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Behavioral characterization of an operant model of binge-like eating in rats

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BEHAVIORAL CHARACTERIZATION OF AN OPERANT MODEL OF BINGE-LIKE EATING IN RATS

by

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DEDICATION

I would like to dedicate this work to Aunt Debbie, Grandma Lee, Grandpa, Christopher, Grandma Gabe, Julie Bryant, Richard Ramey, Matt Butler, Scott Heinig, my family, baby Barrett, and Nathanael.

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I would like to thank Dr. Cottone for giving me the opportunity to work in the Laboratory of Addictive Disorders, Clara Velázquez-Sánchez for teaching me and helping me tremendously with this project, Antonio Ferragud for sharing his knowledge and inspiring my curiosity, and Karen Smith for her contribution to the study.

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BEHAVIORAL CHARACTERIZATION OF AN OPERANT MODEL OF BINGE-LIKE EATING IN RATS

JEFFREY W. SANTOS

ABSTRACT

Binge eating disorder is characterized by excessive consumption of highly palatable food within short periods of time accompanied by loss of control over eating. Extensive evidence provides support for the consideration of binge eating disorder as an addiction-like disorder. In this study, we wanted to determine whether rats undergoing an operant binge-like eating procedure could develop maladaptive forms of conditioned feeding behaviors. For this purpose, we trained male rats to self-administer either a sugary, highly palatable diet (*Palatable* rats) or a chow diet (*Chow* rats) for 1 hour/day. Following escalation and stabilization of palatable food intake, we tested both *Chow* and *Palatable* rats in a *i*) conditioned place preference, a *ii*) second-order schedule of reinforcement and, finally, a *iii*) cue-induced suppression of feeding. In the conditioned place preference task, *Palatable* rats spent significantly more time in the compartment which was paired with the palatable food when compared to *Chow* controls. Furthermore, in the second-order schedule of reinforcement task, *Palatable* rats exhibited active lever responding 4- to 6-fold higher than *Chow* control rats. Finally, in the conditioned suppression of feeding test, while *Chow* control subjects reduced responding by one-third in the presence of the conditioned punishment, *Palatable* rats persevered in responding despite the aversive cue.

These results further characterize our animal model of binge-like eating and provide additional evidence for the addictive properties of highly palatable food.

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LIST OF ABBREVIATIONS

AAALAC .....Association for Assessment and Accreditation of Laboratory Animal Care

ANOVA.....Analysis of Variance

CPP .....Conditioned Place Preference

CR ..... Conditioned Response

CS ..... Conditioned Stimulus

DSM-V.....Diagnostic and Statistical Manual of Mental Disorders, 5th Edition

FI .....Fixed Interval

FR..... Fixed Ratio

IR.....Infrared

LSD .....Least Significant Difference

NA .....Nucleus Accumbens

N.S. ..... Not Significant

UCS.....Unconditioned Stimulus

VTA ..... Ventral Tegmental Area

INTRODUCTION

Binge eating disorder affects more than 10 million people in the United States and is frequently present with obesity, diabetes, cardiovascular diseases, and other psychiatric diseases such as anxiety and depression (Ágh et al., 2015; American Psychiatric Association, 2013; Javaras et al., 2008; Wilfley et al., 2011; Yanovski, 2003). The lifetime prevalence for this disorder is about 2% (Kessler et al., 2013) and it results in a drastically decreased quality of life and increased healthcare cost reaching close to $4,000 per year per binge eating disorder patient (Ágh et al., 2015). Binge eating disorder is characterized by excessive consumption of highly palatable foods within a short period of time without the use of compensatory behaviors such as purging. These symptoms of binge eating disorder culminate in a loss of control over eating (**Table 1**) (American Psychiatric Association, 2013).

An increasing number of behavioral and neurobiological findings provide strong support for the consideration of binge eating disorder as an addiction-like disorder. Addiction is considered a disease caused by dysregulation of the brain's reward system as well as motivation and memory neurobiology (Hyman, Malenka, & Nestler, 2006; Koob, 1992; National Institute on Drug Abuse, 2008; Volkow, Fowler, Wang, & Swanson, 2004). The brain's reward system is primarily composed of the structures making up the limbic system. Specifically, the limbic system is comprised of the hypothalamus, amygdala, hippocampus,

septa nuclei, and anterior cingulate gyrus. The limbic striatum which includes the nucleus accumbens (NA), ventral caudate nucleus and the putamen, is also important in the pathway as is the ventral tegmental area (VTA). The role of the limbic system is to maintain the body's natural order and it controls memory, learning, and emotion. It has also been shown to drive sexual behavior, motivation, and feeding. Drug use alters the balance of the neurotransmitters that these structures release and respond to, which interferes with the overall circuity between all interconnected parts of the brain's reward system (**Figures 1 & 2**)(Hyman et al., 2006; National Institute on Drug Abuse, 2008; Robbins & Everitt, 2002). This interference results in the need to continually participate in the addictive behavior in order to return to a basal healthy state (Hyman et al., 2006; Nestler, 2013). In addition, connections between cortical, hippocampal, and reward centers are affected by drug use in such a way that memory associated with access to the addictive reward results in biological and behavioral responses to external cues which prolong craving, seeking behavior, and relapse from a period of abstinence (Hyman et al., 2006; Robbins & Everitt, 2002). Addiction also deals with a loss of impulse control which includes the fontal cortex in the equation (National Institute on Drug Abuse, 2008). Common characteristics of addiction are featured in **Table 2**.

While addiction is often associated with drugs of abuse, animal studies have shown that calorie-dense highly palatable food causes a release of opioids and dopamine in the limbic systems (Colantuoni et al., 2002). In addition, the

administration of an opioid antagonist, similar to withholding the palatable food, in rats that were given intermittent access causes symptoms consistent with morphine and nicotine withdrawal, thereby showing an addicted state associated with eating sugary, palatable food (Colantuoni et al., 2002; Cottone, Sabino, Roberto, et al., 2009; Cottone, Sabino, Steardo, & Zorrilla, 2008a, 2009; Iemolo et al., 2012, 2013). Animal models have been developed using various experimental procedures to induce binge eating (Blasio, Steardo, Sabino, & Cottone, 2014; Corwin & Buda-Levin, 2004; Cottone et al., 2012; Cottone, Sabino, Steardo, & Zorrilla, 2008b). Further suggesting that binge eating disorder is indeed an addiction-like disorder, models where rats were given access to regular chow diet followed by a macronutrient equivalent palatable chow diet showed associative learning which is crucial for the development of addiction. The animals learned to refrain from eating the standard chow diet in anticipation of the palatable diet that would follow. In addition, nalmefene, an opioid-receptor antagonist, reduced the binge-like eating of the palatable food during its limited access and the animals showed increased anxiety and quickly increased body fat, consistent with an obese state commonly present with binge eating disorder (Cottone et al., 2008b). It has also been shown that the injection of CRF1 receptor antagonist R121919 in the bed nucleus of the stria terminalis, which is the region of the brain thought to be responsible for stress-induced drug seeking and binge eating, decreases stress induced binge eating in rats (Micioni Di Bonaventura et al., 2014).

Binge-Eating Disorder

Diagnostic Criteria **307.51 (F50.8)**

A. Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following:
   1. Eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than what most people would eat in a similar period of time under similar circumstances.
   2. A sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much one is eating).

B. The binge-eating episodes are associated with three (or more) of the following:
   1. Eating much more rapidly than normal.
   2. Eating until feeling uncomfortably full.
   3. Eating large amounts of food when not feeling physically hungry.
   4. Eating alone because of feeling embarrassed by how much one is eating.
   5. Feeling disgusted with oneself, depressed, or very guilty afterward.

C. Marked distress regarding binge eating is present.

D. The binge eating occurs, on average, at least once a week for 3 months.

E. The binge eating is not associated with the recurrent use of inappropriate compensatory behavior as in bulimia nervosa and does not occur exclusively during the course of bulimia nervosa or anorexia nervosa.

Table 1. Binge Eating Disorder Diagnostic Criteria. The Diagnostic and Statistical Manual of Mental Disorders (DSM-V) provides this set of criteria for the diagnosis of binge eating disorder (Taken from (American Psychiatric Association, 2013)).

