Individual Susceptibility to Obesity: Psychological and Neurobiological Mechanisms

by

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Dedication

This doctoral dissertation is dedicated to my mother, Ann Mary Derman who has been an emblem of resiliency and a source of love and wisdom throughout my life, to my husband, closest companion, and cherished interlocutor Michael Allen Coon Jr., and to my two research mentors, Carrie R. Ferrario and Andrew R. Delamater for their steadfast support of my intellectual development, without whom I would not have achieved this triumph.

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Abstract

Global obesity rates have been steadily rising for more than four decades and prevention of obesity has proven challenging. Obesity arises from chronic consumption of hypercaloric diets. Thus, treating and preventing obesity entails promoting adherence to energy appropriate diets. Yet this has proven challenging to the wider population, as is evident by the continually rising rates of obesity. Therefore, identifying factors that promote overeating is essential for the development of effective obesity treatment and prevention plans. Food-seeking and feeding behaviors depend on associative processes including instrumental and Pavlovian mechanisms. Another critical aspect mediating expression of these behaviors is the motivation that drives one to seek out and consume food. In recent years, studies in humans have highlighted the potential contribution of the motivational influence of cues associated with food to the obesity epidemic. In particular, studies have found that food cues elicit stronger brain responses in obese subjects and in normal weight people who subsequently gain more weight in the following year. These findings have led to the idea that enhanced responsiveness to Pavlovian stimuli associated with food may be a pre-existing trait that contributes to obesity susceptibility. The work presented in this dissertation examines the contribution of individual obesity susceptibility to the psychological and neurobiological mechanism underlying Pavlovian motivation.

Using Pavlovian-to-instrumental transfer (PIT), a classic measure of Pavlovian motivation, in Chapters 2-4, I examine differences in the expression of PIT between

obesity susceptible and non-susceptible rats. The results from these studies reveal that in selectively bred rats, obesity-prone rats exhibit stronger Single Outcome PIT (SO PIT) than obesity-resistant rats. Moreover, the expression of SO PIT in obesity-prone rats is mediated by calcium-permeable AMPA receptors (CP-AMPARs) in the Nucleus Accumbens (NAc) Core, but not in obesity-resistant rats. Similarly, in outbred rats the degree to which NAc Core CP-AMPARs contribute to SO PIT expression is strongly correlated with subsequent weight. Jointly these data demonstrate that Pavlovian motivation is enhanced in obesity-prone individuals and that NAc Core CP-AMPARs commonly mediated expression of SO PIT in both selectively bred obesity-prone rats and in outbred obesity susceptible rats. However, given that SO PIT does not distinguish between affective and sensory specific mechanisms of Pavlovian motivation, I subsequently compared the expression of Sensory Specific PIT (SS PIT) versus General PIT in selectively bred and outbred populations. These data reveal that obesity-prone rats exhibit enhanced General PIT versus obesity-resistant rats and that in outbred rats the magnitude of General PIT is strongly positively associated with subsequent weight. This finding explicitly identifies enhanced affective Pavlovian motivation as the primary driver of enhanced PIT in susceptible individuals, prior to obesity.

Chapters 5-6 examined factors that contribute to Pavlovian motivation independent of obesity susceptibility. In Chapter 5, I show that post-training consumption of a palatable 'Junk-Food' diet enhances conditioned food cup approach, independent of individual susceptibility. In Chapter 6, I demonstrate that the expression of SS PIT depends critically on CamKII Basolateral Amygdalar (BLA) neurons.

Collectively, the work presented in this dissertation provides preclinical evidence that obesity susceptibility is accompanied by pre-existing enhancements in Pavlovian motivation which depends on unique neurobiological mechanisms in susceptible versus resistant populations. Moreover, independent of obesity susceptibility, experience with palatable diets can enhance Pavlovian motivation. In summary, the data in this dissertation identify extrinsic and intrinsic factors contribute to Pavlovian motivational processes.

Chapter 1: Introduction

Global obesity rates, as defined by body mass indices above 30 (kg/m^2), have more than tripled within the last four decades (N.C.D.R, 2016). As of 2016, adult obesity rates have reached 10.8% in males and 14.9% in females, and youth obesity is 7.8 in males and 5.6 in females, globally (N.C.D.R, 2017; W.H.O, 2017). These rates are significantly greater in high-income English-speaking countries, reaching an average of 30% (N.C.D.R, 2016). These rates are even higher in the United States, where 39.8% of adults and 18.5% of youth are obese (Hales et al., 2017). In terms of direct toll of obesity on human suffering, obesity is the strongest predictor of all-cause mortality hazard (Krakauer and Krakauer, 2014) and is strongly associated with increased risk for at least 12 different cancers (Pearson-Stuttard et al., 2018). It also levies a hefty economic burden to society; in the US, annual medical spending on obesity has been estimated at $147 billion 2008 USD, where medical spending among obese individuals is 42% higher per capita than non-obese individuals (Finkelstein et al., 2009). Considering the increasing prevalence of this disease, the poor quality of life associated with it, and the economic burden it carries, treating, and perhaps more importantly, preventing obesity is critical for the collective benefit of humanity.

The success of our attempts to treat and prevent obesity depends critically on developing a robust understanding of its, admittedly, complex etiology. At the most basic level obesity arises from chronic consumption of a hypercaloric diet which occurs when energy intake exceeds energy expenditure (Akiyama et al., 1996; Horton et al., 1995; Nascimento et al., 2008). Thus, the cause of weight accumulation and obesity is mediated via at least two large categories of behavior, consumption and energy expenditure. The focus of the work presented in this dissertation explores the psychological and neuronal factors that may contribute to overeating. Ingestive behaviors are influenced by hormonal signals acting in the brain and by food related stimuli within the environment (Belfort-DeAguiar and Seo, 2018). Recent research has placed the spotlight on the contribution

of environmental stimuli to consumption, given that these stimuli can override hormonal influences on the brain and feeding behavior (Belfort-DeAguiar et al., 2016). Conceptually this idea is not unexpected considering that survival depends critically on being able to read signals within the environment to attract organisms to resources and to repel them from mortal threats (Berridge, 1999; Bindra, 1969; Konorski, 1967).

Another reason for the recent focus on the contribution of food related stimuli to the obesity epidemic is the finding that brain and behavioral reactivity to food cues is predicative of outcomes, such as the amount of food consumed and/or weight changes following behavioral or brain imaging tests in humans (Boswell and Kober, 2016). For instance, in humans, food cues can promote feeding (Birch et al., 1989; Cornell et al., 1989; Ferriday and Brunstrom, 2008), and this potentiation of feeding is stronger in overweight and obese people (Halford et al., 2007; Halford et al., 2004; Jansen et al., 2003). Food cues also promote subjective craving which is stronger in overweight individuals (Ferriday and Brunstrom, 2011) and is positively associated with greater subsequent consumption (Fedoroff et al., 1997). In the brain, presentation of food cues elicits activation within reward related nuclei, and the magnitude of these activations positively correlate with the amount of food subsequently consumed in people (Frankort et al., 2015; Lopez et al., 2014; Mehta et al., 2012). Moreover, in healthy weight individuals, food cue elicited activations in regions including the Nucleus Accumbens (NAc) and Amygdala, positivity correlates with subsequent BMI increases in the following year (Demos et al., 2012; Yokum et al., 2014). Collectively, these studies in humans reveal that both behavioral and neuronal sensitivity to food cues is often stronger in overweight and obese individuals, potentiates consumption, and is predictive of subsequent weight gain in healthy weight individuals. This final observation is particularly interesting because it suggests that pre-existing neurobehavioral differences may contribute to obesity susceptibility. However, due to the intrinsic limitations of studies in humans, exploring causality of these associations and examining the mechanisms mediating these associations is not possible. Therefore, use of non-human animal models is required for exploration of causality and mechanism.

The work reported in this dissertation centers on examining factors that influence food-seeking behaviors in the rat. In Chapters 2-4, I explore the contribution of individual

vulnerability to obesity to cue-triggered food-seeking, and the underlying psychological and neuronal processes mediating these effects. In Chapter 5, I examine the influence of diet on cue-triggered food-seeking and consumption. Lastly, in Chapter 6, I examine the role of basolateral amygdala CamKII neurons in the expression of sensory specific food-seeking. Jointly, these studies elucidate some of the intrinsic and extrinsic factors that contribute to food-seeking and that likely play a role in the ongoing obesity epidemic.

1: Models for studying pre-existing traits and mechanisms associated with intrinsic obesity vulnerability.

As the human data discussed above indicate, pre-existing differences in responsivity to food cues seems to precede weight gain and the development of obesity. Due to the intrinsic limitations of studying humans, non-human animal research is necessary for uncovering mechanisms that may drive these effects and to allow and for determining to if this sensitivity is truly a pre-existing trait accompanying obesity vulnerability. One way to examine the contribution of pre-existing differences to obesity is to use selectively bred models of obesity susceptibility and resistance. In rodents, consumption of a high energy diet induces weight gain, but the degree of gain varies across the population (Levin and Dunn-Meynell, 2000). Selective breeding of rats occupying the tail ends of this weight gain spectrum produces two strains of rats, obesity-resistant and obesity-prone rats (Levin et al., 1997). These strains serve as ideal models for exploring pre-existing differences that contribute to the development of obesity for two primary reasons. First, these obesity-prone rats do not spontaneously become obese, but instead must be placed on a high energy diet for their susceptibility to manifest. This is highly relevant to humans, given that obesity is strongly associated with obesogenic environments where calorie rich foods are readily available (Danaei et al., 2013). Secondly, the polygenic nature of the model also parallels the most common form of obesity in humans (Hebebrand and Hinney, 2009; Hinney and Giuranna, 2018; Levin et al., 1997). For these reasons, and given that selective breeding identifies individuals as susceptible or resistant prior to dietary intervention, these strains are particularly valuable for researching pre-existing physiological, neuronal, and psychological differences that may contribute to obesity. However, since the establishment of these selectively bred

lines, much of the research using these rats has explored pre-existing differences within the realm of metabolic physiology and hypothalamic structure and signaling (Bouret et al., 2008; Levin et al., 2004; Levin et al., 2003). The focus of this research on the role of the hypothalamus and homeostatic mechanism of feeding arose from early studies demonstrating that lesions to the sub nuclei of the hypothalamus produced profound effects on feeding behavior. For instance, lesions of the lateral hypothalamus trigger hyperphagia and obesity, whereas lesions of the ventromedial hypothalamus produce aphasia and ultimately starvation (Anand and Brobeck, 1951; Teitelbaum and Epstein, 1962). Yet, as discussed above, sensitivity to food cues appears to be a key feature of obesity vulnerability in humans. In addition, Pavlovian processes have also been identified as critical for foraging behaviors in non-human animals (Couvillon et al., 1983; Menzel et al., 1993; see for extensive review Stephens et al., 2007). Therefore, these selectively bred obesity-prone and obesity-resistant rats may serve as ideal models for exploring pre-existing neuropsychological traits that contribute to obesity vulnerability. In Chapters 2-3, I examine the neuropsychological differences in cue-triggered food-seeking behaviors between these selectively bred obesity-prone and obesity-resistant rats.

An alternative method to examining pre-existing differences in obesity vulnerable, is to take outbred populations, test a given in-vivo manipulation, and then subsequently place these rats on a calorie rich diet in order to identify subsequent individual susceptibility to weight gain and obesity. This is a valuable complementary approach to studies in selectively bred rats, but is limited in a number of important ways. First, these experiments are fairly costly given the requirement for post testing diet manipulations. Second, analysis is typically limited to correlations, given that categorical analyses require sufficiently powered groups and one does not know *a priori* what the distribution of susceptible and non-susceptible individuals will be. Third, basal ex-vivo studies are not possible with this approach because one lacks *a priori* knowledge of a given individual's susceptibility without dietary intervention. Thus, while studies in outbred populations provide important corroborations for data collected in selectively bred rats, experimental approaches are fairly limited. Despite these limitations, here I also employed this approach to provide complementary data to our studies in selectively bred rats and these data are presented in Chapters 3-4.

2: Procedures for studying Pavlovian motivation.

As discussed above stimuli associated with food critically influence food-seeking and in humans they have been shown to elicit craving and enhance consumption. Moreover, sensitivity to these cues is associated with subsequent weight gain. Pavlovian stimulus-outcome associations form between food related stimuli (e.g., packaging, appearance, odor) and the experience of ingestion (e.g., gustatory, hedonic, and satiety effects) with repeated pairing (Bouton, 2011; Pavlov, 1927). The motivation influence of these conditioned stimuli (CS) can be measured directly by looking at the magnitude of conditioned responses (CRs) supported by these CSs. The use of auditory stimuli promotes CRs that are directed toward the site of reward delivery and for all experiments discussed here, auditory stimuli were used. Therefore, conditioned responding was measured as food cup entries throughout all the studies presented here. In Chapter 6, conditioned food cup approach was the primary measure of Pavlovian motivation examined; this study tested the influence of consumption of a highly palatable diet on expression of conditioned approach.

The motivational capacity of CSs can also be measured by their ability to modulate behaviors supported by other associations, including instrumental response-outcome associations (R-O). This latter phenomenon is known as Pavlovian-to-instrumental transfer (PIT), and can be captured as follows: Initially rats are taught an instrumental R-O association (e.g., lever press resulting in food delivery), then separately they are conditioned with a Pavlovian stimulus-outcome (S-O) association (e.g., tone followed by food delivery), and finally in testing they are provided access to the instrumental manipulandum under extinction conditions and the CS is presented to evaluate its ability augment instrumental responding (first demonstrated by Walker, 1942). The degree to which CS presentations invigorate instrumental responding provides a readout of the intensity of the motivational control that a CS has acquired. PIT is a highly sensitive measure that can detect the motivational influence of a CS even once conditioned responses have been extinguished (Delamater, 1996). In addition to the sensitivity of this measure, in recent years, this phenomenon has been demonstrated in humans and a growing number of studies has begun to corroborate various PIT effects found in non-

human animals (Colagiuri and Lovibond, 2015; De Tommaso et al., 2018; Hogarth et al., 2018; Jeffs and Duka, 2017; Nadler et al., 2011; Prevost et al., 2012; Seabrooke et al., 2018a; Seabrooke et al., 2018b; Watson et al., 2014). Moreover, researchers have begun to explore the role of PIT in appetitive disorders in humans, including obesity and internet gaming disorders (Lehner et al., 2017; Vogel et al., 2018). Considering that PIT provides a clean measure of Pavlovian motivation, its high degree of sensitivity of and its ecological validity to human behavior, the vast majority of the studies presented in this dissertation implement variations of PIT. Chapters 2-5 examine the psychological and neuronal processes driving PIT in obesity susceptible individuals. Chapter 6 examines the basic mechanism of Sensory Specific PIT by focusing on the contribution of CamKII basolateral amygdala neurons to its expression.

