In the three days prior to the beginning of the study, rats were handled for 5 min/day and were habituated to consume 10 sugar (fructose/glucose) pellets in their home cages. Each acquisition session began by placing the rat in the central hub of the apparatus for a 1-minute adaptation period. Following adaptation, all guillotine doors were raised and animals were tested for 10 minutes. During this period, they could obtain sugar pellets by entering the arms and by nose poking at the receptacles. Testing was conducted once a day, for a total of 14 consecutive days.

Phase 2: Extinction I

On day 15, rats were placed in the apparatus and were tested as described above, but the pellet dispensers were disconnected from the pellet receptacles. All rats received 3 extinction sessions over 3 consecutive sessions which is how long was needed to reduce nose poking to significantly compared to the first session of extinction and the last session of acquisition. Immediately following behavioural testing on the last extinction session, different groups received different drug treatments (n = 24 each): Vehicle (V), 3 mg/kg JDTic (3/JDTic), 10 mg/kg JDTic (10/JDTic), 0.3 mg/kg Naltrexone (0.3/NTX), and 3 mg/kg Naltrexone (3/NTX). Since JDTic is effective for up to 4 weeks following systemic injection, only one injection was given throughout the experiment (Schank et al., 2012). Immediately following the injection, animals were further assigned to two different feeding conditions (n = 12 each): free feeding (FF) or food restriction (FR). Free feeding involved no specific manipulation. Food restriction involved decreasing the amount of food to achieve 85% of ad-lib body weight. This target was achieved

after 4 days (experimental days 18-21), during which no injections were given and animals were not tested.

Phase 3: Reinstatement induced by food restriction

On day 22, half of the rats in the V group, and rats in the 0.3NTX and 3NTX groups, received the appropriate V or NTX dose. Only half of the rats in each V group were given daily vehicle injections in order to match the daily NTX injections. Since JDTic was only given once, half of the vehicle rats only received one injection to match this condition. Thirty minutes following NTX, V injections, or no injections, all animals (FF & V = 12; FR & V = 12; FF & 3/JDTic = 12; FR & 3/JDTic = 12; FF & 10/JDTic = 12; FR & 10/JDTic = 12; FF & 0.3NTX = 12; FR & 0.3NTX = 12; FF & 3NTX = 12; and FR & 3NTX = 12) were tested in extinction conditions to assess reinstatement of nose poking by food restriction and possible modulation by JDTic or naltrexone.

Phase 4: Reacquisition

Following a day with no injections and no testing, half of the rats in the V group, and rats in the 0.3NTX and 3NTX groups, received the appropriate V or NTX dose and, 30 minutes later, all animals received a single session of self-administration performed as in Phase 1. The objective of this test was to ascertain modulation of reacquisition of nose poking by food restriction, and possible modulations by JDTic or naltrexone.

Phase 5: Extinction II

Twenty-four hours following reacquisition, rats were placed in the apparatus and were tested in extinction conditions as described above. All rats received 3 extinction sessions over 3

consecutive days, and half of the rats in the V group, and rats in the 0.3NTX and 3NTX groups, received the appropriate V or NTX dose 30 minutes prior to each test.

Phase 6: Reinstatement induced by sugar priming

For this last test session, the 8 arms of the apparatus were baited with 1 sugar pellet, half of the rats in the V group, and rats in the 0.3NTX and 3NTX groups, received the appropriate V or NTX dose, and 30 minutes later all animals were tested in extinction conditions. The objective of this test was to assess the effect of food restriction on reinstatement of nose poking by food priming, and possible modulations by JDTic or naltrexone.

Drug and Doses

Naltrexone hydrochloride was purchased from PCCA (Houston, TX) The doses used were 0.3 mg/kg and 3 mg/kg (Bonacchi et al., 2010; Williams & Broadbridge, C. L. 2009) (3R)-7-Hydroxy-N-[(2S)-1-[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethylpiperidin-1-yl]-3-methylbutan-2-yl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (JDTic) was donated by Dr. Ivy Caroll at the Research Triangle Institute, Center for Organic and Medicinal Chemistry (Durham, NC). The doses used were 3 mg/kg and 10 mg/kg (Deehan et al., 2012). All drugs were dissolved in 0.9% physiological saline.

Statistical Analyses

Independent and mixed design Analyses of Variance (ANOVAs) with one or two factors were used as appropriate. Multiple comparisons were performed using the Student Newman Keuls method ($\alpha = 0.05$) when significant interactions or main effects were found. The specific values of non-significant analyses are not reported. All statistical analyses were performed using SigmaStat (version 10.01 for Windows). Planned comparisons between food restricted rats and

free fed rats were made regardless of whether an interaction occurred based on literature suggesting that food-restriction modulates the effects of reinstatement sugar and drug seeking (Nair et al., 2009).

Results

Phase 1: acquisition

The bars in Figure 1A represent mean total number of nose pokes made by all subjects across the 14 sessions of self-administration. The ANOVA was significant [$F(13, 1547) = 46.68$, $p < 0.001$] and multiple comparisons revealed significant increases from session 1 to sessions 9-14. The line in Figure 1A represents percentage of total pellets consumed. The ANOVA was significant [$F(13, 1547) = 80.92$, $p < 0.001$], and multiple comparisons revealed that the percentage of pellets consumed was significantly greater on sessions 2-14 of acquisition compared to session 1.

Phase 2: Extinction 1

Figure 1B represents the mean total nose pokes made during the 3 extinction sessions. The ANOVA was significant [$F(2, 238) = 45.96$, $p < 0.001$], and multiple comparisons revealed significant decreases from sessions E2-E3 in comparison to session E1. Additionally responding on session E3 decreased significantly compared to session E2. Finally responding was significantly reduced compared to session 14 of acquisition.