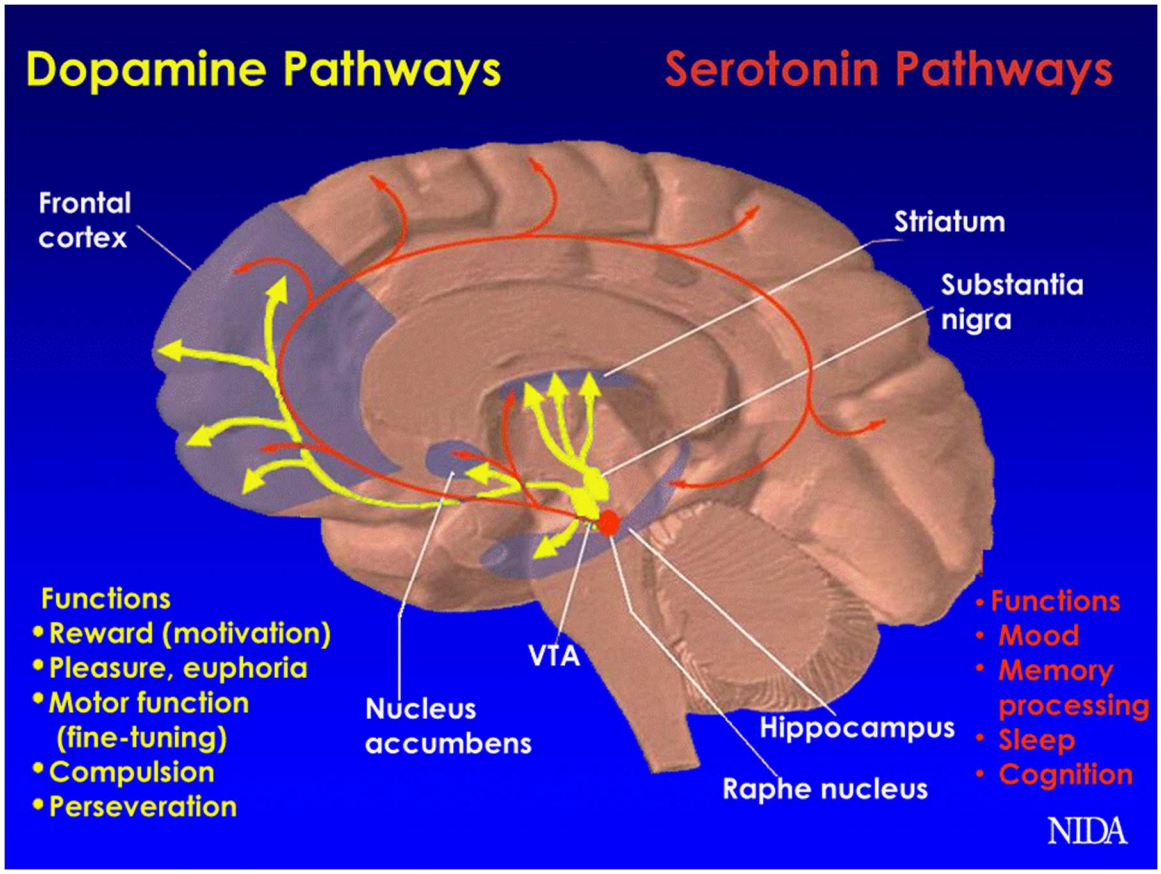

Figure 1. Neurobiology of the Brain's Reward System. The brain's reward system is a connection of several brain centers with various neurotransmitter involvement. Dysregulation of this system leads to the development of addiction (Taken from (National Institute on Drug Abuse, 2008)).

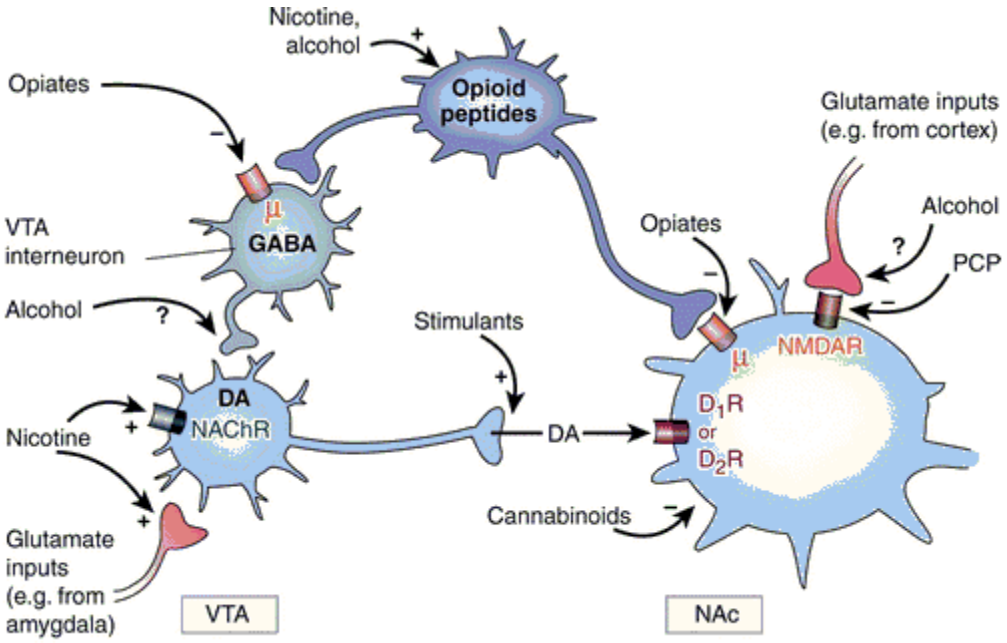

Figure 2. Drug Involvement in the Reward System. Specific drugs impact the brain's reward system from different areas. Since the various brain centers are all interconnected, dysregulation of one affects the others. This is the molecular basis of addiction (Taken from Figure 4 from (Hyman et al., 2006)).

Behavioral Characteristics of Addiction:
Obsession over the addictive substance/activity with constant seeking.
Compulsive behavior towards participation.
Shows withdrawal symptoms with discontinuation of activity.
Loss of control over behaviors.
Denial towards addiction.
Increased anxiety and low self-esteem.

Table 2. Behavioral Characteristics of Addiction. An individual who is addicted displays a stereotypical assortment of behaviors. Most important to the conversion from abuse to dependence is a loss of control over behaviors. (Adapted from (Engs, R.C., 1987)).

In vivo animal models continue to play a major role in understanding the underlying neurobiological substrates of psychiatric diseases and provide predictive information on potential targets for pharmacological treatment. Behavioral models can be developed using various techniques for conditioning leading to either non-associative learning or associative learning. Non-associative learning is exemplified by habituation and sensitization and displays changes in a response due to continuous exposure to a stimulus. In the case of habituation, the response would decrease through prolonged exposure to a stimulus and sensitization would show an increase in response through repeated exposure to a stimulus (Thompson & Spencer, 1966). Associative learning is produced by classical conditioning or operant conditioning. Classical conditioning is when a neutral stimulus is paired with an unconditioned stimulus that leads to an unconditioned response. With repeated pairings, the neutral stimulus becomes a conditioned stimulus and the unconditioned response becomes the conditioned response. As a result, the presentation of the now conditioned stimulus will induce the now conditioned response **(Figure 3)**. This type of conditioning elicits an involuntary action. Operant conditioning is when an action is paired with either a reward or a punishment. The more enjoyable the reward is, the more the animal will perform the action. This type of conditioning elicits a voluntary response (Schachtman & Reilly, 2011). The key differences between classical conditioning and operant conditioning are shown in **Table 3**.