3: Circuitry of conditioned approach and PIT

The broad circuitry controlling reward mediated behaviors is shown in Figure 1 (adapted from Russo and Nestler, 2013). Critical nuclei within this circuitry that play a role in conditioned approach and/or PIT are: the Ventral Tegmental Area (VTA), the Amygdala and the NAc. The VTA is the most upstream nucleus in this system and unsurprisingly mediates the expression of both conditioned approach and PIT (Corbit et al., 2007; Ikemoto and Panksepp, 1996; Murschall and Hauber, 2006). The amygdala is critical for PIT, but does not mediate the acquisition or expression of conditioned food cup approach (Blundell et al., 2001; Gallagher et al., 1990; Hall et al., 2001; Hatfield et al., 1996). As discussed above, Pavlovian stimuli can directly control conditioned responses such as approach to the site of reward delivery and they can independently control the expression of instrumental R-O associations as in PIT. The NAc is critical for the expression of PIT and plays a contributing role in conditioned food cup or goal

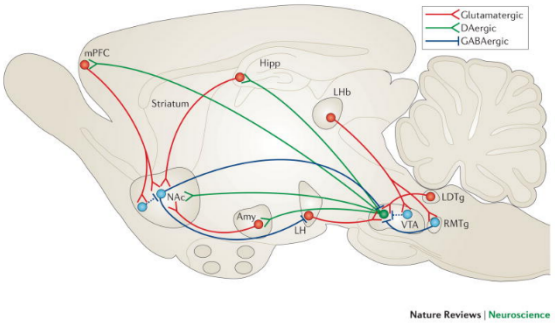

Figure 1: A schematic of the primary reward circuitry in the rat brain. The major nuclei of this circuit include the ventral tegmental area (VTA), the amygdala, and the NAc. The two nuclei investigated within this dissertation are the Amygdala and the NAc. Both receive dopaminergic input from the VTA and play a role in the expression of PIT. Image taken from Ruso and Nestler, 2013.

approach (Corbit et al., 2001; Hall et al., 2001; Parkinson et al., 1999).

In addition to their well-defined role in PIT, the NAc and amygdala are also activated by food cue presentation in humans (Arana et al., 2003; Beaver et al., 2006; Demos et al., 2012; Lopez et al., 2014; Mehta et al., 2012; Murdaugh et al., 2012; Pelchat et al., 2004; Schur et al., 2009; Stoeckel et al., 2008). Moreover, the magnitude of activity in these sites is associated with the incentive value of the food cues (Arana et al., 2003; Schur et al., 2009), with individual sensitivity to reward (Beaver et al., 2006), and with subsequent weight gain (Demos et al., 2012; Yokum et al., 2014). Therefore, the neuronal studies presented in this dissertation focus on the role of the NAc and the amygdala in the mediation of PIT and conditioned food cup approach.

The NAc is a major downstream nucleus that has been implicated in both food and drug seeking behaviors (Corbit and Balleine, 2011; Di Ciano and Everitt, 2004; Fuchs et al., 2004). This site receives convergent dopaminergic input from the VTA and glutamatergic input from diffuse regions including the basolateral amygdala (BLA). Yet the role of NAc glutamatergic transmission in PIT remained to be elucidated. NAc AMPA receptors serve as the primary source of excitatory transmission within the NAc (Di Ciano et al., 2001). These receptors are heterodimers that fall into two major categories calcium-impermeable AMPARs that contain the subunit GluA2, and calcium-permeable AMPARs (CP-AMPARs) that lack GluA2 (Hollmann and Heinemann, 1994; Verdoorn et al., 1991; Wenthold et al., 1996).

Research from the drug addiction literature has highlighted the importance of NAc Core CP-AMPARs in the “incubation of craving” for drug (Wolf, 2016; Wolf and Ferrario, 2010). Incubation of craving refers to the enhanced drug seeking that follows a period of forced abstinence (Grimm et al., 2001). This is captured by initially training animals to lever press to self-administer drug which is co-delivered with a CS. Following training, animals undergo forced abstinence, where they can no longer self-administer drug. After a given period of abstinence, they are tested for lever pressing under drug extinction conditions, were pressing results in CS presentations alone, but no drug delivery. The incubation effect is observed, by increased rates of responding in testing that occur as the window of forced abstinence is lengthened. Importantly, NAc Core CP-AMPARs increase within this abstinence window and blockade of these receptors prevents the

expression of this incubation effects (Conrad et al., 2008; Loweth et al., 2014; Ma et al., 2014; Scheyer et al., 2016; Wolf, 2016; Wolf and Ferrario, 2010). This led to the idea that CP-AMPARs were a unique and defining feature of drug addiction. However, recent data from our lab found that experience with a highly palatable junk-food diet enhances expression of NAc Core CP-AMPARs in outbred rats identified as obesity-susceptible and in selectively bred obesity-prone rats (Oginsky et al., 2016). This finding suggested that these receptors may also play a role in more naturalistic behaviors such as food-seeking. Consistent with this Dingess et al., (2017) found that forced time off a chow self-administration task paralleling the incubation of craving for drug, drives up NAc Core CP-AMPARs. Jointly these latter studies suggest that CP-AMPARs may increase in response to food related experiences and taken together with the drug addiction literature suggest that they may also play a functional role in food-seeking behaviors. Thus, studies in this dissertation I tested whether NAc Core CP-AMPARs mediated the expression of PIT (also referred to as cue-triggered food-seeking), particularly in the framework of obesity vulnerably (Chapters 2 and 4).

Lastly, the BLA is another nucleus that is critical for the expression Sensory Specific PIT and its connection to the NAc Shell has been indirectly implicated in mediating this behavior (Corbit and Balleine, 2005; Shiflett and Balleine, 2010). Given the importance of glutamatergic transmission in the NAc toward food-seeking behavior and that the BLA to NAc connection is glutamatergic (Groenewegen et al., 1999; Sah et al., 2003; Shinonaga et al., 1994), Chapter 6 examined the contribution of CamKII BLA neuron in outbred rats. These data contribute to the development of a finer understanding of the circuitry and cells driving PIT.

4: Primary dissertation focus

All the studies presented within this dissertation center on exploring the psychological and neuronal mechanisms of Pavlovian motivation, with particular emphasis on PIT. The bulk of these studies examine this within the context of obesity vulnerability, by exploring the psychological processes and neuronal mechanisms driving Pavlovian motivation in obesity susceptible individuals. In Chapter 2, I examine the role of NAc Core CP-AMPARs in the expression of Single Outcome PIT in selectively bred

obesity-prone and -resistant rats. Here I show that NAc Core CP-AMPARs mediate PIT in obesity-prone, but not obesity-resistant rats. In Chapter 3, I determine whether intrinsic obesity vulnerability is associated with enhanced expression of Sensory Specific and General PIT in both outbred and selectively bred populations. These data revealed that both outbred and selectively bred obesity vulnerable individuals exhibit enhanced General PIT, a measure of Pavlovian affective motivation. In Chapter 4, I return to the role of NAc Core CP-AMPARs in Single Outcome PIT and determine whether outbred obesity vulnerability is associated with NAc Core CP-AMPAR mediation of Single Outcome PIT. Here I find that indeed, obesity vulnerability is associated with increasing sensitivity of Single Outcome PIT to NAc Core CP-AMPAR blockade. Jointly these studies show that NAc Core CP-AMPARs mediate Single Outcome PIT in both selectively bred and outbred obesity vulnerable rats. Moreover, data from Chapter 2 identify a general affective mechanism as the primarily driver of enhanced PIT in obesity susceptible outbred and selectively bred populations.

In addition to exploring the contribution of individual obesity susceptibility to Pavlovian motivation Chapters 5 and 6 address basic questions of Pavlovian motivation independent of obesity vulnerability. Chapter 5 examines the effect of experience with a palatable diet on Pavlovian motivation. These data reveal that independent of susceptibility, Pavlovian motivation is enhanced by experience with this palatable diet. In Chapter 6, I test the contribution of CamKII BLA neurons to the expression of Sensory Specific PIT. These data reveal that Sensory Specific PIT depends critically on the activity of CamKII BLA neurons. Collectively, in the studies within this dissertation I sought to elucidate factors influencing and mediation the expression of Pavlovian motivational processes.

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Chapter 2: Enhanced Incentive Motivation in Obesity-Prone Rats is Mediated by NAc Core CP-AMPARs

Abstract:

Studies in humans suggest that stronger incentive motivational responses to Pavlovian food cues may drive over-consumption leading to and maintaining obesity, particularly in susceptible individuals. However, whether this enhanced incentive motivation emerges as a consequence of obesity or rather precedes obesity is unknown. Moreover, while human imaging studies have provided important information about differences in striatal responsiveness between susceptible and non-susceptible individuals, the neural mechanisms mediating these behavioral differences are unknown. The Nucleus Accumbens (NAc) mediates cue-triggered reward seeking and activity in the NAc is enhanced in obesity-susceptible populations. Therefore here, we used selectively-bred obesity-prone and obesity-resistant rats to examine intrinsic differences in incentive motivation, and the role of NAc AMPARs in the expression of these behaviors prior to obesity. We found that obesity-prone rats exhibit robust cue-triggered food-seeking (Pavlovian-to-instrumental transfer, PIT). Using intra-NAc infusion of AMPAR antagonists, we show that this behavior is selectively mediated by CP-AMPARs in the NAc core. Additionally, biochemical data suggest that this is due in part to experience-induced increases in CP-AMPAR surface expression in the NAc of obesity-prone rats. In contrast, in obesity-resistant rats PIT was weak and unreliable and training did not increase NAc AMPAR surface expression. Collectively, these data show that food cues acquire greater incentive motivational control in obesity-susceptible populations prior to the development of obesity. This provides support to the idea that enhanced intrinsic incentive motivation may be a contributing factor, rather than a consequence of obesity. In addition, these data demonstrate a novel role for experience-induced up-regulation of NAc CP-AMPARs in PIT, pointing to potential mechanistic parallels between the processes leading to addiction and to obesity.

Please note that the contents of this chapter have been published (Derman and Ferrario, 2018)

1: Introduction

The rise in global obesity has prompted a closer examination of the psychological and neurobiological processes that influence over-eating and enhanced motivation to consume palatable foods. Studies in humans support the idea that cravings triggered by stimuli associated with food (i.e., food cues) may contribute to food-seeking and over-eating leading to obesity (Burger and Stice, 2014; see Stice et al., 2013 for review; Stoeckel et al., 2008). For example, food cues induce feelings of hunger, bias food choice, and increase the amount of food consumed (Fedoroff et al., 1997; Jansen et al., 2003; Watson et al., 2014). These cues include sensory properties of food itself, like the crunching sensation of digging your hand into a bag of potato chips, as well as distal cues like packaging, and branding logos (Bouton, 2011). The ability for food cues to trigger cravings is not unique to obese populations, but rather individuals that struggle to maintain a healthy weight are more sensitive to these motivational properties of food cues (Fedoroff et al., 1997; Ferriday and Brunstrom, 2011; Jansen et al., 2008; Lehner et al., 2017; see Small, 2009 for review). This suggests that brain regions mediating incentive motivation, such as the NAc (Berridge et al., 2010; Berridge et al., 2009; Cartoni et al., 2016; Holmes et al., 2010), differ functionally between obesity-susceptible vs. -resistant populations, thereby contributing to overconsumption in susceptible individuals (Burger and Stice, 2014; Stoeckel et al., 2008; Tomasi and Volkow, 2013). This has prompted vibrant discussion about the degree to which these neurobehavioral differences seen in susceptible individuals are similar vs. different to those driving drug-seeking in addiction (Berridge et al., 2010; Ferrario, 2017; Long et al., 2015; Michaud et al., 2017; Stice et al., 2013; Volkow et al., 2013).

A central question that arises in this discussion is whether enhanced neurobehavioral responses to food cues emerge as a consequence of weight gain, or whether there are intrinsic differences in the motivational responses to food cues that precede weight gain. In support of pre-existing differences, we recently found that outbred rats subsequently identified as susceptible to diet-induced obesity display greater cue-

triggered approach (an indicator of incentive motivation) prior to diet manipulation and weight gain (Robinson et al., 2015). However, identification of susceptible and resistant rats in outbred populations requires the introduction of high-fat diets and weight gain which can themselves alter neural function and behavior (Baladi et al., 2012; Brown et al., 2017; Dingess et al., 2017; Hryhorczuk et al., 2016; Oginsky et al., 2016a). This limitation can, however, be overcome by using established rat lines that were selectively bred for their propensity or resistance to diet-induced obesity (Levin et al., 1997; Vollbrecht et al., 2015). Thus, by using these obesity-prone and obesity-resistant rats in the current study we can know a priori who is susceptible and resistant to obesity without introducing a high-fat diet or weight gain. This allows us to examine intrinsic neurobehavioral differences that precede obesity. Recent studies from our group have shown that NAc function is enhanced in these selectively-bred obesity-prone vs. obesity-resistant rats. For example, basal intrinsic excitability of medium spiny neurons within the NAc is enhanced in adult obesity-prone vs. obesity-resistant rats, in the absence of any diet manipulation (Oginsky et al., 2016b). Furthermore, consumption of a sugary, fatty "junk-food" diet increases the expression and function of NAc calcium-permeable AMPA receptors (CP-AMPARs) in obesity-prone, but not obesity-resistant rats (Oginsky et al., 2016a). This up-regulation of CP-AMPARs is interesting in part because these receptors mediate the "incubation of cocaine-seeking" (Wolf, 2016; Wolf and Ferrario, 2010), consistent with the role of the NAc in incentive motivational processes (Berridge et al., 2010; Berridge et al., 2009; Cartoni et al., 2016). However, whether cue-triggered food-seeking (i.e., Pavlovian-to-instrumental transfer; PIT) is stronger in obesity-prone vs. obesity-resistant rats prior to obesity is unknown. Moreover, while NAc AMPAR-mediated transmission has been indirectly implicated in the expression of PIT (Corbit and Balleine, 2011; Crombag et al., 2008), to date no studies have directly examined the role of endogenous NAc AMPAR-mediated transmission in this behavior. Therefore here, we used PIT, a well-established measure of incentive motivation, to determine whether cue-triggered food-seeking is stronger in obesity-prone vs. obesity-resistant rats. We then examined whether experience during training leading up to PIT testing alters NAc AMPAR expression. Lastly, we determined the role of NAc AMPARs in the expression this behavior.