Phase 3: Reinstatement induced by food restriction

In the first analysis of reinstatement data, stress-induced reinstatement was defined as a significant increase in nose pokes from the last session of extinction (E3) to the test of reinstatement in extinction conditions. Table 1A reports the total nose pokes emitted by FF and

FR animals during these sessions. The ANOVA indicated significant interaction of Session by Food [$F(1, 11) = 30.74$, $p < 0.001$] and of Dose/Drug Group by session [$F(4, 44) = 4.95$, $p < 0.01$]. Similarly, the ANOVA performed in FF rats indicated a significant Session by Dose/Drug Group interaction [$F(4, 44) = 2.71$, $p < 0.05$], as well as a significant main effect of Session [$F(1, 44) = 35.71$, $p < 0.001$]. Multiple comparisons revealed that: 1) after pre-treatment with vehicle, there was reinstatement in FR, but not FF, animals; 2) this effect was not altered by pre-treatment with both doses of JDTic; and 3) this effect was blocked by pre-treatment with both doses of NTX. Additionally, multiple comparisons performed within the reinstatement session, suggested that 3/NTX reduced nose pokes in FF animals, and that both NTX doses reduced nose pokes in FR animals compared to vehicle rats.

In the second analysis of reinstatement data, a difference score was calculated by subtracting nose pokes on E3 from the test of reinstatement in FF and FR rats (Figure 2). The ANOVA indicated a significant main effect of Food [$F(1, 110) = 37.66$, $p < 0.001$], as well as a significant main effect of Dose/Drug Group [$F(4, 110) = 3.81$, $p < 0.01$]. Planned comparisons using ANOVA revealed that: 1) the difference score was significantly larger in FR than FF rats treated with vehicle; 2) this effect was observed in rats treated with both doses of JDTic; and 3) this effect was dose-dependently reduced by NTX.

Table 2A represents general locomotion in the apparatus on the stress-reinstatement test, and the ANOVA indicated a significant main effect of Food [$F(1, 110) = 20.66$, $p < 0.001$], as well as a significant main effect of Dose/Drug Group [$F(4, 110) = 6.02$, $p < 0.001$]. Planned comparisons revealed: 1) no significant difference between vehicle or other Drug/Dose Groups in FR rats; and 2) a significant decrease in FF rats injected with both doses of NTX.

Phase 4: Reacquisition

Figure 3 represents the total nose pokes produced by FF and FR animals on the test of reacquisition. The ANOVA indicated a significant Food by Dose/Drug Group interaction [$F(4, 110) = 5.57$, $p < 0.001$], and significant main effects of Food [$F(1, 44) = 138.70$, $p < 0.001$] and of Dose/Drug Group [$F(4, 44) = 14.03$, $p < 0.001$]. Multiple comparisons revealed that: 1) nose poking was significantly greater in all FR groups; 2) in comparison to the group pre-treated with vehicle, nose poking was significantly lower in FF rats injected with both doses of NTX; 3) the same result was observed in FR animals treated with NTX; and 4) the same was observed in both doses of JDTic in the FF group

Figure 4 represents the total nose pokes produced by FF and FR animals on the test of reacquisition broken into two five minute time bins for each Dose/Drug group. The ANOVAs indicated significant Food/Time interactions for vehicle, [$F(1, 44) = 10.55$, $p < 0.01$], low JDTic, [$F(1, 44) = 12.22$, $p < 0.001$], and high JDTic [$F(1, 44) = 9.29$, $p < 0.01$] but not for either dose of NTX. There were however significant main effects of Food [$F(1, 44) = 43.77$, $p < 0.001$] (0.3/NTX) [$F(1, 44) = 15.03$, $p < 0.001$] (3/NTX) and Time, [$F(1, 44) = 22.25$, $p < 0.001$] (0.3/NTX), [$F(1, 44) = 20.17$, $p < 0.001$] (3/NTX) for both doses of NTX indicating an overall reduction in nose poking for all rats in this group. Multiple comparisons revealed that 1) Nose poking was significantly greater for FF animals compared to FR animals in the second half of the reacquisition session for vehicle as well as both doses of JDTic, 2) This effect was observed in the first half of the reacquisition session for both doses of JDTic 3) Both doses of NTX produced an overall drop in nose poking for both FF and FR animals across the reacquisition session

Table 2B represents general locomotion in the apparatus during reacquisition, and the ANOVA indicated no significant effects.

Phase 5 and 6: Extinction II and reinstatement induced by sugar priming

In the first analysis of reinstatement data, prime-induced reinstatement was defined as a significant increase in nose pokes from the last session of extinction (E6) to the test of reinstatement in extinction conditions (Table 1B). The ANOVA indicated a significant Dose/Drug group by session interaction [$F(4, 44) = 4.32$, $p < 0.01$]. Planned comparisons revealed that: 1) there was significant reinstatement of responding in all Dose/Drug Groups; 2) in both FF and FR conditions, rats treated with 3/NTX responded significantly less than rats pre-treated with vehicle; and 3) in FF rats 10/JDTic produced significantly greater responding than vehicle rats.

In the second analysis of reinstatement data, a difference score was calculated by subtracting nose pokes on E6 from the test of reinstatement in FF and FR rats (Figure 5). The ANOVA indicated significant main effects of Food [$F(1, 110) = 4.55$, $p < 0.05$], and Dose/Drug Group [$F(4, 110) = 3.40$, $p < 0.05$]. Planned comparisons revealed a significant difference between FF and FR rats only after vehicle treatment. The ANOVA however, revealed a significant increase in nose poking in free fed 10/JDTic rats compared to free fed vehicle rats. Both doses of NTX, however appeared to attenuate the effects of food restriction on prime induced reinstatement.

