IMPACT OF GHRELIN RECEPTOR ANTAGONISM ON NICOTINE ADDICTION & CESSATION RELATED WEIGHT GAIN/REGAIN

A Dissertation

by

JUAN ANTONIO RODRIGUEZ

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Paul Wellman
Co-Chair of Committee,	Shoshana Eitan
Committee Members,	Edward Funkhouser
	Arnold LeUnes
Head of Department,	Heather Lench

May 2017

Major Subject: Psychology

Copyright 2017 Juan Antonio Rodriguez

ABSTRACT

Nicotine (NIC) addiction remains a prevalent concern in the US. The number of users continues to climb while cessation success remains low due to the lack of successful available options for quitting. NIC, a well-known weight control substance, is appealing for the obese and those who fear becoming obese. Obesity, too, is a major concern that remains difficult to treat and overcome. One speculation is that NIC cessation-related weight gain provides an increased likelihood of relapse and continued use. Ghrelin (GHR), a gut peptide, has been implicated in the endogenous reward system of appetitive behaviors, including smoking and unhealthy dietary intake. The effects of GHR on feeding and psychostimulant function, accompanied by findings on GHR receptor (GHR-R) manipulation, has lead us to investigate possible pharmacological dual-treatment approaches for both NIC dependence and smoking cessation-related weight gain. The mechanisms of action for NIC and literature supporting GHR's involvement in NIC addiction that favor a dual-treatment hypothesis are investigated here.

Experiment 1 examined the antagonistic effects of the GHR-R antagonist, JMV-2959, and the agonistic effects of GHR on intracranial self-stimulation (ICSS). Experiments 2-3 examined the effects of JMV-2959 on NIC and cocaine intravenous self-administration (IVSA). Experiment 4 examined the effects of GHR on cocaine IVSA. Experiment 5 examined the effects of JMV-2959 on NIC-cessation related high-fat (HIFAT) food intake and weight gain. Experiment 6 examined the potential of JMV-

2959 to induce malaise in a conditioned taste aversion (CTA) task.

The results for Experiment 1 show that JMV-2959 and GHR alone are incapable of mitigating reward-seeking behavior in ICSS. Experiments 2-4's results show that JMV-2959 diminishes the reinforcing effects of NIC and COC IVSA, while GHR alone failed to alter COC IVSA. Experiment 5's results support the notion that JMV-2959 may be a useful tool in curbing the general weight gain and NIC cessation-related weight gain. Experiment 6's results confirm that JMV-2959 does not induce malaise. Collectively, these experiments provide evidence that GHR is involved in reward-behavior and that antagonism of GHR-Rs may provide a serious pathway for the dual-treatment of NIC addiction and weight gain, potentially mitigating drug-seeking for other psychostimulants.

DEDICATION

This dissertation work is dedicated to my parents and their parents,

who paved the way to my success with their blood, sweat and tears…

…and to those I've lost along the way.

Your memory guides me.

May we meet again.

ACKNOWLEDGEMENTS

First, I'm thankful for my committee chair, Paul Wellman, who has been the mentor that I desired when choosing to attend graduate school. Attending graduate school was a fearsome decision and, if it were not for his mentoring, involvement and care for my success, as well as understanding, I would never have made it. He has had patience for my troubles and frustrations along the way and has understood my perspective and approach to life. I want to say thank you for everything along the way.

I also have my committee members to thank: Shoshana Eitan, whose laugh could make anyone's day, while her words and questions motivate one to continue to progress forward. Edward Funkhouser, who has been there since day one of my collegiate run and has always challenged me to look and think outside the box by approaching the seemingly normal in a creative manner regardless of standards. And lastly, Arnold LeUnes, a man who has helped me see life at face value so that it could open my eyes to seeing something and someone bigger ahead. All of you have been patient with me throughout this process and have made yourselves available to me at moment's notice. My success lies in every one of you and in so many more.

I would also thank my friends, Shawn Bates, Michael Emery, and Shane Clifford, for providing a serious work dynamic rich in humor. My girlfriend Emily Zuber who keeps me grounded and pushes me to excel when I encounter doubt and lack of self-confidence. Lastly, I would like to thank my parents, who have done nothing but support me in becoming the person I am today, the person many doubted I could be.

NOMENCLATURE

CHOW..... standard chow

COC ..... cocaine

CPP ..... conditioned place preference

DA ..... dopamine

FR.....fixed ratio

GHR.....ghrelin

GHR-R.....growth hormone secretagogue receptor, ghrelin receptor

HIFAT ..... high fat diet

ICSS ..... intracranial self-stimulation

ICV..... intracerebroventricular

IM ..... intramuscular

IP.....intraperitoneal

IVSA .....intravenous self-administration

LDTg .....laterodorsal tegmental area

MFB ..... medial forebrain bundle

NAc .....nucleus accumbens

nAChR.....nicotinic acetylcholine receptor

NIC.....nicotine

NRT .....nicotine replacement therapy

VTA ..... ventral tegmental area

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of Professor Paul J. Wellman [chair], Associate Professor Shoshana Eitan [co-chair] and Professor Arnold LeUnes [member] of the Department of Psychology and Emeritus Professor Edward Funkhouser [member] of the Department of Biochemistry.

The data for Chapter III were collected with the assistance of Samuel Hughes and Destri Escobar (Schul) of the Department of Psychology. The data for Chapter VI were collected with the assistance of the Nigel Hart of the Department of Psychology.

All other work independently conducted for the dissertation was completed by the student.

Funding Sources

Graduate study was made possible in part by NIDA under Grant Number 5R01DA013188-07 and by internal funds held by PJW. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute of Drug Abuse.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Nicotine (NIC) addiction and obesity are two of the leading health concerns/issues that contribute to progressive increases in health costs as well as increased morbidity rates across the United States (US). Some 10-25% of 12th graders smoke daily and approximately 21% of adults in the US currently smoke cigarettes; a number that is rising with every year (CDC, 2004). Due to the health risks associated with smoking NIC, the need for successful cessation options must be taken into consideration and developed. Briefly, NIC is a tertiary amine alkaloid that is membrane permeable in its uncharged form. It is one of the major ingredients in tobacco and its mechanism of action (i.e. receptor activation) is at nicotinic acetylcholine receptors (nAChR), which it selectively binds to for activation of neurons (Benowitz 2010).

Via nAChRs, NIC increases midbrain dopamine (DA) neuron firing, increases phasic bursts, while primarily elevating prefrontal cortex (PFC) and nucleus accumbens (NAc) DA activity, as well as increasing DA availability in other target regions (DeBiasi et al., 2011; Imperato et al., 1986, Grenhoff et al., 1986, Mameli-Engvall et al., 2006, Pons et al., 2008, Zhang et al., 2009). Of particular interest is that excitation of ventral tegmental area (VTA) DA neurons via NIC binding at nAChRs results in the induction of behavioral sensitization via the NAc (Vezina, 2004) (Figure 1; Berton and Nestler, 2006). This VTA to NAc connection is better known as the brain's reward circuit. Actively pressing a NIC-infusing lever results in the animal acquiring a long-lasting

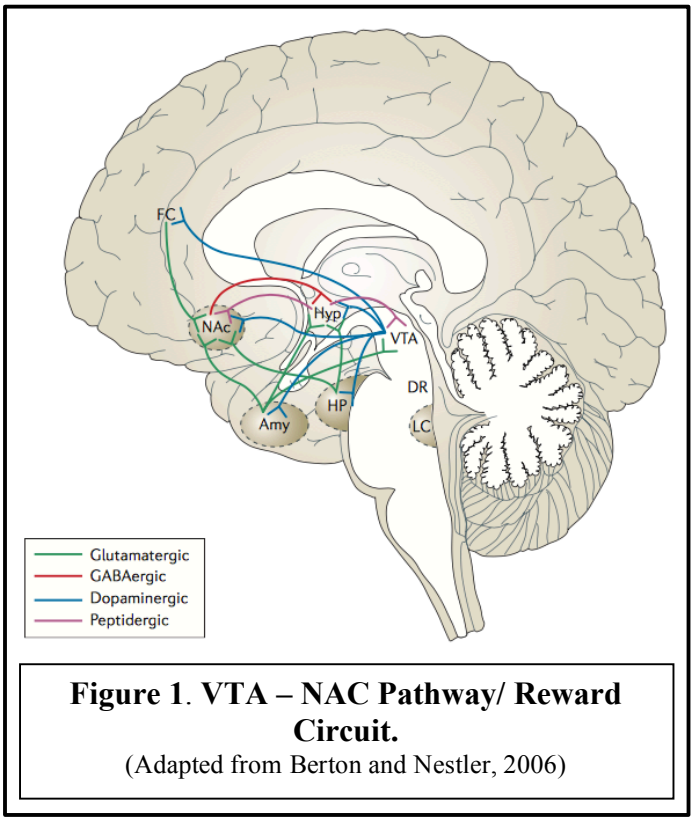


Figure 1. **VTA – NAC Pathway/ Reward Circuit.**
(Adapted from Berton and Nestler, 2006)

sensitization of midbrain DA neuron reactivity that will affect continued NIC-related behaviors. These neuromechanistic changes that result in sensitization play a role in the initiation, maintenance and escalation of NIC drug use (Changeux, 2010).

NIC intake for human users occurs through several routes, primarily including, but not limited to, tobacco products. The most concerning form of use is through smoking tobacco-containing cigarettes. Cigarettes consist of shredded tobacco, rolled into a paper sleeve, with or without a filter, which is then lit by the user and smoked via vapor inhalation through the mouth. The smoke delivers NIC to the lungs, where it is taken up rapidly into the bloodstream and delivered to key areas of action within the

brain. While the other chemical properties of cigarettes are not the focus of this dissertation, it is important to iterate that NIC is not the only component of cigarette smoke.

Tobacco smoke contains around 4000+ chemicals that, when smoked, are generally referred to as "tar", however these chemicals can include, but are not limited to: NIC, tar, carbon monoxide, formaldehyde (a preservative), ammonia (a household cleaner), hydrogen cyanide, arsenic (used in rat poisons), DDT (a banned insecticide), benzene (used to make dyes and synthetic rubber), cadmium (used in batteries), nickel, ethyl furoate (causes liver damage), methoprene (a form of insecticide), polonium (a cancer-causing radioactive substance), methyl isocyanate (a toxin that killed 2000 people in India in 1984) (Agarwal and Bose, 1992), and/or lead (Rabinoff, 2007). While NIC addiction does not lead to severe behavioral and cognitive issues, as does cocaine (COC) and/or methamphetamine, it is well understood that chronic NIC use via tobacco smoking compromises health and leads to early mortality.

Several health issues that arise from chronic smoking include: smoker's syndrome, increased susceptibility to pneumonia, bronchitis, emphysema, cancers of the bladder, esophagus, kidneys, lungs, larynx, mouth, pancreas, and/or stomach, as well as an increased risk of heart attack and/or stroke due to cardiovascular disease. Those exposed to second hand smoke, including the unborn, show an increased likelihood of heart disease, cancer, and premature death (NIH Cancer Institute, 2014).

NIC accounts for 6 million annual deaths, with more than 8 million deaths likely to occur annually by the year 2030 (Jha et al., 2013). This translates to about 1 out of

every 10 deaths being attributable to a NIC related issue (Degenhardt and Hall, 2001). From the perspective of the US, Perkins and Lerman (2014) report that 50 million Americans smoke and that smoking accounts for 20% of the mortality in the US – the latter translating to about 400,000 preventable deaths per year.

Research indicates that most smokers are aware of these health risks and many would like to quit. A major issue is that of those who know and desire this, only a few will actually make the attempt to quit (Perkins and Lerman, 2014). In fact, few will succeed in their quitting attempts. Seventy percent of smokers express a desire to quit, however ~80% attempting to quit relapse into smoking again within a month (Dani et al., 2007). Only 5% or fewer will actually succeed in quitting for up to a year. Most of these individuals used an anti-smoking medication to quit, a topic I will also be discussing in this dissertation (Perkins and Lerman, 2014). Of those who can quit for over a year, only 20% will manage to refrain from smoking for more than 2 years, and only 3% will be completely successful in their cessation attempts (Benowitz, 2010). These numbers strongly document the severity of the dependence to NIC evident in smokers and the difficulty they encounter in cessation compared to other drugs of abuse. Thus, one can see why we seek to understand the addictive properties and mechanisms of NIC and how we may prevent and/or eliminate addiction, preventing untimely death of the user, potential user, and those who encounter second hand smoke.

A second, and less dangerous, route of NIC intake worth mentioning is NIC use through chewing tobacco and snuff/dip. Through this route, the user takes a chunk of cut tobacco and places it in their mouth for chewing or places it in-between their gums

and the wall of their mouth. Here the NIC within the tobacco is absorbed into the saliva and then into blood vessels of the walls and gums of the mouth for delivery to the brain. Typically, the user periodically spits out the saliva, but some users swallow the saliva, which poses a greater health risk for these users beyond the increased likelihood of gum disease that comes with this form of tobacco use.

A few final routes of NIC ingestion discussed here are nicotine replacement therapies (NRTs). NRTs may involve NIC-laden chewing gum that functions like chewing tobacco without compromising the user's health if swallowed, transdermal patches that are placed on the surface of the skin for delivery through permeation, and lastly, electronic cigarettes, or eCigs, in which a chemical mixture containing NIC is vaporized and delivered to the lungs. eCigs are a largely popular substitute for smoking, however their comparable safety is still currently under investigation. These NRTs are primarily sought out and used by individuals who are attempting to quit smoking/dipping.

Obesity, briefly mentioned earlier, has also been and remains a major health concern in the US as well as other Western European societies. In spite of efforts to identify the underlying metabolic, hormonal, and behavioral underpinnings of this disorder, there is evidence that the prevalence of obesity is increasing in American children, adults and the elderly (Fakhouri et al., 2012; Flegal et al., 2012; Ogden et al., 2012, Olshansky et al., 2005). The National Health and Nutrition Examination Survey found that adult (ages starting at 20 and above) obesity prevalence doubled from 1976 to 1980 and then again from 2007 to 2008, with 34.2% of individuals being overweight,

33.8% being obese, and about 5.7% falling into the extremely obese category (CDC, 2004). In fact, prevalence of obesity has almost doubled for white, black, and Mexican-American men from the 1988 to 1994 data to the 2009 to 2010 data, while increasing half-fold to one-fold for women of those same categories (Fryar et al., 2012). Wang et al., 2008 found, given this National Health and Nutrition Examination Survey's data, that current linear projection rates would classify more than 51% of Americans as obesity by 2030. A recent evaluation by Levi et al., in 2012 concurred with this notion, speculating that 44% would be obese by 2030 (Levi, 2013)

When discussing obesity, it is not uncommon for social stigmatizations to be leveled at the obese. Thirty years ago, Vener and Krupka (1985) published findings that focused on these social stigmatizations. They state:

> We have found that college students are more reluctant to marry the obese than to marry cocaine users, ex-mental patients, shop-lifters, the sexually promiscuous, communists, the blind, atheists, marijuana users, and the divorced. More men than women were disinclined to marry the obese. We also discovered that the obese are stigmatized to approximately the same degree as prostitutes and embezzlers. The obese find it difficult to obtain jobs that require attractiveness as a prime criterion of employment, e.g., flight attendant, receptionist, television commentator, hostess, or maître d'hôtel. (Vener and Krupka, 1995)

These social stigmatizations of the obese have not declined with time and indeed likely show higher incidence due to increases in media influences evident on television and the Internet. While all groups are affected by this, the youth and women are often targeted and held to a more unrealistic body weight standard than are adult males, consequently making obesity a more difficult psychological state for the youth and women.

In recent years, considerable effort has sought to determine effective treatments for obesity - including drugs that alter metabolism, diminish nutrient absorption or suppress appetite (Bray, 2000). An important component of chronic NIC use, for many users, is the potential benefit of weight control afforded by NIC use. Due to the negative view of the overweight stated above, many come to discover that smoking assists in weight control and this knowledge lead persons to either start NIC usage or to continue to use of NIC-containing substances.

In knowing that the obese may turn to smoking as a form of weight control, it is not surprising that Pomerleau and Kurth (1996) found that 75% of women versus 35% of men were unwilling to gain more than 5 pounds if it could be attributed to quitting smoking. The numbers were, of course, higher for willingness to gain 10 pounds or more. Sadly, this data is important because cessation of chronic smoking does result in weight gain as well as a number of aversive withdrawal symptoms (Benowitz 2010). Therefore, the individuals who begin to gain weight after NIC cessation will face an increased chance of relapse and may return to chronic NIC use to curb/prevent further weight gain and other withdrawal symptoms.

Smoking cessation, in fact, can lead to a 3-pound weight gain per 7 days of smoking cessation. Pomerlau and Kurth (1996) also found that at any point women who quit smoking would fall into 3 weight gain categories. Approximately 20-30% of women will gain less than 5 pounds, most absurdly, ~50% will gain more than 30 pounds (women who were probably using smoking to control a tendency to overeat), and the remainder will gain between 5 and 15 pounds. Given these facts, the belief that these

Macronutrient Information*	
7001 Teklad 4% Mouse/Rat Diet	
Crude Protein	25.2%
Fat (ether extract)	4.4%
Crude Fiber	3.3%
Energy Density	3.0 kcal/g
Calories from Protein	34%
Calories from Fat	13%
Calories from Carbohydrate	53%

Figure 2. Standard Chow Macronutritional Information.
(adapted from Envigo, 2015)

weight gain issues will likely increase the relapse rate for NIC in these users is not so farfetched.

While it is unknown how smoking is linked to weight regulation, it is speculated to involve a combination of factors including NIC reducing caloric intake, increasing activity, increasing metabolic rate, and reducing the formation of fat (lipogenesis) (Filozof et al., 2004). An uncommon practice in NIC studies, or most drug studies in which food consumption and weight gain are being measured, is a variation in diet type. The US is comprised of a society where multiple fast food restaurants exist within a given square mile in larger cities. Americans are capable of easily accessing and enjoying large quantities of proteins, sugars/high-fructose corn syrup, and fats, all of which in chronic excess can lead to obesity. However, in most animal studies with NIC, standard chow (CHOW) diet (shown in Figure 2; Envigo, 2015) is primarily and,

typically, solely used for the measure of impact of NIC on food intake and meal size. For example, free-feeding rats show a decrease of total CHOW intake and meal size when NIC is administered (Bellinger et al., 2003, 2005; Miyata et al., 2001; Grunberg et al., 1987; Blaha et al., 1998). These findings are reliable, but may not be representative of the effects on NIC on human food intake. For example, let us focus on diets of smokers versus non-smokers. To begin with, smokers generally weigh less, ~10 pounds on average, than nonsmokers with similar or more amounts of food (Perkins, 1992). What does a smoker's typical diet look like?

The study by Dallongeville et al., (1998) which summarize the differences between smokers and non-smokers across a multitude of studies for various diet intakes are presented in Figure 3. In this are presented standardized z-scores of energy, fat, protein, carbohydrates, alcohol, and fiber intake. Symbols that are larger than zero suggest an intake that is greater in smokers whereas a value that is less than zero suggests an intake that is smaller in smokers. In this study, smokers enjoy a greater amount energy intake (+4.9%), as well as a greater amount of fat intake (+3.5%), alcohol intake (+77.5%), saturated fat intake (+8.9%) and cholesterol intake (+10.8%) (not depicted here) relative to non-smokers. Smokers can even neglect fiber (-12.4%) and several essential vitamins to arrive at a weight that is, on average, about 10 pounds less than a non-smoker. This pattern of dietary intake in smokers is believed to result in an increased risk of coronary heart disease and, of course, cancer. These human data are not surprising given that Wellman et al., (2005) noted that NIC had a greater suppressive effect on food intake in rats consuming a HIFAT diet than rats consuming a CHOW diet.

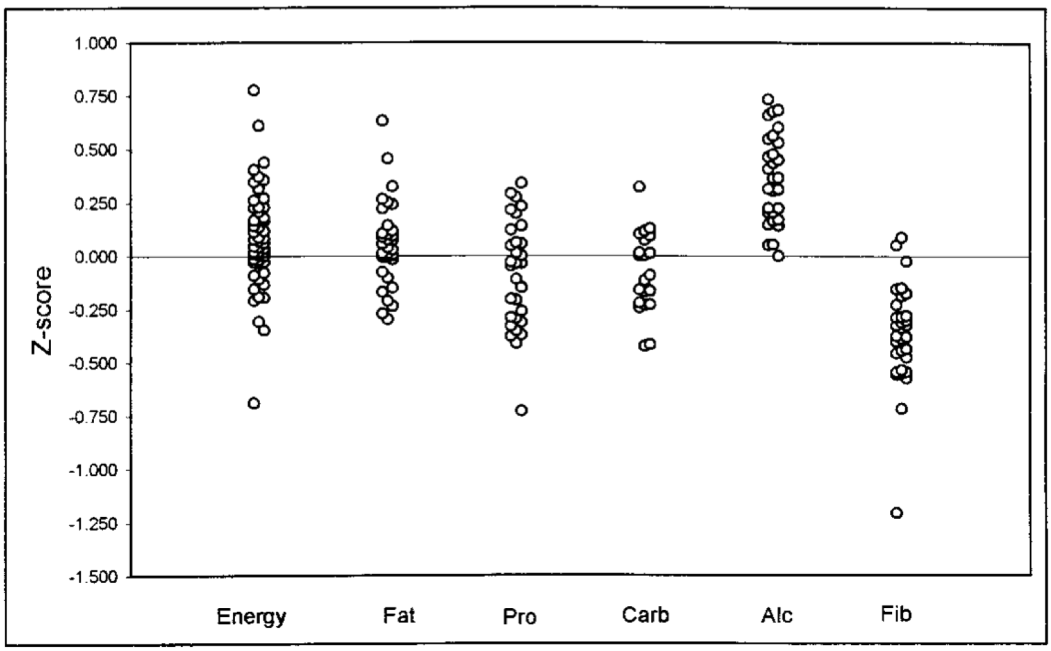


Figure 3. Dallongeville et al., 1998's Typical Smoker's Diet. Standardized difference (Z-score) of energy, fat (Fat), protein (Pro), carbohydrate (Carb), alcohol (Alc) and fiber (Fib) intakes between smokers and nonsmokers. Each circle represents the Z-score of an individual study. Positive Z-score differences indicate higher and negative differences indicate lower reported intakes in smokers than in nonsmokers. (Adapted from Dallongeville et al., 1998)

Moreover, when the NIC treatment was terminated, only rats on the HIFAT diet showed a rebound in food intake. This study remains one of the few animal studies using a palatable high-fat diet to assess the impact of NIC on food intake and body weight.