2: Materials and methods

2.1: Subjects

Obesity-prone (OP) and obesity-resistant rats (OR), originally established by Barry Levin (1997), were bred in house. Breeding was maintained on a Poiley Rotation System using 12 breeding pairs for each line. Breeders were originally purchased from Taconic. Adult males ranging from 65 to 85 days old at the start of the experiment were used (OP N=49; OR N=58; ns for individual experiment are given in results below). Rats were group housed and maintained on a reverse light-dark schedule (12/12); experiments were conducted during the dark phase. All procedures were approved by The University of Michigan Committee on the Use and Care of Animals. For characterization and validation of obesity phenotypes in these lines (see Vollbrecht et al., 2015). Additional details for all procedures and housing can be found at: https://sites.google.com/a/umich.edu/ferrario-lab-publicprotocols/

2.2: Behavioral procedures

Procedures were adapted from Delamater et al., (2017) and Holland and Gallagher, (2003); see Fig. 2.1. Rats were food restricted to 85-90% of their free-feeding bodyweight throughout. They were first trained to press one lever (active, fixed ratio 1: FR1) to earn food pellets (45 mg, Bioserv, #F0021; 0.75 protein, 0.5 fat, 2.36 carbohydrate kCal/g); a second lever (inactive) was present throughout, but had no programmed consequence (40-min sessions). After reaching the acquisition criterion (50 pellets earned within a single session), rats were then switched to a variable interval (VI) reinforcement schedule that was made leaner across training (8, 20-min sessions: 2, VI10"; 2, VI30"; 4, VI60"). Lever responses and food cup entries were recorded throughout. Next, rats underwent Pavlovian conditioning in which one auditory cue (CS+, 2-min) was paired with pellet delivery and a second auditory cue (CS-, 2-min) was presented an equal number of times, but was never paired with pellets (8, 60-min sessions; 4 trials/CS/session; CSs: tone and white-noise CS+/CS- counterbalanced). During CS+ trials 4 pellets were delivered on a VI30" schedule (range: 15-45 sec). A variable 5-min inter-trial-interval (ITI; range 3-7 min) was used. Levers were unavailable throughout Pavlovian conditioning, and pellet delivery was not contingent upon any response. Food cup entries were recorded throughout. Rats were given an instrumental "reminder" session one day prior to PIT testing. During PIT testing, both levers were available for the entire duration of the test session (40 min), but

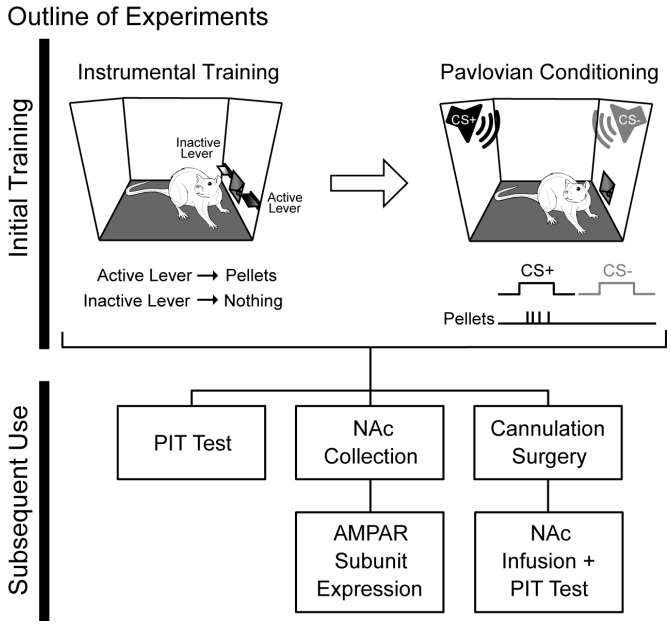


Figure 2.1*:* Schematic of training and timeline of studies. All rats received identical training, first undergoing instrumental training and then Pavlovian conditioning. Following training, rats were either tested for PIT (OP n=20; OR n=19), used to assess NAc AMPAR expression levels (OP-Trained n=10; OP-Control n=6; OR-Trained n=10; OR-Control n=6), or cannulated and subsequently tested for PIT following intra-NAc AMPAR blockade (OP: Vehicle n=11; CNQX n=8; NASPM n=8; OR: Vehicle n=22; CNQX n=20, NASPM n=19).

pellet deliveries were omitted (see Fig. 2.1). After 10 min, each CS was presented 4 times in a quasi-random order with a 2-min fixed ITI. Lever responses and food cup entries were recorded throughout. In addition, videos were made during PIT testing sessions following intra-cranial infusions.

2.3: BS^3 crosslinking and Western blotting

Surface vs. intracellular expression of AMPAR subunits was determined in a separate set of rats using established procedures (Dingess et al., 2017; Oginsky et al., 2016a). Comparisons were made between Trained and untrained Control groups. For the Trained groups, NAc tissue was collected 24 hr. after the final instrumental reminder session. This time point corresponded to the time when PIT testing would have occurred. For untrained Control groups, rats were food restricted, handled, and co-housed with their Trained counterparts. Tissue was rapidly extracted on ice, chopped (400 □m), and incubated in ACSF containing BS^3 (5 mM) for 30 min (4 ºC). Glycine (100 mM) was added to quench the crosslinking reaction after 10 min of BS^3 incubation. Samples were centrifuged for 2 min at 14,000 RPM (4 ºC). The pellet was resuspended in ice cold lysis buffer containing: 25mM HEPES, 500mM NaCl, 2mM EDTA, 1mM DTT, 1mM PMSF 20mM NaF; 1:100 EDTA-free Protease Inhibitor Cocktail (Sigma-Aldrige; 11836170001); and 0.1% Nonidet P-40 [v/v]; pH 7.4) and homogenized by sonication. Samples were stored at -80 ºC until surface and intracellular GluA1 (Thermo-Scientific; PA1-37776; 1:1000 in TBS) and GluA2 (EMD Millipore; AB1768-I; 1:4000 in TBS-T and 5% milk) protein expression levels were determined using SDS-PAGE and Western blotting as previously described (Boudreau et al., 2012). Bands of interest were quantified using Image J (NIH).

2.4: Post-training surgery and intra-NAc infusions

To assess effects of NAc core AMPAR blockade on the expression of PIT, a separate set of rats was trained as described above, and bilateral guide cannulae were implanted above the NAc core (Plastics Ones: C316G; AP: +1.4 mm, ML: ±2.2 mm relative to bregma; DV: -5.5 mm from skull) under isoflurane anesthesia (2.5-5%).

Carprofen was administered pre-operatively and again 24 hr. later (2.5 mg/kg, s.c.). Food restriction was lifted prior to surgery and re-applied after recovery (7 days). Next, rats were given 2 instrumental and 2 Pavlovian reminder sessions identical to pre-surgical training. Bilateral infusions of Vehicle, the general AMPAR antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 0.3μg/0.5μl; 2.58 mM) or the CP-AMPAR selective antagonist 1- Naphthylacetyl spermine trihydrochloride (NASPM: 20μg/0.5μl; 83.35 mM) were administered prior to PIT testing using a within-subject design. Vehicle solutions were artificial cerebro-spinal fluid (ACSF) for CNQX and 6% Dimethyl sulfoxide (DMSO) in ACSF for NASPM. In pilot studies we determined that PIT behavior was stable across three repeated vehicle infusions and re-training sessions, but became variable with additional infusions. Therefore, we limited each rat to a maximum of 3 infusions, with half of the rats receiving ACSF and half of the rats receiving 6% DMSO in ACSF in the vehicle condition. Importantly, we did not see any behavioral differences between rats receiving DMSO or ACSF vehicle infusions. A maximal dose of CNQX was used in the current study and is based on previous studies (Bell et al., 2000; Ferrario et al., 2010; Pierce et al., 1996). Rats were tested in each condition using a counterbalanced design (3 infusion tests per rat). Infusions were delivered at a rate of 0.25μl/min, and the injectors were left in place for 1 additional minute to allow for diffusion. Rats were then left undisturbed for 5 additional minutes before being moved to operant chambers for testing. After each test, rats were left undisturbed in their home cage for a 24 hr. wash out period and were then given reminder sessions as described above. Thus, infusions were separated by 5 days. Cannulae placements and injection sites were confirmed using established histological procedures and anatomical landmarks (Paxinos and Watson, 2007). Analysis of placements was conducted blinded and all data from rats with placements outside of the NAc or with excessive tissue scarring were excluded from all analyses (excluded: OP n=4; OR n=7).

2.5: Statistics

Statistical analyses were performed using GraphPad Prism (Version 7.0c) and included: unpaired and paired t-tests, and one-way and two-way RM ANOVAs. Sidak's multiple comparisons were used for post-hoc and planned comparisons.

3: Results

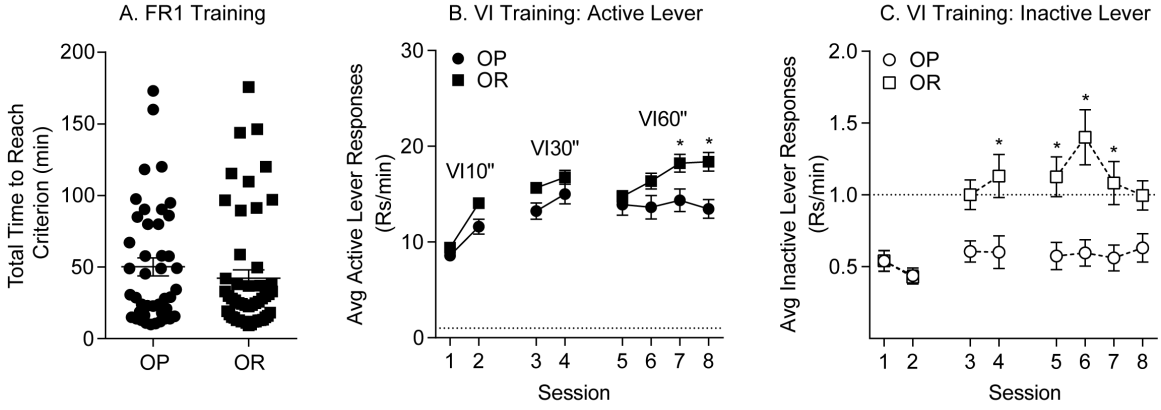
3.1: Behavior during instrumental and Pavlovian training is similar between groups

To assess incentive motivation in the form of cue-triggered food-seeking (i.e., PIT), rats first underwent instrumental and Pavlovian conditioning in separate sessions (see methods). Three cohorts of rats were used for behavioral, biochemical, and pharmacological studies (Fig. 2.1). Behavior during training did not differ significantly across cohorts and therefore the data have been collapsed for ease of presentation in Fig. 2.2 (total rats trained: OP N=43; OR N=52). Prior to food-restriction, obesity-prone rats were heavier than obesity-resistant rats, as expected from previous studies (Vollbrecht et al., 2015 data not shown: OP: 465 ± 13.05; OR: 414 ± 8.38; unpaired two-tailed t-test: $t_{(105)}$=3.39, p<0.01; note this includes untrained Control rats). This difference is within normal variance for adult Sprague Dawley rats and is representative of the tails of expected weight distributions for males of this age (Lillie et al., 1996). During FR1 training, obesity-prone and obesity-resistant rats reached the acquisition criterion within a similar timeframe (Fig. 2.2A: unpaired two-tailed t-test, p=0.32; OP: 1.79 sessions ±0.14; OR: 1.53 sessions ±0.13). When rats were transitioned to a VI schedule of reinforcement, active lever responding increased as a function VI length, as expected (Fig. 2.2B: two-way RM ANOVA; main effect of session, $F_{(7,644)}$=36.91, p<0.01). Additionally, although both groups preferentially responded on the active lever, both active and inactive lever responding was higher in obesity-resistant vs. obesity-prone groups (Fig. 2.2B: main effect of group, $F_{(1,92)}$=6.72, p=0.01; session x group interaction, $F_{(7,644)}$=3.23, p<0.01; Fig. 2.2C: two-way RM ANOVA; main effect of group, $F_{(1,92)}$=12.05, p<0.01; session x group interaction, $F_{(7,644)}$=5.29, p<0.01).

Next, rats underwent Pavlovian conditioning in which pellet delivery was paired with a CS+ (i.e., food cue), but never with a CS- (i.e., control cue). Data in Fig. 2.2D show the average rate of food cup entries during CS presentations (note that food cup entries during the CS+ were recorded in the presence of food). In both groups, the number of food cup entries above baseline (ITI responding; dotted line) were greater during CS+ vs. CS- presentations and this difference increased across training (Fig. 2.2D: two-way RM

ANOVA; OP: main effect of CS, $F_{(1,42)}$=209.8, p<0.01; main effect of session, $F_{(7,294)}$=47.13, p<0.01; session x CS interaction, $F_{(7,294)}$=31.00, p<0.01; OR: main effect of CS, $F_{(1,51)}$=243.2, p<0.01; main effect of session, $F_{(7,357)}$=41.38, p<0.01; session x CS interaction, $F_{(7,357)}$=28.18, p<0.01). Although CS+ responding here was measured in the presence of food, these data are consistent with the development of conditioned discrimination between the CS+ and CS-. In a subset of the rats, food cup entry data were recorded in 10-sec bins during Pavlovian training (OP n=23; OR n=32). Pellet delivery never occurred during the first 10 sec of the CS+. This therefore allowed us to evaluate food cup entries uncontaminated by the pellet and provided a clean measure of conditioned responding (Fig. 2.2E). As expected, rats acquired clear conditioned discrimination that increased across sessions, with both groups preferentially entering the food cup during the first 10 sec of the CS+ vs. the CS- (Fig. 2.2E: OP: two-way RM ANOVA; main effect of CS, $F_{(1,22)}$=21.86, p<0.01; session x CS interaction, $F_{(7,154)}$=4.36, p<0.01; OR: two-way RM ANOVA; main effect of CS, $F_{(1,31)}$=39.16, p<0.01; session x CS interaction, $F_{(7,217)}$=11.64, p<0.01). This conditioned anticipatory discrimination emerged one session earlier in OPs than in ORs. Finally, the rate of entries into the food cup did not differ significantly between groups (Fig. 2.2D: CS+: two-way RM ANOVA; no main effect of group, p=0.15; CS-: no main effect of group, p=0.86; Fig. 2.2E: CS+: two-way RM ANOVA; no effect of group, p=0.60; CS-: two-way RM ANOVA; no effect of group, p=0.87).