Table 2C represents general locomotion in the apparatus during the prime-reinstatement test, and the ANOVA indicated no significant main effects.

Discussion

The primary objective of this study was to test the effects of the non-selective mu-opioid antagonist Naltrexone (0.3 and 3 mg/kg) and selective kappa-opioid antagonist JDTic (3 and 10 mg/kg) on reinstatement of sugar seeking induced by food-restriction and sugar priming. Sprague Dawley male rats were initially trained to nose poke for sugar pellets in an 8-arm apparatus. After 3 extinction sessions, they were treated with JDTic or NTX, and then tested for reinstatement by food restriction. The effects of both drugs were subsequently tested on reacquisition of sugar self-administration, followed by reinstatement by a sugar prime. It was found that NTX, but not JDTic, blocked reinstatement caused by food-restriction. Furthermore, while these two antagonists had opposite effects on sucrose self-administration during reacquisition, NTX attenuated the effects of food restriction on reinstatement caused by the sugar prime, while JDTic dose dependently enhanced nosepoking during prime-induced reinstatement for free fed rats compared to free fed vehicle rats . These data confirm the critical role of opioid systems in sugar consumption and sugar seeking, and further indicate that different antagonists may be more appropriate to prevent relapse induced by different triggers.

Reinstatement induced by food-restriction

Food-restriction has been used to reinstate drug seeking as well as increasing sugar consumption in rats. However food-restriction has not previously been used as a stressor to induce reinstatement of sugar seeking in rats. The pharmacological stressor yohimbine is the only stressor so far that has been shown to reliably induce reinstatement of sucrose pellet seeking (Nair, Gray, & Ghitza, 2006). Food restriction has been shown to reliably reinstate heroin seeking in rats that have previously extinguished responding (Maric, et al., 2011; Maric, et al., 2012; Shalev, 2012). Given the ability of food-restriction to induce reinstatement of drug

seeking and sugar seeking (as found in the current study), as well as the parallels between food restriction and dieting in animals and humans respectively, food-restriction may be an ideal novel method of inducing reinstatement of palatable food seeking. Using pharmacological agents to decrease food-restriction induced reinstatement may have implications for their use in the treatment of relapse to maladaptive eating patterns in humans.

Reacquisition

Reacquisition refers to the resumption of responding for a drug during reinforcement conditions elicited by presentation of the drug following extinction sessions. Reacquisition of sugar consumption may provide useful supplementary knowledge in understanding an animal model of relapse to palatable food. Additionally reacquisition serves as a well understood control method of studying consumption, in a study using two novel methods of reinstating sugar seeking.

Reinstatement induced by priming

Prime-induced reinstatement of sugar seeking has been well studied (Calu, et al., 2014). Methods for priming with sugar pellets tend to adhere to two main designs: 1) Priming with a pellet or multiple pellets at the beginning of a session (typically before or within the first minute of testing) or 2) Priming by intermittent non-contingent presentation of pellets throughout the session. The new method of prime-induced reinstatement used in the current study most closely resembles the second design. In our method rats nose poke under extinction conditions, however each of the eight arms of the self-administration apparatus are baited with a sugar pellet for the rats to find and serve as the prime to induce reinstatement. This method of studying prime-induced sugar seeking has both consumption and seeking elements compared

to the food-restriction induced reinstatement which measured seeking behaviour. This method of studying prime-induced sugar seeking has also produced robust nosepoking that could not be attenuated by either antagonist in the current study.

Naltrexone on reinstatement

The results indicated NTX attenuated food restriction-induced but not prime-induced reinstatement of sugar seeking. No prior studies have investigated the effects of NTX on food restriction-induced reinstatement of sugar seeking in rats, but one study has investigated the effects of NTX on food deprivation-induced drug seeking in rats (Sedki et al., 2014). Sedki et al., 2014 found that kappa, but not mu-opioid antagonists reduced food deprivation induced heroin seeking in rats. These results are opposite to our findings and suggests that mu-opioid antagonists may play a different role in seeking of food than on seeking of drugs. While the role of NTX on food-deprivation induced reinstatement of sugar seeking has not been investigated, NTX has been shown to reduce food deprivation-induced intake of sugar (Bodnar, et al., 1995; Islam, et al., 1994; Koch & Bodnar, 1994; Schaefer, Koch, & Bodnar, 1994) which is consistent with the findings in the current study that NTX decreases food-restriction induced reinstatement of sugar seeking.

While NTX did not prevent prime-induced reinstatement of sugar seeking, it did attenuate the effects of food-restriction on prime-induced reinstatement, further supporting NTX's role on food-restriction induced seeking and consumption of sugar. As found in vehicle rats, food-restriction increased the magnitude of prime-indudced reinstatement compared to free fed rats. These findings are consistent with literature that has found food-restriction to

enhance prime and stress-induced reinstatement of sugar compared to free fed animals (Nair et al., 2009).

Naltrexone on reacquisition

The results indicated that NTX significantly decreased responding during reacquisition of sugar consumption for both food-restricted and free-fed animals compared to vehicle animals. These findings are supported by the extensive literature showing NTX to reduce sugar consumption (Avena, et al., 2014; Glass, Billington, & Levine, 2000; Katsuura & Taha, 2014; Skelly, et al., 2010; Stromberg, et al., 2002; and Wong, Wojnicki, & Corwin, 2009).