While the perceived benefit of weight control due to smoking may seem terribly attractive to some, the negative effects of tobacco use (i.e. greatly increased chance of cancer and other effects resulting in early mortality) far outweigh this positive effect with long term/chronic use and diet/lifestyle doesn't just end when a chronic smoker

decides to quit. The mechanism by which NIC controls weight control is currently not understood and is a topic of active investigation. Given what has been discussed so far, we can conclude that we should focus on smoking cessation treatment methods that target multiple issues, including preventing the rewarding effect of NIC on the brain, preventing NIC withdrawal symptoms, and finally preventing weight gain/regain. Given that food reinforcement and drugs of abuse such as NIC exert a shared common action on the brain reward circuit, it may be possible to develop multiple modality treatments for smoking. If we can prevent the weight gain/regain pharmacologically, or via diet control at a rehabilitation center, while aiding the NIC cessation (e.g. blocking NIC induced reward), a rise in smoking cessation success rates may soon follow.

Current options for aiding cessation in the elimination and prevention of both tobacco addiction and weight gain/obesity, both together and independently of one another, such as the NRTs previously discussed, remain unsuccessful. Of interest to us are current and widely used cessation options that utilize pharmacological approaches. They are intended for those who feel they are unable to quit via weaning or "cold turkey," but have an increased desire to quit for a variety of reasons. These pharmacological approaches include Food & Drug Administration-approved treatments such as NRTs, bupropion, and varenicline. Bupropion is marketed to consumer groups as Wellbutrin®, while varenicline is marketed as Chantix®.

NRTs are available in several delivery forms: polacrilex (NIC gum), transdermal NIC patches, lozenge (oral dissolving tablet), inhaler, and nasal spray (Rose, 2008). Paolini and De Biasi (2011) found that, although many smokers will relapse within 6–12

months, the use of NRTs can almost double quit rates compared to those given a placebo. This finding, however, is challenged by Harvard School of Public Health report in 2012 which showed that the chances of quitting are just as likely for those who quit smoking "cold turkey" versus those who seek out NRT approaches (Alpert, 2012). Adding to this are Rose et al.'s 2012 findings that the transdermal NIC patch is incapable of modifying reward processing, leading to abstinence-induced anhedonia. Anhedonia is the inability to feel pleasure and is often linked to depression and is a symptom of withdrawal commonly seen in NIC cessation. This negative symptom is therefore positioned to increase the likelihood of drug seeking and consequential relapse. Even worse is that some may turn to their feeding habits in an effort to mitigate anhedonia and find pleasure once again, thereby promoting weight gain associated with smoking cessation (Carton et al., 2000; Hughes, 1996).

Varenicline, or Chantix ®, produces significant reduction in negative affect and NIC craving in the absence of NIC (Jorenby et al., 2006), but not in restlessness, insomnia, or appetite, which the latter is a critical component for smoking cessation. Nearly 23% of 12-week varenicline users demonstrated a prolonged effect by abstaining successfully for 52 weeks. The relatively low success rate may be, in part, due to varenicline's side effect profile, as users encounter behavioral changes, feelings of depression, thoughts of self-injury, and suicidal behavior (McNeil et al., 2010; Hays and Ebbert, 2010, Paolini and De Biasi, 2011). Other side effects include: difficulty in breathing, hyperventilation, shortness of breath, wheezing, headaches, nausea, trouble sleeping, etc.

For bupropion, or Wellbutrin ®, it is speculated that the capacity of this drug to increase extracellular DA that normalizes NAc DA levels enough to mitigate the anhedonic and somatic withdrawal effects that come with NIC cessation (Li et al., 2002). However, it is also not side effect free. Commonly encountered side effects include, but are not limited to: anxiety, restlessness, irritability, shaking, dry mouth, irregular heartbeats, shortness of breath, hyperventilation, and sleeping difficulty. Other potential side effects during initial bupropion use include: constipation, a decrease in appetite, dizziness, an increase sweating, stomach pain, trembling and some weight loss. Bupropion is the only option, of the two, that has the ability to decrease appetite to allow for continued weight control upon smoking cessation.

A study published by Luis M. Tuesta et al. in 2011 summarizes the statistical success of these two options, however also stating the need to develop more effective drugs. Twenty-three percent of varenicline versus 16% of bupropion users succeed in smoking cessation for over a 1-year, while only 9% given a placebo have such success rates (Knight et al., 2010). Drugs like varenicline and bupropion are therefore useful, however the incidence of success in smoking cessation is still too low. Therefore, the mechanisms that allow for NIC addiction should be investigated so that we can develop better drugs, with more specificity that will allow for greater success. Overall, it can still be argued that, while there is little success, the benefits of both drugs, even when considering the withdrawal effects, outweigh the heightened risk of morbidity that eventually leads to the early death of half of all chronic and long-term tobacco users.

While both varenicline and bupropion are statistically effective in promoting

quitting, they do not address or target the weight gain among withdrawal symptoms generated upon smoking cessation. As mentioned, bupropion is the only pharmacological approach that has some effect in attenuating weight gain, and it does this poorly or temporarily with prevalence primarily in individuals that encounter depression upon cessation. Overall, all NRT's and pharmacological/prescription drug approaches fall short of what is important to our lab, fighting the NIC addiction **and** preventing the weight gain/regain associated with smoking cessation, something the current approaches have not focused on.

This has led us to investigate possible common grounds shared by NIC and unhealthy food-intake habits. This common ground would allow us to utilize a dual-treatment approach for reducing the health issues and morbidity rates of the NIC using population that may or may not also be battling obesity. Findings covered here and stated by Tuesta et al. (2011) have led us to investigate possible pharmacological approaches with greater specificity resulting in reduced side effects and dual-treatment capabilities.

A speculation was previously made above that the reinforcing properties of drugs and food might function via shared circuits. A study by Di Chiara et al. (2004) supports this notion in showing that DA overflow in the VTA to NAc reward circuit can be consequent to either psychostimulant or highly palatable foods and may have an additive effect when presented together. This brain region is also understood to be involved in the reinforcement of motivated behaviors and therefore places it as a prime target area

for this dual-treatment approach. Of the various manipulations that may alter VTA to NAc activity, a change in ghrelin (GHR) signaling is an ideal candidate.

GHR is secreted peripherally from the stomach and gut in a gradient fashion, higher levels from the stomach and lower levels as one travels along the gut. GHR enters the blood stream and then makes its way to the brain where it is known to primarily function as an orexigenic peptide (28 amino-acid; see Figure 4; St.Pierre et al., 2003) (Cummings et al., 2001, Kojima and Kangawa, 2005, Hosoda et al., 2006) by binding to the ghrelin receptor (GHR-R), a 7 transmembrane Gq protein coupled receptor (Howard et al., 1996; Damian et al., 2012, Uchida et al., 2013). GHR-R can be found on the vagus nerve, putting GHR in a position to alter vagus nerve signaling as well as entering through the blood brain barrier to act on brain GHR-Rs. The brain areas in which GHR-Rs are found include the VTA, hippocampus, arcuate nucleus of the hypothalamus, NAc, amygdala, and even the Edinger-Westphal nucleus which lies just dorsal to the VTA (Kaur and Ryabinin, 2010). Of specific interest are those GHR-Rs located on brain DA neurons found in the reward circuit coursing from the VTA to the NAc (Guan et al., 1997, Naleid et al., 2005, Abizaid et al., 2006, Diano et al., 2006). Most importantly, and discussed further below, is that GHR has been implicated in the modulation of drug and food reinforcement.

In regards to feeding behavior, GHR increases food intake and shifts preference to foods high in fat (Tschop et al., 2000; Shimbara et al., 2004). Chronic GHR induces

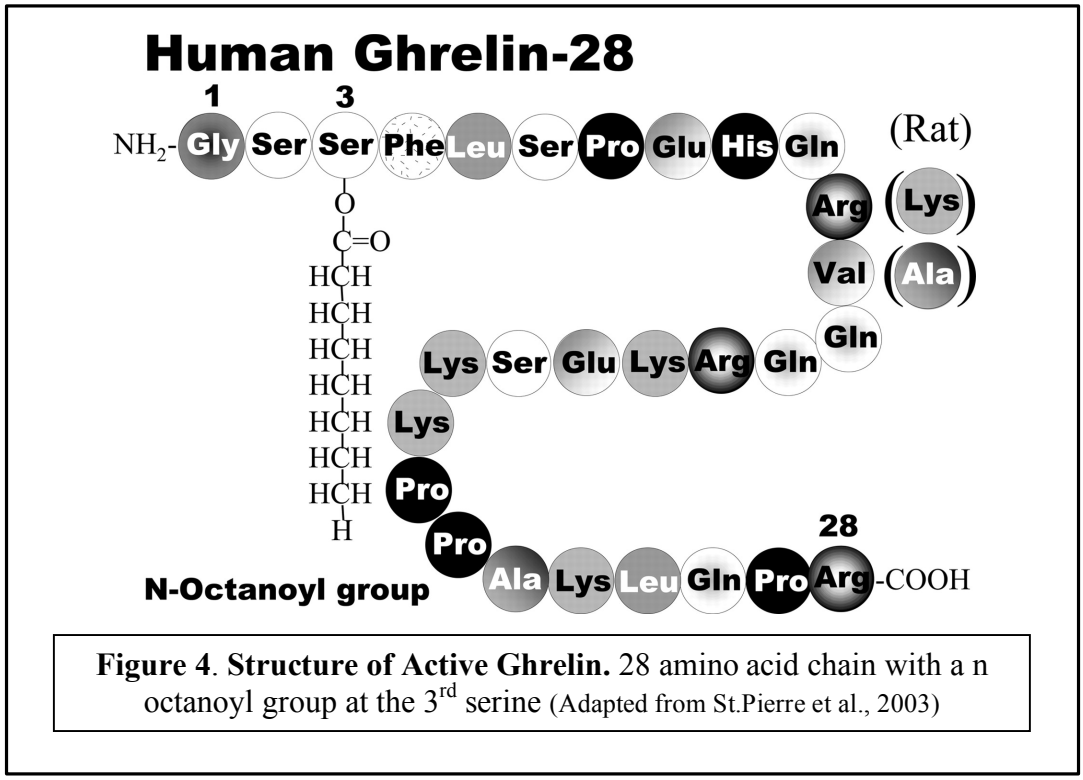


Figure 4. **Structure of Active Ghrelin.** 28 amino acid chain with a n octanoyl group at the 3rd serine (Adapted from St.Pierre et al., 2003)

overeating and can lead to obesity in high fat fed rats (Tschop et al., 2000). Antagonism of GHR-Rs reduces gastric emptying, reduces food intake, and leads to a loss of body weight (Asakawa et al., 2003). Human GHR levels progressively rise before a meal, where they peak and consequently fall following the meal. Anticipation of a large meal results in higher peak levels of GHR prior to the eating (Drazen et al., 2006). Systemic (IP) injections of 6 nMol GHR increase food-seeking behaviors, an effect comparable to that induced by 24-hour food deprivation (Davidson et al., 2005).

Injection of GHR directly into the VTA and/or the NAc can stimulate food intake. Intra-VTA GHR also increases sucrose reward seeking, but injection into the

NAc does not, suggesting the VTA is a locus for food motivation and reinforcing the notion that GHR acts on feeding through multiple pathways (Figlewicz and Sipols, 2010, Dickson et al., 2011, Skibicka and Dickson, 2011, Skibicka et al., 2011a). When the VTA is damaged in rats, GHR administration fails to initiate increases in peanut butter consumption, however regular chow consumption remains unaffected (Egecioglu et al., 2010). ICV infusion at least 1 nMol GHR causes animals to increase their food intake (Nakazato et al., 2001) and also results in an increased preference for high fat foods that leads to an increase in fat consumption (Shimbara et al., 2004). Chronic ICV administration of GHR leads to weight gain and adiposity (Wren et al., 2001). Infusion of 1 nMol GHR directly into the third ventricle results in increases in food seeking behaviors as well (Davidson et al., 2005) and also increases food intake to a larger degree in already fat rats as opposed to lean rats. It is notable that fat rats had significantly higher GHR-R mRNA present in the hypothalamus (Brown et al., 2007). When infused into the lateral ventricle or fourth ventricle, GHR stimulates food intake and increases expression of NPY mRNA (Kinzig et al., 2006, Spinedi et al., 2006), which suggests that GHR might exert a secondary action via activation of NPY orexigenic circuits.

Increased GHR levels, via food restriction, increase the ability of NIC and COC to increase locomotion and to produce conditioned place preference (CPP) (Bell et al., 1997, Davis et al., 2007) as well as augmenting lateral hypothalamic (LH) ICSS reward effects. When discussing and investigating the central reward circuit, the LH is a key brain focus (Cabeza De Vaca et al., 1998, Fulton et al., 2010).

Convergent studies support the hypothesis that GHR administration increases the locomotion and hyperactivity effects of psychostimulants (Wellman et al., 2005) while both GHR administration alone and concomitant administration with psychostimulants increases accumbal DA overflow in the reward circuit (Jerlhag et al., 2006a). Repeated daily administration of feeding-relevant doses of GHR (5 or 10 nMol) has been shown to result in a cross sensitization to COC when co-administered; also increasing locomotor responding to COC (Wellman et al., 2008a). This appears to be made possible through GHR's capacity to reorganize excitatory inputs in the reward circuit starting at the VTA, resulting in enhanced neural activation (Abizaid et al., 2006).

The usual standard for investigating addiction is using a task in which a rewarding drug is self-administered via a jugular catheter. Drug self-administration is studied in multiple phases (Carroll et al., 2004). The first phase is acquisition, which involves being sensitized to the value of the reward and conditioned to respond for a reward. In this phase, rats are shaped to lever press for the reward and over days show a progressive increase in drug intake. Next is the maintenance phase, which is demonstrated by reward-seeking behavior and continued response for the reward, typically a plateaued response rate. In this phase, the rat is seeking to maintain a fixed blood level of drug – as their response rates progressively increase when the drug infusion amount is cut from say 0.5 mg/kg/infusion to 0.25 mg/kg/inf to 0.125 mg/kg/inf. Lastly is the extinction phase where responding on a lever no longer leads to drug infusion. During extinction, lever pressing gradually diminished to near zero. Once drug responding is extinguished, it is possible to examine reinstatement - where

there is restoration of the reward seeking behavior often due to exposure to the previous conditioned stimulus/the reward becoming available again. Rats with higher serum GHR levels show higher incidence of reinstatement in COC self-administration after conditioned stimulus exposure compared to rats with lower GHR levels (Tessari et al., 2007). Self-administration of a reinforcer does not necessarily require exposure to drugs as GHR (also) increases sucrose, peanut-butter-flavored sucrose, and high fat pellet lever-responding in rodents (Perello et al., 2010, Finger et al., 2011, Skibicka et al., 2011a; 2011b; Perello and Zigman, 2012).

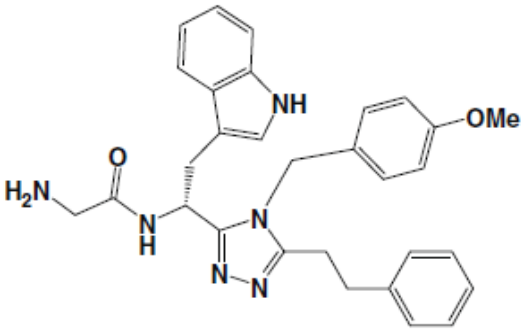
In 1954, James Olds reported that low level electrical stimulation of rat brain could be used as a reinforcer to guide behavior, described more below and in detail in Chapter III, Section 3.1. This finding resulted in the ICSS task, which involves implanting an electrode into a brain area such as the medial forebrain bundle (MFB) of the LH, a collection of neurons that communicate between the NAc and the VTA, and allowing a rat to press a lever which in turn stimulates this area with pleasurable pulses of electrical current (Olds and Milner, 1954).

The ICSS task is thought to tap into brain reinforcement mechanisms and procedures have been devised to examine how drugs of abuse alter ICSS patterns. In one procedure, termed the rate-frequency procedure, the intensity of the electrical current is held constant while the frequency of the stimulation is systematically reduced downward each minute for 15 minutes. The effect is that high rates of responding are noted at high frequency (e.g. 141 Hz) while rate of responding diminishes as frequency is reduced. A notable advantage of this procedure is that it allows for the separation of

maximal rate of responding versus the shape of the curve. A rewarding dose of COC (5 mg/kg, for example) would not only increase the maximal rate of responding (the 100% value) but would also move the curve to the left such that the rat would continue to respond at frequencies that would be non-rewarding after vehicle (VEH). This is also called a shift in the 50% response rate. Were one only to look at 30-minute total responses, no such separation of action could be discerned.

The above studies take the approach of investigating the impact of GHR system activation on reward function; a second approach involves GHR system inactivation. Our laboratory is interested in investigating what occurs to drug and/or food reward upon activation and inactivation of the GHR system, particularly at the level of the reward circuit. While activation of the GHR system can be accomplished using natural GHR (acylated or active GHR) and synthetic GHR (such as hexarelin) and were used in the experiments described above, several strategies have been developed to inactivate the GHR system (see Wellman et al., 2013 for a review). In the present experiments, we focus on inactivating the GHR system through the use of the GHR-R antagonist, JMV-2959 (Moulin et al., 2007, 2012; Salome et al., 2009a, 2009b).

JMV-2959 is a derivative of the triazole structure (see Figure 5; Moulin et al., 2012) that has low nanomolar affinity for GHR-Rs and is not a mixed agonist (Salome et al., 2009b, Wellman et al., 2012). ICV infusion of JMV-2959 suppressed GHR induced food intake and blocked the increased food intake induced by periods of food deprivation (Bell et al., 1997, Wellman et al., 2012). Central administration of JMV-2959 suppresses GHR induced increases in body weight and fat mass, and blocks



JMV 2959
IC_{50} (binding) = 32 nM
GHR-R Antagonist

Figure 5. Structure of JMV-2959.
(Adapted from Moulin et al., 2012)

the GHR induced decreases in energy use (Salome et al., 2009b). Further, drugs of abuse such as NIC, COC and amphetamine cause increases in accumbal DA release and increases in locomotion, which can be blocked by pretreatment with JMV-2959 (Jerlhag et al., 2010, Jerlhag and Engel, 2011). It has also been shown that rats treated with another GHR-R antagonist show less peanut butter or Ensure® intake, but show no change in standard chow consumption when given a choice (Egecioglu et al., 2010). It is also important to note that mice lacking the GHR-R do not show a development of obesity when fed a high fat diet (Zigman et al., 2005). Given these results, it can be speculated that the use of a GHR-R antagonist, like JMV-2959, would yield the same result and prove to be a practical approach to tackling overeating and obesity, independent of NIC use and cessation.

In this dissertation work, the focus was on examining the role of GHR-Rs on ICSS and IVSA of NIC or COC through GHR-R activation using acylated GHR and GHR-R inactivation using JMV-2959. We also investigated weight gain following cessation of chronic NIC exposure via GHR-R antagonism and conclude with a focus on characterizing JMV-2959 by investigating the possibilities of it causing malaise when administered alone and/or in combination with NIC compared to known negative, malaise-inducing, control LiCl and positive control VEH.

Our hope was to generate evidence to support our belief of GHR modulation's role in drug reward in general and specifically to document that drugs such as JMV-2959 may offer a possible dual-treatment approach for assisting in smoking cessation and withdrawal related weight gain. The ability of JMV-2959 to prevent/mitigate any potential weight increases that are common in chronic smokers who attempt to quit, which is unfound in currently available options for assistance in quitting smoking, would be pivotal for this line of research.

CHAPTER II

GENERAL METHODS

2.1 Subjects

The experiments utilized male Sprague-Dawley rats (Envigo). All rats were allowed to adjust to the Psychology building vivarium for a minimum of 7 days on arrival before the start of any procedure or experiment. All rats received continuous food and water and were housed on a 12:00 hour day/night cycle with the lights on at 8:30 am and off at 8:30 pm for all experiments except Experiment 5, which utilized a reverse day/night light cycle. All procedures were approved by Texas A&M University's Institutional Animal Care and Use Committee.

2.2 Housing

All rats were singly housed in standard polycarbonate cages. The vivarium temperature remained between 68 and 72 °F (~21 +/- 1 °C), while the humidity was maintained at 60-70%.

2.3 Drug Solutions

All drug solutions were kept sterile via filtration.

2.3.1 Nicotine

Nicotine hydrogen tartrate salt ≥98% (HPLC) (Sigma item: SML1236) was calculated as the free base and dissolved into 0.9% saline to generate several doses:

30 μg/kg/infusion, 1.4 mg/kg/day, and 0.4 mg/kg. Each NIC solution was balanced to a ~7.0 pH using sodium hydroxide.

2.3.2 Cocaine

Cocaine was dissolved into 0.9% saline to generate several doses: 0.5 mg/kg/.16 ml 6 second infusion, 0.25 mg/kg/inf, 0.125 mg/kg/inf. All doses were calculated as the salt.

2.3.3 JMV-2959

JMV-2959 was a kind gift from Jean-Alain Fehrentz of the Institut des Biomolécules Max Mousseron, Faculté de Pharmacie. It was dissolved into 0.9% saline to generate a 3.0 mg/kg dose and a 6.0 mg/kg dose. An IP administration volume of 1.0 ml/kg was utilized.

2.3.4 Ghrelin

A 5 nMol and a 10 nMol/rat acylated GHR (Phoenix Pharmaceuticals, Burlingame, CA, No. 031-31) concentration were dissolved into 0.9% saline and administered IP in a fixed volume of 0.5 ml.

2.3.5 Sodium Pentobarbital

Sodium pentobarbital was prepared by diluting a stock solution (Beuthanasia-D; Merck Animal Health, Madison, NJ) with 0.9% saline to a dose of 100 mg/ml at a volume of 1 mg/ml for IP administration and a volume of 7.5 mg/kg for IV administration via the jugular vein of those animals in Experiments 2-4, which also tested catheter line patency.

2.3.6 Lithium Chloride

LiCl was calculated as the salt and a 32 mg/kg dose dissolved into a 0.9% saline solution was calculated. An IP administration volume of 1.0 ml/kg was utilized.

2.4 Consumables

2.4.1 High Fat Diet

A HIFAT diet was freshly made every third day of Experiment 5 and consisted of two parts ground chow (Teklad 8604 Rodent Diet; Madison, WI) and one part melted vegetable shortening (Crisco®; Orrville, OH) to create a 35.9% fat/16.3% protein mix with an energy content of 5.28 Kcal/g (see Wellman et al., 2005). The diet was prepared by melting shortening and then mixed this into the ground chow. The mixture was then allowed to cool to room temperature. The HIFAT diet is richer in fat than is the CHOW by 23%, making it more attractive to the rats, contributing a rewarding property to the meal that will allow for greater/overconsumption versus typical CHOW consumption.