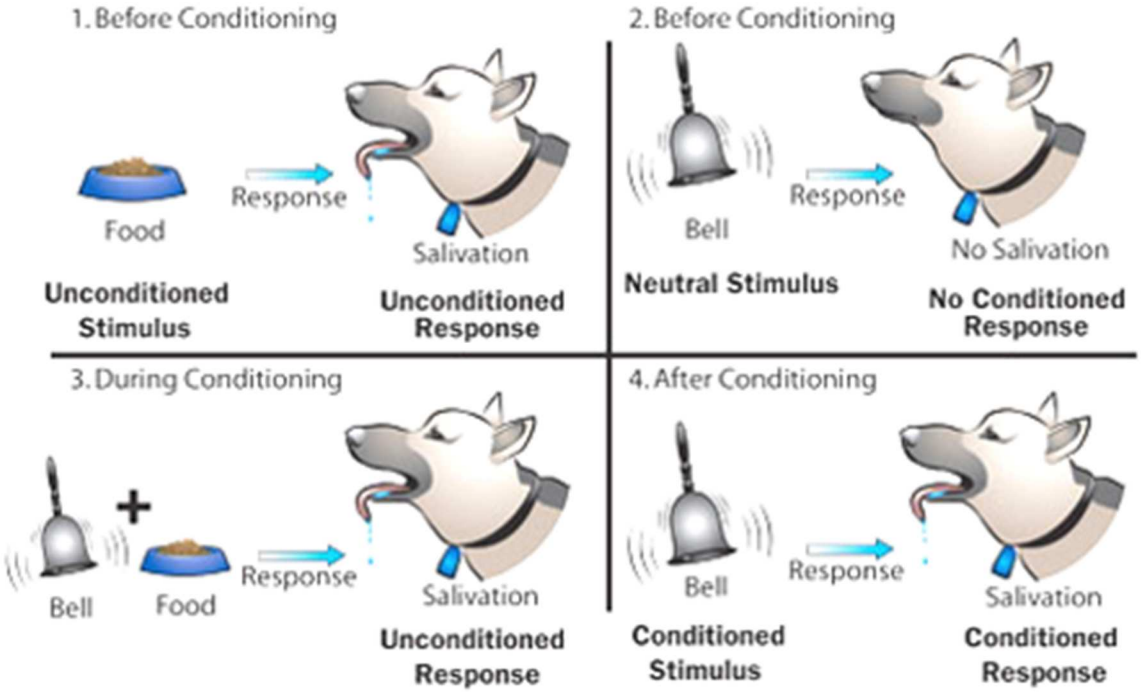

Figure 3. Development of Classical Conditioning. The animal displays an involuntary response to an unconditioned stimulus. An outside cue is introduced with the previous unconditioned response and stimulus. After repeated exposures, the outside cue alone is able to elicit the involuntary response (Adapted from (Harris, Hannah, 2006)).

TABLE 8.2 COMPARISON OF CLASSICAL AND OPERANT CONDITIONING

	Classical Conditioning	Operant Conditioning
Response	Involuntary, automatic	"Voluntary," operates on environment
Acquisition	Associating events; CS announces UCS.	Associating response with a consequence (reinforcer or punisher).
Extinction	CR decreases when CS is repeatedly presented alone.	Responding decreases when reinforcement stops.
Cognitive processes	Subjects develop expectation that CS signals the arrival of UCS.	Subjects develop expectation that a response will be reinforced or punished; they also exhibit latent learning, without reinforcement.
Biological predispositions	Natural predispositions constrain what stimuli and responses can easily be associated.	Organisms best learn behaviors similar to their natural behaviors; unnatural behaviors instinctively drift back toward natural ones.

Table 3. Classical and Operant Conditioning. The keys difference between classical and operant conditioning is the type of response, either involuntary or voluntary, that is being expressed and the driving force for the behavior. Classical conditioned behaviors are an association between behavior and stimuli and operant conditioned behaviors are an association between an action and a consequence or reward (Taken from Table 8.2 Comparison of Classical and Operant Conditioning from ("Classical -vs- Operant Conditioning," n.d.)).

We have recently developed an operant rat model of binge-like eating which is based on a 1 hour per day limited access to a highly palatable, sucrose diet (Blasio et al., 2014; Cottone et al., 2012; Velázquez-Sánchez et al., 2014). This model pairs the action of the rat poking their nose through a swinging gate with the illumination of a light overhead and the delivery of food. Under these limited access conditions, rats escalate responding for the palatable diet up to four times compared to control rats responding for a standard chow diet. When tested in a progressive ratio schedule of reinforcement, the animal had to perform an increased number of responses in order to receive each subsequent pellet. The number of responses needed to receive a pellet follows an equation: response ratio = $[4 \cdot (e^{\#\text{ of reinforcer} * 0.075}) - 3.8]$, rounded to the nearest integer. Binge eating rats exhibit a heightened motivation to obtain the sugary food as seen with a measured breakpoint in the progressive ratio schedule of reinforcement (Blasio et al., 2014; Velázquez-Sánchez et al., 2014). Binge eating rats also display a loss of control over food (compulsive eating) shown by the continuation of consuming highly palatable food during exposure to aversive conditions, as measured by time spent and food consumed in the open field of a light/dark conflict test (Cottone et al., 2012; Velázquez-Sánchez et al., 2014). In addition, we have recently shown by evaluating increased food intake, motivation, and compulsive-like eating as it relates to impulsivity, an approach similar to the one used to prove cocaine addiction-like behavior in the rat (Belin, Mar, Dalley, Robbins, & Everitt, 2008; Deroche-Gamonet, Belin, & Piazza, 2004), that not all

the rats exposed to the highly palatable food respond the same way and that only some rats develop a phenotype which is suggestive of addiction-like behavior (Velázquez-Sánchez et al., 2014). This phenotype represents a highly translational aspect of the model as it mimics what is observed in Western societies where although palatable foods are omnipresent, only some individuals develop pathological overeating.

A critically important aspect in the study of addictive disorders is represented by the development of maladaptive conditioned behaviors. Stimuli conditioned to alcohol, drugs of abuse, or food can exert a powerful control over behavior and can elicit craving and precipitate a relapse or binge (Everitt & Robbins, 2000; Robinson, Yager, Cogan, & Saunders, 2014). In addition, aversive environmental stimuli which signal punishment and readily suppress appetitive behavior, such as conditioned suppression (Bouton & Bolles, 1980), become ineffective in addicted individuals proving the compulsive nature of the disorders (Johnson & Kenny, 2010; Vanderschuren & Everitt, 2004).

Therefore, in this study we wanted to determine whether rats undergoing the operant model of binge-like eating procedure could develop maladaptive forms of conditioned feeding behaviors. For this purpose, we tested both palatable eating rats and chow responding control rats in a *i*) conditioned place preference, which is a task used to measure the rewarding properties of stimuli associated with food (Tzschentke, 2007), a *ii*) second-order schedule of reinforcement in which food seeking responding is maintained by the

presentation of conditioned stimuli (Everitt & Robbins, 2000) and finally, a *iii*) cue-induced suppression of feeding test used to measure the perseverance of instrumental responding in the presence of conditioned aversive stimuli (Belin et al., 2008; Deroche-Gamonet et al., 2004; Johnson & Kenny, 2010).

METHODS

Subjects

Three separate cohorts of male Wistar rats, 45 days old and 180g on arrival (Charles River, Wilmington, MA), were used as subjects for the individual conditioned place preference, second-order schedule of reinforcement, and cue-induced suppression of feeding experiments. The animals were triple housed in wire-topped plastic cages (27 × 48 × 20 cm^3) in a 12:12 hour reverse light cycle (lights off at 11:00 am), AAALAC-approved humidity- (60%), and temperature-controlled (22 degrees Celsius) vivarium. The animals were allowed to habituate to the research facility for four days and were given ad labitum access to corn-based chow (Harlan Teklad LM-485 Diet 7012) (65% [kcal] carbohydrate, 13% fat, 21% protein, metabolizable energy 341 cal/100g); Harlan, Indianapolis, IN) and water. Procedures adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Principles of Laboratory Animal Care, and were approved by Boston University Medical Campus Institutional Animal Care and Use Committee. No experimental procedures involved food or water restriction or deprivation.