Naltrexone on locomotion

The findings in the current study indicated that while NTX appeared to reduce locomotion, there was only a significant difference found in free-fed rats injected with NTX in the food restriction-induced reinstatement session compared to vehicle rats, however nose poking did not significantly decrease in this group compared to vehicle. Since the eight-arm self-administration apparatus requires the rat to exit an arm entirely following a nose poke, total hub and arm entries will decrease as responding decreases. Consistent with our findings, a study showed NTX to reduce consumption of sugar in food-deprived rats, while not significantly reducing locomotion (Cooper & Turkish, 1989). Additionally, while mu-opioid agonism has been shown to increase high fat intake in rats, it does not affect generalized locomotion which is consistent with our findings (Pritchett, et al., 2010).

JDTic or other kappa antagonists on reinstatement

Our results indicated that JDTic did not attenuate food-restriction or prime-induced reinstatement of sugar seeking. The highest dose of JDTic significantly increased nosepoking

during prime induced reinstatement in free fed animals compared to free fed animals pretreated with vehicle. JDTic, has been found to attenuate stress and cue-induced reinstatement of drug seeking (Beardsley, et al., 2005; Beardsley, et al., 2010; Deehan, et al., 2012; Schank et al., 2012). Additionally other kappa-opioid antagonists such as arodyn and nor-BNI have been shown to attenuate food deprivation-induced reinstatement of cocaine seeking and heroin seeking (Carey et al., 2007; and Sedki et al., 2014). Schank et al., 2012 found that JDTic had no effect on the consumption of 10% sucrose solution. These findings are consistent with our findings that JDTic does not reduce sugar seeking. These findings are also interesting in the JDTic appears to dose dependently increase the difference score for nose poking for free fed rats during seeking conditions compared to vehicle treated free fed rats.

JDTic or other kappa antagonists on reacquisition

Our findings indicated nosepoking in first five minutes of the reacquisition session was significantly increased in food restricted animals compared to free fed animals for both doses of JDTic. This effect was not observed in rats pretreated with vehicle. These findings indicate a possible enhancement of food restriction nose poking and a role for the kappa opioid receptors. Few studies have investigated the effects of kappa opioid antagonists on sugar consumption. It has been found that the kappa antagonist nor-BNI reduces high fat diet selection when rats are given choice but this is in combination with enterostatin making it difficult to compare to sugar seeking (Ookuma, Barton, York, & Bray, 1997). Another study showed that the kappa agonist U50,488H increased palatable food consumption in rats, while the kappa agonists EKC, reduced consumption of palatable food (Cooper, et al., 1985). U50,488H has higher affinity for kappa-opioid receptors while EKC has been shown to have high affinity for mu-opioid receptors in

addition to its weak affinity for kappa opioids. These findings and the modulation of the free feeding enhancement in prime reinstatement suggest it is important to consider experimental design when interpret effects of kappa opioid receptors on free feeding compared to food restriction and the resulting effects on food consumption and seeking. These findings are also interesting in that JDTic appears to enhance nose poking in food restricted rats during consumption conditions at the beginning portion of the reacquisition session.

JDTic or other kappa antagonists on locomotion

Our results indicated that JDTic did not affect locomotor activity. This is consistent with the only study that tested the effects of JDTic on locomotor activity (Schank et al., 2012). There is very little literature connecting the effects of kappa antagonists to locomotion. One study has shown the kappa-opioid antagonist nor-BNI to decrease cocaine induced locomotor activity in rats (Allen, Zhou, & Leri, 2013). However this does not tell us the effects of kappa opioid antagonists on generalized locomotion in rats and the involvement of sugar and food restriction. Nor-BNI was found to not affect ethanol induced locomotor activity in rats (Arias, Molina, & Spear, 2010; Pautassi, Nizhnikov, Fabio, & Spear, 2012). Additionally the kappa-opioid agonist RU-51599 did not affect locomotor activity in rats trained to self-administer heroin (Marinelli, Le Moal, & Piazza, 1998). These findings are consistent with our findings that JDTic did not affect generalized locomotor activity.

Implications for knowledge about opioids and reinstatement and reacquisition of sugar seeking

This study is the first to investigate the effects of NTX and JDTic on food restriction-induced reinstatement of sugar seeking as well as reacquisition of sugar consumption. Food

restriction-induced reinstatement may serve as an alternative method of inducing reinstatement of sugar seeking than pharmacological stressors such as yohimbine. It may also be beneficial to use a human analogous stressor such as food restriction to imitate real world relapse to palatable food consumption through hunger and stress (i.e breaking a diet by binge eating).

While other studies have used sugar pellet primes to reinstate sugar pellet seeking, their methodology differs from ours in that sugar pellets were either given at the start of the session or delivered non-contingently throughout the session (Calu, et al., 2014; Nair et al., 2009). Our method of prime-induced reinstatement produced reliable and robust responding that was not attenuated by opioid antagonists. However, while neither antagonist could prevent reinstatement by priming in this case, NTX and JDTic reduced the effects of food restriction on prime-induced reinstatement of sugar seeking. Nair et al., (2009) suggested that food restricted rats reinstate sugar seeking by a prime more reliably and less variably than free fed rats. Our findings suggest that NTX may be useful in attenuating the effects of dieting which appear to enhance liking and wanting of palatable food in humans as well as animals. Additionally JDTic appeared to enhance food restricted nosepoking in rats during the first five minutes of the reacquisition session. Furthermore JDTic dose dependently increased the difference score in free fed animals compared to free fed animals pretreated with vehicle injection. These findings indicate a possible modulation of feeding by kappa receptors that has not been previously observed.