2.4.2 Sodium Saccharin

0.1% saccharin solutions were prepared fresh by dissolving saccharin chloride (Sigma Chemical) into tap water.

2.5 Surgical Procedures

2.5.1 ICSS Model

For the ICSS experiments, a single stimulating electrode was implanted into the LH of each rat. The pre-surgery injection regiment (IP) consisted of separate injections

of filtered: atropine sulfate (0.4 mg/kg), ketamine (60 mg/kg) and xylazine (20 mg/kg) for each rat.

Upon verification of surgical plane of anesthesia, each rat was injected IM with sodium penicillin (200,000 units), prepared for surgery (body placed in a surgical sock; scalp shaved and cleaned) and its head mounted in the frame of a Kopf stereotaxic instrument. Styptic gel (Kwik-Stop) was used to stop skull/scalp bleeding and lidocaine jelly (2%) was used as a local anesthetic. The periosteum was pulled away with sterile clamps to allow for the necessary workspace.

> A bipolar, 0.125-mm wire diameter, stimulating electrode (Plastics One, Roanoke, VA; No. 303/3) was implanted into each brain. The incisor bar was set at −2.7 mm, and coordinates were 3.2 mm posterior to bregma, 1.7 mm lateral to the sagittal suture, and 8.3 mm ventral to the skull surface to place the electrode at the MFB at the level of the LH. (Wellman et al., 2008b) (Figure 6, Paxinos and Watson, 2004)

Three skull screws were placed in skull quadrants outside the quadrant in which the electrode was placed. The screws were then secured to the skull using cyanoacrylate glue and dental acrylic (Lang Dental; Wheeling, IL), allowing electrode anchoring onto the skull and providing protection from environmental damage. Gentamicin sulfate ointment (0.1%) (E. Fougera; Melville, NY) was then applied to the incision, which was then closed using sterile cyanoacrylate (Vetbond: 3M; Maplewood, MN).

A subcutaneous (SC) injection of filtered buprenorphine (0.05 mg/kg: Dolorex) followed the surgery and a 7-day recovery was allowed. Rats were weighed on a daily basis to monitor recovery for the remainder of the experiment.

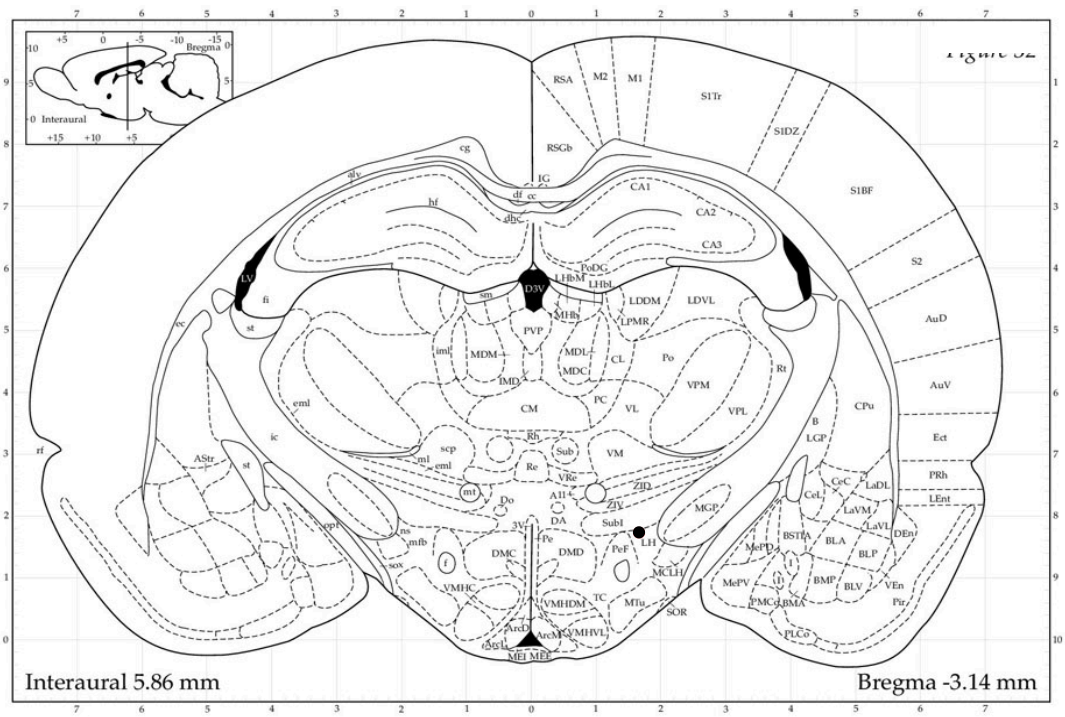


Figure 6. Lateral Hypothalamic (Electrode Target) Location on Atlas Plate. Coordinates for MFB at level of LH: 3.2 mm posterior to bregma, 1.7 mm lateral to the sagittal suture, and 8.3 mm ventral to the skull surface. The black dot on the right half of the panel shows the intended brain target.
(Adapted from Paxinos and Watson, 2004)

2.5.2 IVSA Model

For the IVSA experiments, indwelling jugular catheters were surgically implanted. The pre-surgery injection regiment consisted of separate injections of filtered: atropine sulfate (0.4 mg/kg), ketamine (60 mg/kg) and xylazine (20 mg/kg), with each dose injected via IP into each ICSS rat.

Chronic indwelling jugular catheters (Silastic tubing; 0.25-mm ID; Dow Corning; Midland, MI) were implanted into each rat. Using sterile technique, an

incision was made at the neck above the right jugular vein, as well as an incision at the back just beneath the shoulder blades (Nation et al., 2003; 2004). Lidocaine jelly (2%) was applied to these incisions served as a local anesthetic. The catheter was placed within right jugular vein and anchored in place via suture to the periphery muscle of the vein. The catheter was then routed from the neck area to the back between the scapulae subcutaneously with guidance using a stainless-steel tube (11-gauge). The polyester mesh-based back-plate possessed a threaded cylindrical shaft with stainless steel tubing port to allow for connectivity to an infusion tether in the test box (Plastics One, Roanoke, VA; No. 313-000BM-10/SP). Gentamicin sulfate ointment (0.1%) (E. Fougera; Melville, NY) was then applied to the incisions before being sealed with sterile cyanoacrylate.

The back-plate was attached to a spring leash (Plastics One, Roanoke, VA; No. C313CS) in the test chamber that allowed the catheter to extend from the back-plate at one end to the other at a single channel plastic swivel positioned at the top of the test chamber (Instech, Plymouth Meeting, PA; SKU: 375/22PS). The swivel interlocked with a connecting arm of the test chamber, allowing for free movement with the rat in the test chamber and for delivery of NIC or COC solution via tubing that continued on from the top of the swivel over to an infusion pump (Razel Scientific, Saint Albans, VA; Model R-E). Depression of the left lever activated the pump via GraphicState software (Coulbourn Instruments, Whitehall, PA) while depression of the right lever was without effect.

Post-surgical discomfort was treated SC with 0.05 mg/kg buprenorphine and followed by a 7-day recovery. Rats were weighed on a daily basis and given catheter flushing via infusions of sterile heparinized saline (1.25 U/ml) daily in the home cage to maintain catheter patency. The catheters were also flushed before and after each daily test sessions with 0.20 mls of heparinized saline to prevent clotting and catheter blockage. Upon completion of the study, catheter patency was tested using an infusion of sodium pentobarbital (7.5 mg/kg), resulting in a rapidly induced loss of righting.

2.6 Histology Procedures

At the completion of the ICSS experiments, sodium pentobarbital (100 mg/kg, IP) was used to euthanize each rat. Following cessation of the heartbeat, a perfusion via the heart was performed using phosphate buffered saline (0.9%) and then formalin (10%). For all other procedures, the rats were euthanized with CO_2 to effect. Following extraction, the brains of each ICSS rat were placed in 10% formalin/30% sucrose solution ≥72 hours. Alternate 80µ frozen sections were then sliced, cover-slipped, electrode placements were verified using the Paxinos and Watson (2004) rat brain atlas.

2.7 Apparatus

2.7.1 ICSS Apparatus

The ICSS test chamber (Cambden Instruments) is a 28x22x22 cm stainless steel and Plexiglass box fitted with two levers mounted on opposite ends of one wall. The right lever, when pressed, yielded no outcome, while the left lever depression delivered a

train of 300-ms monophasic rectangular pulses and lit a lamp above the lever. A Grass S88 stimulator (Quincy, MA) and constant current stimulator (Digitimer, Model: DS3; Hertfordshire, England) were used to deliver a 0.3 second train of constant current pulses (at a selected frequency between 141 and 28 Hz) to the brain via a commutator and cable (Plastics One; Model No. SL2C and 305-305, respectively). An oscilloscope (Jameco Electronics, Model: 645280; Belmont, CA) was used to monitor all stimulation activity.

2.7.2 IVSA Apparatus

The IVSA test chamber (Cambden Instruments) is a 28x22x22 cm stainless steel and Plexiglass box fitted with two levers mounted on opposite ends of one wall. The right lever, when pressed, yielded no outcome, while the left lever lit a lamp above the lever and delivered a single drug infusion (0.1 mL) via a single speed infusion pump (MedAssociates, St. Albans, VT; No. PHM-100) to the jugular via a single fluid channel flexible connector assembly (Plastics One; No. C313CS/SPC). All administration parameters were monitored on an IBM compatible computer using the Graphic State software.

2.7.3 Feeding Behavior/CTA Apparatus

Rats in the feeding behavior study were individually housed and monitored on a DACB pad positioned under a wire grid floor while those in the CTA experiment remained in their standard individually housed home-cage environment (sawdust bedding).

CHAPTER III

EXPERIMENT 1: EFFECT OF JMV-2959 AND GHRELIN ON INTRACRANIAL SELF-STIMULATION

3.1 Background

Examining drug-induced locomotor sensitization can provide information about the activational effects of psychostimulants (Wellman et al., 2013). However, such a task provides little information into the rewarding effects of psychostimulants as assessed in drug dose self-administration protocols. While few studies have focused on the role of GHR in drug self-administration, rises in GHR serum levels have been shown to be associated with enhanced subsequent reinstatement for COC (Tessari et al., 2007). Yet there are other ways to look at drug-related reward other than looking at how drugs are self-administered.

ICSS is a measure of brain reinforcement – namely the maintaining of operant behavior in those areas relating to reward. Lever responding, as mentioned above, results in a delivery of constant-current pulses via an electrode capable of being implanted within any brain region, with our work focusing on reinforcement circuits (Wise, 1996; Kenny et al., 2003; Carlezon and Chartoff, 2007; Wellman et al., 2008b), particularly the MFB between the VTA and NAc that course through the LH (Burkey and Nation, 1994).

On the first day in the testing apparatus, the electrode-implanted rat's threshold of minimal current needed to promote ICSS is estimated. Once the current has been

determined, a shaping period is commenced in which the rat is trained to press the lever until it has developed a consistent rate of responding at a fixed frequency of 141 Hz, typically being capable of responding at a consistent rate above 20 responses per minute. The rat is run through trials to establish a baseline responding rate and then, using IP injections of psychostimulants, we can measure the behavioral influence of the drug on the reward circuit and ICSS. Psychostimulants are understood to decrease the reward threshold and increase responding, whereas withdrawal states do the opposite (Markou and Koob, 1992).

ICSS stimulation intensity can range from 70-300 μA (depending on the electrode placement). In the rate frequency procedure, responding is recorded each minute and frequency of stimulation is decreased from 141 Hz to 28 Hz in 0.05 log steps. Decreases in frequency (at a constant current stimulator) occurs every minute over a 15-minute period, creating 15 data points per pass (Carlezon and Chartoff, 2007; Zheng et al., 2013). Four passes are conducted for each rat and the first pass is omitted due to potential confounds resulting from acclimation to the test chamber at the beginning of every test period. The value of reward is related to a suitable wave frequency in this case, decreases in frequency result in reduced rates of responding and leads to active lever responding decreases to a point that the effort required to produce the desired reward is too high to continue seeking out the reward. Thus, responding on the active lever typically decreases with lower stimulation frequencies.

Here, GHR, GHR-R antagonists, and/or psychostimulants can be administered to measure concomitant effects of both electrical and pharmacological stimulation along

varying frequencies for those rats with an established baseline of response for a set current amplitude. Antagonism of DA activity at the MFB and/or LH area allows us to see the effects of inhibition on the ICSS response behavior alone, as well as the effect on the augmented response of COC or NIC on ICSS. Both are important for understanding the reward circuit and investigating potential cessation approaches for addiction.

In fact, it has been shown that NIC lowers reward thresholds in an ICSS test (Kornetsky et al., 1979; Kornetsky and Esposito, 1979; Ivanova and Greenshaw, 1997; Markou, 2008). This relationship of increased reward activation inversely reduces the threshold for reward seeking behavior in ICSS. A reduction in reward threshold motivates the animal to respond, or to continue to respond for frequencies typically unattractive, as the effort input formerly was not worth the reward output. It can also be shown that, alike withdrawal as a result of psychostimulant cessation, reduction of endogenous reward system activation leads to increased reward thresholds in the ICSS paradigm.

In a recent unpublished study by our laboratory, rats were trained and tested in the ICSS paradigm with NIC. Put simply, psychostimulant drugs typically decrease thresholds and increase the rate of responding. This NIC dose both increased the 100% response rate and induced a slight leftward shift of the curve (consistent with augmented reinforcement) (Figure 7). In contrast, pretreatment with JMV-2959 reversed the leftward shift and generally suppressed ICSS responding. A statistical measure of

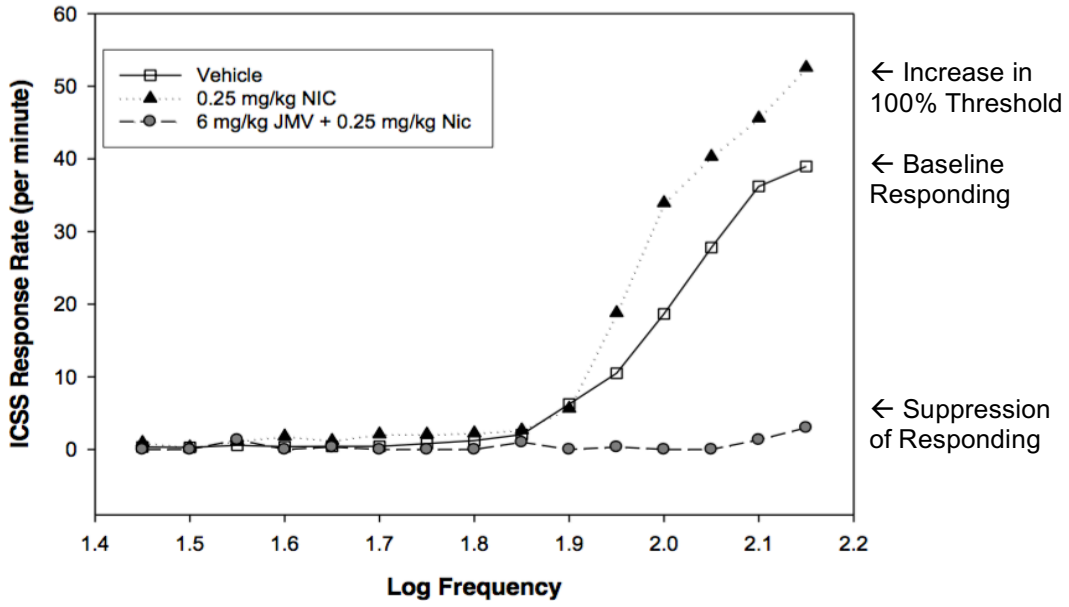


Figure 7. Impact of JMV-2959 on NIC Augmented ICSS Responding. The results show rate frequency curves for rats treated with VEH, NIC (0.25 mg/kg) or a combined pretreatment of JMV-2959 and NIC. NIC both increased the 100% response rate and induced a slight leftward shift of the curve (consistent with augmented reinforcement). In contrast, pretreatment with JMV-2959 reversed the leftward shift and generally suppressed ICSS responding. [N=5] (Unpublished data from the Wellman laboratory)

the leftward shift is to compare the 50% figure metric between the curves to confirm that the drug is encouraging the animal to work harder and longer for rewards that previously held little to no value to them.

In this study, we considered the impact of GHR-R inactivation (JMV-2959) and activation (5 and 10 nMol acylated ghrelin) on ICSS responding. Our focus was to determine if GHR and/or JMV-2959 alone have the capacity to alter responding for

ICSS, and if the changes previously seen in the NIC ICSS experiment in Figure 7 are only possible when JMV-2959 and NIC are concomitantly administered. In an earlier preliminary study from our lab, it was noted that GHR reduced total ICSS responding over a 30-minute test period. That result, however, was difficult to interpret and this experiment sought to clarify the effect of GHR using the rate-frequency testing procedure.

3.2 Experimental Procedures

3.2.1 Subjects

This experiment utilized 15, ~250-275g, Sprague-Dawley male rats (Envigo)

Surgery	Recovery	Shaping Period	Base-line	Test Day 1	Inter-trials	Test Day 2: JMV-2959		
Electrodes Implanted	7 Days Recovery	10 Rats Shaped on FR1	2 Days	VEH	2 Days	3 mg/kg n=5 or 6 mg/kg n=5		
Surgery	Recovery	Shaping Period	Base-line	Test Day 1	Inter-trials	Test Day 2: Ghrelin	Inter-trials	Test Day 3: Ghrelin
Electrodes Implanted	7 Days Recovery	5 Rats Shaped on FR1	2 Days	VEH	2 Days	10 nmol n=5	2 Days	5 nmol

Table 1. Experiment 1: Effect of GHR and JMV-2959 on ICSS

3.2.2 Behavioral Analysis

Table 1 outlines the timeline of the experiment. After recovery from surgery, we determined each rat's preferred current for lever responding and shaped them to lever-press for ICSS. The shaping occurred at a fixed-ratio of 1 (FR1). The minimum current

sufficient to incite lever responding for the rat was determined (usually between 70-300 μA) by increasing the intensity. Once lever pressing was established (responding at 10% day to day variation), a series of 60-minute daily baseline trials commenced. Each trial consisted of separate 15-minute passes in which the frequency of stimulation was lowered each minute from 141 Hz to 28 Hz (decreasing in 0.05 log units) and the current intensity remained constant. During testing, each rat ran in multiple trials on separate days. The first group (n=5) were injected with VEH on Test Day 1, given two days to reestablish baseline and injected on a final day with 3 mg/kg JMV-2959. The second group (n=5) were injected with VEH on Test Day 1, given two days to reestablish baseline and injected with 6 mg/kg JMV-2959. In a final group (n=5) each rat ran in multiple trials on separate days and were injected with VEH on Test Day 1, given two days to reestablish baseline and injected with 10 nmol GHR on Test Day 2, given two days to reestablish baseline and injected with 5 nmol GHR on Test day 3. All injections were given via IP 5 minutes prior to commencing each trial. The amount of active lever pressing was recorded for every 1-min interval for each rat throughout each 15 min pass for all 4 passes. Following the study, all electrode placements were confirmed to be within the LH (see Chapter II, Section 2.5.1).

3.2.3 Data Analysis

The first pass of every daily trial was deemed an environmental habituation period and consequently discarded (Carlezon and Chartoff, 2007). The total number of lever responses for each rat was taken and averaged by group condition (JMV-2959 dose and GHR dose or VEH) for the last 3 daily passes and a rate-frequency curve was

generated. Separate ANOVAs were calculated in investigating the effect of treatment, frequency, and the interaction of treatment and frequency for JMV-2959 (3 and 6 mg/kg) groups (and their respective VEH control groups), while a repeated measures analysis was utilized for the GHR (5 and 10 nmol) groups on lever-responding for ICSS. The maximal response rate (100% response rate) and 50% response rate for each rat were computed for each group. The initial ANOVAs of response rate for each drug condition were followed up by planned contrasts comparing group differences in 100% values and 50% values. A risk factor of $p < 0.05$ was considered statistically significant.

3.3 Results

3.3.1 JMV-2959

To evaluate the impact of JMV-2959 dose on ICSS responding, the study first assessed the impact of 6 mg/kg JMV-2959 on ICSS responding versus a VEH control condition (n=5). Because of concerns that this dose of JMV might represent the high end of the dose effect curve, we identified the need for an assessment of a lower dose (3 mg/kg) of JMV-2959 on ICSS (n=5 rats). Separate ANOVAs for both 3 and 6 mg/kg JMV-2959 showed a significant effect of frequency ($F(1,14) = 28.329$, $p < 0.001$ and $F(1,14) = 17.91$, $p < 0.001$, respectively). Neither JMV-2959 dose exerted a significant effect of treatment or a significant interaction between treatment and frequency. A 100% response rate analysis revealed no significant difference in ICSS responding in 3 or 6 mg/kg JMV-2959 pretreated rats compared to VEH. Analysis of the 50% response rate also showed a no significant difference in responding following 3 or 6 mg/kg JMV-2959

administration compared to VEH. Put simply, neither 3 mg/kg JMV-2959 (Figure 8A) nor 6 mg/kg JMV-2959 (Figure 8B) exerted any significant effect on ICSS responding across the rate-frequency curve.

3.3.2 GHR

Pretreatment with 5 or 10 nmol GHR failed to augment or diminish brain stimulation as the data shows identical response rates when compared to the VEH condition (see Figure 9). A 100% response rate analysis revealed no difference in ICSS responding in 5 nor 10 nmol GHR pretreated rats compared to the VEH condition. Analysis of the 50% response rate revealed the same result in both 5 and 10 nmol GHR pretreated rats compared to VEH pretreated rats. Put simply neither dose of GHR exerted any significant effect on ICSS responding across the rate-frequency curve.

3.4 Interim Discussion

The results of this experiment indicate that neither JMV-2959, at either 3 or 6 mg/kg doses, nor GHR, at either 5 or 10 nmol doses, are capable of affecting reward seeking in the ICSS paradigm. Neither drug is capable of increasing responding at higher frequencies, nor encouraging responding at frequencies typically seen as unattractive. More specifically, neither JMV-2959 nor GHR pretreatment resulted in a change, much less a significant change in the 50% and 100% response rate of responding in rats.

Our previous unpublished results showed that NIC, when given alone, is capable of increasing the 100% and 50% response rate. This result is often described as an increase in threshold and a left-ward shift in rate of responding, indicating an

augmentation in reward seeking. Alternatively, pretreatment with JMV-2959 prior to administration of NIC has been shown to reverse the left-ward shift in the rate-frequency curve of responding and to generally suppress ICSS responding when given in combination (Figure 7). Those results lead us to this experiment to investigate the effects of JMV-2959 alone on ICSS responding.