Acquisition of lever pressing for food (A) is similar between groups, but lever responding is generally greater in obesity-resistant than in obesity-prone groups on both active (B) and inactive levers (C).



Acquisition of Pavlovian conditioned discrimination is generally similar between groups.

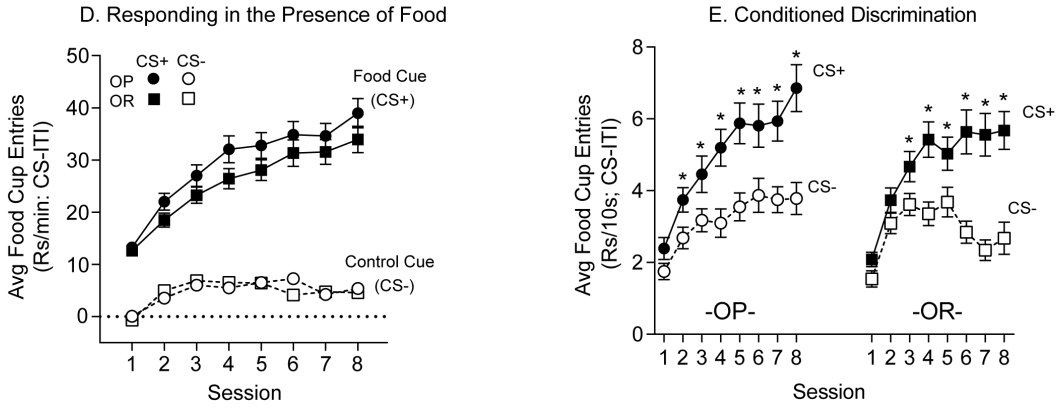


Figure 2.2: Acquisition of instrumental and Pavlovian responding was similar between obesity-prone and obesity-resistant groups. **A)** The total time to reach the acquisition criterion during FR1 training was similar between groups. Values for each subject (circles, squares) and the mean (horizontal line) are shown. **B)** Average active lever responding during VI training. The average rate of active lever responding increased across sessions in both groups, but the rate of responding was greater in obesity-resistant rats in the final 2 sessions of VI training. **C)** Average inactive lever responding during VI training. The average rate of inactive lever responding was very low compared to active responding (note difference in scale of y-axis between panels B and C). However, rates of responding were greater in obesity-resistant vs. obesity-prone groups. **D)** Average food cup entries above baseline (CS-ITI; dotted line = ITI) during Pavlovian conditioning. Rates of responding during the entire CS period were greater during CS+ (closed symbols) vs. CS- (open symbols) presentations, and rates of entries were similar between groups. **E)** Average food cup entries during the first 10 sec of each CS presentation. Both groups rapidly acquire the discrimination, preferentially responding during the CS+ vs. CS-, although discrimination emerged one session earlier in the obesity-prone vs. obesity-resistant group. * = Sidak's post-test, $p < 0.05$. All data shown as average ± SEM unless otherwise noted.

3.2: Obesity-prone rats show robust Pavlovian-to-instrumental transfer

After training, rats were tested for cue-triggered food-seeking (i.e., PIT; Fig. 2.3A; OP n=20; OR n=19), a classic measure of incentive motivation (Berridge and Robinson, 2003; Wyvell and Berridge, 2000). Pellets were omitted during testing, and the degree to which the CS+ invigorated active lever pressing above baseline relative to the CS- provided a clean measure of the motivational influence of the food cue (Cartoni et al., 2016; Holmes et al., 2010; Lovibond, 1983; Morse and Skinner, 1958; Rescorla and Lolordo, 1965). Obesity-prone rats exhibited significantly greater PIT than obesity-resistant rats, making more active lever responses during CS+ presentations than obesity-resistant rats (Fig. 2.3B: two-way RM ANOVA; main effect of CS, $F_{(1,37)}=52.80$, $p<0.01$; group x CS interaction, $F_{(1,37)}=5.95$, $p=0.02$). In addition, the magnitude of PIT (i.e., the difference in active lever responding elicited by the CS+ vs. CS-) was significantly greater in obesity-prone vs. obesity-resistant groups (Fig. 2.3C: unpaired two-tailed t-test: $t_{(37)}=2.27$, $p=0.03$). This difference was also apparent when the magnitude of PIT was examined across trials (Fig. 2.3D: two-way RM ANOVA; main effect of group, $F_{(1,37)}=5.14$, $p=0.03$). Specifically, obesity-prone rats showed a classic pattern of robust CS+ triggered responding during early trials that slowly declined across testing; whereas PIT in obesity-resistant rats was variable and short lived. In contrast, conditioned discrimination, (i.e., the difference in food cup entries elicited by the CS+ vs. CS-) was similar between groups (Fig. 2.3E: two-way RM ANOVA; main effect of CS, $F_{(1,37)}=92.25$, $p<0.01$; no effect of group, $p=0.67$; no group x CS interaction $p=0.18$). Thus, although the CS+ acquired the same predictive significance in both groups, the ability of the CS+ to invigorate food-seeking behavior was stronger in obesity-prone rats.

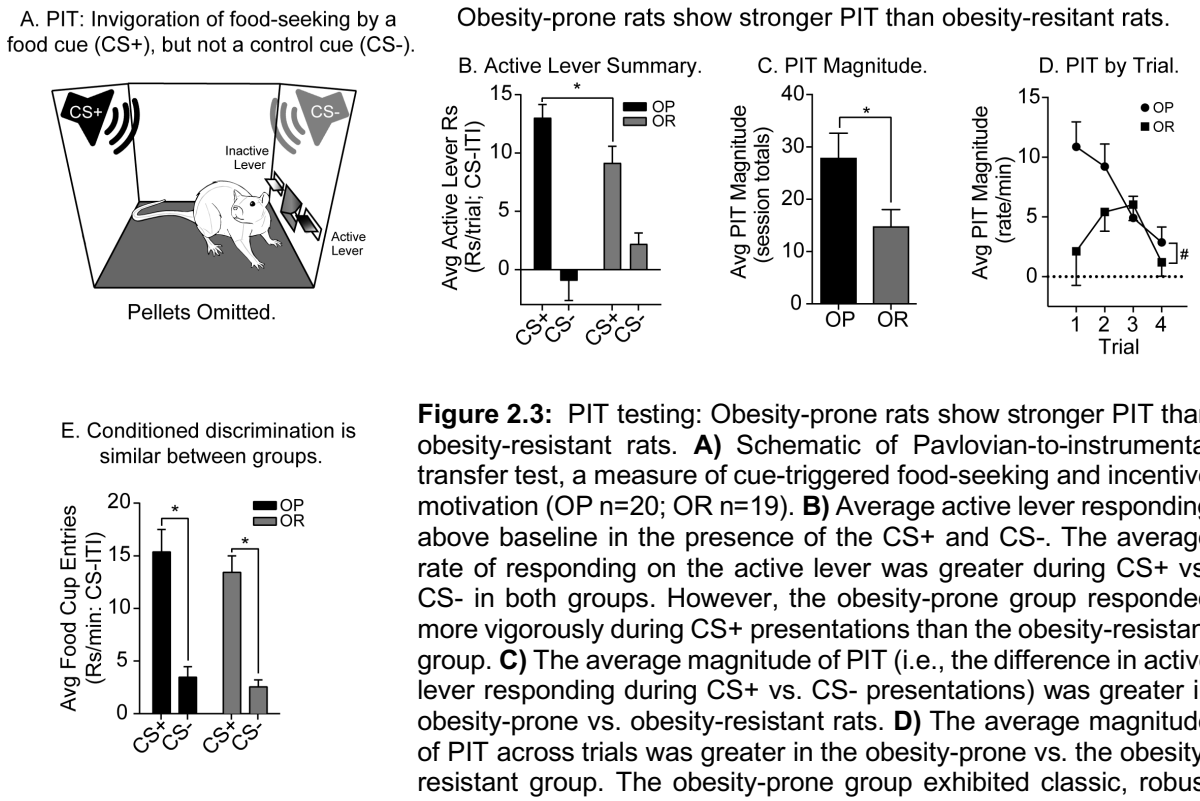


Figure 2.3: PIT testing: Obesity-prone rats show stronger PIT than obesity-resistant rats. **A)** Schematic of Pavlovian-to-instrumental transfer test, a measure of cue-triggered food-seeking and incentive motivation (OP n=20; OR n=19). **B)** Average active lever responding above baseline in the presence of the CS+ and CS-. The average rate of responding on the active lever was greater during CS+ vs. CS- in both groups. However, the obesity-prone group responded more vigorously during CS+ presentations than the obesity-resistant group. **C)** The average magnitude of PIT (i.e., the difference in active lever responding during CS+ vs. CS- presentations) was greater in obesity-prone vs. obesity-resistant rats. **D)** The average magnitude of PIT across trials was greater in the obesity-prone vs. the obesity-resistant group. The obesity-prone group exhibited classic, robust PIT, which is most prominent in early trials, but slowly declines across repeated CS presentations, whereas the obesity-resistant group showed weak PIT that was variable, and short-lived. **E)** Conditioned discrimination during PIT testing was present in both groups, with rats preferentially entering the food cup during CS+ vs. CS- presentations. The rate of responding during CS+ and CS- presentations and the magnitude of discrimination was similar between the groups. All data shown as average rate of responding above baseline (ITI) ± SEM; *=p<0.05; #=Main effect of group, p<0.05.

3.3: Initial training experience is sufficient to increase CP-AMPAR surface expression in obesity-prone, but not obesity-resistant rats

As stated above, NAc CP-AMPARs mediate the "incubation of cocaine-seeking" (Conrad et al., 2008; Lee et al., 2013; Loweth et al., 2014; Ma et al., 2014; Wolf, 2016), and CP-AMPAR up-regulation occurs more readily in obesity-prone vs. obesity-resistant rats (Oginsky et al., 2016a). However, whether CP-AMPARs are involved in PIT is unknown. Therefore, we next determined whether experience during initial training differentially alters surface and intracellular expression of NAc AMPAR subunits in obesity-prone vs. obesity-resistant rats (OP-Trained n=10; OP-Control n=6; OR-Trained n=10; OR-Control, n=6). Expression of GluA1 and GluA2 differed between untrained Control groups, with greater GluA1 surface expression and lower GluA2 intracellular expression in obesity-resistant vs. obesity-prone control groups (Fig. 2.4A: Surface GluA1: unpaired two-tailed t-test: $t_{(9)}$=4.42, p<0.01; Fig. 2.4B Intracellular GluA2: unpaired two-tailed t-test; $t_{(10)}$=4.96, p<0.01). Given these differences in the control groups, comparisons were made between Trained and Control groups within obesity-prone and obesity-resistant groups (Fig. 2.5).

NAc GluA1 and GluA2 expression differs between obesity-prone and obesity-resistant control groups.

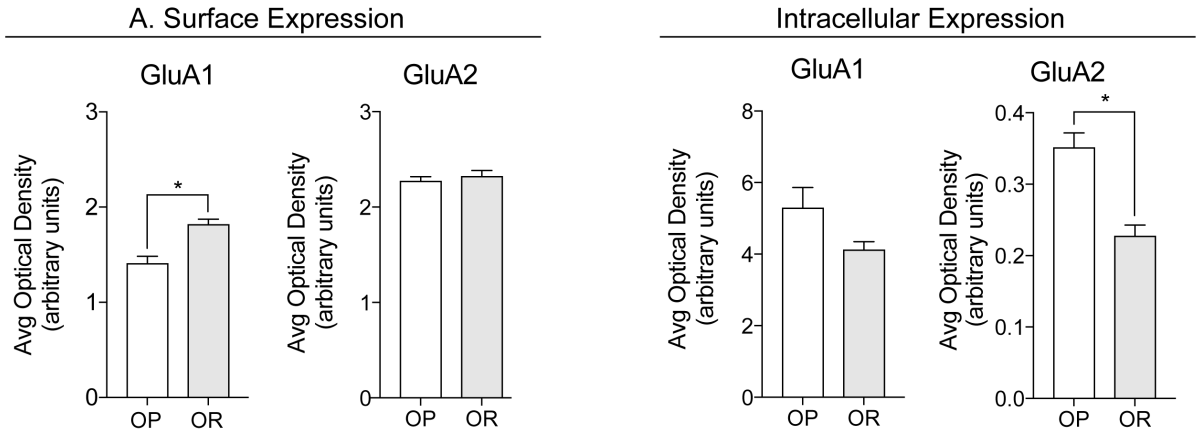


Figure 2.4*:* AMPAR subunit expression pattern differs between obesity-prone and obesity-resistant control groups. A) GluA1, but not GluA2 surface expression is lower in obesity-prone rats. B) Intracellular GluA1 is similar between groups, but GluA2 intracellular expression is lower in obesity-resistant rats. All data shown as average ± SEM; *=p<0.05.