It is likely that NTX modifies palatability/hedonic value and incentive salience of palatable food such as sugar (Bertino, Beauchamp, & Engelman, 1991; Fantino, Hosotte, &

Apfelbaum, 1986; Langleben, et al., 2012; Parker et al., 1992; Waldhoer, Bartlett, & Whistler, 2004). These studies have shown that NTX decreases perceived craving and the intensity of sweetness of sugar in humans as well as decreasing positive oral-facial responses in the taste reactivity test in rats. An interesting double dissociation was found when a study investigated the effects of local injections of the mu-opioid antagonist naloxone into the shell of the NAc and basolateral amygdala (BLA) on food-deprivation induced lever pressing (i.e. incentive value or wanting) and hedonic licking (liking) in rats (Wassum et al., 2009). They found that while injections of naloxone into the shell of the NAc reduced food-deprivation induced hedonic licking, it did not reduce deprivation-induced lever pressing for sucrose. Additionally, while injections of naloxone into the BLA reduced deprivation-induced lever pressing for sucrose, it failed to suppress deprivation-induced hedonic licking in rats. These findings suggest that wanting and liking of palatable food may be governed by two distinct opioid neural circuits.

Limitations

Given that sugar is both sweet, and has post-ingestional consequences, it is difficult to discern which motivational aspects (i.e. liking compared to wanting) are driving the consumption and seeking of sugar. Additionally because NTX and JDTic are administered systemically, it is difficult to know exactly which pathways and neural structures are mediating the effects of opioid antagonists on consumption and seeking of sugar.

Some studies have suggested that nausea is the mechanism by which NTX acts in reducing palatable food consumption, however these studies are inconsistent and in some cases, nausea is not rated as significantly different than nausea ratings in the placebo group, so it is unlikely that NTX's entire effect on palatable food consumption is due to nausea (Yeomans

& Gray, 2002). Additionally previous findings have shown that while systemic injections of NTX reduce positive orofacial responses to sweet solutions in rats, they do not increase aversive gaping reactions further suggesting that NTX can reduce palatibility of sugar without causing nausea (Parker et al., 1992).

The order of presentation of the different phases of testing may also be a concern. However, given the exploratory nature of the food-restriction induced reinstatement of sugar seeking procedure, it was important to not confound this particular phase of the procedure with possible carryover effects from the other phases of testing. It also difficult to tease apart the effects of hunger and stress in food restriction induced reinstatement.

JDTic also could be given at a different time point to adjust for serum and brain presence of the drug in addition to antagonism of the kappa receptors.

Future directions

It could be interesting to investigate how NTX affects yohimbine induced reinstatement since yohimbine has been frequently used as a stressor for inducing reinstatement of sugar seeking (Nair et al., 2009). Using a common stressor such as yohimbine could be useful for making comparisons to food restriction induced reinstatement to see if the stressing effects of yohimbine may be reduced in a similar fashion by NTX as food restriction.

Repeating this study with saccharin pellets may provide information to tease apart wanting vs liking since saccharin is sweet but calorically devoid. If calorically devoid palatable pellets are able to reinstate seeking by food restriction and also attenuated by NTX, then there would be a strong argument for the importance of the hedonic aspect of palatable food in relapse influenced by dieting or fasting in humans.

As previously stated, Wassum et al., (2009) found a double dissociation between liking and wanting by giving mu-opioid antagonist (naloxone) microinfusions into the NAc shell and BLA. They found that NAc shell infusions reduced the food deprivation induced hedonic licking while not influencing deprivation induced lever pressing for sugar. The BLA infusions produced the opposite effects on deprivation induced responding. It would be interesting to modify the current study to investigate this double dissociation. As in Wassum et al., 2009 a taste reactivity task would be given following pre-treatment of NTX and vehicle into the NAc and BLA. Assuming our results were consistent with previous findings, NAc infusions should reduce taste reactivity licking while maintaining a high level of responding during reacquisition while an intra-BLA infusion should produce opposite effects. Assuming these findings occurred then the effects of these infusions on food-restriction induced reinstatement, and prime-induced reinstatement could be tested and perhaps inferences about wanting and liking could be drawn as well. It is expected that both BLA and NAc, NTX infusions would reduce prime induced reinstatement as it is likely that this phase of the experiment is influenced by wanting and liking. The effects on food-restriction induced reinstatement and reacquisition could also be tested using an operant chamber as well with a camera attached to the spout to measure licking for a sucrose solution.

Summary

The following predictions were made: 1) Since food restriction elicits reinstatement of drug seeking, the same effect is expected to be observed for sugar (Epstein et al., 2006). It was found that food restriction reinstated seeking of sugar.

2) Since JDTic attenuates stress but not prime induced reinstatement of drug seeking, the same should be observed for food restriction and prime induced reinstatement of drug

seeking (Beardsley et al., 2005). It was found that JDTic did not attenuate stress or prime induced reinstatement of drug seeking

3) Since naltrexone reduces food restriction induced intake of sugar, and cue-induced reinstatement of sugar seeking, it is expected that naltrexone will reduce food-restriction and prime-induced reinstatement of sugar seeking (Bodnar, et al., 1995; Burton, et al., 2011) It was found that naltrexone blocked food restriction induced reinstatement of sugar seeking but not prime-induced reinstatement of sugar seeking.

4) Since reinstatement of sugar induced by priming and yohimbine is reduced in free fed animals compared to food-restricted animals, the same should be observed in all tests of seeking and consumption in the current study (Nair et al., 2009). It was found that food-restriction increased responding compared to free fed rats treated with vehicle in both food and prime-induced reinstatement of sugar seeking. This effect was attenuated by both doses of naltrexone for food-restriction induced and prime induce reinstatement of sugar seeking. The highest JDTic dose appeared to enhance reinstatement by priming in free fed rats. And JDTic appeared to enhance food restriction induced nose poking during the first half of the reacquisition session.

The method of studying food-restriction induced reinstatement of sugar seeking in rats may provide insight into why and how humans also relapse to palatable consumption and seeking when attempting to reduce their intake. Furthermore, this study further demonstrates possible clinical benefits of using NTX to treat problematic eating and suggests further research is needed to better determine a role for kappa opioid receptors in feeding related behaviour.