It was important to determine the capacity of JMV-2959 and GHR to alter ICSS responding alone because it not only allows us to confirm that ICSS is capable of functioning independent of GHR activation when placed in the LH, but that, specifically, JMV-2959 and GHR alter drug effects on ICSS via an action at GHR-R's located

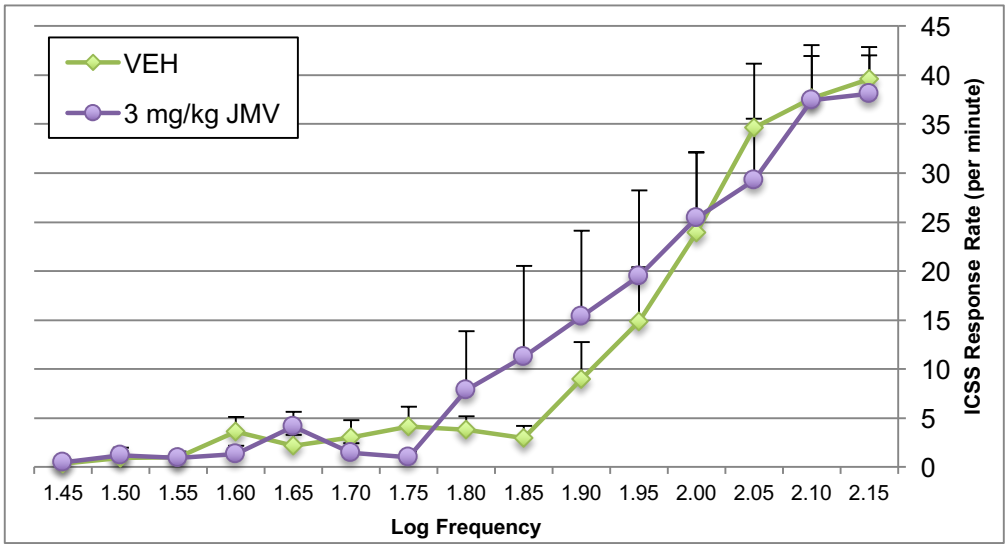


Figure 8A. Impact of 3 mg/kg JMV-2959 on ICSS Responding. The results show rate frequency curves for rats treated with VEH and then 3 mg/kg JMV-2959. JMV-2959 (3 mg/kg) failed to increase the 100% response rate and failed to shift the curve (increase the 50% responding rate). [N=5]

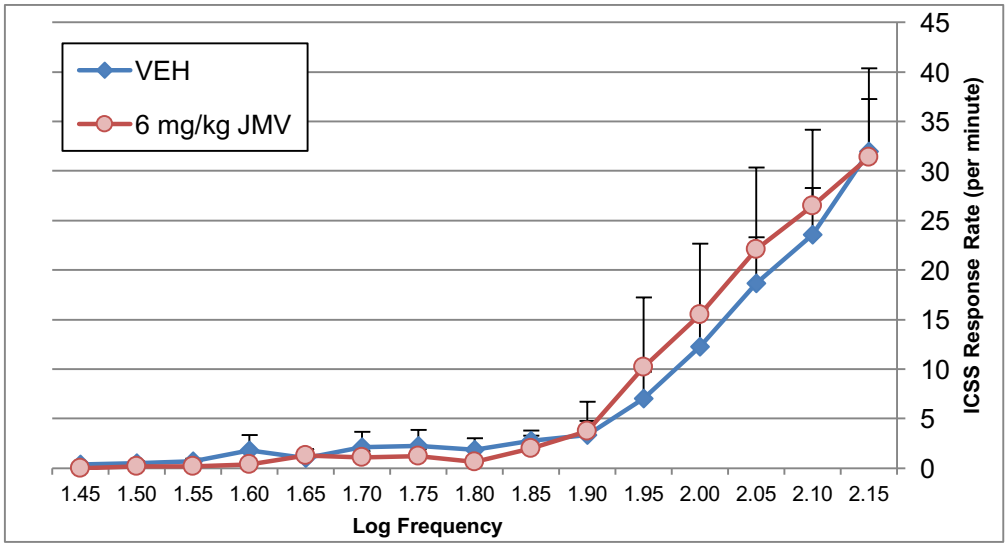


Figure 8B. Impact of 6 mg/kg JMV-2959 on ICSS Responding. The results show rate frequency curves for rats treated with VEH and then 6 mg/kg JMV-2959. JMV-2959 (6 mg/kg) failed to increase the 100% response rate and failed to shift the curve (increase the 50% responding rate). [N=5]

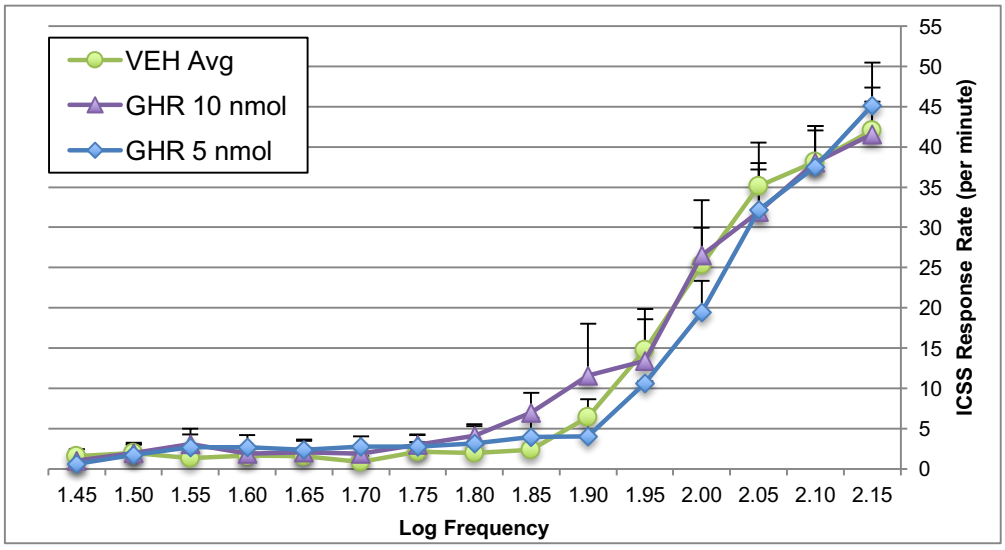


Figure 9. Impact of GHR on ICSS Responding. The results show rate frequency curves for rats treated with VEH followed by 10 then 5 nmol GHR. Both doses of GHR failed to increase the 100% response rate and failed to shift the curve. [N=5]

upstream from the electrode placement, likely at the VTA as shown by Abizaid et al., 2006.

The capacity of JMV-2959 to only be capable of suppressing the reward-seeking when given in combination with NIC is a curious one when assessing our results. Psychostimulants also function at or just before the level of the VTA and, when JMV-2959 is given prior to NIC, reward-seeking behavior for ICSS is mitigated. Perhaps it is the case that psychostimulants act on neurons possessing GHR receptors within the VTA to enhance the VTA-NAc reward circuit – thus JMV-2959 can antagonize NIC-enhanced responding (at the level of the VTA). In the absence of NIC, reward is elicited by activation of the reward circuit starting at the MFB – a nexus that has no GHR receptors. Preliminary studies in our laboratory have shown that JMV-2959 is incapable of blocking the expression of NIC developed locomotor sensitization. Our preliminary experiment only utilized acute exposure to NIC (only 1 day), however in a chronic tast, prior to pre-treatment with JMV-2959 just before NIC administration. Perhaps JMV-2959 would be incapable of preventing ICSS lever responding when given to rats pretreated with JMV-2959 following chronic exposure to NIC. The next 2 experiments provide some insight into the effect of JMV-2959 on chronic exposure to NIC or COC, which may provide evidence for what to expect if we were to investigate JMV-2959 pretreatment following chronic exposure to NIC or COC on IVSA.

CHAPTER IV

EXPERIMENT 2: EFFECT OF JMV-2959 ON INTRAVENOUS SELF-ADMINISTRATION OF NICOTINE

4.1 Background

In the next two experiments, we examined the impact of the GHR-R antagonist JMV-2959 on self-administration of the psychostimulants NIC or COC. As described previously, neither locomotor experiments, nor ICSS experiments, as they are a poor proxy for reinforcement, provide as much information into the rewarding effects of psychostimulants as do drug self-administration studies (Wise, 1996).

As explained previously, in the IVSA paradigm, the animal is implanted with an intravenous catheter within the jugular vein in the neck that allows the animal to be trained to lever-press for a IV delivery of a psychostimulant infusion, which in turn increases DA levels in reward areas (Di Chiara et al., 2004). This allows for assessment of responding for the stimulant and the impact of an agonistic or antagonistic pretreatment on responding for the stimulant infusion (Carroll, 1985). This reinforcement model allows for the rapid onset of NIC or COC ingestion comparable to that of humans who can ingest these drugs through the lungs.

For NIC or COC IVSA, the number of responses required to obtain the drug is typically set to a fixed ratio (FR) where the animal receives the NIC infusion upon X amount of successive responses, where X is value chosen by the experimenter. On an FR1 schedule, the animal receives an infusion of NIC per one response of the active

lever; (FR2) requires two responses, etc. In the present study, the FR value was set to 2 to allow for a moderate level of responding against which to judge the impact of pretreatment with 3 mg/kg JMV-2959. What is important to our laboratory about this paradigm is that once the animal has acquired the appetitive behavior and is responding at a baseline rate, we can seek out pharmacological methods to enhance, alter, or ablate the drug-seeking behavior.

In this experiment, we considered the impact of JMV-2959 on NIC IVSA responding in order to evaluate JMV-2959 as a potential treatment for maintenance phase drug addiction, a phase that is difficult to eliminate.

4.2 Experimental Procedures

4.2.1 Subjects

This experiment utilized 6, ~300g, Sprague-Dawley male rats (Envigo) implanted with intravenous catheters (See Chapter II, Section 2.5.2).

Surgery	Recovery	Shaping Period	Days 1-3	Day 4	Days 5-6
Catheter Implanted	7 Days Recovery	Rats Shaped on FR1	Baseline FR2 NIC (30 µg/kg/inf)	JMV-2959 (3 mg/kg) 5 min prior to NIC trial	Reestablishment FR2 NIC (30 µg/kg/inf)

Table 2. Experiment 2: Effect of JMV-2959 on IVSA of NIC

4.2.2 Behavioral Analysis

Table 2 outlines the timeline of the experiment. Once FR1 lever-pressing for NIC was acquired, the schedule was raised to FR2 and the rats were run through 7 days of 60-minute baseline trials to establish a baseline response rate. During each trial, the NIC dose of 30 μg/kg/infusion remained constant (Paterson et al., 2010). Once baseline was established, each rat was pretreated with an IP injection of JMV-2959 (3 mg/kg) or a VEH injection 5-min before the next trial on the following day. Following this test day, rats continued baseline trials (with VEH injections) for 2 days to reestablish baseline response rates and confirm that JMV-2959 has no lingering or permanent behavioral effects.

4.2.3 Data Analysis

For each trial, the total number of responses and infusions were analyzed for each rat. An ANOVA was computed to determine differences between VEH and JMV-2959 and recovery day conditions. Mean group differences were examined using planned t-test contrasts and a $p < 0.05$ established statistical significance.

4.3 Results

Figure 10 shows the number of active lever responses for NIC during VEH baseline, following pre-treatment of JMV-2959, and 2 consecutive recovery days. On VEH baseline days, rats averaged 30 lever presses for every 60-minute session for 30 μg/kg/infusion of NIC. On the JMV-2959 pretreatment day, active lever pressing for NIC was almost non-existent. On the next 2 days, the rate of responding for NIC was decreased (in the absence of JMV-2959). Repeated measure ANOVA revealed a

significant effect of days ($F(3,12) = 4.5$, $p < 0.024$). On the 2 subsequent recovery days, responding for NIC increased over that noted after JMV-2959, but had not reached VEH baseline levels.

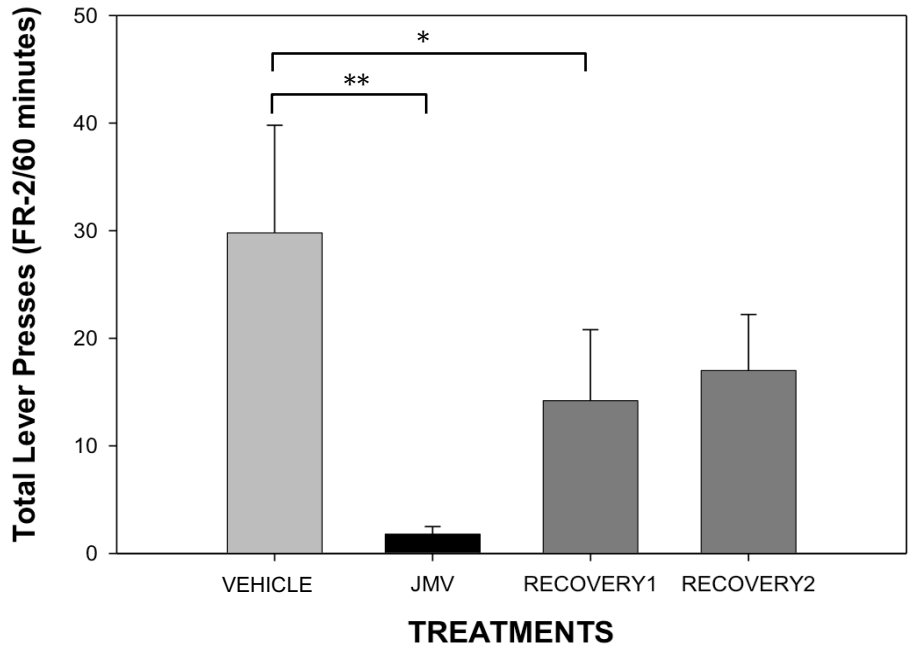


Figure 10. Impact of JMV-2959 on NIC IVSA Responding. Mean group total active lever presses on an FR-2 schedule of reinforcement for 30 µg/kg/infusion NIC for rats treated on successive days with VEH, 3 mg/kg JMV-2959, VEH and then VEH. Repeated measure ANOVA revealed a significant effect of days ($F(3,12) = 4.5$, $p < 0.024$). [N=6]

4.4 Interim Discussion

The results for this experiment demonstrate the capacity of JMV-2959 to ablate continued NIC drug-seeking during maintenance phase addiction. Prior to JMV-2959

pretreatment, the rats in this experiment were capable and willing to press an active lever for an infusion of NIC ~29 times during a 60-minute session. The decrease in motivation to drug-seek can be seen by the consequential decrease in responding for NIC following JMV-2959 and can be confirmed to be an effect of the drug by the observations that followed on the Recovery Days.

The purpose and hope of this study was that our investigations would lead us to find that JMV-2959 can mitigate or eliminate the continued drug-seeking in maintenance phase of addiction to NIC. This investigation is important because, until now, all previous experiments that have sought to investigate the effects of JMV-2959 on reward-seeking with psychostimulant modifications have done so with only acute administration of the psychostimulants (Jerlhag et al., 2009, 2010; Dickson et al., 2011; Jerlhag and Engel, 2011; Skibicka and Dickson, 2011; Skibicka et al., 2011a, 2011b). The preparation for an IVSA experiment allows for the development of an addiction and the observation of an effect by a drug like JMV-2959 on the maintenance phase of addiction. After all, it is those individuals who are having trouble quitting and that are at risk of health issues and early mortality that we must aim to treat them. In the IVSA paradigm, the animal learns to press a lever for the drug. It gets to choose to take the drug, a measure not taken into account in any of the previous JMV-2959 literature. These animals are allowed to develop a dependence and constant drug-seeking behavior in the context of the drug.

The results herein demonstrate that JMV-2959 has the ability to almost entirely ablate lever responding for NIC in the context of chronic exposure when compared to

VEH baseline lever responding for NIC (Figure 10). These results support our notion that JMV-2959 may be a suitable candidate in suppressing active drug/reward-seeking behavior in chronic psychostimulant users, specifically smokers.

While JMV-2959 has a half-life of 6 hours, the results for Recovery Day 1 and 2 suggest that JMV-2959 had a slight residual effect that may be a result in a neuromechanistic change in these drug/reward-seeking circuits. Further investigation into the temporal course of JMV-2959 is recommended, but the current results are the first to provide evidence JMV-2959 may be a suitable drug, in comparison to other currently available pharmacological options, in assisting chronic smokers in that are attempting to quit.

CHAPTER V

EXPERIMENT 3: EFFECT OF JMV-2959 ON INTRAVENOUS SELF-ADMINISTRATION OF COCAINE

5.1 Background

In compliment of the previous experiment, this experiment considered the GHR-R antagonist JMV-2959's impact on COC IVSA responding in order to evaluate JMV-2959 as a potential treatment for maintenance phase COC addiction.

5.2 Experimental Procedures

5.2.1 Subjects

This experiment utilized 9, ~300g, Sprague-Dawley male rats (Envigo) implanted with intravenous catheters (See Chapter II, Section 2.5.2).

Surgery	Recovery	Shaping Period	Days 1-3	Day 4
Catheter Implanted	7 Days Recovery	Rats Shaped on FR1	Baseline FR2 COC (0.5 mg /kg/inf)	JMV-2959 (3 mg/kg) 5 min prior to COC trial

Table 3. Experiment 3: Effect of JMV-2959 on IVSA of COC

5.2.2 Behavioral Analysis

Table 3 outlines the timeline of the experiment. Once FR1 lever-pressing for COC was acquired, the schedule was raised to an FR2 and the rats were run through 7

days of 120-minute VEH baseline trials to establish a baseline response rate. During each trial the COC dose (0.5 mg/kg/0.16 ml 6 second infusion) remained constant. Once baseline responding was established, each rat was pretreated with an IP injection of JMV-2959 (3 mg/kg) 5-min before the next trial the following day.

5.2.3 Data Analysis

For each trial, the total number of responses and infusions were analyzed for each rat. Single sample t-tests were used to determine differences between VEH and JMV-2959 conditions. Mean group differences were examined and a $p < 0.05$ established statistical significance.

5.3 Results

Figure 11 shows the number of active lever responses for COC during VEH baseline, and then again following pre-treatment of JMV-2959. On VEH baseline days, rats averaged approximately 60 lever presses for every 120-minute session for 0.5 mg/kg/infusions of COC. On the JMV-2959 pretreatment day, active lever pressing for COC was greatly reduced. A planned within group t-test compared active lever pressing after SAL pre-treatment was significantly higher than lever pressing after 3 mg/kg JMV-2959 ($t(8) = 6.7$, $P< 0.001$).

5.4 Interim Discussion

The results for this experiment compliment those of the previous experiment as JMV-2959 proved capable of mitigating chronic drug-seeking behavior for COC (Figure

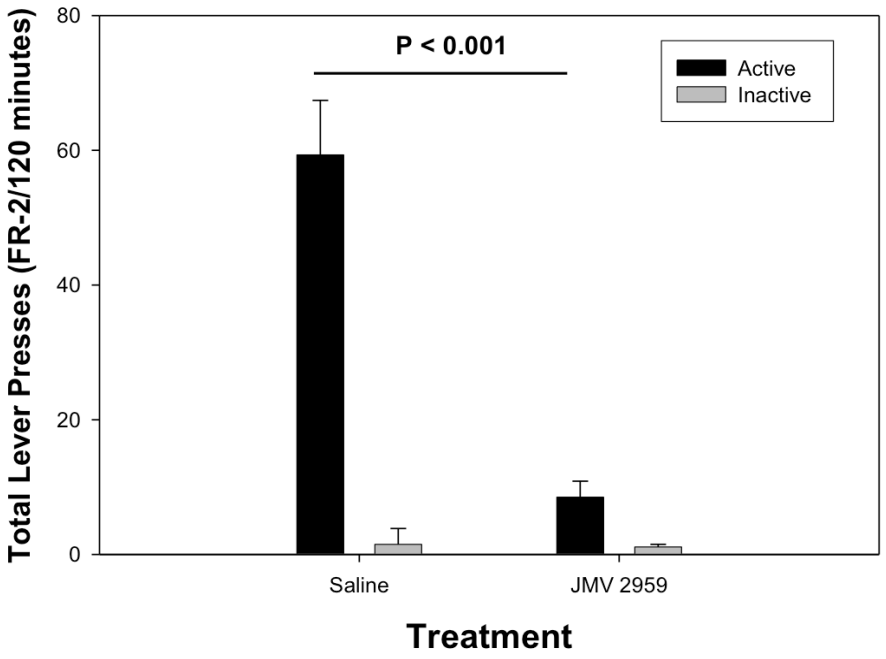


Figure 11. Impact of JMV-2959 on COC IVSA Responding. Mean group total active (black bar) and inactive (gray bar) lever presses on 0.5 mg/kg/infusion cocaine FR2. Vehicle vs. 3 mg/kg JMV-2959 responding $t(8) = 6.7$, $P < 0.001$. [N=9]

11). The rationale for this experiment, as in the previous experiment, was that psychostimulants activate the dopaminergic reward circuit and therefore should produce comparable results if JMV-2959 is functioning on the same area at the level of the VTA to NAc.

The potential for JMV-2959 to be capable of mitigating drug-seeking for both NIC and COC is an exciting one because it not only tells us that antagonism of GHR-Rs may be suitable for the treatment and elimination of the addiction of many psychostimulants, but that this drug may offer an avenue by which to treat COC

addiction. There are currently no Food and Drug Administration approved treatments for COC addiction. Indeed, treatment for COC addiction mostly focuses on supportive measures that aim to stabilize the symptoms of COC overdose (American Psychiatric Association, 2010). As for the therapies currently available for treating NIC addiction, as discussed earlier, those available are not very successful and cause side effects that are likely to drive many to relapse.

Again, these results support the findings in Experiment 2 and also support our notion that JMV-2959 may not only be a suitable candidate in suppressing active chronic drug-seeking behavior in smokers, but also suitable as a treatment for maintenance addiction for many psychostimulants. A drug like JMV-2959 might not just be useful to help someone stop use, but also be capable of preventing future incidence and the potential of relapse likely instigated by a drug-taking context, visual, and odorant cues that often associated with heightened desire to use and relapse.

It is important to continue investigating JMV-2959 in combination with other psychostimulants, and, as discussed before with ICSS, in models where psychostimulant exposure has occurred for chronic periods of time. It is not currently known if JMV-2959 is capable of inducing mechanistic changes with long-term treatment, such as already established sensitization, but given the results for these 2 experiments, it would be of interest to investigate the potential of JMV-2959 with long-term treatment.