Apparatus for self-administration procedures

The individual operant test chambers (30×24×29 cm) (Med Associates Inc., St. Albans, VT) had a stainless steel grid floor and were located in ventilated, sound-attenuating enclosures (66×56×36 cm) (Blasio et al., 2014;

Cottone et al., 2012). Food reinforcers were delivered by a pellet dispenser into a nose-poke aperture accessed through a swinging gate located on the front side of the right wall and water reinforcers (100 microliters in volume) were delivered by a solenoid into a liquid cup nose-poke receptacle located in the middle of the right wall directly next to the gated pellet receptacle. Two retractable levers were placed on the opposite wall of the chamber. 28-V stimulus cue-lights were located above each lever and above the food receptacle. The delivery of a pellet was paired with a light-cue lasting 0.3 sec. All responses were recorded automatically by a microcomputer with 10 ms resolution.

Operant model of binge-like eating in rats

After the four day acclimation period, the standard Harlan Teklad chow diet was replaced with an AIN-76A-based diet (5TUM diet formulated as 4-5g extruded pellets, 65.5% [kcal] carbohydrate, 10.4% fat, 24.1% protein, metabolizable energy 330 cal/100 g; TestDiet, Richmond, IN), which will be referred to as “chow” for the remainder of the paper. The animals were given access to the new chow diet in their home-cage beginning three days prior to any procedures. The configuration of the operant chambers used in the operant binge-like eating procedure is shown in **Figure 4**. For this procedure, all rats were individually placed in operant chambers overnight and trained to self-administer, through nose-poking, 45-mg chow pellets which were identical in composition to the diet the rats received in their home-cages as 5g extruded

pellets, and water on a fixed ratio 1 (FR1) continuous schedule of reinforcement, where each nose-poke resulted in the delivery of one pellet or 100 microliters of water. Once the animals learned to nose-poke, they were allowed to self-administer pellets and water in the operant chambers during 1 hour/day sessions. Levers were retracted during the entire session in this procedure and pellet delivery was paired with a light-cue located above the nose-poke hole. After reaching a stable responding for standard chow, rats were assigned to either a "*Chow*" control group and continued receiving the same 45-mg chow pellets offered during the training phase or a "*Palatable*" group and instead received 45-mg chocolate-flavored, high sucrose (50% Kcal) pellets similar in macronutrient composition and energy density to the chow 45-mg food (chocolate-flavored Formula 5TUL: 66.7% [kcal] carbohydrate, 12.7% fat, 20.6% protein, metabolizable energy 344 cal/100 g; formulated as 45 mg precision food pellets; TestDiet, Richmond, IN) during the operant chamber sessions. The animals underwent daily 1 hour sessions under an FR1 schedule of reinforcement for 12 days. The different testing procedures were performed in *Palatable* rats which fully escalated and stabilized their intake and the *Chow* control counterparts as previously shown (Blasio et al., 2014; Cottone et al., 2012; Velázquez-Sánchez et al., 2014).

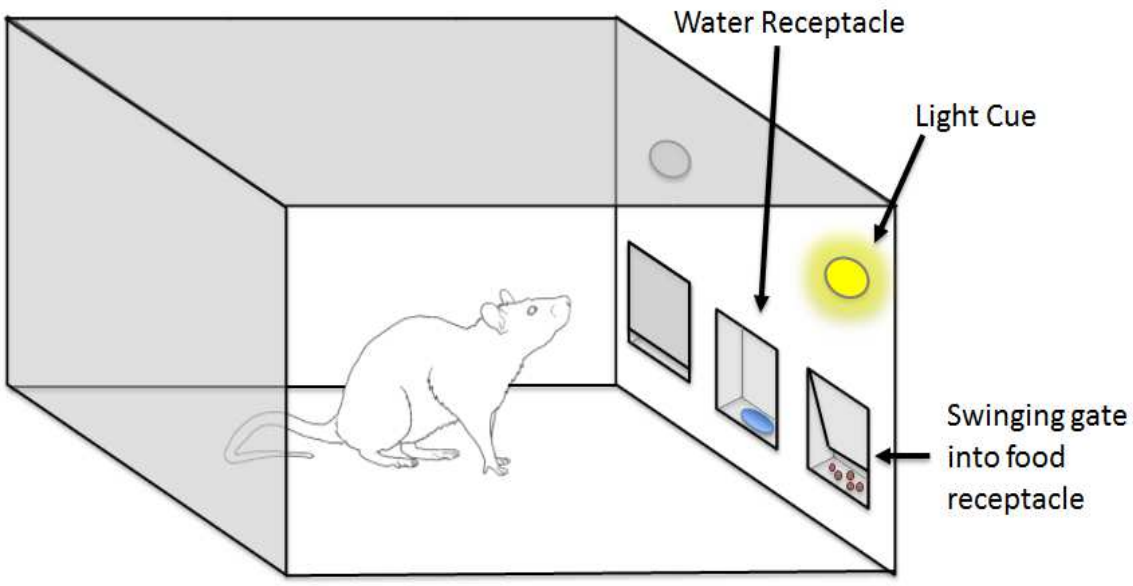

Figure 4. Configuration of the Operant Chamber During the Binge-like Eating Procedure. While in the operant chamber, the rat nose-pokes through a swinging gate that reveals a food receptacle. The nose-poke action is paired with an illumination of a light overhead and results in the delivery of food.

Conditioned place preference

The conditioned place preference (CPP) apparatus (Med Associates, Georgia, VT) consisted of two equally sized black and white outer chambers (27.5×20.6×21.5 cm) connected by a smaller gray center chamber (11.9×20.6×21.5 cm) separated by automatic guillotine doors. The white chamber contained wire mesh flooring and the black chamber had stainless steel rod flooring. IR photobeam detectors were placed in each chamber to measure the time spent in each chamber using Med PC software. The light intensity of each chamber was adjusted to counter any extreme unconditioned chamber bias. The CPP procedure ran in three different phases: pre-conditioning, conditioning, and testing. During the pre-conditioning phase, rats were placed in the center gray chamber as the guillotine doors opened allowing the animal to explore the entire apparatus for 15 minutes. The time spent in each chamber was recorded to determine which outer chamber was favored by each animal. Animals that showed extreme unconditioned chamber bias (spent greater than 400 seconds in any chamber) or did not show a reliable preference for either outer chamber (less than a 20 second difference between time spent in the outer two chambers) were excluded from the experiment. In the biased conditioning phase (Wang et al., 2014), rats were confined on alternating days to their initial preferred chamber with an empty food bowl or in their initial un-preferred chamber with 150g of food (either chow (*Chow* group) or palatable diet (*Palatable* group)) placed in a food bowl. Conditioning sessions were performed

daily, lasted 25 minutes and continued for 16 days (eight pairings each). Twenty-four hours after the last conditioning session, the animals were tested following the same procedure as the pre-conditioning test. The time spent in each chamber was recorded and CPP was determine by calculating a CPP score (time in paired chamber – time in unpaired chamber) (Lax, George, Ignatz, & Kolber, 2014; Velázquez-Sánchez et al., 2015; Wang et al., 2014; Xue et al., 2014).

Second-order schedule of reinforcement

Food-seeking behavior using a second-order schedule of reinforcement is a method in which responding is sustained by the reliant display of stimuli which have been paired with food and serve as conditioned reinforcers of instrumental behavior (Everitt & Robbins, 2000; Giuliano, Robbins, Nathan, Bullmore, & Everitt, 2012). The configuration of the operant chambers used in the second-order schedule of reinforcement experiment is shown in **Figure 5**. For this procedure, the animals were trained in FI1 (Fixed Interval of 1 minute), FI2, FI3, FI4, and FI5 training sessions which determined how long the animal needed to wait until a response on the active lever would result in the delivery of a pellet. The training taught the animals to press an active extended lever on the opposite wall of the pellet receptacle and use light-cues above the lever and pellet receptacle to communicate the delivery of pellets. Once the animals learned the task, food seeking was measured with a second-order schedule of reinforcement test. The second-order schedule of reinforcement test is a FI5(FR10:S)

procedure. This means that there is a Fixed Interval of five minutes (FI5) before pellets are available and the active lever must be pressed 10 times (Fixed Ratio 10, FR10) before a stimulus is presented. The test began in the FI5 phase where 10 active lever presses resulted in a one second illumination of light-cues above both the active lever and the food receptacle. The animals did not receive any pellets during the FI phases in either the training sessions or the test. There were no penalties for pressing the inactive lever during the procedure but the measure was recorded to assess lever bias and any differences in motor activity. Upon completion of the Fixed Interval of five minutes (FI5), pellets became available and the tenth active lever press resulted in the delivery of 20 pellets (45-mg chow pellets for *Chow* rats or 45-mg sucrose pellets for *Palatable* rats) into the food receptacle. The delivery of pellets was accompanied by the retraction of both the active and inactive levers and the illumination of the light-cues above the active lever and the food receptacle for a 20 second time out. Once the time out elapsed, the light-cues above the active lever and the food receptacle turned off and the two levers extended back into the chamber. The second-order schedule of reinforcement session consisted of seven FI5 phases and lasted 40 minutes.