In obesity-prone rats, experience during training increased GluA1 surface expression and decreased GluA2 surface expression compared to Controls (Fig. 2.5A: two-way RM ANOVA; main effect of training: $F_{(1,26)}=11.17$, $p<0.01$; subunit x training interaction: $F_{(1,26)}=65.46$, $p<0.01$). This was accompanied by a reduction in intracellular GluA1 in Trained vs. Control groups (Fig. 2.5B: two-way RM ANOVA; main effect of training, $F_{(1,26)}=10.35$, $p<0.01$; subunit x training interaction, $F_{(1,26)}=7.92$, $p<0.01$). This surface increase in GluA1 expression along with reductions in GluA2 surface expression suggests an increase in GluA2-lacking, CP-AMPARs (Conrad et al., 2008; Oginsky et al., 2016a; Wenthold et al., 1996). In contrast, in obesity-resistant groups training did not alter GluA1 surface expression, but instead produced a significant reduction in GluA2 surface expression (Fig. 2.5D: two-way RM ANOVA: main effect of training, $F_{(1,25)}=14.61$, $p<0.01$; subunit x training interaction, $F_{(1,25)}=33.07$, $p<0.01$). On the intracellular level, training in obesity-resistant groups increased GluA1, without altering GluA2 expression (Fig. 2.5E: two-way RM ANOVA: main effect of training, $F_{(1,25)}=110.10$, $p<0.01$; subunit x training interaction, $F_{(1,25)}=102.60$, $p<0.01$). This pattern in obesity-resistant rats is not typical of CP-AMPAR increases, but may suggest an intracellular accumulation of GluA1-containing AMPARs (see also section 4.3).

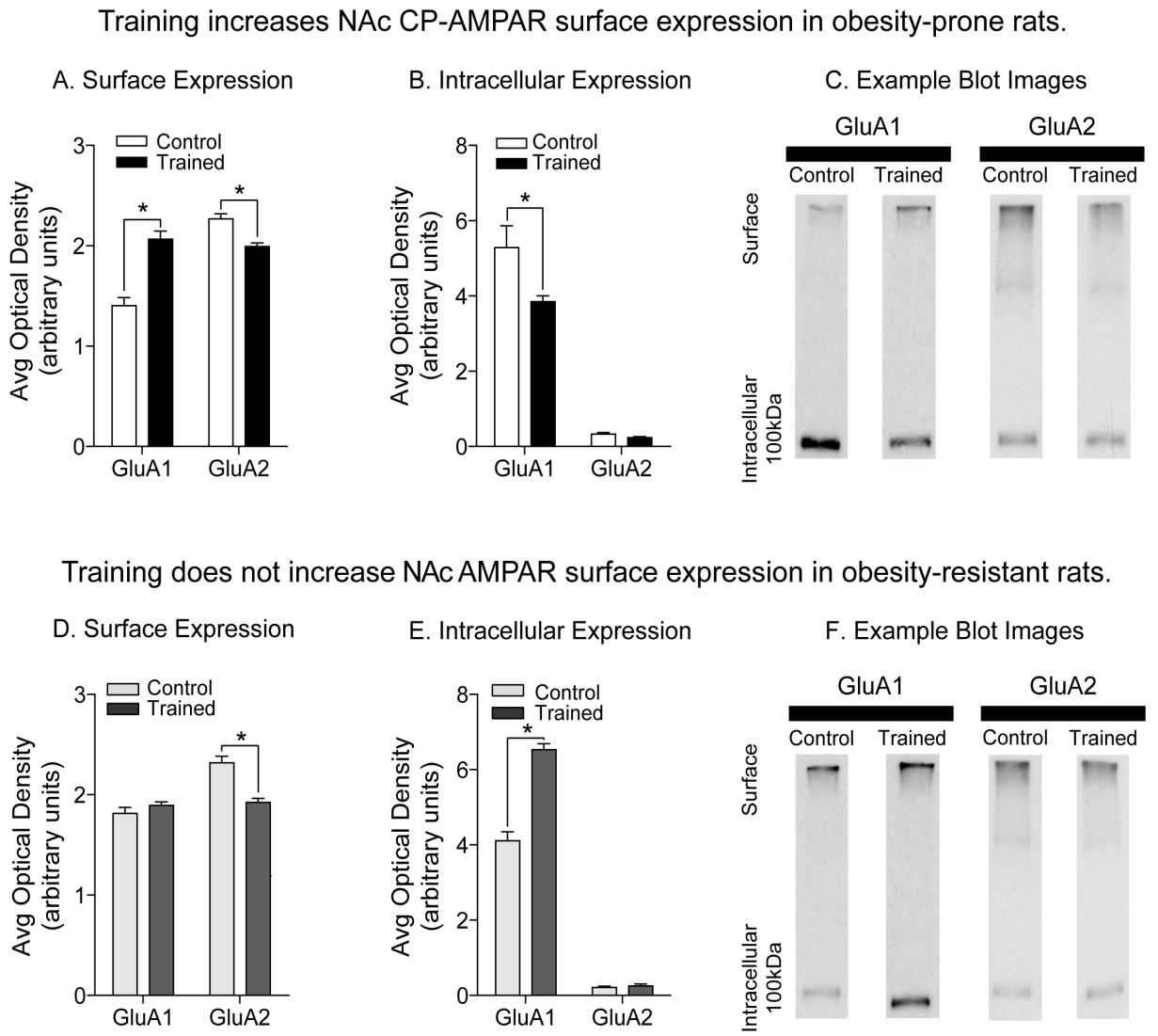


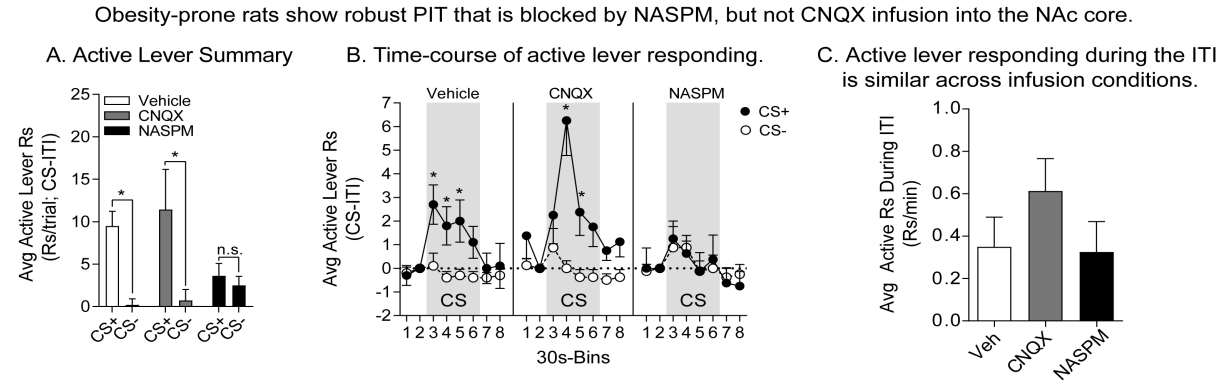
Figure 2.5*:* Experience during training increases NAc CP-AMPAR surface expression in obesity-prone, but not obesity-resistant rats. **A)** Average surface expression of GluA1 and GluA2 subunits in obesity-prone groups: Relative to the Control group (white bars), surface GluA1 expression was increased, whereas surface GluA2 expression was decreased following training (black bars). This pattern is consistent with an increase in GluA2-lacking CP-AMPARs. **B)** Average intracellular expression of GluA1 and GluA2 subunits in obesity-prone groups. Intracellular GluA1 levels were decreased following training in obesity-prone rats. **C)** Representative images of GluA1 and GluA2 expression in crosslinked NAc tissue from obesity-prone groups. **D)** Average surface expression of GluA1 and GluA2 subunits in obesity-resistant rats. GluA1 surface expression did not differ between Control (light gray bars) and Trained (dark gray bars) groups, but GluA2 surface expression was decreased in the Trained vs. Control groups. **E)** Average intracellular expression of GluA1 and GluA2 subunits in obesity-resistant rats. Intracellular GluA1 expression was increased following training, with no differences observed in intracellular GluA2 expression. **F)** Representative images of GluA1 and GluA2 expression in crosslinked NAc tissue from obesity-resistant groups. OP-Trained n=10; OP-Control n=6; OR-Trained n=10; OR-Control n=6; *=p <0.05.

3.4: Blockade of CP-AMPARs in the NAc core prevents the expression of PIT in obesity-prone rats

Given that obesity-prone rats exhibit robust PIT (Fig. 2) and that PIT relies on excitatory transmission in the NAc, we next determined whether AMPAR blockade in the NAc core would prevent the expression of PIT. Although obesity-resistant rats had not displayed robust PIT, nor were increases in GluA1 or GluA2 surface expression found after training, obesity-resistant rats were none-the-less included in these studies because it is still possible that reductions in GluA2 surface expression seen in obesity-resistant rats could alter AMPAR-mediated transmission. Intra-NAc infusions were conducted using a counterbalanced, within-subjects design, however, not all animals are represented in each condition due to unsuccessful bilateral infusions (OP: Vehicle, n=11; CNQX n=8; NASPM n=8; OR: Vehicle n=22; CNQX n=20, NASPM n=19). Importantly, there were no statistical differences between behavior during post-operative vs. pre-operative instrumental and Pavlovian sessions and no significant order effects of infusion were found (data not shown). However, active lever responding during the first 10 min of testing (prior to cue presentation) and during the ITI under Vehicle conditions was significantly higher in obesity-resistant vs. obesity-prone rats (data not shown: pre-cue period OP vs. OR: unpaired two-tailed t-test: $t_{(31)}=4.24$, $p<0.01$; ITI OP vs. OR: unpaired two-tailed t-test: $t_{(31)}=2.89$, $p<0.01$). Due to these differences in baseline responding, the effects of AMPAR blockade on PIT were evaluated within each group separately (Figs. 2.6 and 2.7). Importantly, this did not impede the ability to assess PIT and effects of antagonists, as PIT is defined by differences in responding above baseline in the presence of the CS+ vs. the CS-.

Total active lever responding during the first CS+ and CS- presentation following Vehicle, CNQX, and NASPM infusion in obesity-prone rats is shown in Fig. 2.6A, and the time-course of this responding is shown in Fig. 2.6B (30 sec bins). Effects of infusion on the first presentation of each cue were examined to avoid potential confounds of extinction from repeated CS presentation (see Fig. 2.3D) and to examine behavior most proximal to drug infusion. Importantly, the order of CS+ vs. CS- presentation was counter-balanced across infusion conditions. Consistent with behavior in intact rats, obesity-prone

rats exhibited PIT following Vehicle infusion (Fig. 2.6A: Sidak's planned comparison: CS+ vs. CS-: Vehicle, $t_{(22)}$=3.40, p<0.01). Analysis of the time-course of their behavior shows that the CS+ elicited robust active lever responding that began immediately at CS+ onset, whereas active lever responding was unaltered by presentation of the CS- (Fig. 2.6B left: Vehicle: two-way RM ANOVA: main effect of CS, $F_{(1,9)}$=16.56, p<0.01; CS+, closed; CS-, open symbols). Infusion of CNQX did not block the expression of PIT, whereas infusion of NASPM did (Fig. 2.6A: Sidak's planned comparison: CS+ vs. CS-: CNQX, $t_{(22)}$=3.27, p=0.01; NASPM, $t_{(22)}$=0.36, p=0.97). Furthermore, when the rate of active lever responding was compared between Vehicle and drug infusion conditions, NASPM, but not CNQX, significantly reduced responding during the CS+ with no effect on CS- responding (Fig. 2.6A: Sidak's planned comparison: Vehicle vs. CNQX: CS+, p=0.81; CS-, p=0.99; Vehicle vs. NASPM: CS+, $t_{(32)}$=3.15, p<0.01; CS-, p=0.40). This blockade by NASPM was consistent throughout CS+ presentation (Fig. 2.6B right: two-way RM ANOVA; NASPM: no effect of CS, p=0.98, no CS x time interaction p=0.92). Although CNQX infusion did not produce robust effects on PIT (Fig. 2.6A and B middle), the onset of responding was delayed compared to Vehicle conditions, with significant increases in lever pressing only emerging 30 sec after CS+ onset (Fig. 2.6B middle; two-way RM ANOVA: CNQX: main effect of CS, $F_{(1,7)}$=10.75, p=0.01). Importantly, infusion of either antagonist did not produce any general motoric effects; active lever responding during the first 10 min of testing (data not shown: two-way ANOVA: no effect of drug, p=0.77) and during the ITI between CS presentations (Fig. 2.6C; one-way ANOVA: no effect of drug, p=0.96) was similar across infusion conditions. In addition, inspection of videos did not reveal any overt motor effects on behavior. Furthermore, NASPM infusion did not alter conditioned discrimination in approach to the food cup during CS+ vs. CS- presentations (Fig. 2.6D: two-way RM ANOVA: main effect of CS, $F_{(1,23)}$=11.69, p<0.01; no effect of drug, p=0.65), demonstrating that the effect of NASPM infusion was specific to the expression of PIT. This also confirms that the effect of NASPM is not the result of a loss of discrimination between the cues in obesity-prone rats, but rather is due to an attenuation of the ability of the CS+ to invigorate food-seeking (i.e., incentive motivation).



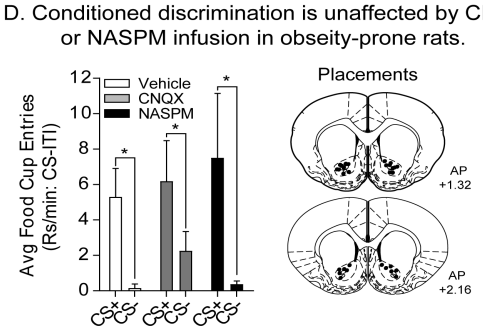


Figure 2.6: Infusion of NASPM into the NAc core blocks the expression of PIT in obesity-prone rats without altering conditioned discrimination. **A)** The average rate of active lever responding above baseline during the first CS+ and CS- presentation of PIT testing following infusion. Obesity-prone rats showed robust PIT following Vehicle infusion (n=11), with presentation of the CS+, but not the CS- eliciting increases in active lever responding (white bars). Infusion of CNQX (0.3μg/0.5μl/hemisphere; 2.58 mM; n=8) did not alter the expression of PIT (gray bars), but infusion of NASPM (20μg/0.5μl/hemisphere; 83.35 mM; n=8) blocked PIT by reducing responding during CS+ presentations to levels similar to responding during the CS- (black bars). **B)** Time-course of active lever responding. Following Vehicle infusion, CS+ presentation elicited an immediate increase in the average rate of active lever responding, whereas the CS- did not. CNQX infusion produced a slight delay in the onset of responding following CS+ presentation, but did not eliminate the expression of PIT. In contrast, NASPM infusion selectively blocked CS+ triggered active lever responding throughout the entire CS presentation period. **C)** Active lever responding during the ITI was similar across infusion conditions. **D)** Average food cup entries during CS+ and CS- presentation. Conditioned discrimination was unaffected by infusion conditions. Following Vehicle, CNQX, and NASPM infusions food cup entries were greater during CS+ vs. CS- presentation. Infusion placements are shown at the right; *=p<0.05, CS+ vs. CS-.