CHAPTER VI

EXPERIMENT 4: EFFECT OF GHR ON INTRAVENOUS SELF-ADMINISTRATION OF COCAINE

6.1 Background

It is well understood that elevated GHR levels are associated with increases in reward seeking behavior. It has been shown that GHR levels correspond to the reinstatement of COC IVSA in rats in that naturally increased GHR levels, or if increased via GHR injection, can augment the reinforcement of COC (Tessari et al., 2007). In this experiment, we considered the impact of systemically injected GHR on COC IVSA responding. This allowed us to investigate the potential of increasing GHR levels on the possible consequent increases in lever responding for COC in rats in the maintenance phase of addiction.

6.2 Experimental Procedures

6.2.1 Subjects

This experiment utilized 6, ~300g, Sprague-Dawley male rats (Envigo) implanted with intravenous catheters (See Chapter II, Section 2.5.2).

Surgery	Recovery	Shaping	Day 1	Day 2	Day 3	Day 4	Day 5
Catheter Implanted	7 Days Recovery	Rats Shaped on FR1	Baseline	Baseline	VEH	GHR (30 nmol)	Washout
			COC (0.5 mg/kg/inf)	COC (0.5 mg/kg/inf)	COC (0.5 mg/kg/inf)	COC (0.5 mg/kg/inf)	COC (0.5 mg/kg/inf)
Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
VEH	GHR (30 nmol)	Washout	VEH	GHR (30 nmol)	Washout	Baseline	Terminate
COC (0.25 mg/kg/inf)	COC (0.25 mg/kg/inf)	COC (0.25 mg/kg/inf)	COC (0.125 mg/kg/inf)	COC (0.125 mg/kg/inf)	COC (0.125 mg/kg/inf)	COC (0.5 mg/kg/inf)	

Table 4. Experiment 4: Effect of GHR on IVSA of COC

6.2.2 Behavioral Analysis

Table 4 outlines the timeline of the experiment. After recovery from surgery, each rat was trained to lever-press for COC (0.5 mg/kg/inf) on a FR1 schedule. Following acquisition of FR1 responding behavior, the schedule was raised to FR2 and the rats were run through 7 days of 120-minute baseline trials to establish a baseline response rate. During each training and baseline trial, the COC dose of 0.5 mg/kg/inf remained constant. Once baseline was established, each rat was pretreated with an IP injection of GHR (10 nmol), VEH, or nothing 5-minutes just before beginning each of the next 4 trials in which the COC dose was systematically halved over trial days to create 3 COC dose samples; 0.5, 0.25, and 0.125 mg/kg/inf.

6.2.3 Data Analysis

Two separate analyses were conducted for the active lever data of this experiment. In the first, planned contrasts (t-tests) were used to determine the dose-dependence of the COC self-administration procedure. COC dosing started at 0.5

mg/kg/inf and over subsequent days was reduced by half (i.e. to 0.25 mg/kg/inf and then to 0.125 mg/kg/inf. The expectation was that such dose changes would result in enhanced responding. The last dose change was back to 0.5 mg/kg/inf and served to assess the reliability of the responding. A second analysis consisted on planned contrasts comparing, at each COC dose, differences in responding after ghrelin pretreatment relative to vehicle pretreatment. Between group differences of $p < 0.05$ were deemed statistically significant.

6.3 Results

The rate of responding for COC as the dose was decreased resulted in the expected dose-dependent increases in responding (see Figure 12). Planned contrasts indicated a significant increase in responding when COC dose was shifted from 0.5 mg/kg/infusion to 0.25 mg/kg/infusion ($t(4)=3.13$, $p < 0.01$) and a significant decrease in responding when COC dose was increased from 0.125 mg/kg/infusion back to 0.5 mg/kg/infusion ($t(4)= 5.1$, $p < 0.009$). The shift from 0.25 mg/kg/infusion to 0.125 mg/kg/infusion only slightly increased response rate ($p=0.68$). Pre-treatment with GHR failed to affect IVSA responding for each dose of COC 0.5, 0.25, and 0.125 mg/kg COC, relative to the respective VEH baseline. A final trial at the training dose of 0.5 mg/kg COC demonstrated the return to VEH baseline responding. Collectively, these results indicate that GHR administration does not alter COC IVSA once maintenance phase behaviors/drug-seeking has been established.

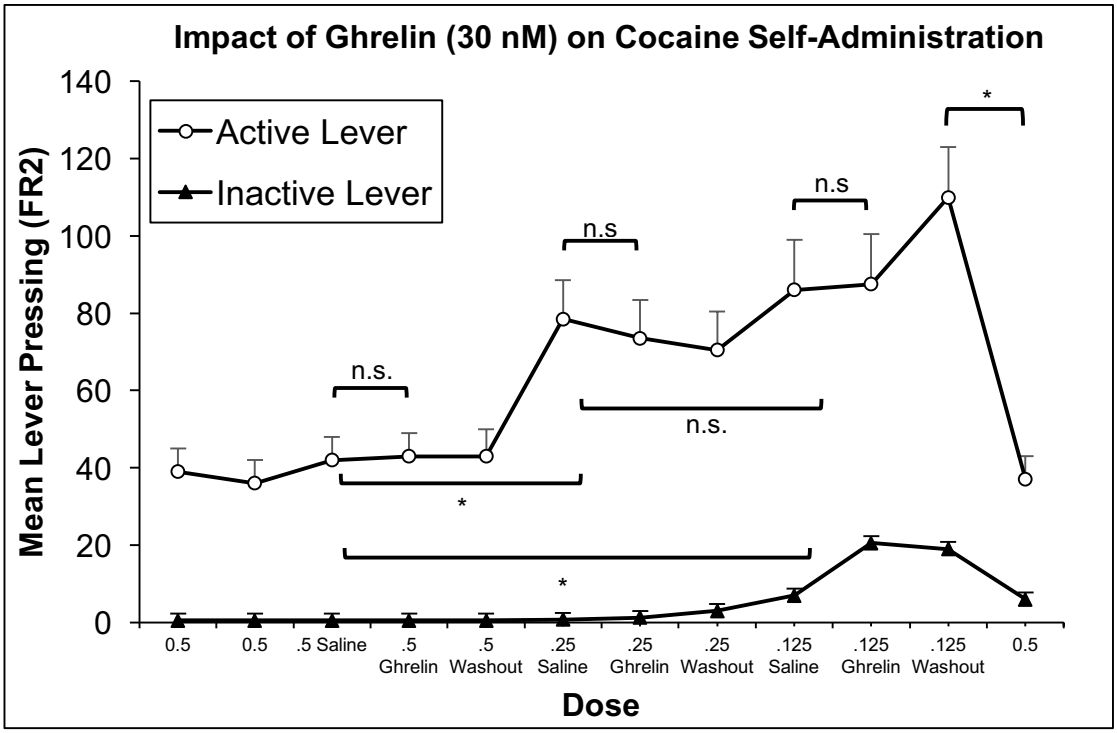


Figure 12. Impact of GHR on COC on IVSA Responding. GHR (30 nM) did not significantly alter self-administration rates for any dose of COC ranging from 0.5 to 0.125 mg/kg/infusion. [N=6]

6.4 Interim Discussion

The results for this experiment show that, while systematically decreasing the COC dose from 0.5 to 0.25 and then to 0.125 over days alone augments responding for the drug, pretreatment with GHR fails to alter the rate of responding for any of these doses (see Figure 12).

The rationale for this experiment was based on previous studies that have shown that intra-VTA GHR administration results in changes in DA release in the NAc (Jerlhag

et al., 2006, Jerlhag et al., 2007, Quarta et al., 2009). Consistent with these studies, our laboratory has examined changes in the behavioral actions of COC in rats given acute supplemental doses of GHR. Systemic GHR administration facilitates acute COC hyper locomotion in rats (Wellman et al., 2005) and induces behavioral sensitization to COC (Wellman et al., 2008a) but, as seen in Experiment 1, GHR alone fails to increase frequency thresholds and response rates in the ICSS paradigm. Given these results, we speculated that GHR pretreatment for rats chronically exposed to COC and in the maintenance phase would show an increase in the rate of responding for COC during the 120-minute session. These results would provide a compliment to the findings of the previous two experiments.

However, the results for this experiment show that rats that have already acquired COC IVSA do not vary their response rate when injected with GHR, suggesting that GHR may not augment chronic COC IVSA. This may be due to the fact, again, that previous experiments utilizing JMV-2959 and psychostimulants have done so by exposing the animals to the psychostimulants only acutely. Our testing here was an adequate test. It involved a chronic exposure design. The rats in this experiment and the previous 2 had been exposed to the psychostimulant at least 10 days before JMV-2959 or GHR was administered. We utilized 3 doses of COC, all within the standard doses administered in the literature. We also used a dose of GHR that is among the highest doses typically used. The results simply failed to show any evidence of a treatment effect.

Perhaps it is the case that JMV-2959 and GHR can alter acute psychostimulant effects, but once the exposure becomes chronic a divergence occurs in the capacity of both substances to have an effect on reward behavior. For example, it has been show that GHR pretreatment alters acute locomotor COC sensitization, augmenting it, but it fails to alter ICSS or COC IVSA, both chronic tasks.

It is the case that GHR-Rs have a degree of constitutive activity – meaning that in the absence of a GHR agonist, these receptors exhibit a near-maximal degree of activity (Holst et al., 2003; 2004). An implication of such activity is that administration of an antagonist drug may reveal an action on reward whereas administration of an agonist might be without effect as was noted in the present study in which systemic GHR injection has no effect on COC IVSA.

Future studies should investigate the effect of GHR on chronic NIC IVSA as well as self-administration paradigms utilizing sucrose pellets to determine if the effects of JMV-2959 and GHR are tied to psychostimulants, the paradigm, or reward in general. It would also be of interest to determine if JMV-2959 pretreatment prior to GHR administration in the ICSS paradigm would have any effect given that GHR has previously been shown to cross-sensitize to COC when administered acutely.

CHAPTER VII

EXPERIMENT 5: EFFECT OF JMV-2959 ON NICOTINE-WITHDRAWAL RELATED BODY WEIGHT GAIN

7.1 Background

It has been established that smoking is often used as a weight control strategy that allows users to increase caloric intake while remaining, on average, 10 pounds lighter than the average non-smoker (Dallongville et al., 1998) (Figure 13). Cessation of chronic smoking results in an average weight gain of 3 pounds per 7 days of cessation in many users. Which in turn can drive them back to smoking as 75% of women smokers nor 35% men smokers are not willing to gain more than 5 pounds if attributed to smoking cessation (Pomerleau and Kurth, 1996). In this experiment, we used Wellman et al., 2005 experiment as a model.

The Wellman et al., 2005 study was a 2x2 experiment in which animals were on one of 2 diets: HIFAT or CHOW and one of 2 drug treatments: SAL or NIC, forming 4 groups: CHOW-SAL, CHOW-NIC, HIFAT-SAL, HIFAT-NIC. Rats were initially acclimated to a reverse day-night cycle upon arrival and then to their assigned diet for 3 days prior to the treatment period with SAL or NIC. Rats are nocturnal creatures and therefore mimicking human drug taking would require treatment during their waking cycle, in the dark. Smokers only smoke while they are awake and smoke at typically regular intervals across the day. The experiment modeled this smoker's behavior by

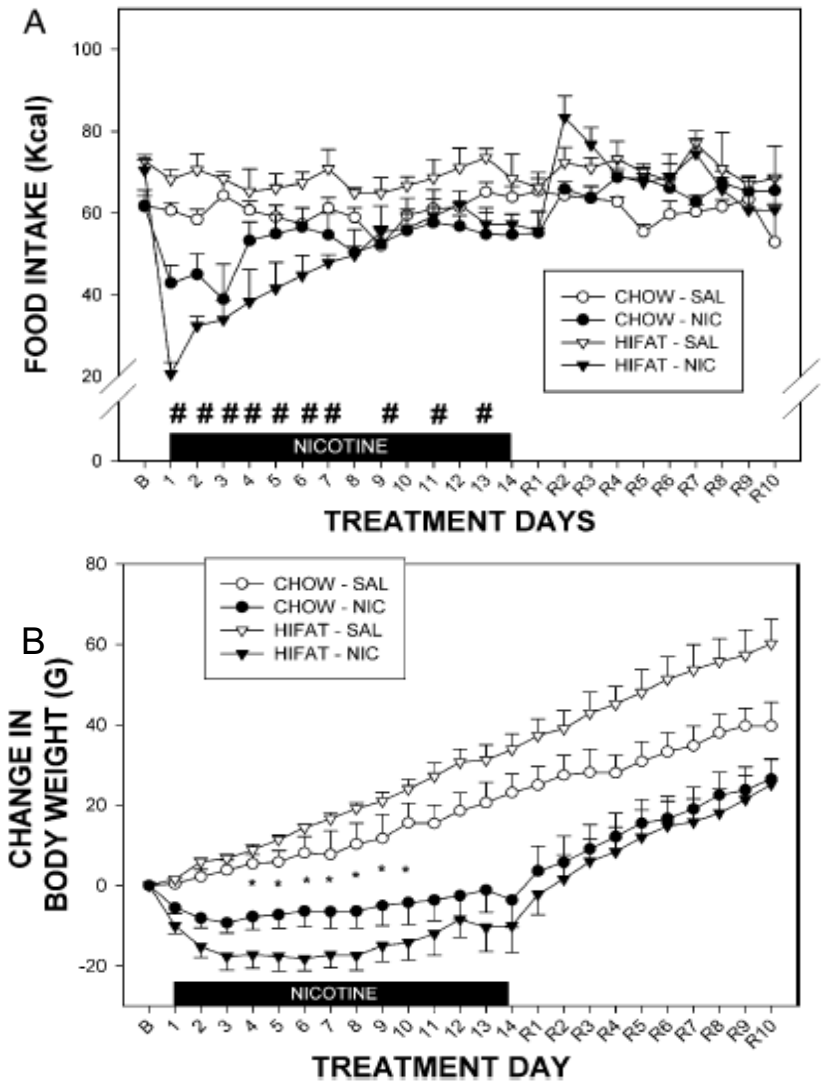


Figure 13. Wellman et al., 2005:Food Intake & Weight Gain After NIC. Panel A). Mean group caloric intake in kcals in male rats treated for 14 days with either 0.9% saline (VEH) or 1.4 mg/kg free base nicotine (NIC) maintained on either a chow (CHOW) or high-fat diet (HIFAT). During the 10 days after cessation of NIC, all rats were injected daily with VEH. The vertical line above or below each symbol represents the SEM. Panel B). Mean group changes in body weight (g) in male rats treated for 14 days with either VEH or NIC maintained on either CHOW or HIFAT. During the 10 days after NIC, all rats were injected daily with VEH. Means T S.E.M. An * denotes a significant ($p < 0.05$) difference. Total daily caloric intake; Panel Treatment days: B=Baseline day; 1–14=drug treatment days; R1–R10=Recovery days. (Adapted from Wellman et al., 2005)

administering the treatment across the active, night, period. Treatment occurred for 14 days in which, food intake, water intake, and body weights were also recorded. After 14 days, Treatment was discontinued while all other measures remained and continued to be recorded.

A key issue from this study was that NIC induced weight loss and weight control during the Treatment period, with the loss and control being greater for rats on a HIFAT diet versus a CHOW diet. Cessation of NIC resulted in an initial spike in food intake in both HIFAT-NIC and CHOW-NIC groups, with those in the HIFAT-NIC group demonstrating a larger effect. Cessation was also followed by comparable linear increases in weight gain in both HIFAT-NIC and CHOW-NIC groups. The rate of weight increase over days for these groups was almost identical to those in the HIFAT-SAL group, which displayed a consistent linear increase in weight gain over the entire experiment.

The experiment here sought to evaluate the impact of JMV-2959 on NIC-withdrawal related feeding behaviors, particularly the spike in food intake, and body weight change like those seen upon NIC cessation in the Wellman et al., 2005 study in order to determine the potential use of GHR-R antagonism tin preventing NIC cessation related increases in food intake and body weight gain or regain. In this experiment, a shorter time period (7 days) was utilized.

7.2 Experimental Procedures

7.2.1 Subjects

This experiment utilized 20, ~300g, Sprague-Dawley male rats (Envigo) and followed the experimental procedure described in Section 2.1 of Chapter II with the reverse day/night light cycle.

<table>
<tr><th>Days 1-3</th><th>Day 4</th><th>Days 5-7</th><th>Treatment Days 1-7</th><th>Recovery Days 1-5</th></tr>
<tr><td rowspan="4">Housing & HIFAT Diet Adaptation</td><td rowspan="4">HIFAT diet only 23hr access</td><td rowspan="4">Baseline</td><td rowspan="2">VEH</td><td>VEH</td></tr>
<tr><td>JMV (3 mg/kg)</td></tr>
<tr><td rowspan="2">NIC (1.4 mg/kg/day administered)</td><td>VEH</td></tr>
<tr><td>JMV (3 mg/kg)</td></tr>
<tr><td colspan="5">Table 5. Experiment 5: Effect of JMV-2959 on NIC-Withdrawal Weight Gain</td></tr>
</table>

7.2.2 Behavioral Analysis

Table 5 outlines the timeline of the experiment. Upon habituation to individual housing on DACB pad with wired grid floored housing, the rats were placed on a HIFAT diet (n = 20) (diet described below) and allowed 23 h per day access to the diet for the entirety of the experiment. The wired floors and DACB pads were used in order to measure food spillage (to the nearest 0.1 g) by the rats and consequently subtracted from the rat's daily food intake measurement.

Rats were acclimated to a spaced dark-phase IP injection design protocol of VEH (0.9% saline at 1 ml/kg volume injection) on baseline days 5,6, and 7, (VEH, 1 ml/kg)

administered at 0700, 1000, 1300, 1600, and 1900 h daily. Days 1–7 were the drug Treatment period, in which rats were assigned (randomly) to an injection group of VEH (HIFAT-VEH group [n=10]) or NIC (1.4 mg/kg/day freebase; HIFAT-NIC group [n=10]). The dose was determined by the weight of the rat prior to the first injection of the day (see Bellinger et al., 2003 for procedure). We chose the dose of 1.40 mg/ kg NIC given that Wellman et al., 2005 states,

> One factor is that 70–75% of NIC given by the IP route used in the present study is removed by the liver during a single pass (Svensson, 1987) and thus did not reach the brain. Humans smoking one to three packs of cigarettes per day take a total daily dose of approximately 0.3 – 0.5 mg/kg/day of NIC through their lungs (Benowitz and Jacob, 1984; Perez-Stable et al., 1998). Therefore, the effective NIC dose in the present study [1.4 mg/kg in 5 injection/day] that reached the rat brain was about 0.42 mg/kg/day and is in the range of that used spontaneously by human smokers. (Wellman et al., 2005)

After the Treatment period, all rat groups were split into two subgroups and given either VEH or JMV-2959 (3 mg/kg) injections (at the 0700, 100, and 1900-hour marks) during the subsequent 5-day recovery period to monitor food consumption during Treatment cessation. This created 4 Post-Treatment groups: saline Treatment – saline Post-Treatment (VEH-VEH), saline Treatment – JMV-2959 Post-Treatment (VEH-JMV), NIC Treatment – saline Post-Treatment (NIC-VEH), and NIC Treatment – JMV-2959 Post-Treatment (NIC-JMV) [n=5 per group].

At 0600 h of each test day, each food cup and rat were weighed, to the nearest 0.1g and gram respectively. The DACB pad below the grid floor collected food spillage. It was weighed, recorded (nearest 0.1 g), and subtracted from the food intake for the day. Injections commenced with the dark cycle at 0700 hours.

7.2.3 Data Analysis

7.2.3.1 Food Intake

An ANOVA for the comparison of food intake during the Treatment period investigated a between-group factor (Treatment: VEH vs. NIC) and within group factor (Treatment Days 1-7) for food intake. A second ANOVA was performed to determine the differences between Post-Treatment subgroups (VEH-VEH, VEH-JMV, NIC-VEH, NIC-JMV) during Post-Treatment Days 1-5. A series of planned between-group t-tests were conducted to investigate food intake differences between Treatment groups (VEH vs. NIC) for each Treatment Day (1-7). A series of planned between-group t-tests also investigated differences between NIC-VEH and NIC-JMV Post-Treatment groups, as well as VEH-VEH and VEH-JMV Post-Treatment groups on Post-Treatment Days 1 and 2, as Treatment cessation in Wellman et al., 2005 showed and initial spike in food intake following Treatment cessation. We were interested in mitigating this effect. Between group differences were examined using the Bonferroni measure and a $p < 0.05$ established statistical significance.

7.2.3.2 Body Weight

An ANOVA was utilized for the comparison of change in body weight during the Post-Treatment period, specifically for the last day of Post-Treatment, day 5, JMV-2959 between Post-Treatment groups (VEH-JMV and NIC-JMV) and VEH Post-Treatment groups (VEH-VEH and NIC-VEH). Between group differences were examined using the Bonferroni measure and a $p < 0.05$ established statistical significance.