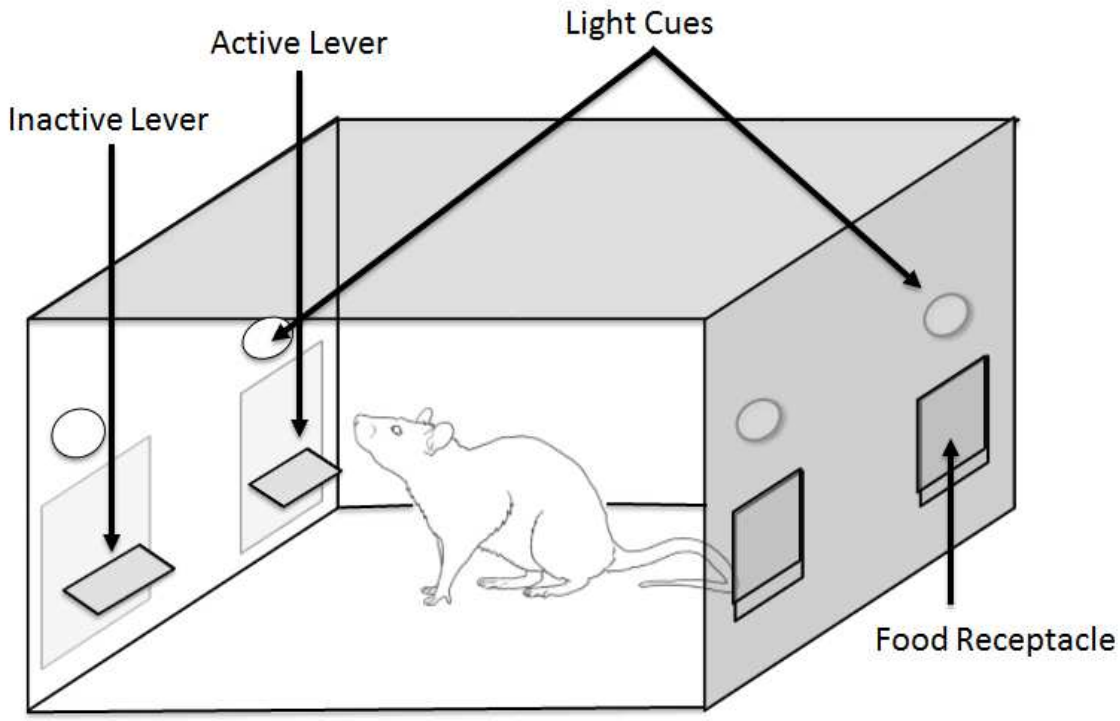

Figure 5. Configuration of the Operant Chamber for the Second Order Schedule of Reinforcement Test. While in the operant chamber, the rat will press the extended lever on the opposite wall of the food receptacle. Once the correct lever becomes active, a press will result in the retraction of both levers, illumination of the light cues above the active lever and the food receptacle, and the delivery of pellets. The rat will use the light cues to signal the availability of pellets.

Cue-induced suppression of feeding

The conditioned suppression of feeding task was modified from (Johnson & Kenny, 2010). Following stabilization of intake in the binge-like eating procedure, the operant sessions were shortened to 30 minutes until a new stable baseline of responding was established. Under these conditions, *Palatable* rats still show an intake greater than 2-fold that of *Chow* rats (not shown). The procedure consisted of three 30 minute phases: pre-conditioning, conditioning, and post-conditioning. During the 30 minute pre-conditioning session, rats were allowed to obtain food pellets (45-mg chow pellets for *Chow* rats or 45-mg sugar pellets for *Palatable* rats) and a tone was activated for 10 minutes, turned off for 10 minutes, and then turned back on for 10 minutes. Levers were retracted during the entire session in this procedure. Pellet delivery was paired with a light-cue located above the nose-poke hole. Four daily 30 minute conditioning sessions were performed in different self-administration boxes where no pellet dispensers were available. During the session, the same tone was activated for 10 minutes, turned off for 10 minutes, and then turned back on for 10 minutes. Rats received foot shock only during presentation of the cue tone (0.5 mA for 1.0 s; 10 stimulations with 1-minute intervals). During the 30 minute post-conditioning session, rats were allowed to obtain food pellets and the tone was activated for 10 minutes, turned off for 10 minutes, and then turned back on for 10 minutes. No foot shock was delivered. Food responses were recorded in the pre- and post-conditioning sessions as the main dependent variables.

Statistical analyses

Data was analyzed by simple or factorial analyses of variance (ANOVAs). Pairwise post-hoc comparisons were made using the Fisher's least significant difference (LSD) test and Student-*t* test was used to compare two groups. Statistical significance level was set at $\alpha \leq 0.05$.

RESULTS

Development of an Operant Model of Binge-like Eating in Rats

Rats (n = 59) allowed to self-administer the highly palatable diet for one hour per day rapidly increased their intake compared to the control group which had access to the chow diet (Food: $F(1,57) = 67.68$, $p \leq 0.001$, Food*Day: $F(11,627) = 23.86$, $p \leq 0.001$, **Figure 6**). As we previously showed (Cottone et al., 2012; Velázquez-Sánchez et al., 2014), body weights did not differ between *Palatable* fed rats and *Chow* fed rats (not shown).

Palatable rats show conditioned place preference for palatable food

In the conditioned place preference experiment ($n = 8$), the analysis of the CPP scores indicated that *Palatable* rats spent significantly more time in the initial non-preferred compartment (biased procedure), which was paired with the palatable food, when compared to either the pre-conditioning or the *Chow* control group (Food: $F(1,6) = 7.87$, $p \leq 0.05$; Day: $F(1,6) = 6.08$, $p \leq 0.05$; Food*Day: $F(1,6) = 5.70$, $p \leq 0.05$, **Figure 7**). No effect was observed in *Chow* rats.