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Tables and figure legends

Table 1

A: *Stress-induced reinstatement total nose pokes*. Mean (SEM) nose pokes in rats on extinction session 3 and the food restriction-induced reinstatement session. Half of the rats were free fed (n=60) and half of the rats were food restricted (n=60). The drugs and doses were divided into five groups: Vehicle (n=24), 3/JDTic (n=24), 10/JDTic (n=24), 0.3/NTX (n=24), and 3/NTX (n=24). The # indicates a significant decrease in nosepoking in NTX compared to vehicle rats within the same feeding condition. The * indicates a significant increase in nosepoking on the test of reinstatement compared to the extinction responding within food restricted rats.

B: *Prime-induced reinstatement total nose pokes*: Mean (SEM) nose pokes in rats on extinction session 6 and the sugar prime-induced reinstatement session. The # indicates a significant decrease in nosepoking in NTX compared to vehicle rats within the same feeding condition. The * indicates a significant increase in nosepoking on the test of reinstatement compared to the extinction responding.

Table 2

A: *Stress-induced reinstatement total entries*. Mean (SEM) total entries for rats on extinction session 3 and the food restriction-induced reinstatement session. Half of the rats were free fed (n=60) and half of the rats were food restricted (n=60). The drugs and doses were divided into five groups: Vehicle (n=24), 3/JDTic (n=24), 10/JDTic (n=24), 0.3/NTX (n=24), and 3/NTX (n=24). The # indicates a significant decrease in total entries in NTX compared to vehicle rats within the same feeding condition.

B: *Reacquisition total entries*. Mean (SEM) total entries for rats on reacquisition session.

C: *Prime-induced reinstatement total entries*. Mean (SEM) total entries for rats on extinction session 6 and the sugar prime-induced reinstatement session.

Figure 1

A: Mean (SEM) nose pokes in rats in 14 sessions of acquisition responding. All rats were free fed (N=120). The # indicates a significant increase in % pellets consumed compared to session 1 of self-administration. The * indicates a significant increase in mean nose pokes compared to session 1 of self-administration

B: Mean (SEM) nose pokes in rats in 3 sessions of extinction responding. All rats were free fed (N=120). The * indicates a significant decrease in responding compared to E1.

Figure 2: Mean (SEM) difference score in rats generated from subtracting nosepoking on extinction session 3 from the food restriction-induced reinstatement session. Half of the rats were free fed (n=60) and half of the rats were food restricted (n=60). The drugs and doses were divided into five groups: Vehicle (n=24), 3/JDTic (n=24), 10/JDTic (n=24), 0.3/NTX (n=24), and 3/NTX (n=24). The # indicates a significant decrease in nosepoking in NTX compared to vehicle rats within the same feeding condition. The * indicates a significant increase in nosepoking difference scores for food restricted rats compared to the vehicle group.

Figure 3: Mean (SEM) nose pokes in rats for the reacquisition session. Half of the rats were free fed (n=60) and half of the rats were food restricted (n=60). The drugs and doses were divided

into five groups: Vehicle (n=24), 3/JDTic (n=24), 10/JDTic (n=24), 0.3/NTX (n=24), and 3/NTX (n=24). The # indicates a significant decrease in nosepoking in NTX compared to vehicle rats within the same feeding condition. The * indicates a significant increase in nosepoking for food restricted rats free fed rats.

Figure 4: Mean (SEM) nose pokes divided into two five-minute time bins in rats for the reacquisition session. The * indicates a significant increase in nosepoking for food restricted rats compared to free fed rats within each time bin at each dose.

Figure 5: Mean (SEM) difference score in rats generated from subtracting nosepoking on extinction session 6 from the sugar prime-induced reinstatement session. Half of the rats were free fed (n=60) and half of the rats were food restricted (n=60). The drugs and doses were divided into five groups: Vehicle (n=24), 3/JDTic (n=24), 10/JDTic (n=24), 0.3/NTX (n=24), and 3/NTX (n=24). The * indicates a significant increase in difference scores for food restricted rats in the vehicle group compared to free fed rats in the vehicle group.

Appendices

Table 1

(A) *Food restriction-induced reinstatement total nose pokes*

	Free Fed				Food Restricted			
	Extinction 3		Reinstatement		Extinction 3		Reinstatement	
Dose/Drug Group	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Vehicle	21.42	2.10	19.75	2.23	21.25	1.70	28.08*	0.94
3/JDTic	21.08	1.87	13.25	1.23	21.00	2.50	26.17*	1.77
10/JDTic	20.83	2.19	18.25	2.05	21.67	1.76	26.92*	1.65
0.3/NTX	17.92	1.63	16.25	1.29	17.92	1.76	20.25#	1.11
3/NTX	18.17	2.23	11.50#	1.56	18.25	2.40	16.17#	1.65

(B) *Prime-induced reinstatement total nose pokes*

	Free Fed				Food Restricted			
	Extinction 6		Reinstatement		Extinction 6		Reinstatement	
Dose/Drug Group	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Vehicle	22.00	2.20	27.50*	1.86	27.17	2.35	38.08*	1.79
3/JDTic	15.75	2.07	23.75*	1.25	26.67	1.10	38.42*	1.62
10/JDTic	15.42	1.36	28.33*	1.47	29.25	1.29	40.50*	1.34
0.3/NTX	13.42#	1.72	20.00*	2.91	22.50	2.02	33.50*	2.16
3/NTX	12.42#	1.36	17.50*#	1.59	23.00	3.18	28.75*#	2.46