The pattern of behavior in obesity-resistant rats was very different from that seen in the obesity-prone group. First, obesity-resistant rats did not exhibit robust PIT following infusion of Vehicle, CNQX, or NASPM and differences in the magnitude of responding during either cue did not reach statistical significance (Fig. 2.7A: Sidak's planned comparison: CS+ vs. CS-: Vehicle, $t_{(59)}$=2.19, p=0.09; CNQX, $t_{(59)}$=2.23, p=0.09; NASPM, $t_{(59)}$=2.18, p=0.10). The time-course of active lever responding following Vehicle infusion is shown in Fig. 5B. Active lever responding was elevated at the onset of both the CS+ and CS- (Fig. 2.7B: two-way RM ANOVA: main effect of time, $F_{(7,147)}$=13.38, p<0.01; no effect of CS, p=0.50). Furthermore, compared to Vehicle conditions, both CNQX and NASPM infusion reduced overall rates of active lever responding during CS presentations (Fig. 2.7A: two-way RM ANOVA: main effect of drug, $F_{(2,59)}$=8.29, p<0.01), during the ITI (Fig. 2.7C: one-way ANOVA: main effect of drug, $F_{(2,59)}$=4.84, p=0.01) and during the 10 min pre-cue period (Data not shown: two-way ANOVA: main effect of drug, $F_{(2,59)}$=7.94, p<0.01). Thus, in obesity-resistant rats, infusion of either antagonist resulted in general motor suppressant effects. These motor effects were also apparent when entries into the food cup were examined (Fig. 2.7D: two-way RM ANOVA: main effect of drug, $F_{(2,59)}$=3.38, p=0.04), but were not visually apparent in video recordings. Despite these general effects, the number of entries during the CS+ were significantly greater than during the CS-, regardless of infusion condition (Fig. 2.7D: two-way RM ANOVA: main effect of drug, $F_{(1,59)}$=22.28, p<0.01). Thus, although obesity-resistant rats discriminate between the CS+ and CS-, both cues increased active lever responding. The latter is not indicative of invigoration by the food cue (CS+) per se, but rather of a generalized effect of stimuli on responding.

Following Vehicle infusion, obestiy-resistant rats did not exhibit PIT and infusion of CNQX and NASPM each produced generalized motor supression, without disrupting conditioned discrimination.

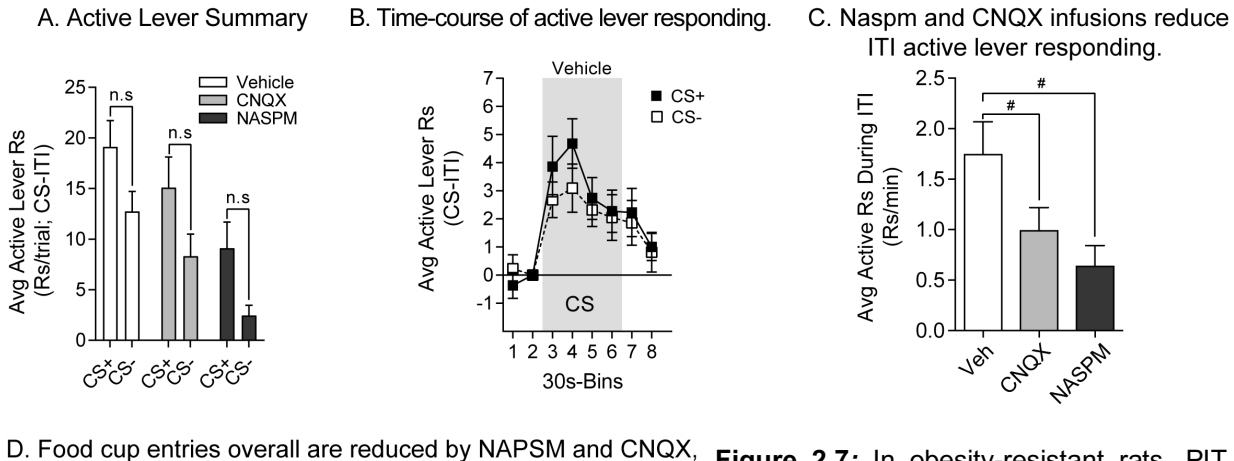


Figure 2.7*:* In obesity-resistant rats, PIT was absent following all three infusion conditions, but conditioned discrimination was maintained throughout. **A)** The average rate of active lever responding above baseline during the first CS+ vs. CS- presentation did not differ significantly following Vehicle (n=22), CNQX (0.3μg/0.5μl/hemisphere; n=20), or NASPM (20μg/0.5μl/hemisphere; n=19) infusion. **B)** Time-course of active lever responding following Vehicle infusion. The average rate of active lever responding was increased by both CS+ and CS- presentation. Thus, PIT was absent in the obesity-resistant group and a non-specific responding to stimulus presentation was observed. **C)** Active lever responding during the ITI was significantly reduced by infusion of CNQX and NASPM, consistent with a generalized motoric depression by AMPAR blockade. *=Sidak's post-test, $p<0.05$. **D)** Average food cup entries during CS+ and CS- presentation. Conditioned discrimination was present under all three infusion conditions. Rats preferentially entered the food cup during CS+ vs. CS- presentation. In addition, overall rates of entry were reduced by infusion of CNQX and NASPM. *=Main effect of CS, $p<0.05$; $=Main effect of drug; #=Main effect of drug. Infusion placements are shown at the right.

In sum, the data above demonstrate that obesity-prone rats exhibit more reliable, robust PIT that is mediated by NAc CP-AMPARs, and that training produces an increase in surface expression of GluA1-containing AMPARs in this group. In contrast, in obesity-resistant rats, the expression of PIT was relatively weak and variable, despite reliable discrimination between the CS+ vs. CS-, and there was no clear evidence for AMPAR increases. Furthermore, in both groups, conditioned discrimination in approach to the

food cup was unaffected by NAc AMPAR blockade, supporting a selective role for NAc CP-AMPARS in the transfer of Pavlovian incentive motivation to food-seeking behavior.

4: Discussion

Studies in humans suggest that in obesity-susceptible individuals, stronger motivational responses elicited by food cues drive over-consumption that leads to and maintains obesity (see chapter introduction) and may share neurobehavioral features with drug addiction (Berridge et al., 2010; Dagher, 2009; Ferrario, 2017). However, to date only one preclinical study has examined potential intrinsic differences in cue-triggered motivation in models of susceptibility to obesity (Robinson et al., 2015), and the underlying mechanisms are poorly understood. Differences in motivational responses to food cues may arise from alterations in NAc function, as cue-triggered food- and drug-seeking require NAc excitatory transmission (Corbit and Balleine, 2011; Di Ciano and Everitt, 2004; Fuchs et al., 2004). Here, we found that obesity-prone rats exhibited robust PIT (i.e., incentive motivation) that was mediated by NAc core CP-AMPARs. Additionally, biochemical data suggest that this is due in part to experience-induced increases in NAc CP-AMPAR surface expression. In contrast, obesity-resistant rats displayed weak and variable PIT that was not associated with CP-AMPAR upregulation. These data demonstrate that incentive motivational responses to food cues are stronger in obesity-prone rats prior to obesity, and establish a novel role for the up-regulation of NAc CPAMPARs in this form of incentive motivation. Together these data substantiate the idea that enhanced cue-triggered food “craving” is a feature of susceptibility to obesity that may lead to over-eating and weight gain.

4.1: Obesity-prone rats display robust PIT

In two separate cohorts, we found that obesity-prone rats exhibited robust PIT, where presentation of the food cue (CS+), but not the control cue (CS-), selectively invigorated food-seeking in the absence of food itself (Figs. 2.3 and 2.6). In contrast, the magnitude of PIT was weak to absent in obesity-resistant rats (Figs. 2.3 and 2.7). These differences

in PIT expression are not explained by differences in learning, as acquisition of the instrumental and Pavlovian tasks was similar between groups (Fig.2.2). Moreover, both groups showed clear conditioned discrimination between the cues during testing, preferentially approaching the food cup during CS+, but not CS- presentations (Figs. 2.3E, 2.6D and 2.7D). Thus, weaker PIT in obesity-resistant groups is not due to an inability to understand the predictive significance of each cue.

We previously found that the magnitude of conditioned approach was greater in outbred rats subsequently identified as susceptible to obesity compared to resistant rats (Robinson et al., 2015). However, here we did not find group differences in conditioned discrimination. This is likely due to the use of food restriction in the current study, which is sufficient to eliminate differences in approach in outbred rats (see Robinson et al., 2015 for discussion). Additionally, several procedural differences may also contribute (e.g., use of a prolonged CS+ and the inclusion of a CS- here, but not in our previous study; see Silva and Timberlake, 1997 for discussion). Nonetheless, incentive motivation in the form of CS+ driven food-seeking (PIT) was more robust in obesity-prone rats. This is consistent with enhanced cue-triggered motivation found previously in outbred populations that are susceptible to weight gain (Robinson et al., 2015).

4.2: Experience-induced increases GluA1 surface expression in obesity-prone rats

The expression of PIT relies on activation of the NAc (Corbit and Balleine, 2011), although the role of NAc AMPARs in PIT has not previously been examined. Here, we found that NAc GluA1 surface expression was increased, while GluA2 surface expression was decreased following training in obesity-prone, but not obesity-resistant rats (Fig. 3). This is consistent with an increase in GluA2-lacking CP-AMPARs (i.e., GluA1/1 or GluA1/3 containing AMPARs) and with the role of CP-AMAR up-regulation in the “incubation of cocaine craving” effect (Ferrario et al., 2010; Scheyer et al., 2016; Wolf, 2016). In contrast, in obesity-resistant rats increases in intracellular GluA1 without changes in GluA2 expression were found, suggesting a possible accumulation of intracellular CPAMPARs. CP-AMPARs can be rapidly recruited to synapses to enhance

neurotransmission (Clem and Huganir, 2010). Thus, it is possible that the intracellular accumulation of GluA1 in obesity-resistant rats represents an internal pool of CP-AMPARs, but that either their recruitment and/or retention at the synapse are insufficient for their accumulation at the surface in obesity-resistant rats (see Ferrario et al., 2011 for discussion of retention of CP- vs. Non-CP AMPARs at synaptic sites).

We also found greater GluA1 surface and lower GluA2 intracellular expression in obesity-resistant vs. obesity-prone untrained control groups. This was surprising because we previously reported similar basal NAc GluA1 surface expression between obesity-prone and obesity-resistant rats (Oginsky et al., 2016a). However, in our previous study rats were fed *ad libitum*, whereas in the current experiment rats were mildly food deprived. Indeed, recent studies have shown that food restriction itself is sufficient to produce modest increases in NAc GluA1 expression (Ouyang et al., 2017; Peng et al., 2015). Therefore, the basal differences found here may have arisen from the differential impact of food restriction in obesity-prone vs. obesity-resistant groups. This raises the intriguing possibility that dieting may produce undesired effects in obesity-susceptible populations that will be investigated in future studies. While interesting, this difference between control groups does not interfere with the primary objective of this experiment, which was to assess the effects of training on NAc AMPAR expression in these selectively-bred lines of rats.

4.3: NAc CP-AMPARs mediate enhanced incentive motivation in obesity-prone rats

Consistent with biochemical data, infusing the CP-AMPAR antagonist NASPM blocked the expression of PIT in obesity-prone rats (Fig. 2.6). Importantly, NASPM did not affect active lever responding during any other phase of testing and left conditioned discrimination intact. Thus, the effect of NASPM was selective to the expression of PIT. CP-AMPAR mediated enhancements in incentive motivation in obesity-prone rats is similar to alterations that drive withdrawal-dependent increases in cocaine-seeking, a key feature of addiction (Conrad et al., 2008; Wolf and Tseng, 2012). This is consistent with the overlap in neural systems underlying incentive motivational responses to food and

drug-associated cues, but also raises questions about the degree to which recruitment of NAc CPAMPARs represents aberrant vs. normal plasticity. In support of aberrant plasticity, the “incubation” of cocaine, but not sucrose craving is associated with increases in NAc CP-AMPAR expression and function in outbred rats (Conrad et al., 2008; Counotte et al., 2014). However, arguing against aberrant plasticity, the expression of PIT is absent in transgenic mice in which synaptic insertion of GluA1-containing AMPARs throughout the brain is prevented (Crombag et al., 2008). This latter study suggests that the recruitment of CP-AMPARs may be part of normal plasticity underlying incentive motivation.

Surprisingly, infusion of the general AMPAR antagonist CNQX did not block the expression of PIT in obesity-prone rats. Although speculative, this may be due to the fact that CNQX is a competitive antagonist, whereas NASPM is not. Thus, the efficacy of CNQX, but not NASPM, is reduced by the presence of glutamate. In addition, the affinity and efficacy of CNQX are altered by AMPAR auxiliary subunits, which likely differ between AMPAR populations (Kawai, 1991; Kott et al., 2009; Maclean and Bowie, 2011; Menuz et al., 2007). Thus, in cases when CP-AMPARs dominate synapses, CNQX may be less effective at blocking AMPAR-transmission. Regardless of these possibilities, the selective loss of PIT following NAc CPAMPAR blockade in obesity-prone rats is consistent with the upregulation of these receptors following training (see above) and with the role of these receptors in enhanced incentive motivation for other reinforcers like cocaine (Huang et al., 2015; Wolf, 2016; Wolf and Tseng, 2012).

In the obesity-resistant group, the expression PIT following Vehicle infusion was weak, with both the CS+ and CS- invigorating active lever responding (Figs. 2.3 and 2.7). Given the absence of reliable PIT, it is not surprising that neither NASPM nor CNQX had any selective effects on behavior in this group. Instead, we found that infusion of either drug produced a general suppression of lever responding and food cup entries during all phases of testing. Although the mechanistic reason for this effect is unclear, one would expect sufficient blockade of excitatory transmission in the NAc to produce a reduction in general behavioral output. In addition, it is worthwhile to note that at no time did obesity-resistant rats appear lethargic or uncoordinated in their movements (based on videos

recorded during testing). However, similar to obesity-prone rats, conditioned discrimination persisted following infusion of either drug. While behavioral dissociations between conditioned discrimination and PIT responding have been established (Delamater, 1996; Delamater et al., 2017; Lichtenberg et al., 2017), to our knowledge, results here are the first to demonstrate receptor-mediated dissociations between these two behaviors.