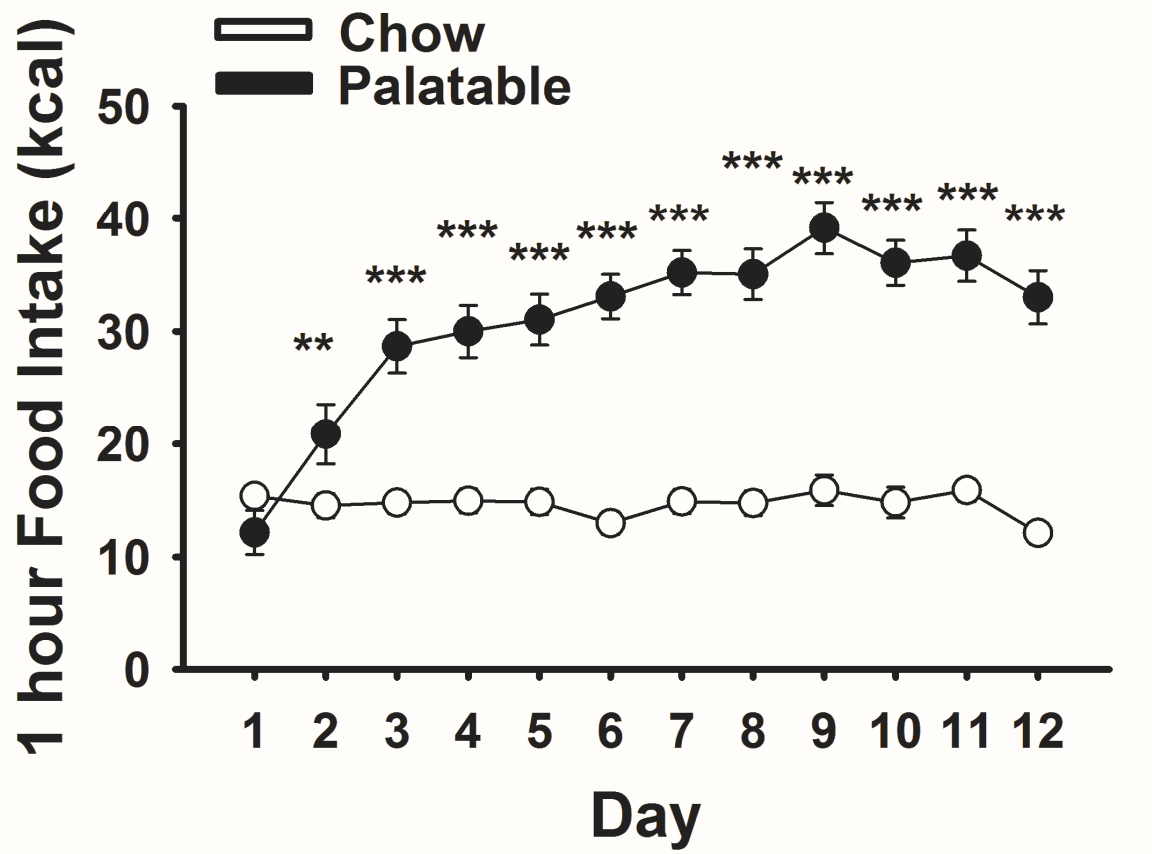

Figure 6. Rats Escalate Their Intake When Exposed to Highly Palatable Food. Effects of daily 1 hour per day self-administration of a highly palatable diet on food intake in male Wistar rats. Data show M ± SEM. $^{**}p \leq 0.01$, $^{***}p \leq 0.001$ *Chow* vs *Palatable*.

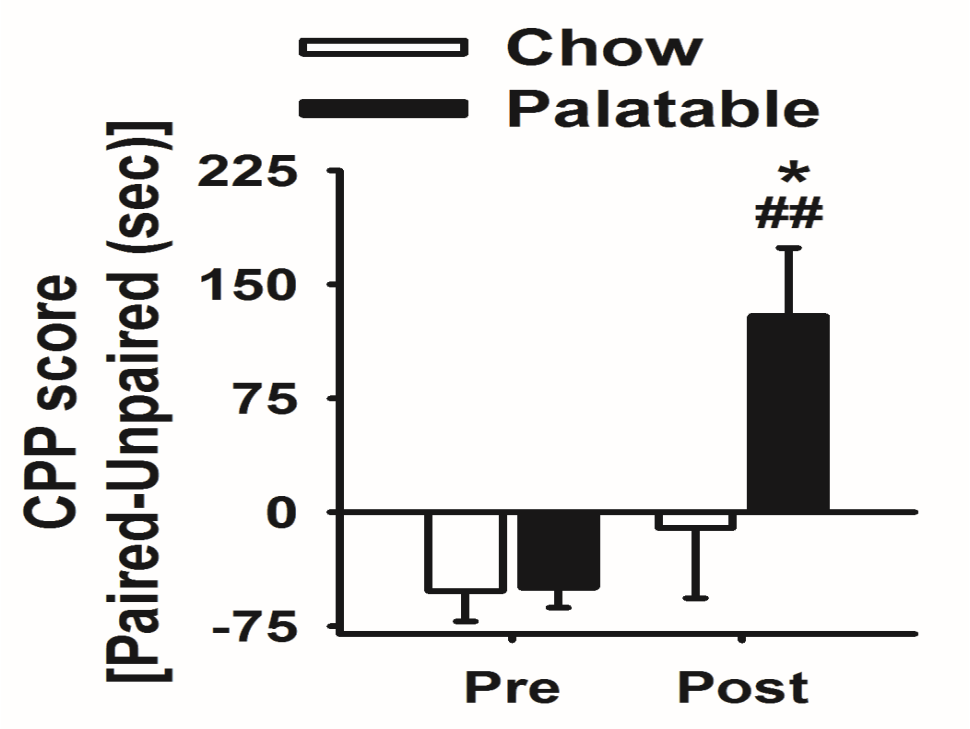

Figure 7. Increased Place Preference in Rats Exposed to Highly Palatable Food. *Palatable* animals showed enhanced conditioned food reward compared to *Chow* control animals when tested in a conditioned place preference paradigm in which the food was located in the least preferred compartment. Data show M ± SEM. *$p \leq 0.05$ **$p \leq 0.01$, ***$p \leq 0.001$ *Chow* vs *Palatable*. ##$p \leq 0.01$, Postconditioning vs preconditioning.

Palatable rats show heightened seeking behavior for palatable food

In the second order schedule of reinforcement task (*n* = 14), ANOVA analysis showed that *Palatable* rats performed a higher number of responses on the active lever compared to the *Chow* group in both the first interval and the remaining intervals (Food: $F(1,12) = 8.99$, $p \leq 0.02$, **Figure 8**). No difference in the number of inactive lever responses were observed (Food: $F(1,12) = 2.40$, *n.s.*).

Palatable rats show resistance to conditioned punishment

In the conditioned suppression of feeding test (*n* = 37), when exposed to the tone which was previously paired with the foot shock, *Chow* control animals significantly decreased their intake compared to their intake during the pre-conditioning phase (i.e. when the tone was still a neutral stimulus). Conversely, the conditioned punishment did not exert any effect on responding for the sucrose diet in *Palatable* rats (Food: $F(1,35) = 102.86$, $p \leq 0.001$; Day: $F(1,35) = 4.00$, $p \leq 0.05$, **Figure 9**). Specifically, control *Chow* rats showed a ~32% reduction in the intake of the standard chow diet while *Palatable* rats were resistant to the aversive properties of the cue showing a reduction of just ~3% ($t(35) = 3.13$, $p \leq 0.005$).

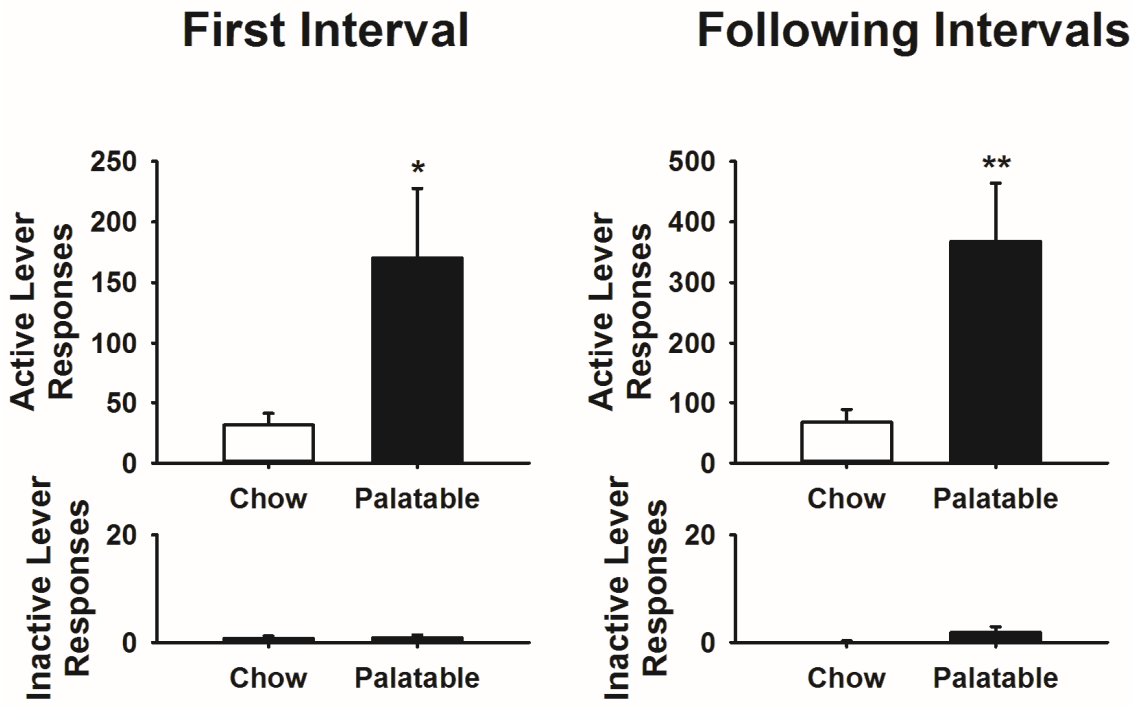

Figure 8. Increased Food Seeking in Rats Exposed to Highly Palatable Food. Under a second order schedule of reinforcement, *Palatable* rats show a 4 to 6-fold increase in food seeking responding (both during the first and the following intervals) compared to control *Chow* rats. No effect was observed during the first or during the following trials in the number of inactive lever responses. Data show M ± SEM. *$p \leq 0.05$ **$p \leq 0.01$, ***$p \leq 0.001$ *Chow* vs *Palatable*. ##$p \leq 0.01$, Postconditioning vs preconditioning.