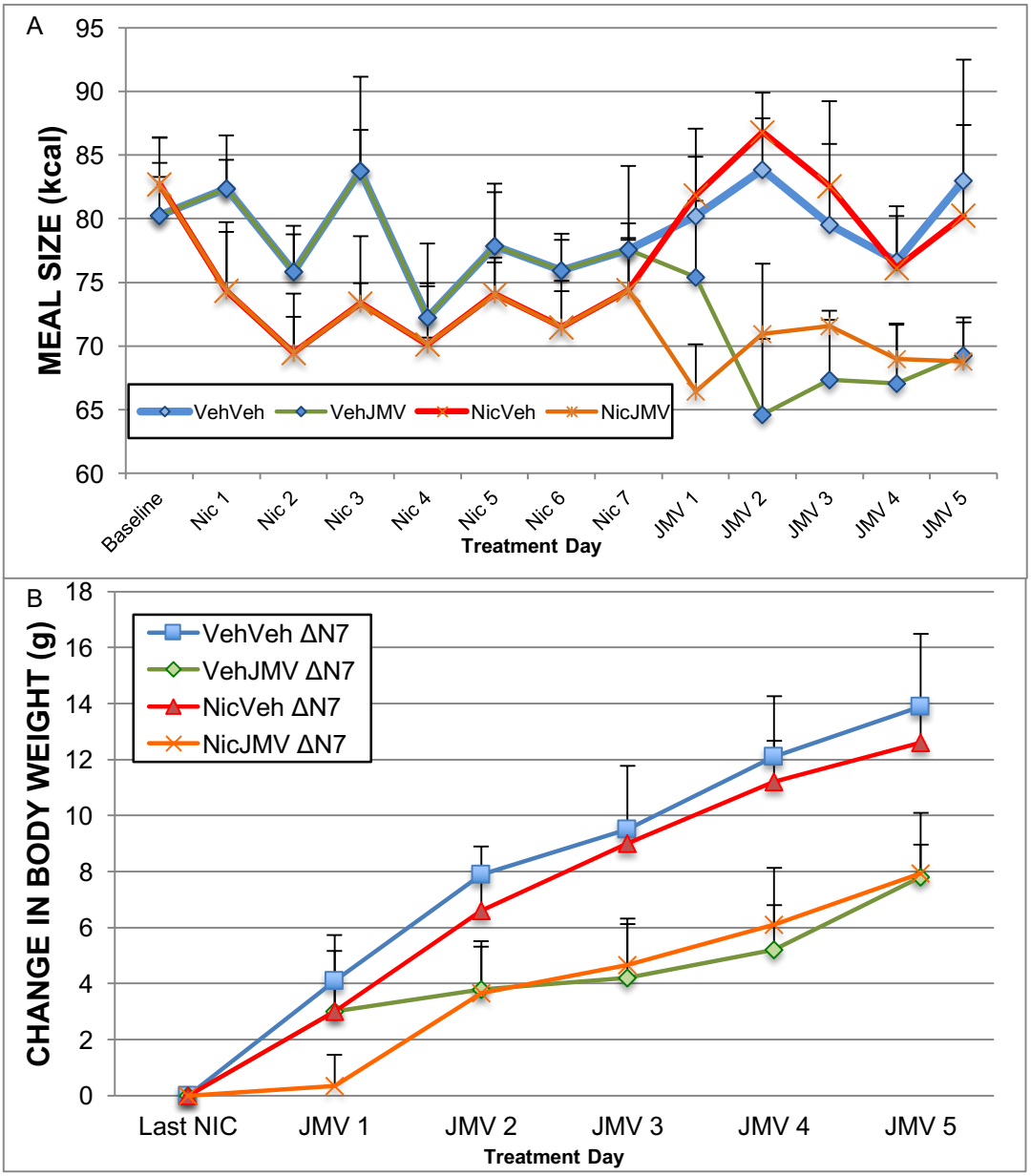


Figure 14. Food Intake & Weight Change Properties of NIC on JMV-2959. Panel A). Mean group caloric intake in kcals in male rats treated for 7 days with either 0.9% saline (VEH) or 1.4 mg/kg free base nicotine (NIC) maintained on high-fat diet (HIFAT). During the 5 days after cessation of NIC, all rats were injected daily with VEH or JMV-2959 (3 mg/kg). Panel B). Mean group changes in body weight (g) in male rats Treated for 7 days with either VEH or NIC and Post-Treated with VEH or JMV-2959 relative to Treatment cessation.

Panel descriptors: NIC 1–7 = Treatment days; JMV 1-5 = Post-Treatment days. [N=20]

7.3 Results

7.3.1 Food Intake

An ANOVA procedure revealed a significant difference between VEH and NIC Treatment groups on Treatment Days 1-7 ($F(2,18) = 4.953$, $p = 0.039$). A second ANOVA revealed a significant difference between VEH-VEH, VEH-JMV, NIC-VEH, and NIC-JMV Post-Treatment groups during Post-Treatment Days 1-5 ($F(4,16) = 5.874$, $p = 0.007$).

For all groups, with the exception of the NIC-JMV group, caloric intake increased over days (Figure 14: Panel A). Upon NIC cessation and the beginning of the Post-Treatment period, rats in the VEH Post-Treatment groups (VEH-VEH and NIC-VEH) showed a greater increase in caloric intake per day on Post-Treatment Days 1-5, while rats in the JMV-2959 Post-Treatment groups (VEH-JMV and NIC-JMV) displayed the opposite effect, with an immediate decrease in caloric intake on Day 1 of Post-Treatment that remained constant and persisted for the remainder of the study.

Planned between-group t-tests revealed differences in Treatment groups' (VEH vs. NIC) food intake during each Treatment Day (1-7). However, this difference was only significant on Treatment Day 3 ($t(8) = 2.268$, $p = 0.036$).

A planned between-group t-tests was utilized to assess the expected food intake spike, as seen by Post-Treatment Day 2 in Wellman et al., 2005, following Treatment cessation, for Post-Treatment groups (VEH-VEH, VEH-JMV, NIC-VEH, NIC-JMV) on Post-Treatment Days 1 and 2. A planned comparison revealed significant differences between NIC-VEH and NIC-JMV groups on Post-Treatment Days 1 and 2 ($t(8) = 2.329$,

$p = 0.048$ and $t(8) = 2.478$, $p = 0.038$, respectively). Of interest, by Post-Treatment Day 2, planned comparison also revealed significant differences between VEH-VEH and VEH-JMV groups ($t(8) = 2.642$, $p = 0.029$).

7.3.2 Body Weight

Figure 14 (Panel B) shows that after NIC cessation, rats in the JMV-2959 Post-Treatment groups (VEH-JMV and NIC-JMV) displayed a reduced body weight increase relative to that of rats in the VEH Post-Treatment groups (VEH-VEH and NIC-VEH). These changes in body weight were confirmed in an ANOVA with a significant group difference on Day 5 of the Post-Treatment period ($F(1,18) = 8.605$). NIC Treatment cessation resulted in weight gain for the NIC-VEH group, however, this body weight remained lower than those rats in the VEH-VEH group. The weight gain expressed by the NIC-VEH group was not noted in the NIC-JMV group.

7.4 Interim Discussion

The results for changes in food intake for this experiment were not at the magnitude of the NIC effect during the Treatment period as noted by Wellman et al., 2005. This experiment only focused on the effects of NIC on HIFAT intake (i.e. no CHOW group) and NIC exposure was halved to 7 days (as opposed to 14). The results herein show that the NIC Treated rats show an initial suppression in food intake versus VEH Treated rats and that NIC and VEH groups have a slight positive linear trend over Treatment Days 1-7. Once NIC is removed, however, and Post-Treatment begins, a divergence of food intake is noted. Those subgroups Post-Treated with JMV-2959

(VEH-JMV, NIC-JMV) show a decrease in food intake that persists over the Post-Treated VEH groups (VEH-VEH, NIC-VEH), which continue to progress in a linear fashion over the remainder of the experiment.

In regards to changes in body weight during the Post-Treatment period, the body weight of those rats Post-Treated with VEH (VEH-VEH, NIC-VEH) displayed a linear increase in weight that is likely related to their linear increase in food intake after the cessation of NIC (Post-Treatment Days 1-5). The rats Post-Treated with JMV-2959, however, showed a suppression of weight gain following NIC cessation.

As stated previously, the focus of this dissertation work was to not only investigate the capacity of JMV-2959 to prevent continued drug seeking for NIC, but to also investigate the capacity of JMV-2959 to alter food intake in an effort to prevent the onset of obesity typically seen in individuals who quit smoking, individuals likely utilizing tobacco cigarettes as a weight control substance.

Previous studies have shown that GHR administration is capable of stimulating food intake, increasing sucrose reward seeking (Figlewicz and Sipols, 2010, Dickson et al., 2011, Skibicka and Dickson, 2011, Skibicka et al., 2011a, Nakazato et al., 2001) and also results in an increased preference for HIFAT foods that leads to an increase in fat consumption (Shimbara et al., 2004) and leads to weight gain and adiposity (Wren et al., 2001). Conversely, GHR-R antagonism results in less peanut butter or Ensure® intake, but no change in CHOW consumption when given a choice (Egecioglu et al., 2010) and VTA damage results in a failure of GHR initiate increases in peanut butter consumption, however regular chow consumption remains unaffected (Egecioglu et al., 2010). These

results lead to the conclusion that JMV-2959 may be suitable for the treatment of not only NIC cessation-associated weight gain, but also for the treatment of obesity alone.

The data herein supports this notion, in that JMV-2959 alone is able to suppress free feeding and limit body weight gain, suppressing overconsumption of high fat food to normal CHOW feeding levels seen in Wellman et al., 2005 (~65 kcal/day in both NIC-JMV and VEH-JMV subgroups). This decrease in consumption appears to curtail the continued weight gain in rats not exposed to NIC (VEH-JMV) as well as the weight gain, and the spike in food intake, associated with NIC cessation seen both in the Wellman et al., 2005's HIFAT-NIC subgroup and in the NIC-VEH subgroup in this experiment.

It is important to note that JMV-2959, however, is unlikely to work on the same mechanisms that suppress feeding through the use of NIC. It is currently not understood how NIC alters feeding behavior and metabolism beyond its initial toxic effects, but the change in intake and body weight following cessation confirm that NIC exerts an inhibitory action on feeding and body weight, both in Wellman et al., 2005 and in the present study. The HIFAT diet possesses a rewarding property, the fat, that makes it attractive and leads to its overconsumption. JMV-2959 does not completely eliminate feeding, but instead suppresses feeding to homeostatic required levels, which implies that JMV-2959 is removing the rewarding value of the food and the rat is only consuming food to maintain energy homeostasis.

This experiment is the first to investigate the effect of JMV-2959 on NIC-cessation associated changes in food intake and weight gain as well as weight gain

typically seen following poor dieting leading to obesity. The results lay the foundation to understanding JMV-2959's potential as an obesity/overeating treatment strategy and potentially a dual-treatment method to fight NIC cessation relapse and NIC cessation related weight gain.

CHAPTER VIII

EXPERIMENT 6: EFFECT OF JMV-2959 ON CONDITIONED TASTE AVERSION INDUCED BY NICOTINE

8.1 Background

In previous experiments of this dissertation, JMV-2959 has suppressed reward-seeking behavior in a psychostimulant enhanced ICSS task and the psychostimulant IVSA task. This may occur because JMV-2959 blocks relevant reward circuits or because JMV-2959 induces malaise, which in turn nonspecifically suppresses behavior.

In the CTA task, water intake is measured during a 30-minute period on 6 successive days. On a training day, each rat is offered a novel saccharin solution during the 30-minute period which is then followed by an injection of either VEH or a drug solution. The saccharin solution, although possessing no caloric properties, is more appealing and the rats will typically consume more of it during this 30-minute period versus those bouts with just water. When the treatment injection is a malaise causing agent, such as LiCl, the rat will exhibit an avoidance behavior towards the saccharin on subsequent presentations when it is presented again (Niijima and Yamamoto, 1994). As a comparison, this is similar to eating a particularly appetizing dish and then coming down with a food-borne illness. Consequently, the dish might be avoided for quite some time, if not indefinitely. On days following the injection, both water and saccharin solution are made available during each 30-minute trial and the consumption volumes for both are recorded. This allows for the calculation of the percent of saccharin of the

total volume consumed to determine the effects of the agent that was injected on the subsequent seeking and consummatory behavior of the saccharin. No treatments are given on the subsequent days, often termed the Extinction Period. Rats exposed to malaise inducing agents will typically display a high-preference for the water over the saccharin with an increasing trend favoring the saccharin over trials.

In this CTA experiment, we aimed to assess the malaise properties of JMV-2959 (with and without NIC) relative to LiCl (Kumar, Pratt, and Stolerman, 1983; McMahon et al., 1998; Garcia et al., 1968).

8.2 Experimental Procedures

8.2.1 Subjects

This experiment utilized 25, ~300g, Sprague-Dawley male rats (Envigo) and followed the experimental procedure described in Section 2.1 of Chapter II.

<table>
<tr><th>Days 1-6 (Baseline)</th><th>Day 7 (Saccharin Exposure)</th><th>Days 8-11 (Extinction)</th></tr>
<tr><td rowspan="5">Open access to tap water from a drinking bottle</td><td>VEH</td><td rowspan="5">Open access to 0.1% saccharin and tap water in separate bottles (bottle position alternated daily)</td></tr>
<tr><td>NIC (.4 mg/kg)</td></tr>
<tr><td>JMV (3 mg/kg)</td></tr>
<tr><td>JMV (3 mg/kg) + NIC (.4 mg/kg)</td></tr>
<tr><td>LiCl (32 mg/kg)</td></tr>
<tr><td colspan="3">Table 6. Experiment 6: Effect of JMV-2959 on CTA induced by NIC</td></tr>
</table>

8.2.2 Behavioral Analysis

Table 6 outlines the timeline of the experiment. During testing, standard housing water bottles were replaced with 100-ml graduated drinking bottles (Wahmann). The rats were water deprived, daily, for 4 hours prior to each 30-min drinking session. Following a 30-min session, fluid intakes (nearest 1.0 ml) and body weight were recorded for each rat. Fluid volumes were not corrected for spillage. All sessions were conducted within the same time frame across days. Food was continuously available throughout the studies.

Days 1-6 were baseline days in which baseline fluid (water) drinking intakes were recorded. Five groups (n=5 for each) of comparable daily average fluid intake were formed using the baseline values recorded during Baseline Days 1-6. Rats were then randomly assigned to a treatment group: VEH, NIC (0.4 mg/kg), JMV-2959 (3 mg/kg)(JMV), JMV-2959 pre-treatment (3 mg/kg) plus NIC (0.4 mg/kg), or LiCl (32 mg/kg) group.

On Day 7, the rats were offered a saccharin solution (0.1%) (Sigma Aldrich) in place of the standard water bottle. Immediately after this session, each saccharin bottle was removed and the rats were injected with their assigned group drug schedule (listed above). An Extinction Period followed for Days 8-11. During each Extinction Day session, rats were allowed access to a water bottle and a saccharin bottle without further injections. The positions of the bottles were alternated on a daily basis to control for position preference.

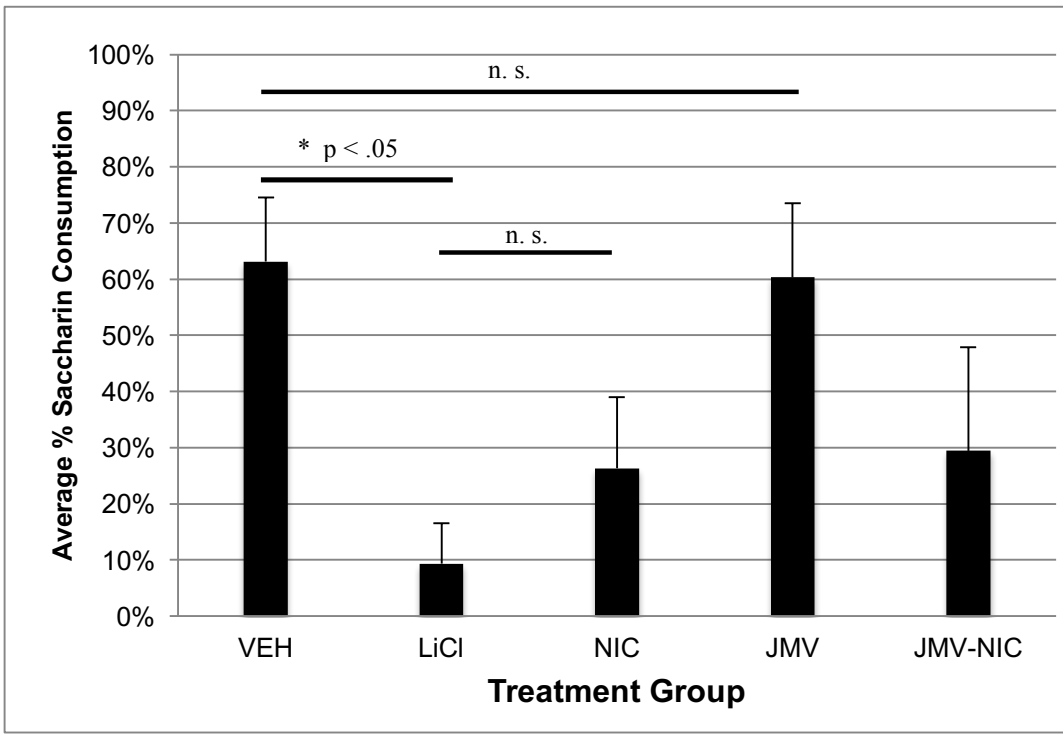


Figure 15. Induction of CTA by LiCl in Contrast to the Effects of JMV-2959. The figure depicts average percent consumption of saccharin across groups on the day following exposure to only saccharin and treatment with either VEH, LiCl (32 mg/kg), NIC (0.4 mg/kg), JMV-2959 (3 mg/kg), or NIC and JMV-2959 combined. [N=20]

8.2.3 Data Analysis

Three separate one-way ANOVAs were calculated (SPSS Version 23, St. Louis, MO). The first was utilized to investigate differences across treatment groups for average baseline consumption of water for Baseline Days 1-6. A second was utilized to investigate differences in saccharin consumption on the treatment day, prior to treatment injection (IP) (Drug Treatment: VEH, LiCl, NIC, JMV-2959, NIC + JMV-2959). A

final one-way ANOVA sought differences in the percentage of saccharin consumption versus total consumption when rats were given a choice of water or saccharin during the 30-minute session on the first day of extinction. A series of a priori t-tests were also computed to analyze planned comparisons between treatment groups. A risk level of $p < 0.05$ was set to establish statistical significance.

8.3 Results

A one-way ANOVA confirmed that all treatment groups consumed comparable levels of water during the baseline period, as well as saccharin on the Treatment Day (data not depicted). A one-way ANOVA for baseline water consumption revealed no differences across all 5 groups, $F(4,20) = 0.337$, $p = 0.849$ (data not depicted). A second one-way ANOVA for saccharin consumption prior to treatment exposure also found no difference across the 5 groups, $F(4,20) = 0.488$, $p = 0.745$. A final one way ANOVA looked at the treatment effect on percent saccharin consumption when given the option of water or saccharin during the 30-minute session (Figure 15) and found the effect to be significant, $F(4,20) = 3.106$, $p = 0.027$.

Planned between-group t-tests for Extinction Day 1 determined a significant difference in saccharin consumption between VEH and LiCl groups ($t(8) = 3.955$, $p = 0.004$), as well as between JMV-2959 and LiCl groups ($t(8) = 4.039$, $p = 0.004$). Saccharin consumption for the JMV-2959 group was comparable that of the VEH group, showing no significant difference. This confirms that JMV-2959 alone is unlikely to alter reward behavior as a side effect of inducing malaise. While the VEH group almost

did ($t(8) = 2.146$, $p = 0.0642$), the JMV-2959 group ($t(8) = 2.067$, $p = 0.0363$) consumed significantly more saccharin than did the NIC treated rats. However, VEH treated animals did not consume significantly more saccharin than animals co-administered NIC with JMV-2959 ($t(8) = -1.550$, $p = 0.160$).

Two final t-tests determined that NIC treatment alone showed an effect of malaise comparable to that of LiCl ($t(8) = 1.158$, $p = 0.280$), which was seen also when comparing the NIC group to the group treated with a combination of JMV-2959 and NIC ($t(8) = 1.576$, $p = 0.190$). These two tests support the notion not only is NIC capable of inducing malaise similar to LiCl, but that JMV-2959 is not capable of making NIC more aversive when co-administered. This confirms that JMV-2959 does not induce malaise contingent on its combination with NIC, an effect that would alter the interpretation of previous research evaluating JMV-2959/psychostimulant combinations across paradigms.

8.4 Interim Discussion

In an unpublished study by our laboratory, JMV-2959 attenuated the rewarding action of NIC in the ICSS task, as well as NIC and COC-seeking in the IVSA tasks of Experiments 2 and 3. This may occur because JMV-2959 blocks the rewarding substrate or because JMV-2959 produces malaise, which in turn suppresses behavior. The present study confirms that the results of experiments utilizing JMV-2959, including all above, are not a result of JMV-2959 inducing malaise. Figure 16 show the results in which LiCl was utilized as a malaise-inducing control, in causing avoidance of the saccharin in

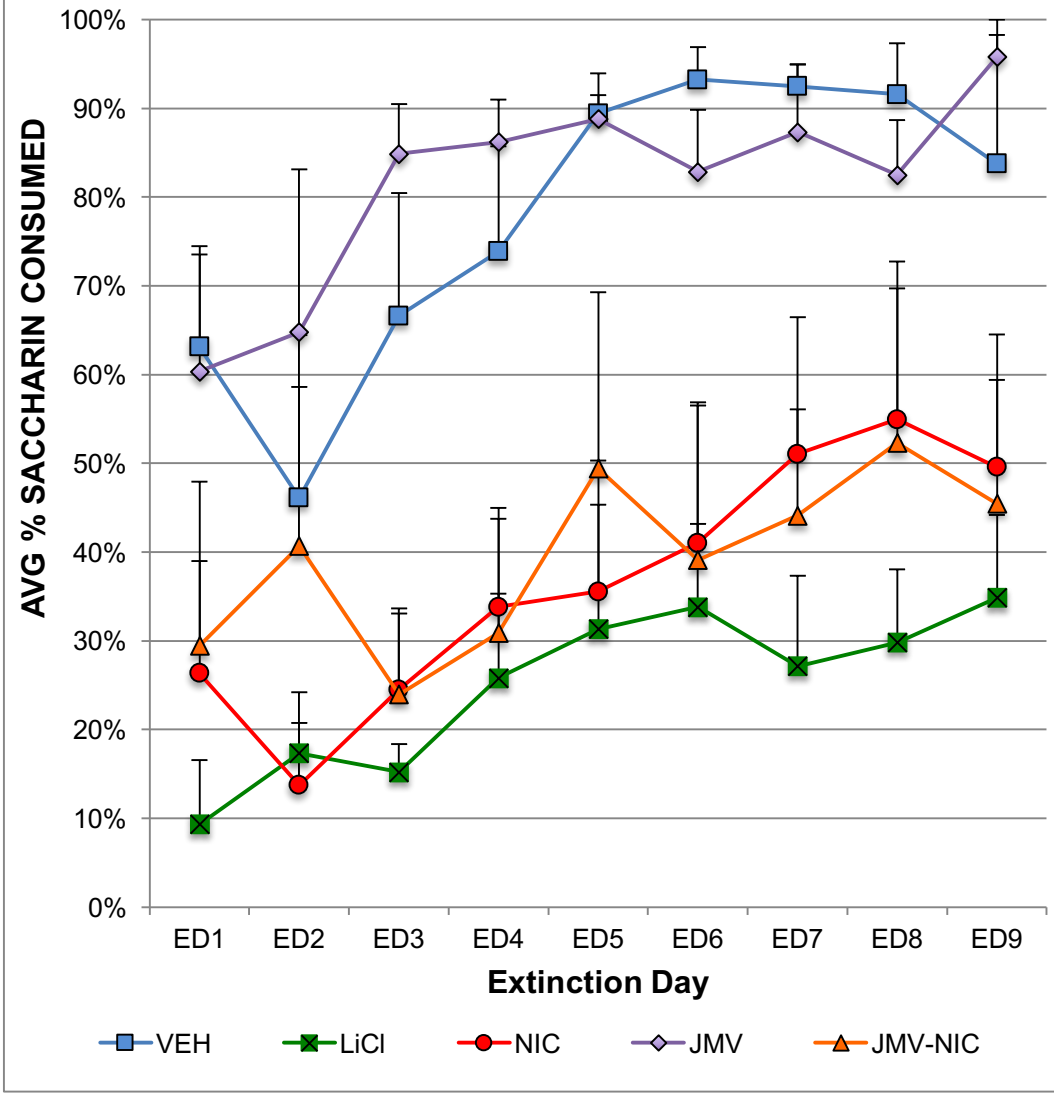


Figure 16. Average Percent Saccharin Consumed Over Days. The average percentage of saccharin consumed increased at a linear rate for all groups over extinction trial days (ED). Animals treated with JMV-2959 (3mg/kg) showed consumption rates comparable to those in the positive vehicle control group. [N=20]

future bouts, and VEH utilized as a regular control, for which rats prefer to consume saccharin whenever it is presented as an option. NIC alone resulted in consumption levels falling between that of the LiCl and VEH controls, an understandable finding given that initial NIC exposure is understood to induce nausea, which in turn would result in malaise (Mishra et al., 2015). An important finding of this study was that JMV-2959 alone did not alter total fluid consumption on Extinction Day 1. More importantly, however, was that JMV-2959 also did not alter the aversiveness of NIC when combined with NIC. This is important because the studies here, and previous studies by other labs investigating GHR have seen suppression of behavior with JMV-2959, primarily when JMV-2959 injections were coupled with psychostimulant treatment. This study strongly suggests that behavioral suppression in those situations in which JMV-2959 is coupled with a psychostimulant is not due to malaise.