Table 2

(A) *Food restriction-induced reinstatement total entries*

	Free Fed		Food Restricted	
Dose/Drug Group	Mean	SEM	Mean	SEM
Vehicle	67.25	4.75	70.33	3.92
3/JDTic	53.67	3.81	77.50	4.72
10/JDTic	60.17	5.51	71.08	2.77
0.3/NTX	49.42#	4.10	60.00	4.12
3/NTX	47.92#	3.93	58.41	2.44

(B) *Reacquisition total entries*

	Free Fed		Food Restricted	
Dose/Drug Group	Mean	SEM	Mean	SEM
Vehicle	98.50	5.54	88.92	9.02
3/JDTic	83.92	10.08	100.0	8.22
10/JDTic	94.42	10.78	86.67	9.72
0.3/NTX	73.00	9.98	64.50	4.26
3/NTX	69.50	6.15	63.58	6.64

(C) *Prime-induced reinstatement total entries*

	Free Fed		Food Restricted	
Dose/Drug Group	Mean	SEM	Mean	SEM
Vehicle	72.00	5.42	87.25	6.10
3/JDTic	71.58	4.64	92.08	3.04
10/JDTic	79.58	4.52	91.33	4.07
0.3/NTX	55.75	6.84	81.75	6.60
3/NTX	54.50	4.51	70.75	5.47

Figure 1

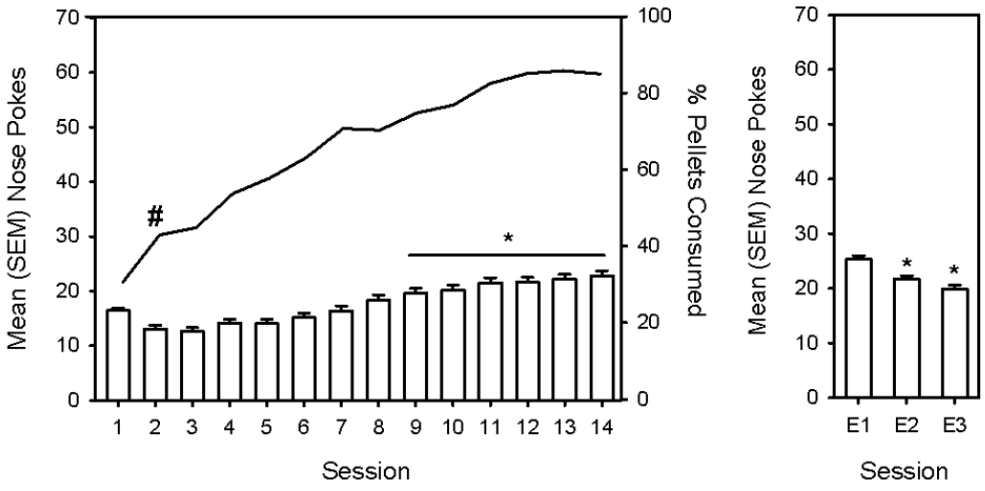

Mean (SEM) Nose Pokes
% Pellets Consumed
Session
#
*
Mean (SEM) Nose Pokes
Session
E1
E2
E3
*
*

Figure 2

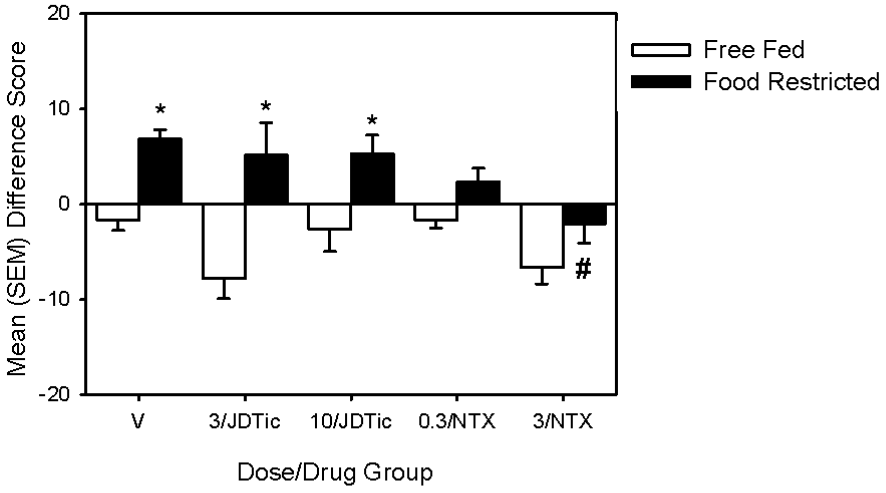

20
10
0
-10
-20
Mean (SEM) Difference Score
*
*
*
#
V
3/JDTic
10/JDTic
0.3/NTX
3/NTX
Dose/Drug Group
Free Fed
Food Restricted

Figure 3

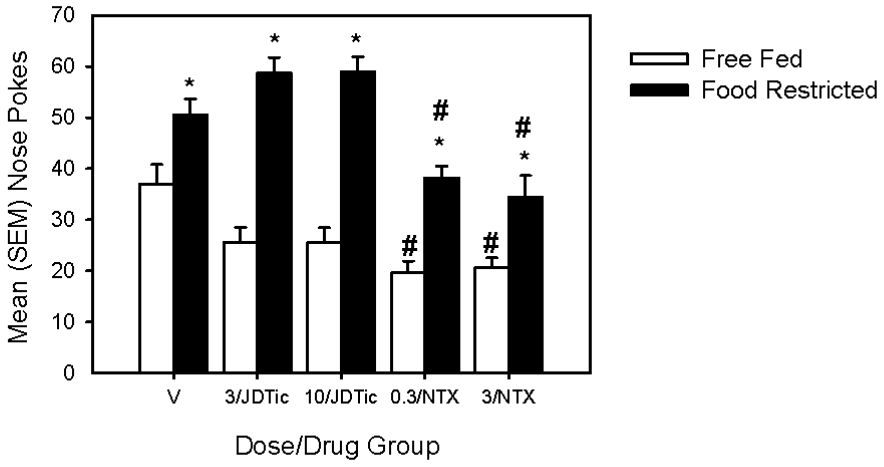

Mean (SEM) Nose Pokes
70
60
50
40
30
20
10
0
V
3/JDTic
10/JDTic
0.3/NTX
3/NTX
Dose/Drug Group
Free Fed
Food Restricted