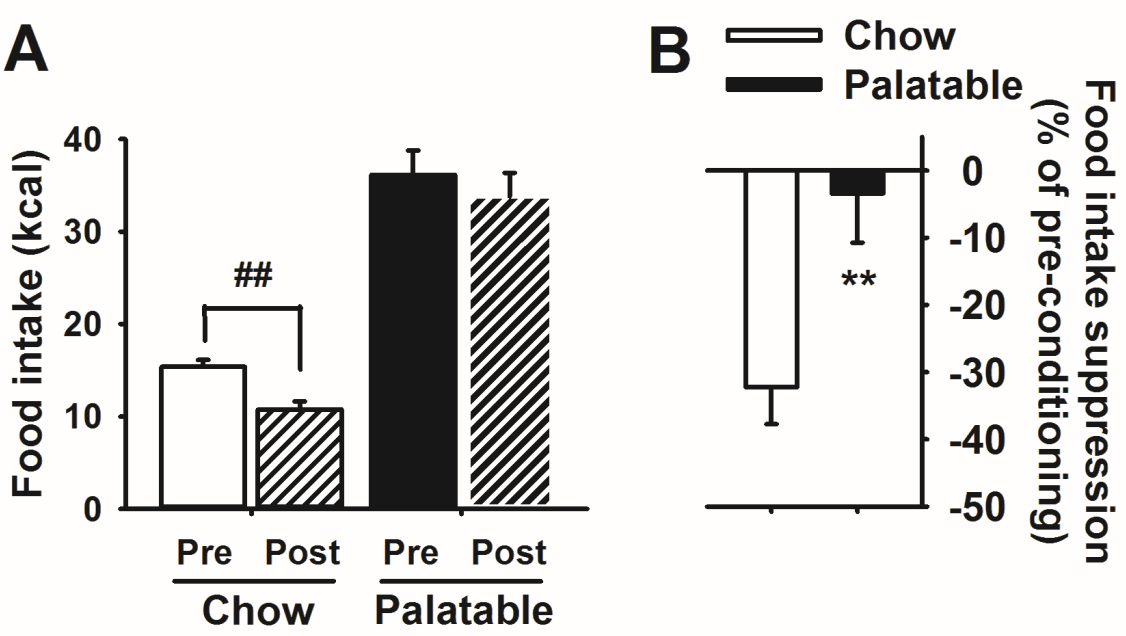

Figure 9. Resistance to Cue-induced Suppression of Feeding in Rats Exposed to Highly Palatable Food. *Palatable* rats showed resistance to the suppression of feeding behavior by a cue previously associated with a foot shock. The presentation of an aversive cue decreased the intake of control *Chow* rats by ~32%. Conversely, *Palatable* rats' intake was resistant to the aversive properties of the cue (~3% decrease). Data show M ± SEM. *$p \leq 0.05$ **$p \leq 0.01$, ***$p \leq 0.001$ *Chow* vs *Palatable*. ##$p \leq 0.01$, Postconditioning vs preconditioning.

DISCUSSION

Rats undergoing operant limited access to a sucrose diet dramatically escalate responding and show excessive food intake in a short period of time, heightened motivation for food, and loss of control over intake (Blasio et al., 2014; Cottone et al., 2012; Velázquez-Sánchez et al., 2014). We found that *Palatable* rats showed increased conditioned place preference, heightened seeking behavior, and developed inflexible responding for palatable food compared to *Chow* control rats. The results of this study further characterize the phenotype of *Palatable* rats providing evidence that binging rats show multiple maladaptive forms of conditioned feeding behavior.

Conditioned place preference is a form of Pavlovian conditioning or classical conditioning. This type of conditioning pairs a neutral stimulus with an unconditioned stimulus that transfers its effects to the new conditioned stimulus. In the case of the conditioned place preference paradigm, the chamber is the neutral stimulus and the rewarding properties of the substance being consumed are transferred to the previously neutral tactile and visual cues of the paired chamber (Bardo, Rowlett, & Harris, 1995; Cunningham, Gremel, & Groblewski, 2006). When re-exposed to the paired chamber in the absence of substance consumption, the animal has associated the tactile and visual cues of the specific chamber with the previously felt reward resulting in the animal spending more time in the paired chamber (Kummer et al., 2014). If the substance has aversive effects on the animal, the opposite will occur and less time with be spent in the

paired chamber due to association with negative properties (Cunningham et al., 2006; Sun et al., 2014). Conditioned place preference has been extensively used to evaluate the rewarding properties of drugs of abuse. In studies using animal models of drug abuse and addiction, it has been reliably demonstrated that cocaine and other opiates cause an increased conditioned place preference in test animals compared to control animals (Liddie & Itzhak, 2014; Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982). Ethanol has also been shown to induce conditioned place preference in rats (Carnicella, Ron, & Barak, 2014).

In the present study, we show that in *Palatable* eating rats, tactile and visual cues associated with the palatable diet can acquire rewarding properties as measured in a conditioned place preference paradigm. Under the same conditions, cues associated with the regular chow diet fail to elicit place preference. Conditioned place preference investigates the effects of rewards on the salience of contextual stimuli in which they are experienced; therefore, contextual neutral stimuli, through an associative learning process, acquire rewarding properties that are expressed in the absence of the reward. Hence, conditioned place preference measures an associative conditioned process that is fundamentally distinct from drug/food self-administration. It has been extensively used to study the salience of drugs/food associated contextual cues that set the stage for behaviors to be engendered (Bardo & Bevins, 2000). A limitation of the biased procedure is that the results could be potentially confounded by a reduced aversion for the non-preferred compartment; however,

a reduction in aversion is a positive experience which could be argued as one of the components that elicit the conditioned preference. Nevertheless, both drugs and natural rewards generally have similar effects in both biased and unbiased procedures (Tzschentke, 2007). It is relevant to mention that it is unlikely that the conditioned place preference results are due to differences in locomotor activity because we have previously showed that *Chow* and *Palatable* rats do not differ in this measure (Cottone et al., 2012).

A second order schedule of reinforcement test is a result of operant conditioning. The animals are trained in such a way that they learn to lever press and wait for a light to signal the availability of the substance. This procedure is commonly used to evaluate drug seeking behavior which is one of the main characteristics of drug addiction. When rats are exposed to addictive substances such as heroin or cocaine, they display strong drug seeking behavior in a second order schedule of reinforcement (Economidou, Dalley, & Everitt, 2011; Giuliano, Robbins, Wille, Bullmore, & Everitt, 2013; Murray, Belin, & Everitt, 2012).

In this study, *Palatable* animals trained in a second order schedule of reinforcement for palatable food showed a very high rate of responding, exhibiting a ~4 to ~6 fold increase in seeking behavior compared to control chow responding rats. The higher rate of responding was evident in both the first interval, before any intake of food has occurred, as well as in the following intervals. The second order schedule of reinforcement is a widely used task to measure seeking behavior for food and drugs of abuse in which responding is

maintained not only by the primary reinforcer but also by contingent presentation of food-paired stimuli that serve as conditioned reinforcers of instrumental behavior (Everitt & Robbins, 2000; Giuliano et al., 2012).