When looking at the progressive effects of the saccharin and injection exposure (Figure 16), we note that the consumption of the saccharin consumption remained low for those groups which experienced a degree of malaise (LiCl, NIC, NIC plus JMV-2959), but shows some recovery at a positively increasing linear rate over days. Average percent saccharin consumption levels of VEH and JMV-2959 administered alone begin at 60% and climb to almost a 90% of all fluid consumption within 3 days of extinction versus malaise associated groups which remain under 50% for a majority of the study.

CHAPTER IX

GENERAL DISCUSSION AND CONCLUSION

The results herein show that the GHR antagonist, JMV-2959, was capable of preventing the capacity of psychostimulants, NIC and COC, to be self-administered during maintenance phase addiction. These two psychostimulants are thought to act via activation of DA cells within the VTA, in which GHR-R are located, while JMV-2959 is thought to block this activation of DA cells within the VTA (Abizaid et al., 2006, Skibicka et al., 2011b, Wellman et al., 2012). In an unpublished pilot study, JMV-2959 was also capable of blocking the excitatory effect of NIC on ICSS. However, JMV-2959 (3 or 6 mg/kg) is not capable of altering ICSS responding rates when administered independent of NIC. These results suggest that JMV-2959 is capable of altering enhancements, not only produced by psychostimulants administered acutely, but also capable of mitigating continued drug seeking in paradigms utilizing chronic NIC or COC administration, but not capable of affecting chronic ICSS activity when administered alone. The latter is likely due to the ICSS electrodes placement downstream from GHR-R receptors of VTA neurons that JMV-2959 and GHR work on, allowing for ICSS reward-seeking to remain unaffected.

Moreover, GHR (30 nmol) pretreatment did not alter the reinforcing actions of COC in a self-administration task, regardless if COC was given at a large dose (0.5 mg/kg/inf), a medium dose (0.25 mg/kg/inf), or a small dose (0.125 mg/kg/inf). One explanation for these results is that COC acts on a GHR-dependent reward mechanism

such that augmenting GHR tone does not enhance reward whereas blockade of GHR tone does – this is consistent with the notion that GHRs have a degree of constitutive activity (Akamizu et al., 2004), meaning that, in most situations, GHR-Rs are always at a high level of tone and, thus, augmenting GHR may fail to increase response behavior.

JMV-2959 was also noted to suppress HIFAT intake and weight gain in VEH treated rats as well as under conditions following NIC treatment, which was also administered over days while the same HIFAT was made available. Each outcome suggests a useful feature of the JMV-2959 molecule. It can, not only, diminish appetite by itself, preventing overeating and slowing weight gain associated with poor, typically HIFAT, dieting, but it can also help to diminish the appetite spikes typically seen in smokers attempting to cease NIC use and as seen in our lab's previous findings (Wellman et al., 2005), which would mitigate, or at least slow, the onset of weight gain seen following NIC-cessation when a HIFAT choice is available.

A key concern of the JMV-2959 literature is that this molecule may be capable of mitigating behaviors, such as those the IVSA and food experiments above, due to aversive properties that can diminish responding for food or drug-related reward. This proposition is false for two reasons – the first is that JMV-2959 did not alter ICSS responding at doses that block NIC-enhanced ICSS when presented alone (unpublished data). Secondly, a formal experiment herein compared the aversive properties of NIC, JMV-2959 and LiCl in a CTA task. Whereas NIC treatment (0.4 mg/kg) produced evidence of CTA similar to that noted in rats treated with the prototypical aversive agent LiCl (32 mg/kg); JMV-2959 (3 mg/kg) alone did not. One might ask if, perhaps, JMV-

2959, when coupled with NIC or COC, augments the toxicity of the drug, as typically seen in NIC, during its initial use and as seen here in the CTA experiment. However, concomitant administration of JMV-2959 and NIC resulted in comparable saccharin consumption on the day following treatment as those rats treated with NIC alone. Therefore, JMV-2959 is incapable of augmenting or synergizing the toxic effect of NIC, capable of inflicting a toxic effect in JMV-2959, nor capable of rescuing its toxic effects seen with NIC.

Many routes of NIC ingestion are available, however, smoking is the greatest form of concern when considering the health issues and morbidity associated with NIC use. While many chronic users will attempt to quit smoking, via NRTs, cold turkey, or currently available pharmacological approaches, many will fall far short and their effort will not persist. Tobacco companies are in the business of making money and do nothing more to prevent current and new users from falling into a financially and life-threatening habit outside of slapping a small government-required warning label of, "SURGEON GENERAL'S WARNING: Smoking Causes Lung Cancer, Heart Disease, Emphysema, and May Complicate Pregnancy." The choice and will is left in the hands of the smoker to acknowledge those warnings and give up the habit, a will most do not have.

Of course, a better strategy would be to prevent individuals from ever engaging in smoking and eventually becoming sensitized to NIC addiction. While little is done, particularly pharmacologically, to prevent the onset of NIC addiction, it is important to note that prevention by any means (e.g. great parenting/parental control and good, legal,

business practice) may help prevent smokers, especially the young, from continuing use before becoming fully sensitized and transitioning into an addictive state in which they possess the compulsive urge to seek out and maintain the drug-induced state.

Upon deciding to quit, users experience a rough period of withdrawal, with reoccurring symptoms so aversive that even a person who has experienced the occasional migraine might agree that productivity and quality of life might depreciate enough to turn back to smoking just to return to normalcy. As discussed, for some, smoking is purely a desire by the user to obtain the reinforcing properties of the cigarette. For others, however, it allows for the continuance of very poor feeding habits, typically heavy consumption of calories in the form of fat and alcohol (Dallongeville et al., 1998). The benefit of the NIC use is that it also allows for a person to consume calorically dense fat and alcohol poor feeding habits without much consequence and for such users to weigh less than non-smokers (Klesges et al., 1989; Zoli and Picciotto, 2012) - that is, until they try to quit smoking. Withdrawal issues result in difficulty with treatment compliance, typically via NRTs, for those who see weight gain and are affected by stigmatizations and/or have weight-related health concerns (Pomerleau and Saules, 2007). Sadly, there are only a few interventions tailored to assist in the smoking cessation while also mitigating the weight gain (Farley et al., 2012).

This has led laboratories, like that of our own, to attempt to find a dual-treatment that tackles the urge to return to NIC from primary withdrawal symptoms and the weight gain from it. Currently available methods for cessation do not assess the weight gain and those available pharmacological approaches that may have some promise in this area

fall short due to a lack of specificity that leads to symptoms that are just as horrendous as those that come with NIC withdrawal, if not worse. It is therefore important to find a dual-treatment approach with a greater specificity with less to no symptoms generated that would outweigh the symptoms of NIC withdrawal typically leading the user back to smoking.

The current literature strongly supports a key role for GHR and GHR-Rs within the VTA for the induction of reinforcement for multiple classes of reinforcers including food and perhaps other natural rewards, drugs of abuse and novelty (Jerlhag et al., 2009, 2010; Perello et al., 2010; Dickson et al., 2011; Egecioglu et al., 2011; Jerlhag and Engel, 2011; Skibicka and Dickson, 2011; Hansson et al., 2012; Wellman et al., 2012). This suggests that there may be a therapeutic approach to addiction via these GHR-R receptors. These GHR-Rs, along with cholinergic receptors, can be found on cholinergic neurons projecting from the laterodorsal tegmental area (LDTg) to the VTA. Activation of the VTA then results in increased DA release within the NAc (Jerlhag et al., 2006b, Dickson et al., 2011). This leads to the speculation that NIC may have the capacity to alter the activity of VTA neurons via the cholinergic receptors, while GHR antagonism can non-competitively block target the GHR-Rs to inactivate the same neurons. This notion is supported by findings showing that GHR infusions into the LDTg enhance locomotion and induces NAc DA release (Jerlhag et al., 2007). Conversely, acute GHR-R antagonism blocks this effect, as well as the development of CPP by NIC, and the consequential DA increases within the NAc (Jerlhag and Engel, 2011). While there is a mechanistic difference of action, all routes seem to lead back to the VTA and reward/DA

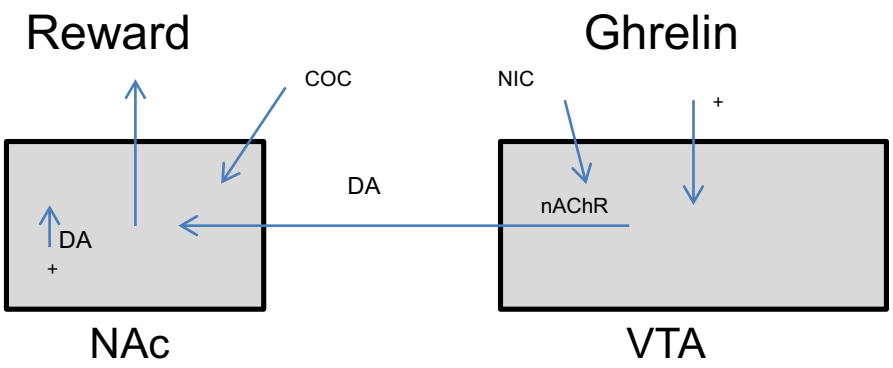


Figure 17. Theorized GHR Influence on Reward Circuit.

modulation circuit. Figure 17 shows our theorized model of GHR influences on the reward circuit given the current findings in the literature.

Our preliminary behavioral studies (Wellman et al., 2011, 2012) support the notion that JMV-2959, and drugs of similar structure, may provide an option for blocking psychostimulant-induced reinforcement. In regards to our focus here, this would be of value when considering options for smoking cessation. The literature regarding GHR-R antagonism for the prevention and mitigation of drug-seeking behavior is small and consists of only studies utilizing mice and rats and therefore requires, not only confirmation of these observations, but also demonstration that GHR-R antagonists like JMV-2959 can aid in smoking abstinence.

With regards to feeding behavior, injections of GHR into the VTA and the NAc, both acutely and chronically, can induce feeding (Naleid et al., 2005; King et al., 2011; Skibicka and Dickson, 2011; Skibicka et al., 2011a). Palatable foods possess rewarding

properties and the consumption of these foods is GHR-dependent, primarily for sweet and fatty foods (Egecioglu et al., 2010; Perello et al., 2010; Skibicka and Dickson, 2011; Skibicka et al., 2011b). This suggests that GHR-R antagonism and GHR's role here may not be limited to drug use, but may also be useful in the assistance to those who wish to control palatable food overconsumption. This would set GHR-R antagonists up as a potential treatment method for those who are overweight and obese. Experiment 5 shows that this is indeed plausible, as JMV-2959 following NIC Treatment cessation, or VEH cessation slowed weight gain for the HIFAT diet rats. This makes the reward circuit a prime candidate for investigation and supports our belief that GHR-R drug antagonists may have a dual utility for the treatment of NIC addiction: the first being antagonism of the rewarding action of NIC; the second being suppression of the weight gain often noted following smoking cessation (Wellman et al., 2013).

The experiments herein support the possible use of drugs like JMV-2959 in mitigating drug and food seeking during the maintenance phase of addiction while also mitigating the rewarding properties of food after NIC exposure, and potentially independent of NIC exposure, and preventing weight gain, as has been shown here. The results for these experiments strengthen the notion that not only is GHR key to reward, but that GHR-R antagonism is a strong candidate for dual-treatment with possibly no side effects and greater specificity in eliminating NIC addiction while also mitigating the possible weight that is a major contributor to relapse.

Future studies should venture into investigating several areas. First, as stated in the introductory chapter, is due to the general concern that women may be more

susceptible to smoking relapse due to standards created and pushed by mainstream media that women must be skinny in order to be considered attractive. As noted, Vener and Krupka (1985) found that the obese are more likely to be stigmatized for being overweight/obese, while Pomerleau and Kurth (1996) found that women were unwilling to gain more than 5 pounds within a given month as a result of smoking cessation. The current experiments only utilized male rats. Due to the concern for women stated above and the ability of JMV-2959 to control body weight in male rats, investigations, particularly the feeding study, should be conducted utilizing only female rats. Recent findings (unpublished 2016) by Carrie Ferrario's laboratory have shown that the estrous cycle phase modulates cue-triggered motivation for food in obesity-prone but not in obesity-resistant rats. This, of course, is not a contributing factor when investigating males. Because of this, a drug like JMV-2959 may have the capacity to block this motivation and assist females that are obesity-prone. It would, therefore, be of particular interest to investigate differences in HIFAT food intake and determining if female rats along different time points of the estrous cycle following and independent of NIC exposure.

Another area of focus for future studies should seek to investigate the capacity of JMV-2959 to alter drug-seeking for substances with abuse potentials that are not classified as psychostimulants. The experiments discussed herein only focused on two compounds, NIC and COC, but it may also be the case that GHR-R blockade may alter the reinforcing action and drug-seeking of ethanol, another highly concerning addictive substance with little to no pharmacological options for assistance in treatment. Doyon et

al.'s (2013) investigations of the promotion of drinking via smoking reported that smokers have increased alcohol intake as well as a reciprocal interaction (Barrett et al., 2006; Harrison et al., 2008; McKee et al., 2007; Weitzman and Chen, 2005). In fact, 83% of alcoholics also smoke (Burling and Ziff, 1988; Batel et al., 1995; DiFranza and Guerrera, 1990) and alcohol binging occurs more regularly in smokers versus non-smokers (Harrison et al., 2008; Weitzman and Chen, 2005). Given our findings, this would be the first logical step in investigating JMV-2959 on other drug systems. Previous studies by Uri Shalev's laboratoy have determined that GHR has a limited role in heroin seeking and, therefore, should not be further investigated with JMV-2959 (Maric et al., 2011), however, methamphetamine and THC may be of interest.

Investigations should also seek to utilize other pharmacological compounds and methods of inactivation of GHR and/or GHR receptors. For instance, CF801, a novel peptide, reduces circulating acylated GHR levels by inhibiting ghrelin-O-acyltransferase, the only known enzyme capable of acylating (activating) GHR so that it is capable of binding to and activating neurons via GHR-Rs. CF801 was recently shown to, acutely, decrease rebound feeding after an overnight fast, chronically decrease weight gain and adiposity without affecting caloric intake, and change in diet preference away from HIFAT (Wellman et al., 2015). Given these early findings, it would be of interest to conduct, once again, an experiment similar to Wellman et al., 2005 and Experiment 5 here with the use of CF801 instead of JMV-2959 to determine if CF801 has the capacity to reduce or eliminate the initial spike in food consumption and weight that occur upon NIC cessation. Investigating this, as well as CF801's capacity to alter ICSS and

psychostimulant IVSA would support the notion that GHR-R inactivation or simply the reduction of GHR availability via drugs like JMV-2959 and CF801, respectively, offer a possible dual-treatment solution for NIC addiction and weight gain associated with NIC-cessation or general poor food diets.

Finally, Prader-Willi Syndrome is a genetic disorder that affects 1 in 10,000 to 30,000 people worldwide (Genetics Home Reference, 2017). While in infancy, this disorder is characterized by hypertonia and poor weight gain, along with mental retardation and growth hormone insufficiency (Cassidy and Driscoll, 2009), however, beginning in early childhood and into adulthood, it is associated with elevated acylated GHR levels, consistent with hyperphagia, that lead to obesity in these individuals (Hagg et al., 2008; Purtell et al., 2011; Kuppens et al., 2015). A mouse model of Prader-Willi syndrome has been developed by Jeffrey Zigman's laboratory and, because of the elevated levels of GHR that lead to hyperphagia and obesity, as well as the results found in Experiment 5 here, it would be a suitable model for investigating what drugs like JMV-2959 may have to offer individuals with this disorder, as well as allow for a better understanding of the role of GHR in Prader-Willi Syndrome and in general.

Since 1998 our understanding of GHR and the literature has grown tremendously. GHR is no longer simply "The Hunger Hormone." It has been implicated in neuroprotective roles when blood glucose levels drop, in mood, depression, stress and reward-seeking behavior for drug-reinforcers and natural reinforcers. There remains plenty to understand about GHR and its role in other symptoms. I look forward to continuing to contribute to this science for the years to come.

REFERENCES

Abizaid A, Liu ZW, Andrews ZB, Shanabrough M, Borok E, Elsworth JD et al. (2006) Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. J Clin Invest 116(12):3229-39.

Agarwal DK and Bose M (1992) Inhalation toxicity of methylisocyanate: Assessment of germ cell mutagenicity and reproductive effects in rats. Indian J Exp Biol 30(6):504-8.

Akamizu T, Takaya K, Irako T, Hosoda H, Teramukai S, Matsuyama A et al. (2004) Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. Eur J Enocrinol 150(4):447-55.

Alpert H, Biener L, and Connolly G (2012) Nicotine replacement therapies may not be effective in helping people quit smoking. Harvard School of Public Health. www.hsph.harvard.edu/news/press-releases/nicotine-replacement-therapies/

American Psychiatric Association (2010) Practice guideline for the treatment of patients with substance use disorders: Second edition. APA. https://psychiatryonline.org/pb/assets/raw/sitewide/practice_guidelines/guidelines/substanceuse.pdf

Asakawa A, Inui A, Kaga T, Katsuura G, Fujimiya M, Fujino MA, and Kasuga M (2003) Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. Gut 52(7):947-52.

Barrett SP, Tichauer M, Leyton M, and Pihl RO (2006) Nicotine increases alcohol self-administration in non-dependent male smokers. Drug Alcohol Depend 81(2):197–

204.

Batel P, Pessione F, Maitre C, and Rueff B (1995) Relationship between alcohol and tobacco dependencies among alcoholics who smoke. Addiction 90(7):977-80.

Bell SM, Stewart RB, Thompson SC, and Meisch RA (1997) Food-deprivation increases cocaine-induced conditioned place preference and locomotor activity in rats. Psychopharmacology 131(1):1-8.

Bellinger L, Cepeda-Benito A, and Wellman PJ (2003) Meal patterns in male rats during and after intermittent nicotine administration. Pharmacol Biochem Behav 74(2):495-504.

Bellinger L, Wellman PJ, Cepeda-Benito A, Kramer PR, Guan G, Tillberg CM et al. (2005) Meal patterns in female rats during and after intermittent nicotine administration. Pharmacol Biochem Behav 80(3):437-44.

Benowitz NL (2010) Nicotine addiction. Engl J N Med 362(24):2295–303.

Benowitz NL and Jacob P 3rd (1984) Daily intake of nicotine during cigarette smoking. Clin Pharmacol Ther 35(4):499–504.

Berton O and Nestler EJ (2006) New approaches to antidepressant drug discovery: Beyond monoamines. Nat Rev Neurosci 7(2):137-51.

Blaha V, Yang ZJ, Meguid M, Chai JK, and Zadak Z (1998) Systemic nicotine administration suppresses food intake via reduced meal sizes in both male and female rats. Acta Medica 41(4):167-73.

Bray, GA (2000). A concise review on the therapeutics of obesity. Nutrition 16(10):953-60.

Burkey RT and Nation JR (1994) Brain stimulation reward following chronic lead exposure in rats. Behav Neurosci 108(3):532-6.

Burling TA and Ziff DC (1988) Tobacco smoking: A comparison between alcohol and drug abuse inpatients. Addict Behav 13(2):185-90.

Cabeza De Vaca S, Holiman S, and Carr KD (1998) A search for the metabolic signal that sensitizes lateral hypothalamic self-stimulation in food-restricted rats. Physiol Behav 64(3):251-60.

Carlezon, WA Jr. and Chartoff EH (2007) Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. Nat Protoc 2(11):2987-95.

Carroll ME (1985) The role of food deprivation in the maintenance and reinstatement of cocaine-seeking behavior in rats. Drug Alcohol Depend 16(2):95-109.

Carroll ME, Lynch WJ, Roth ME, Morgan AD, and Cosgrove KP (2004) Sex and estrogen influence drug abuse. Trends Pharmacol Sci 25(5):273-9.

Carton S, Le Houezec J, Lagrue G, and Jouvent R (2000) Relationships between sensation seeking and emotional symptomatology during smoking cessation with nicotine patch therapy. Addictive Beh 25(5):653-62.

CDC. Health, US (2004) 2004: With chartbook on trends in the health of Americans. National Center for Health Statistics. DHHS Publication No.2004-1232. cdc.gov/nchs/data/hus/hus04trend.pdf#069

Changeux, JP (2010) Nicotine addiction and nicotinic receptors: Lessons from genetically modified mice. Nat Rev Neurosci 11(6):389-401.

Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, and Weigle DS (2001) A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50(8):1714-9.

Dallongeville J, Marecaux N, Fruchart JC, and Amouyel P (1998) Cigarette smoking is associated with unhealthy patterns of nutrient intake: A meta-analysis. J Nutr 128(9):1450-7.