4.4. Conclusions:

In sum, enhanced incentive motivation in obesity-prone rats is mediated by NAc CP-AMPARs. These neurobehavioral differences may render obesity-susceptible populations more sensitive to the motivational influence of food cues, producing more intense, focused, “wanting” that may limit the ability to divert behavior towards healthier alternatives. These data also demonstrate that in addition to mediating the intensification of cocaine-seeking (Huang et al., 2015; Wolf, 2016), NAc CP-AMPARs also mediate the expression of PIT for a food cue. This raises important questions about whether CP-AMPAR up-regulation represents aberrant vs. normal neural processes that underlie cue-triggered reward seeking behaviors, and the degree to which susceptibility to obesity shares features of addiction.

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Chapter 3: General Pavlovian-to-Instrumental Transfer is Commonly Enhanced in Outbred and Selectively Bred Obesity Susceptible Individuals

Abstract:

Within the last four decades global obesity rates have steadily risen, presenting a major challenge to society. Efforts to uncover the drivers of this epidemic have highlighted the contribution of Pavlovian motivational processes. In humans, brain and behavioral reactivity to food related stimuli positively correlates with subsequent weight gain. In concordance with this, selectively bred obesity-prone rats exhibit stronger cue-triggered food-seeking via single outcome Pavlovian-to-instrumental transfer (SO PIT) than obesity-resistant rats. These data show that Pavlovian motivation is stronger in selectively bred obesity-prone individuals. However, whether PIT is enhanced in outbred rats identified as obesity susceptible remains to be determine. Moreover, given that PIT can arise via two neurobehaviorally dissociable processes, a sensory specific versus a general affective process and that SO PIT does not distinguish between these, it is unclear which process contributes to the enhanced PIT observed in obesity-prone rats. Therefore, here we determine whether outbred obesity susceptibility is associated with enhanced Sensory Specific (SS) PIT or General PIT and separately whether selectively bred obesity-prone rats differ from obesity-resistant rats in expression of these variants of PIT. Our results demonstrate that in outbred rats, obesity susceptibility is strongly positively correlated with the magnitude of General PIT prior to weight gain. In selectively bred rats, General PIT was stronger in obesity-prone versus obesity-resistant rats. Jointly these data show that obesity susceptibility is linked with enhanced Pavlovian affective motivation. This has important implications for obesity prevention and for the neural circuitry mediating enhanced food-seeking in vulnerable individuals.

1: Introduction:

Obesity rates the world over continue to rise presenting both a major health concern and economic burden to society (Finkelstein et al., 2009; N.C.D.R, 2016). At its most basic level, the cause of obesity is understood: chronic consumption of hypercaloric diets leads bodyweight and fat accumulation which left unchecked results in obesity (Akiyama et al., 1996; Horton et al., 1995; Nascimento et al., 2008). Thus, our endeavor to reverse the obesity trends of the recent decades depends essentially upon uncovering factors that drive obesity. Hence, elucidating factors that contribute to heightened food-seeking and consumption is a critical step toward understanding the etiology of obesity (Matikainen-Ankney and Kravitz, 2018; Stice et al., 2013). Another important potential contributor to obesity is genetic vulnerability, which researchers have identified as strongly predictive of bodyweight and body mass indices (BMI), so much so that in some populations, this contribution has been estimated to account for up to 80% of the population variance in these traits (Hur et al., 2008; Maes et al., 1997). Consistent with this idea, in outbred rat populations, obesogenic diets induce differing levels of weight gain and fat accumulation across individuals (Levin et al., 1997). Understanding what traits render some individuals vulnerable to obesity is critical. Given that overeating is a necessary antecedent to obesity, it would seem likely that differences in factors that control food-seeking and feeding behaviors may render certain individuals more vulnerable to obesity. Recent work has suggested that individual variance in the degree to which Pavlovian stimuli influence brain function and behavior may play an important role in obesity vulnerability (Boswell and Kober, 2016). In humans, the magnitude of brain activity evoked by presentation of food related stimuli is greater in overweight and obese versus healthy weight individuals, particularly in nuclei related to Pavlovian motivation such as the amygdala and the Nucleus Accumbens (NAc; Rothemund et al., 2007; Stoeckel et al., 2008). Moreover, the magnitude of activation in the amygdala, NAc and the ventral pallidum elicited by food related stimuli is predictive of subsequent weight gain among healthy weight individuals (Burger and Stice, 2014; Demos et al., 2012; Yokum et al., 2014). These latter data in particular suggest that differences in brain responses to food stimuli precede weight gain in vulnerable individuals.

Food-seeking behaviors manifest via two primary associative learning processes, instrumental and Pavlovian learning (Bouton, 2007). Instrumental learning entails the formation of an association between actions or responses and their outcomes (R-O; Thorndike, 1898; Tolman, 1938). For a naturalistic example, consider an animal learning to pick fruit from a tree, with experience an R-O association forms between the act of picking and the ingestive experience of consuming the fruit. Pavlovian learning in contrast, involves the formation of an association between predictive stimuli and their outcomes (S-O; Pavlov, 1927). Consider the same animal eating the fruit from the tree; as it consumes the fruit it is learning associations between the colors and fragrant odors of ripe fruit and the positive effects of its ingestion. Thus, both instrumental and Pavlovian learning processes are critical for basic survival skills like foraging for food, but these processes also extend to a wide range of behaviors including mating, nesting and avoiding predation. One special feature of Pavlovian learning is that these S-O associations can also guide and influence instrumental behaviors. Continuing with our example, an animal moving through the woods might be drawn to a given tree by the scent of ripened fruit via an S-O association (odor-fruit), and then be led to climb the limbs and pick the fruit via an R-O association (picking action-fruit). This phenomenon of Pavlovian influence, is known as Pavlovian-to-Instrumental transfer (PIT) and can be capture in laboratory settings (as first demonstrated by Walker, 1942). PIT testing can therefore provide a quantitative measure of the degree to which a Pavlovian stimulus has acquired motivational control over instrumental food-seeking.

Consistent with the human studies described above, we found that selectively bred obesity-prone rats exhibited stronger single outcome PIT, than obesity-resistant rats prior to the onset of obesity (Derman and Ferrario, 2018). This suggests that Pavlovian motivation is stronger in rats selectively bred for obesity susceptibility. However, PIT can emerge via two neuronally and behaviorally independent processes, a sensory specific process and a general affective process (Corbit and Balleine, 2005; Corbit and Balleine, 2011; Corbit et al., 2007). Sensory Specific PIT (SS PIT) occurs when the sensory properties of an outcome shared by an R-O and S-O association drives the expression of PIT. This form of PIT is captured by contrasting the effects of a conditioned stimulus (CS) on two separate instrumental responses, where one response shares an outcome with

the CS, but the other does not (e.g., CS1: R1>R2; CS2: R2>R1; Colwill and Motzkin, 1994). On the other hand, General PIT arises when transfer emerges via the general affective properties shared by an R-O and S-O association. This effect can be observed when a CS enhances instrumental responding above baseline for an outcome other than that previously predicted by the CS (Balleine, 1994; Corbit and Balleine, 2005). Importantly, these variants of PIT are differentially influenced by psychological state. General PIT tends to be more labile; for instance, hunger can promote, whereas satiation can abolish General PIT; in contrast, SS PIT is difficult to disrupt, remaining intact despite changes in hunger state (Balleine, 1994; Corbit et al., 2007). In addition to the behavioral/psychological dissociations described above, SS PIT and General PIT are also mediated by dissociable neural circuitry. Specifically, intact function of the central amygdala (CA) and the Nucleus Accumbens (NAc) Core are both needed for the expression of General PIT, whereas the basolateral amygdala (BLA) and the NAc Shell are required for SS PIT (Corbit and Balleine, 2005; Corbit and Balleine, 2011).

As mentioned above, single outcome PIT is enhanced in selectively bred obesity-prone versus obesity-resistant rats (Derman and Ferrario, 2018). However, this PIT procedure does not distinguish between sensory specific and general motivational PIT processes, thus the precise psychological mechanism driving this effect is unknown. Consequently, an outstanding question that we address here, is whether one or both of these processes (SS and General PIT) is enhanced in obesity vulnerable individuals. Moreover, it has yet to be determined whether PIT expression is enhanced in obesity vulnerable individuals from outbred populations in a manner similar to that seen in selectively bred obesity-prone individuals. Therefore, in the current study we addressed these questions using a more sophisticated PIT paradigm that enables us to measure the magnitude of SS and General PIT within subjects (adapted from Corbit and Balleine, 2005). In Experiment 1, we determine whether basal difference in SS and General PIT correlates with subsequent diet induced weight gain in outbred male rats. In Experiment 2, we determine if selectively bred obesity-prone rats exhibit enhancements in both SS and General PIT compared to obesity resistant rats. These data provide key insights into the psychological aspects obesity vulnerability and point to specific neural circuits that may play a crucial role in this vulnerability.

2: Materials and methods:

2.1: Subjects

Adult Sprague Dawley male rats purchased from Envigo were used for Experiment 1 (N=39). Adult male obesity-prone (OP: N=15) and obesity-resistant rats (OR: N=15) were used for Experiment 2. Obesity-prone and obesity-resistant rats were bred at the University of Michigan using in a Poiley rotation system with 12 breeding pairs per line. These rat lines were started using rats purchased from Taconic and were originally developed by Barry Levin (1997). Rats were housed in groups of two or three and maintained on a reverse light-dark circadian cycle (12/12). Experiments were conducted during the dark phase of this cycle. All procedures were approved by the University of Michigan Institutional Animal Care and Use Committee. Further details for all procedures and housing can be found at: https://sites.google.com/a/umich.edu/ferrario-lab-public-protocols/.

2.2: Behavioral training chambers:

Training and testing were conducted in standard Med Associates operant chambers housed within sound attenuating cabinets. Each chamber was outfitted with a recessed food cup into which 45mg pellets could be delivered via a tube attached to externally housed food hoppers. The food cup was equipped with an infrared emitter receiver unit that detected entries into the food cup. Two deflection-sensitive retractable levers flanked the food cup. Two speakers were mounted on the wall opposite to the food cup, one delivered a tone stimulus, and the other a noise stimulus. In addition, a click generator was also mounted externally on this same wall. LED red and infrared light strips were used as house lights to enable video recording of training and testing sessions via mini cameras mounted overhead (Surveilzone, CC156).

2.3: Behavioral training

The training procedures used in Experiment 1 and 2 were identical. Prior to training, rats were food restricted to 85-90% of their *ad libitum* weights and maintained at this weight until the end of behavioral training and testing. Instrumental training, Pavlovian

conditioning, and PIT testing were all adapted from Corbit and Balleine (2005; 2011). Table 3.1 provides details for the experimental design of this training and testing.

2.4: Food cup training

Rats were initially trained to retrieve food pellets from the food cups within the operant chambers in three separate sessions, using three distinctly flavored 45 mg pellets (Bioserv: Unflavored #F0021; Banana #F0059; Chocolate #F0299). Each session lasted 20 min, during which 20 pellets of one flavor were delivered on a variable time (VT) schedule of 60 sec (range, 30”-90”)

2.5: Instrumental training

Rats were next trained to press two separate levers to earn two different outcomes (R1-O1; R2-O2; see Table 3.1). Each lever was trained in isolation. Of the three pellets introduced during food cup training, two were used for these distinct R-O associations. Lever-outcome assignments were counterbalanced across rats. At first, lever pressing was reinforced on a schedule of continual reinforcement (CRF), such that every press earned a single pellet. In this phase, rats were required to reach an acquisition criterion of earning 50 pellets within 40 min for each lever. In the next phase, rats were transitioned to variable interval (VI) schedules of reinforcement. VI training sessions lasted for 45 min. For the first 20 min of these sessions one lever was available. This lever was then retracted and after a five min break during which neither lever was present, the other lever was inserted and remained available for the final 20 min of the session. During these sessions, VI reinforcement schedules were executed as follows: after passage of a pre-selected interval of time the first lever press to occur resulted in delivery of two pellets, triggering selection and initiation of a new interval. The VI schedules were increased slowly across 8 sessions of training in the following sequence: VI10” (range: 5”-15”), VI30” (range: 15”-45”), VI45” (range: 30”-60”), and VI60” (range: 45”-60”). Rats were trained for two sessions under each VI schedule. The first lever

Instrumental Training	Pavlovian Conditioning	PIT Testing
R1-O1	CS1-O1	CS1: R1 v R2
R2-O2	CS2-O2	CS2: R1 v R2
	CS3-O3	CS3: R1 v R2

Table 3.1: Experimental design of training and testing.

trained of the day was counterbalanced across session using a double alternating pattern (e.g., first lever trained of the day: L1, L2, L2, L1, L1…etc.).

2.6: Pavlovian conditioning

Following instrumental training, rats were conditioned to associate three unique CSs with three different food pellet outcomes (CS1-O1; CS2-O2; CS3-O3). Importantly, CS1 and CS2 share a common outcome with the instrumental R1 and R2, therefore these associations were designed to capture SS PIT (see below). In contrast, CS3 is paired with O3, an outcome not shared by either lever and therefore this CS3-O3 association was designed to capture General PIT. All three CSs were auditory stimuli presented for 120 sec. During CS presentations, four food pellets were randomly delivered into the food cup on a VT20" schedule (range 11-30). This delivery schedule ensured that pellets were never delivered within the first 10 sec of CS presentation; this allowed us to measure anticipatory conditioned food cup approach without interference of consummatory behaviors. A white noise (60 dB), a tone (57 dB), and a click train (20 Hz) were each used as the CSs. Each CS was trained in isolated sessions that lasted 30 min and consisted of four CS-O trials separated by a variable five min inter-trial-interval (ITI; range: 3'-7'). CS-O assignments were counterbalanced to ensure that each stimulus and flavor was evenly represented in an SS and General CS-O associations within each group. Each session was separated by ~40 min and rats underwent three separate sessions per day (one for each CS). Throughout Pavlovian conditioning, levers were unavailable and pellet delivery was not contingent upon any behavioral response. Food cup entries were recorded throughout.