Both the conditioned place preference for food and the food seeking behavior tasks are highly relevant in the investigation of the mechanisms underlying maladaptive behaviors driven by stimuli associated with palatable food. Indeed, it is well established that palatable food-associated environmental cues exert a powerful control over feeding behavior in people and have the power to override energy-homeostasis signals and trigger binge eating episodes (Ng & Davis, 2013).

It is also relevant to discuss the important role that cues play in drug relapse. The use of addictive substances such as cocaine has been shown to activate the limbic system and cause the user to associate memories with drugs of abuse (Childress et al., 1999; Garavan et al., 2000). These memories allow for cue-induced continuation of drug use or relapse from abstinence as seen in animal models (Guercio, Schmidt, & Pierce, 2015). By showing the presence of cue-induced behaviors in food seeking and conditioned place preference following our operant model of binge-like eating, we have captured this important aspect of addiction. Our model instills these cue-induced memories that drive addiction, thereby further characterizing it as a model for binge eating disorder.

The progression from substance abuse to addiction is defined as a loss of control over the behavior (American Psychiatric Association, 2013). In humans,

this could be determined by the behavior costing the individual their job, having a negative effect on their family, or continued participation regardless of any other extreme aversive results. In the present study, we examined this idea of a loss of control by conditioning rats to receive a foot shock when a specific tone is made within the operant boxes. After conditioning, we allowed the animals to receive pellets in the operant boxes while playing the same tone but without a foot shock. The results show that *Palatable* rats develop inflexible responding for the highly palatable, sucrose diet measured as resistance to the behavioral suppressing properties of a conditioned aversive stimulus. The persistence of instrumental behavior despite the presence of conditioned aversive stimuli is one of the strategies used to operationalize the construct of loss of control in addictive disorders as it depicts the compulsive nature of the disease (Johnson & Kenny, 2010; Vanderschuren & Everitt, 2004). Previous findings have shown that rats with a history of cocaine exposure became inflexible and resistant to conditioned punishment (Vanderschuren & Everitt, 2004). Similarly, intake of rats that had a daily extended access to a palatable, cafeteria diet became insensitive to the anorexic effects of a cue predicting punishment (Johnson & Kenny, 2010). Our results show that inflexible responding occurs in rats with limited operant access to a highly palatable diet.

Although we assumed that a certain history of palatable food is necessary to develop the behavioral profile observed in the *Palatable* animals, we cannot completely exclude the possibility that the palatable pellet itself supports stronger

food seeking across the different behavioral tests. It is possible that single exposure or short access to this type of food might produce some of the effects observed to a certain degree. Further experiments would be needed to address this possibility.

Unlike previous reports (Delamater, Sclafani, & Bodnar, 2000; Giuliano et al., 2012), in the present study, food restriction or deprivation was not used; this feature minimizes any possible confounding factor that might be driven by a negative energy balance, and it also allows us to dissociate the mechanisms underlying behaviors triggered by the rewarding properties of the palatable diet from behaviors induced by purely homeostatic needs. An operant model of binge-like eating in non-deprived subjects, therefore, better models what occurs in Western societies where palatable food is omnipresent and overeating is triggered by environmental cues (Wardle, 1990).

The importance of continuing to characterize our operant model of binge-like eating lies in its application. Recently, our laboratory has used this model to establish the potential use of memantine in the treatment for binge eating disorder (Smith et al., 2014). We have also used it to examine the significance of impulsivity as a driving factor for addiction and to discover neural pathways involved in excessive eating behavior (Cottone et al., 2012; Iemolo et al., 2013; Velázquez-Sánchez et al., 2014). By showing that rats display these maladaptive forms of feeding behavior, as seen in the current study, after being subjected to our operant model, we bring more evidence that this is a valuable model to work

with to continue searching for answers in the field of binge eating disorder. The validation of an animal model for binge eating disorder is important because, as of now, there is only one drug recently approved by the Food and Drug Administration (FDA) for treating binge eating disorder with unknown long term efficacy (McElroy et al., 2015; “Press Announcements - FDA expands uses of Vyvanse to treat binge-eating disorder,” n.d.). With a model such as ours, we can safely continue searching for more treatment options.

In summary, in this study we continued the characterization of a novel operant model of binge-like eating induced by providing limited access to a highly palatable, sucrose diet. This animal model is “multisymptomatic” (Belin & Deroche-Gamonet, 2012) as *Palatable* rats show multiple excessive food-related behaviors which mimic the different aspects of the symptomatology observed in subjects affected by binge eating disorder. Our results will help to further characterize our binge-like animal model and provide additional information regarding the potential addictive properties of highly palatable food. Better understanding of the underlying neurobiological substrates of binge eating disorder will help improve the identification of novel potential targets for pharmacological intervention.

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CURRICULUM VITAE

JEFFREY W. SANTOS

942 7th Street ● Hermosa Beach, CA 90254 ● (925) 522-1164
jeffwsantos@gmail.com ● DOB: 1988

SKILLS

- Strong academic background in biological sciences with laboratory experience and biostatistics.
- Has internship and work experience with patients in a clinical setting.
- Quickly learns and masters new techniques and skills; works equally well in a team or alone; is competent with computers and programs such as MS Word, PowerPoint, and Excel.

EDUCATION

Boston University School of Medicine – Boston, MA — Expected May 2015
Master of Science, Medical Sciences

University of California, Davis – Davis, CA — March 2011
Bachelor of Science, Exercise Biology
GPA: 3.59

EXPERIENCE

Laboratory Experience

Post-graduate Researcher – Laboratory of Addictive Disorders at Boston University School of Medicine in Boston, MA

May 2014 to Present

- Studies Binge Eating Disorder as an addiction-like disorder using rat animal models.

Volunteer Experience

Emergency Department Volunteer – Boston Medical Center in Boston, MA

January 2014 to Present

- Assists emergency department staff with preparing stretchers and consoling patients.

Server and Food Pantry Volunteer – Rosie's Place in Boston, MA

October 2013 to Present

- Helps prepare and serve meals to the homeless. Assists with food distribution in the food pantry.

Employment

Graduate Medical Science Tutor – Boston University School of Medicine in Boston, MA

September 2014 to December 2014

- Tutored graduate students in Physiology and Biochemistry and Cell Biology.

Physical Therapy Aide - Ho Physical Therapy in Beverly Hills, CA

June 2011 to December 2012

- Set up patients on hot packs and cold packs, administered ultrasound, muscle stimulation, spinal traction, myofascial release, and other rehabilitation techniques and modalities.
- Helped with patient scheduling, charting, inventory monitoring and ordering, and other general office tasks.

Office Assistant – Inner Action Sports Rehab in Walnut Creek, CA

January 2011 to April 2011

- Performed Patient scheduling and assisted with patient flow in and out of the office.
- Taught patients how to properly and safely perform rehabilitation exercises.
- Assisted with recording new patient information such as personal and family medical history.

Internship

UC Davis Student Athletic Trainer — August 2008 to June 2010

- Provided injury evaluation, rehabilitation and sports taping for UC Davis intercollegiate athletes under supervision of Certified Athletic Trainers.

- Documented all injuries and rehabilitation programs using SOAP notes (Subjective, Objective, Assessment, and Plan)

- Worked in a rehabilitation clinic and on field during practices and home games of all UC Davis teams as well as traveled with the baseball team during the 2010 season.
- Maintained CPR and First Responder certifications.

UCDMC Physical Therapy Clinic – Outpatient April 2009 to June 2009

- Assisted and observed physical therapists at the UC Davis Medical Center.

Shadowing

Dr. Manuel Diaz, D.O. – Woodland, CA January 2010 to March 2010

- Observed a Family Practice doctor and learned how to apply assessment and documentation skills to a broader range of injuries, illnesses, and procedures.

Surgery Observation

Dr. Kirk Lewis, M.D. – UCDMC in Sacramento, CA March 3, 2010

- Observed the removal of a foreign body in a knee joint that was causing an inflammatory response and a rotator cuff repair.