Figure 4

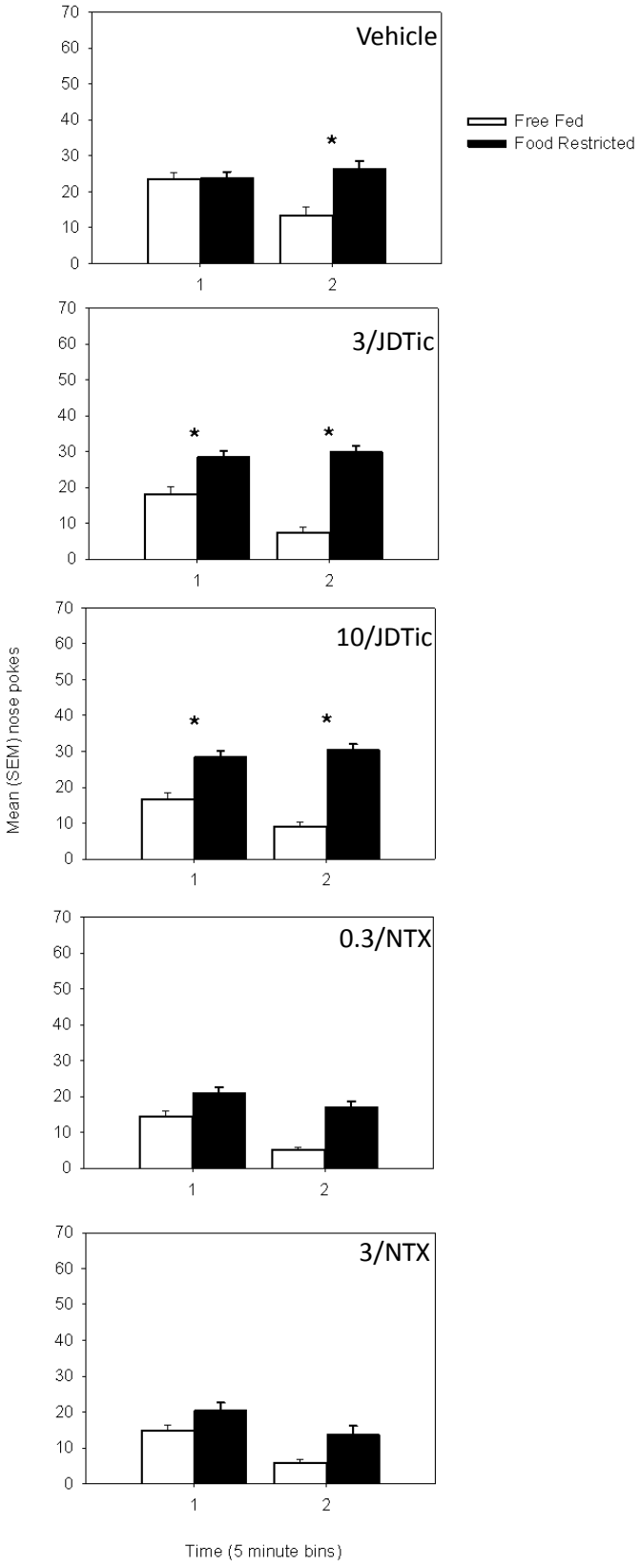
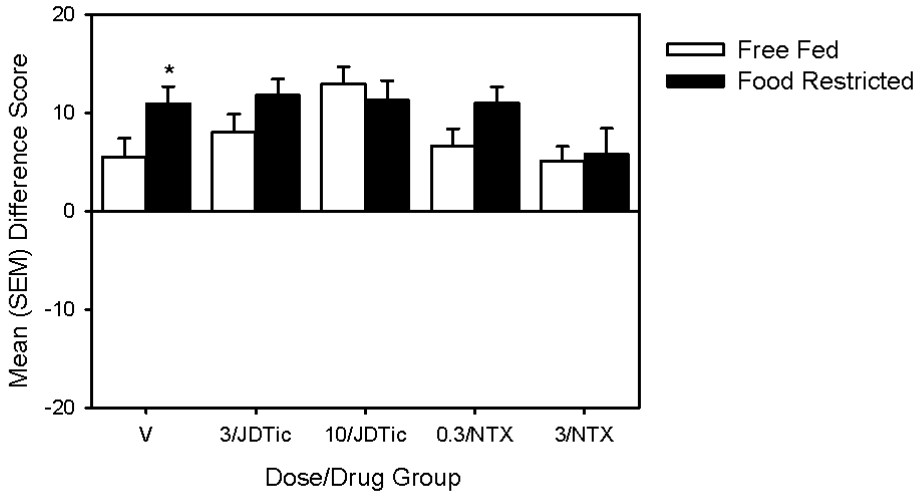

Vehicle
3/JDTic
10/JDTic
0.3/NTX
3/NTX
Free Fed
Food Restricted
Mean (SEM) nose pokes
Time (5 minute bins)

Figure 5


20
10
0
-10
-20
Mean (SEM) Difference Score
*
V
3/JDTic
10/JDTic
0.3/NTX
3/NTX
Dose/Drug Group
Free Fed
Food Restricted