Damian M, Marie J, Leyris JP, Fehrentz JA, Verdie P, Martinez J et al. (2012) High constitutive activity is an intrinsic feature of Ghrelin receptor protein: A study with a functional monomeric GHS-R1a receptor reconstituted in lipid discs. J Biol Chem 287(6):3630-41.

Dani JA and Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol 47:699–729.

Davidson TL, Kanoski SE, Tracy AL, Walls EK, Clegg D, and Benoit SC (2005) The interoceptive cue properties of ghrelin generalize to cues produced by food deprivation. Peptides 26(9):1602-10.

Davis KW, Wellman PJ, and Clifford PS (2007) Augmented cocaine conditioned place preference in rats pretreated with systemic ghrelin. Regul Pept 140(3):148-52.

De Biasi M and Dani JA (2011) Reward, addiction, withdrawal to nicotine. Annu Rev Neurosci 34:105-30.

Degenhardt L and Hall W (2001) The relationship between tobacco use, substance-abuse disorders and mental health: Results from the National Survey of Mental Health

and Well-being. Nicotine Tob Res 3(3):225-34.

Di Chiara G, Bassero V, Fenu S, De Luca MA, Spina L, Cadoni C et al. (2004) Dopamine and drug addiction: The nucleus accumbens shell connection. Neuropharmacology. 47 Suppl 1:227-41.

Diano S, Farr SA, Benoit SC, McNay EC, da Silva I, Horvath B et al. (2006) Ghrelin controls hippocampal spine synapse density and memory performance. Nat Neurosci 9(3):381-8.

Dickson SL, Egecioglu E, Landgren S, Skibicka KP, Engel JA, and Jerlhag E (2011) The role of the central ghrelin system in reward from food and chemical drugs. Mol Cell Endocrinol 340(1):80-7.

DiFranza JR and Guerrera MP (1990) Alcoholism and smoking. J Stud Alcohol 51(2):130-5.

Doyon WM, Thomas AM, Ostroumov A, Dong Yu, and Dani JA (2013) Potential substrates for nicotine and alcohol interactions: A focus on the mesocorticolimbic dopamine system. Biochem Pharm 86(8):1181-93.

Drazen DL, Vahl TP, D'Alessio DA, Seeley RJ, and Woods SC (2006) Effects of a fixed meal pattern on ghrelin secretion: Evidence for a learned response independent of nutrient status. Endocrinology 147(1):23-30.

Egecioglu E, Skibicka KP, Hansson C, Alvarez-Crespo M, Friberg PA, Jerlhag E et al. (2011). Hedonic and incentive signals for body weight control. Rev Endocr Metab Disord 12(3):141-51.

Egecioglu E, Jerlhag E, Salome N, Skibicka KP, Haage D, Bohlooly-Y M et al. (2010)

Ghrelin increases intake of rewarding food in rodents. Addict Biol 15(3):304-1.

Envigo (2015) 8604: Teklad rodent diet. http://www.envigo.com/resources/data-sheets/8604-datasheet-0915.pdf

Fakhouri TH, Ogden CL, Carroll MD, Kit BK, and Flegal KM (2012) Prevalence of obesity among older adults in the United States, 2007–2010. NCHS Data Brief (106):1–8.

Farley AC, Hajek P, Lycett D, and Aveyard P (2012) Interventions for preventing weight gain after smoking cessation. Cochrane Database Syst Rev 1:CD006219.

Filozof C, Fernandez Pinilla MC, and Fernandez-Cruz A (2004) Smoking cessation and weight gain. Obes Rev 5(2):95-103.

Figlewicz DP and Sipols AJ (2010) Energy regulatory signals and food reward. Pharmacol Biochem Behav 97(1):15-24.

Finger BC, Dinan TG, and Cryan JF (2011). Diet-induced obesity blunts the behavioural effects of ghrelin: Studies in a mouse-progressive ratio task. Psychopharmacology (Berl) 220(1):173-81.

Flegal KM, Carroll MD, Kit BK, and Ogden CL (2012) Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999–2010. JAMA 307(5):491-7.

Fryar CD, Carroll MD, and Ogden CL (2012) Prevalence of overweight, obesity, and extreme obesity among adults: United States, Trends 1960-1962 through 2009-2010. Health & Stats 2012. www.cdc.gov/nchs/data/hestat/obesity_adult_09_10/obesity_adult_09_10.pdf

Fulton S (2010) Appetite and reward. Front Neuroendocrinol 31(1):85-103.

Garcia J, McGowan BK, Ervin FR, and Koelling RA (1968) Cues: Their relative effectiveness as a function of the reinforcer. Science 160(3829):794-5.

Genetics Home Reference (2017) Prader-Willi Syndrome. https://ghr.nlm.nih.gov/condition/prader-willi-syndrome#

Grenhoff J, Aston-Jones G, and Svensson TH (1986) Nicotinic effects on the firing pattern of midbrain dopamine neurons. Acta Physiol Scand 128(3):351-8.

Grunberg NE, Winders SE, and Popp KA (1987) Sex differences in nicotine's effects on consummatory behavior and body weight in rats. Psychopharmacology 91(2):221-5.

Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ et al. (1997) Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. Brain Res Mol Brain Res 48(1):23-9.

Hansson C, Shirazi RH, Naslund J, Vogel H, Neuber C, Holm G et al. (2012) Ghrelin influences novelty seeking behavior in rodents and men. PLoS ONE 7(12):e50409.

Harrison EL, Desai RA, and McKee SA (2008) Nondaily smoking and alcohol use, hazardous drinking, and alcohol diagnoses among young adults: Findings from the NESARC. Alcohol Clin Exp Res 32(12):2081-7.

Hays JT and Ebbert JO (2010) Adverse effects and tolerability of medications for the treatment of tobacco use and dependence. Drugs 70(18):2357–72.

Holst B, Cygankiewicz A, Jensen TH, Ankersen M, and Schwartz TW (2003) High

constitutive signaling of the ghrelin receptor–identification of a potent inverse agonist. Mol Endocrinol 17(11):2201-10.

Holst B, Holliday ND, Bach A, Elling CE, Cox HM, and Schwartz TW (2004) Common structural basis for constitutive activity of the ghrelin receptor family. J Biol Chem 279(51):53806-17.

Hosoda H, Kojima M, and Kangawa K (2006) Biological, physiological, and pharmacological aspects of ghrelin. J Pharmacol Sci 100(5):298-410.

Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI et al. (1996) A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273(5277):974-7.

Hughes JR (1996) The future of smoking cessation in the United States. Addiction 91(12):1797-802.

Imperato A, Mulas A, and Di Chiara G (1986) Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. Eur J Pharmacol 132(2-3):337-8.

Ivanova S and Greenshaw AJ (1997) Nicotine-induced decreases in VTA electrical self-stimulation thresholds: Blockade by haloperidol and mecamylamine but not scopolamine or ondansetron. Psychopharmacology (Berl) 134(2):187–92.

Jerlhag E and Engel JA (2011) Ghrelin receptor antagonism attenuates nicotine-induced locomotor stimulation, accumbal dopamine release and conditioned place preference in mice. Drug Alcohol Depend 117(2-3):126-31.

Jerlhag E, Egecioglu E, Dickson SL, and Engel JA (2010) Ghrelin receptor antagonism

attenuates cocaine and amphetamine-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference. Psychopharmacology (Berl) 211(4):415-22.

Jerlhag E, Egecioglu E, Landgren S, Salome N, Heilig M, Moechars D et al. (2009) Requirement of central ghrelin signaling for alcohol reward. Proc Natl Acad Sci USA 106(27):11318-23.

Jerlhag E, Egecioglu E, Dickson SL, Douhan A, Svensson L, and Engel JA (2007) Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. Addict Biol 12(1):6-16.

Jerlhag E, Grøtli M, Luthman K, Svensson L, and Engel JA (2006a) Role of the subunit composition of central nicotinic acetylcholine receptors for the stimulatory and dopamine-enhancing effects of ethanol. Alcohol 41(5):486-93.

Jerlhag E, Egecioglu E, Dickson SL, Andersson M, Svensson L, and Engel JA (2006b) Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward. Addict Biol 11(1):45-54.

Jha P, Ramasundarahettige C, Landsman V, Rostron B, Thun M, Anderson RN et al. (2013) 21st Century Hazards of Smoking and Benefits of Cessation in the United States. New England Journal of Medicine 368(4):341-50.

Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE et al. (2006) Efficacy of varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial

agonist, vs placebo or sustained-release bupropion for smoking cessation: A randomized controlled trial. JAMA 296(1):56–63.

Kaur S and Ryabinin AE (2010) Ghrelin receptor antagonism decreases alcohol consumption and activation of perioculomotor urocortin-containing neurons. Alcohol Clin Exp Res 34(9):1525-34.

King SJ, Isaacs AM, O'Farrell E, and Abizaid A (2011) Motivation to obtain preferred foods is enhanced by ghrelin in the ventral tegmental area. Horm Behav 60(5):572-80.

Kinzig KP, Scott KA, Hyun J, Bi S and Moran TH (2006) Lateral ventricular ghrelin and fourth ventricular ghrelin induce similar increases in food intake and patterns of hypothalamic gene expression. Am J Physiol Regul Integr Comp Physiol 290(6):R1565-9.

Kenny PJ, Koob GF, and Markou A (2003) Conditioned facilitation of brain reward function after repeated cocaine administration. Behav Neurosci 117(5):1103-7.

Klesges RC, Meyers AW, Klesges LM, and La Vasque ME (1989) Smoking, body weight, and their effects on smoking behavior: A comprehensive review of the literature. Psychol Bull 106(2):204-30.

Knight C, Howard P, Baker CL, and Marton JP (2010) The cost-effectiveness of an extended course (12+12 weeks) of varenicline compared with other available smoking cessation strategies in the United States: An extension and update to the BENESCO model. Value Health 13(2):209-14.

Kojima M and Kangawa K (2005) Ghrelin: Structure and function. Physiol Rev 85(2):495-522.

Kornetsky C, Esposito RU, McLean S, and Jacobson JO (1979) Intracranial self-stimulation thresholds: A model for the hedonic effects of drugs of abuse. Arch Gen Psychiatry 36(3):289–92.

Kornetsky C and Esposito RU (1979) Euphorigenic drugs: Effects on the reward pathways of the brain. Fed Proc 38(11):2473–6.

Kumar R, Pratt JA, and Stolerman IP. (1998) Characteristics of conditioned taste aversion produced by nicotine in rats. Br J Pharmacol 79(1):245-53.

Levi J (2013) F as in fat: How obesity threatens America's future 2013. Trust for America's Health. http://healthyamericans.org/report/108/

Li SX, Perry KW, and Wong DT (2002) Influence of fluoxetine on the ability of bupropion to modulate extracellular dopamine and norepinephrine concentrations in three mesocorticolimbic areas of rats. Neuropharmacology 42(2):181–90.

Mameli-Engvall M, Evrard A, Pons S, Maskos U, Svensson TH, Changeux JP, and Faure P (2006) Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. Neuron 50(6):911-21.

Maric T, Sedki F, Ronfard B, Chafetz D, and Shalev U (2011) A limited role for ghrelin in heroin self-administration and food deprivation-induced reinstatement of heroin seeking in rats. Addict Biol 17(3):613-22.

Markou A (2008) Neurobiology of nicotine dependence. Philos Trans R Soc Lond B Biol Sci 363(1507):3159–68.

Markou A and Koob GF (1992) Construct validity of a self-stimulation threshold paradigm: Effects of reward and performance manipulations. Physiol Behav 51(1):111–9.

McKee SA, Falba T, O'Malley SS, Sindelar J, and O'Connor PG (2007) Smoking status as a clinical indicator for alcohol misuse in US adults. Arch Intern Med 167(7):716-21.

McMahon LR, Jones SL, Gilliland TR, Hall WD, and Wellman PJ (1998) Effects of ephedrine enantiomers on conditions taste aversion and kaolin intake in rats. Pharmacol Biochem Behav 63(1):119-24.

McNeil JJ, Piccenna L, and Ioannides-Demos LL (2010) Smoking cessation-recent advances. Cardiovasc Drugs Ther 24(4):359–67.

Mishra A, Chaturvedi P, Datta S, Sinukumar S, Joshi Poonam, and Garg A (2010) Indian J Med Paediatr Oncol 36(1):24–31.

Miyata G, Meguid MM, Varma M, Fetissov SO, and Kim HJ (2001) Nicotine alters the usual reciprocity between meal size and meal number in female rat. Physiol Behav 74(1-2):169-76.

Moulin A, Brunel L, Boeglin D, Demange L, Ryan J, M'kadmi, C et al. (2012) The 1,2,4-triazole as a scaffold for the design of ghrelin receptor ligands: Development of JMV-2959, a potent antagonist. Amino Acids 44(2):301-14.

Moulin A, Ryan J, Martinez J, Fehrentz JA (2007) Recent developments in ghrelin receptor ligands. ChemMedChem 2(9):1242-59.

Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, and Matsukura S

(2001) A role for ghrelin in the central regulation of feeding. Nature 409(6817):194-8.

Naleid AM, Grace MK, Cummings DE, and Levine AS (2005) Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. Peptides 26(11):2274-9.

Nation JR, Cardon AL, Heard HM, Valles R, and Bratton GR (2003) Perinatal lead exposure and relapse to drug-seeking behavior in the rat: A cocaine reinstatement study. Psychopharmacology (Berl) 168(1-2):236-43.

Nation JR, Smith KR, and Bratton GR (2004) Early developmental lead exposure increases sensitivity to cocaine in a self-administration paradigm. Pharmacol Biochem Behav 77(1):127-35.

NIH Cancer Institute (2014) Harms of Cigarette Smoking and Health Benefits of Quitting. cancer.gov/about-cancer/causes-prevention/risk/tobacco/cessation-fact-sheet.

Niijima A and Yamamoto T (1994) The effects of lithium chloride on the activity of the afferent nerve fibers from the abdominal visceral organs in the rat. Brain Res Bull 35(2):141-5.

Ogden CL, Carroll MD, Kit BK, and Flegal KM (2012) Prevalence of obesity in the United States, 2009–2010. NCHS Data Brief (82):1-8.

Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J Comp Physiol Psychol 47(6):419-27.

Olshansky SJ, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J et al. (2005) A potential decline in life expectancy in the United States in the 21st century. N Engl J Med 352(11):1138-45.

Paolini M and De Biasi M (2011) Mechanistic insights into nicotine withdrawal. Biochem Pharmacol 82(8):996-1007.

Paterson NE, Min W, Hackett A, Lowe D, HananiaT, Caldarone B, and Ghacami A (2010) The high-affinity nAChR partial agonists varenicline and sazetidine-A exhibit reinforcing properties in rats. Prog Neuropsychopharmacol Biol Psychiatry. 34(8):1455-64.

Paxinos G and Watson C (2004) The rat brain in stereotaxic coordinates. Amsterdam: Elsevier Academic Press.

Perello M, Sakata I, Birnbaum S, Chuang JC, Osborne-Lawrence S et al. (2010) Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner. Biol Psychiatry 67(9):880-6.

Perello M and Zigman JM (2012) The role of ghrelin in reward-based eating. Biol Psychiatry, 72(5): 347–353.

Perkins KA and Lerman C (2014) An efficient early phase 2 procedure to screen medications for efficacy in smoking cessation. Psychopharmacology (Berl) 231(1):1–11.

Perkins KA (1992) Effects of tobacco smoking on caloric intake. Br J Addict 87(2):193-205.

Pomerleau CS and Kurth CL (1996) Willingness of female smokers to tolerate

postcessation weight gain. J Subst Abuse 8(3):371-8.

Pomerleau CS and Saules K (2007) Body image, body satisfaction, and eating patterns in normal-weight and overweight/obese women current smokers and never-smokers. Addict Behav 32(10):2329-34.

Pons S, Fattore L, Cossu G, Tolu S, Porcu E, McIntosh JM et al. (2008) Crucial role of alpha4 and alpha6 nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. J Neurosci 28(47):12318-27.

Quarta D, Di Francesco C, Melotto S, Mangiarini L, Heidbreder C, and Hedou G (2009) Systemic administration of ghrelin increases extracellular dopamine in the shell but not the core subdivision of the nucleus accumbens. Neurochem Int 54(2):89-94.

Rabinoff M (2007) Pharmacological and Chemical Effects of Cigarette Additives. Am J Public Health 97(11):1981-91.

Rose EJ, Ross TJ, Salmeron BJ, Shakleya DM, Huestis M, and Stein EA (2012) Chronic exposure to nicotine is associated with reduced reward-related activity in the striatum but not the midbrain. Biol Psychiatry 71(3):206-13.

Rose JE (2008) Disrupting nicotine reinforcement: From cigarette to brain. Ann N Y Acad Sci 1141:233-56.

Salome N, Haage D, Perrissoud D, Moulin A, Demange L, Egecioglu E et al. (2009a). Anorexigenic and electrophysiological actions of novel ghrelin receptor (GHS-R1A) antagonists in rats. Eur J Pharmacol 612(1-3):167-73.

Salome N, Hansson C, Taube M, Gustafsson-Ericson L, Egecioglu E, Karlsson-Lindahl L et al. (2009b) On the central mechanism underlying ghrelin's chronic pro-obesity effects in rats: New insights from studies exploiting a potent ghrelin receptor antagonist. J Neuroendocrinol 21(9):777-85.

Shimbara T, Mondal MS, Kawagoe T, Toshinai K, Koda S, Yamaguchi H et al. (2004) Central administration of ghrelin preferentially enhances fat ingestion. Neurosci Lett. 369(1):75-9.

Skibicka KP and Dickson SL (2011) Ghrelin and food reward: The story of potential underlying substrates. Peptides 2(11):2265-73.

Skibicka KP, Hansson C, Alvarez-Crespo M, Friberg PA, and Dickson SL (2011a) Ghrelin directly targets the ventral tegmental area to increase food motivation. Neuroscience 180:129-37.

Skibicka KP, Hansson C, Egecioglu E, and Dickson SL (2011b) Role of ghrelin in food reward: Impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression. Addict Biol 17(1):95-107.

Spinedi E, Voirol MJ, Verdumo C, Giacominni M, Pralong F, and Gaillard RC (2006) Hypothalamic ghrelin treatment modulates NPY-but not CRH-ergic activity in adrenalectomized rats subjected to food restriction: Evidence of a novel hypothalamic ghrelin effect. Endocrine 29(3):477-84.

St.Pierre DH, Wang L, Taché Y (2003) Ghrelin: A novel player in the gut-brain regulation of growth hormone and energy balance. News Physiol Sci 18:242-6.

Svensson CK (1987) Clinical pharmacokinetics of nicotine. Clin Pharmacokinet

12(1):30-40.

Tessari M, Catalano A, Pellitteri M, Di Francesco C, Marini F, Gerrard PA et al. (2007) Correlation between serum ghrelin levels and cocaine-seeking behaviour triggered by cocaine-associated conditioned stimuli in rats. Addict Biol 12(1):22-9.

Tschop M, Smiley DL, and Heiman ML (2000) Ghrelin induces adiposity in rodents. Nature. 407(6806):908-13.

Tuesta LM, Fowler CD, and Kenny PJ (2011) Recent advances in understanding nicotinic receptor signaling mechanisms that regulate drug self-administration behavior. Biochem Pharm 82(8):984-95.

Uchida A, Zigman JM, and Perello M (2013) Ghrelin and eating behavior: Evidence and insights from genetically-modified mouse models. Front Neurosci 7:121.

Vener AM and Krupka LR (1985) Over the counter anorexiants: Use and perceptions among young adults. Clin Pharm and Therapeutics 5:33.

Vezina, P (2004) Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. Neurosci Biobehav Rev 27(8):827-39.

Wang Y, Beydoun M, Liang L, Caballero B, and Kumanyika S (2008) Will all Americans become overweight or obese? Estimating the progression and cost of the U.S. obesity epidemic. Obesity 16(10):2323-30.

Weitzman ER and Chen YY (2005) The co-occurrence of smoking and drinking among young adults in college: National survey results from the United States. Drug Alcohol Depend 80(3):377-86.

Wellman MK, Patterson ZR, MacKay H, Darlin JE, Mani BK, Zigman JM et al. (2015) Novel regulator of acylated ghrelin, CF801, reduces weight gain, rebound feeding after a fast, and adiposity in mice. Front Endocrinol (Lausanne) 6:144.

Wellman PJ, Clifford PS, and Rodriguez JA (2013) Ghrelin and ghrelin receptor modulation of psychostimulant action. Front Neurosci 7:171.

Wellman PJ, Clifford PS, Rodriguez JA, Hughes S, Di Francesco C, Melotto S et al. (2012) Brain reinforcement system function is ghrelin dependent: Studies in the rat using pharmacological fMRI and intracranial self-stimulation. Addict Biol 17(5):908-19.

Wellman PJ, Clifford PS, Rodriguez J, Hughes S, Eitan S, Brunel L et al. (2011) Pharmacologic antagonism of ghrelin receptors attenuates development of nicotine induced locomotor sensitization in rats. Regul Pept 172(1-3):77-80.

Wellman PJ, Hollas CN, and Elliott AE (2008a) Systemic ghrelin sensitizes cocaine-induced hyperlocomotion in rats. Regul Pept 146(1-3):33-7.

Wellman PJ, Elliott AE, Barbee S, Hollas CN, Clifford PS, and Nation JR (2008b) Lobeline attenuates progressive ratio breakpoint scores for intracranial self-stimulation in rats. Physiol Behav 93(4-5):952-7.

Wellman PJ, Davis KW, and Nation JR (2005) Augmentation of cocaine hyperactivity in rats by systemic ghrelin. Regul Pept 125(1-3):151-4

Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA et al. (2001) Ghrelin causes hyperphagia and obesity in rats. Diabetes 50(11):2540-7.

Wise RA (1996) Addictive drugs and brain stimulation reward. Annu Rev Neurosci

19:319–340.

Zhang L, Doyon WM, Clark JJ, Phillips PE, and Dani JA (2009) Controls of tonic and phasic dopamine transmission in the dorsal and ventral striatum. Mol Pharmacol 76(2):396-404.

Zheng D, Liu S, Cabeza De Vaca S, and Carr KD (2013) Effects of time of feeding on psychostimulant reward, conditioned place preference, metabolic hormone levels, and nucleus accumbens biochemical measures in food restricted rats. Psychopharmacology (Berl) 227(2):307-20.

Zigman JM, Nakano Y, Coppari R, Balthasar N, Marcus JN, Lee CE et al. (2005) Mice lacking ghrelin receptors resist the development of diet-induced obesity. J Clin Invest 115(12):3564-72.

Zoli M and Picciotto MR (2012) Nicotinic regulation of energy homeostasis. Nicotine Tob Res 14(11):1270-90.