2.7: Pavlovian to instrumental transfer testing

PIT testing was conducted 2 and 4 days after the last Pavlovian conditioning session. Rats were given an instrumental "reminder" training identical to training sessions described above, the day prior to each PIT test. PIT testing lasted for 44 min, both levers were available throughout, but no pellets were delivered within the session. The session began with simultaneous insertion of both levers into the chambers. After 10 min, each of the three CSs was presented three times in a quasi-random order, with presentations

separated by a fixed 2-min ITI. Lever presses, food cup entries and video footage were recorded throughout. Each rat was tested two times with one day of instrumental reminder training in between.

These training and testing procedures were designed to capture two distinct forms of PIT, SS and General PIT (e.g., Corbit and Balleine, 2005). SS PIT is observed when presentation of the sensory specific CSs (CS1 or CS2) results in greater responding on the lever that previously generated the same outcome versus the other lever that generated a different outcome than that predicted by the CS. Thus, the critical behavioral feature defining SS PIT is the differential influence of CS presentation on the rate of lever responding between the 'Same' and 'Different' levers. In contrast, General PIT is observed when presentation of a CS augments lever responding for an outcome not explicitly predicted by that CS (illustrated in Fig 3.2B). In the current experimental design, rats were explicitly trained with a General CS (CS3) that was paired with an outcome that was never paired with an instrumental response. This procedure was designed to maximize our ability to observe General PIT, by measuring the effect of CS3 presentations on lever responding. However, it is important to note that General PIT can also be observed during presentation of the sensory specific CSs (CS1 and CS2), as responding on the 'Different' lever greater than pre-CS rates of responding. The 60 sec immediately preceding CS presentation was defined as the pre-CS period.

2.8: Experiment 1: Individual differences in outbred rats

The goal of Experiment 1 was to determine whether obesity vulnerability was linked to pre-existing differences in the expression of sensory specific versus affective motivation (i.e., SS versus General PIT, respectively). To assess this, outbred male rats were trained and tested as described above, immediately following testing, rats were relieved from food restriction and placed onto a moderately fatty palatable 'Junk-Food' diet with *ad libitum* access. The purpose of this diet manipulation was to identify individual propensity to weight gain to determine obesity vulnerable individuals (as in Robinson et al., 2015). The Junk-Food (JF) diet was a mash consisting of Chips Ahoy! chocolate chip cookies (16% w/w; 260g), Frito Lays potato chips (5% w/w; 80g), Jif peanut butter (16% w/w; 260g), Nestle Nesquik chocolate powder (16% w/w; 260g), Test Diet, 5001 (25%

w/w; 400g) and water (22% w/w; 355ml). Food intake (per cage) and body weight were recorded daily. In addition, nuclear magnetic resonance analyses (NMR) were conducted on a subset of rats prior to being placed on the JF diet and again following 5 weeks of JF consumption. These scans were performed by the Metabolism, Bariatric Surgery and Behavior Core at the University of Michigan. NMR data was gathered using a Bruker Minispec LF 90II device to measure lean mass, fat mass and body fluids. Body weight at the end of the 5 weeks of JF diet access were used for correlational analyses. As control, parallel correlational analyses were conducted comparing pre-training *ad libitum* weights.

2.9: Experiment 2: Differences in selectively bred obesity-prone and -resistant rats.

The goal of Experiment 2 was to determine whether SS and/or General PIT were enhanced in selectively obesity-prone than obesity-resistant rats, to help clarify our previous finding that obesity-prone rats exhibited enhanced single outcome PIT. To achieve this, we trained and tested selectively bred obesity-prone and obesity-resistant rats using identical procedures to those in Experiment 1. No post-training diet manipulation was used in this experiment because the identity of individual obesity susceptibility was known a priori via selective breeding (chacterized in, Levin et al., 1997; Vollbrecht et al., 2015).

2.10: Experimental design and statistical analysis

Data was processed and organized with Microsoft Excel (Version 16.16.16) and statistical analyses were performed using the GraphPad statistical software suite Prism (Version 8.02). Data were analyzed using, student's t-tests, One-way and Two-way repeated measures ANOVAs (RM ANOVAs) and HS multiple comparison tests for planned and post-hoc multiple comparisons. Correlational analyses were conducted using Pearson product-moment correlation coefficients.

CRF training data were analyzed using the total time to reach the acquisition criteria per lever. The total time to acquire was summed across sessions for each lever. For instrumental responding, data were analyzed by obtaining average rates per session, and then averaging these rates across all sessions within each VI. Lever pressing data are presented as average responses per min (Rs/min) and pellets earned as averages

per session. Behavior during Pavlovian conditioning was analyzed by obtaining session averages of anticipatory conditioned approach and latencies to approach the food cup following CS onset and offset. Anticipatory conditioned approach was evaluated by subtracting the number of food cup entries during the 10 sec pre-CS period from the first 10 sec of CS presentations (Rs/10s). Data from PIT testing were analyzed as responses per minute, with 60 sec pre-CS responding subtracted from CS responding when relevant and then averaged across trials, and tests.

3: Results: Experiment 1: Individual differences in outbred rats

3.1: Instrumental training

After learning to retrieve food pellets from recessed food cups within the operant chamber, rats were trained to acquire two distinct R-O associations, where they learned to lever press to earn different pellets on two separate levers (Lever1-O1 and Lever2-O2). Each lever was trained in isolation, such that both levers were present at the same time in training (Fig 3.1A). Initially pressing was reinforced on a continual reinforcement (CRF) schedule. This schedule of reinforcement was terminated once rats had reached the acquisition criterion of earning 50 consecutive pellet deliveries in less than 40 min. The average time to reach the acquisition criteria for both levers was 21.27 min (SEM: 19.32). Rats reached the acquisition criterion slightly more quickly for Lever 2 than Lever 1 (Fig 3.1B: Paired t test, Lever 1 v Lever 2, $t_{(37)}=3.16$, $p<0.01$). During the next phase of instrumental training, rats were shifted to a variable interval (VI) schedule of reinforcement where the VI lengths were increased over 8 days of training. As expected by thinning the reinforcement schedule, the rate of lever pressing steadily increased as the VI lengths increased (Fig 3.1C: Two-way RM ANOVA: main effect of schedule: $F_{(3,114)}=126.3$, $p<0.01$). While we did observe slight differences in response rates between the levers, the outcome assignments and position of these levers was counterbalanced across rats therefore it is unlikely this effect reflects a meaningful difference between these responses (Fig 3.1C: Two-way RM ANOVA: main effect of lever: $F_{(1,38)}=6.75$, $p=0.01$; no schedule x lever interaction, $p=0.20$). Moreover, the number of pellets earned between the levers was similar across training and by the final VI schedule of training there was no hint of a

difference between the pellets earned on each lever (Fig 3.1D: Two-way RM ANOVA: no main effect of lever, p=0.08; HS multiple comparison, VI60: Lever 1 v Lever 2, p=0.87). The number of pellets earned across training decreased systematically as the schedule of reinforcement thinned (Fig 3.1D: Two-way RM ANOVA: main effect of schedule: $F_{(3,114)}$=317.1, p<0.01; no lever x schedule interaction, p=0.15). Together these data demonstrate stable acquisition of instrumental lever responding across VI training for two distinct R-O associations.

3.2: Pavlovian conditioning:

After instrumental training, rats were conditioned to associate three distinct CS-O relationships (Fig 3.1E). On each trial, the CS was presented for 120 secs during which four pellets were delivered into the food cup, but delivery never occurred within the first 10 sec (Fig 3.1F; grey box). In this way, responding in the first 10 sec was uncontaminated by the presence of the outcome and therefore provided a true measure of conditioned anticipatory approach. This anticipatory approach behavior rapidly increased between first two sessions and then stabilized for the remaining sessions; responding was similar across CSs (Fig 3.1G: Two-way RM ANOVA: main effect of session: $F_{(8,304)}$=18.95, p<0.01; no effect of CSs: p=0.42; no session x CS interaction, p=40). As an additional measure of conditioning, we also examined the latencies to approach the food cup following CS onset and offset. The latency to approach the food cup following CS onset was significantly more rapid than approach following CS offset (Fig 3.1H: Two-way RM ANOVA: main effect of phase, CS1: $F_{(1,38)}$=92.88, p<0.01; CS2: $F_{(1,38)}$=137.4, p<0.01; CS3: $F_{(1,38)}$=67.19, p<0.01). While initially the approach latencies between CS onset and offset were comparable, across conditioning sessions the approach latencies following CS offset slowed dramatically (Fig 1H: Two-way RM ANOVA: phase x session interaction, CS1: $F_{(8,304)}$=5.18, p<0.01; CS2: $F_{(8,304)}$=11.79, p<0.01; CS3: $F_{(8,304)}$=7.73, p<0.01). Collectively these data show acquisition of three distinct CS-O associations as supported by the steady emergence of conditioned anticipatory responding across conditioning.

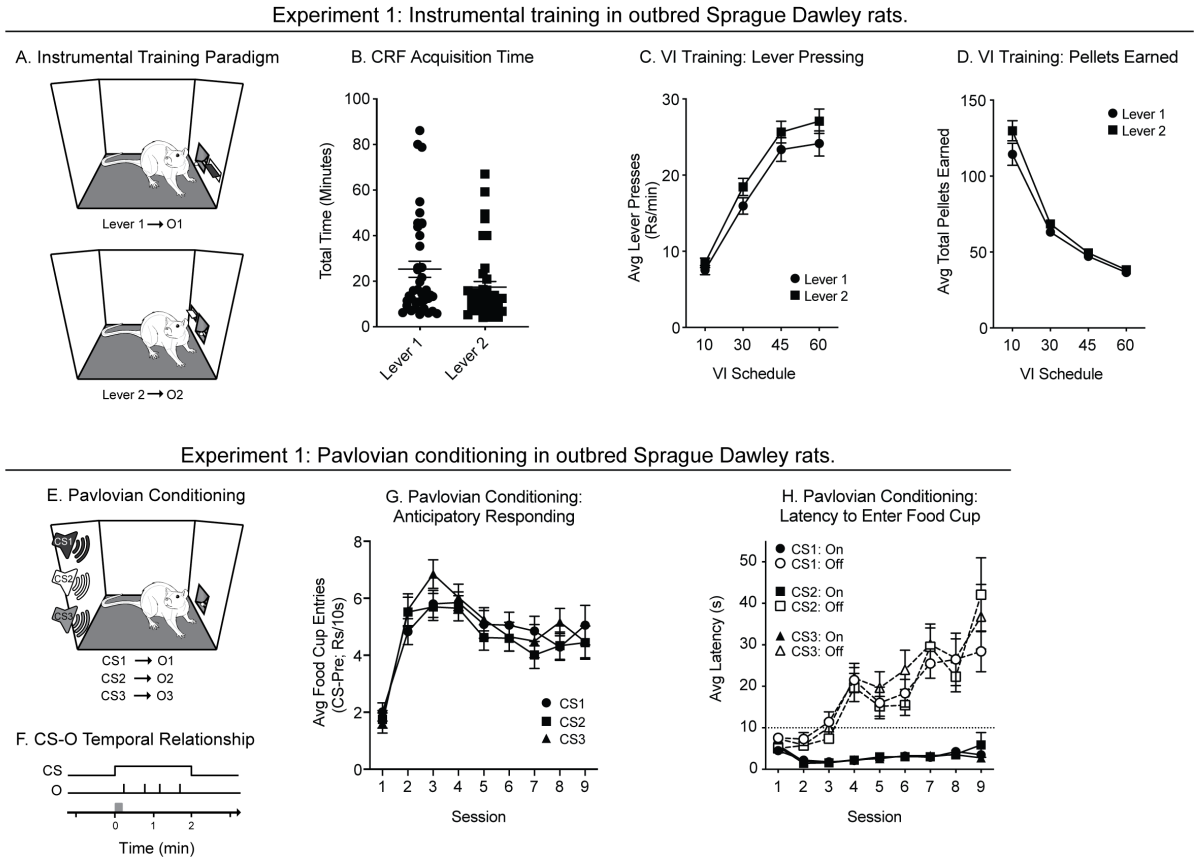

Figure 3.1: Experiment 1, Instrumental and Pavlovian conditioning. **A)** Schematic of instrumental training. **B)** Total time to reach acquisition criterion during continual reinforcement training. **C)** The average rate of lever responding during variable interval instrumental training increased as the VI lengths increased. **D)** The average number of pellets earned decreased across VI training, as the schedule grew leaner. **E)** Schematic of Pavlovian training. **F)** Schematic of the CS-O temporal relationship depicting the delivery of four pellets within each 2-min CS presentation. The grey box over the timescale illustrates the10-sec window during no pellets are delivered following CS onset, when anticipatory conditioned responding is measured. **G)** Pavlovian anticipatory responding during the first 10 seconds of CS presentation increased between session 1 and 2 and remained stable thereafter and was similar between CSs. **H)** The latency to enter the food cup following CS onset was rapid and stable across training, whereas the latency to enter following CS offset increased across training. (All data are shown as averages ±SEM, unless otherwise noted).

3.3: Pavlovian to instrumental transfer testing

Following instrumental and Pavlovian conditioning, rats were tested for PIT under extinction conditions. Each test session began with simultaneous insertion of both levers, which remained available throughout testing. After an initial instrumental extinction phase which lasted 10 min, rats were presented with each CS, three times, delivered in a quasi-random order. Lever responding steadily declined across the first 10 min of testing as

expected and rates of responding were similar between both levers across time (Fig 3.2C: Two-way RM ANOVA: main effect of time: $F_{(9,342)}$=91.84, p<0.01; no effect of lever, p=0.07; no lever x time interaction, p=0.97). For the remainder of the session, CSs were presented intermittently to measure PIT. Classically, SS PIT is observed when presentation of a CS results in preferential instrumental responding for the Same outcome versus a Different outcome than that predicted by the CS (Same>Diff). Trials presenting CS1 and CS2 where designed to capture this effect. In contrast, General PIT is observed when presentation of a CS augments lever pressing for an outcome not specifically predicted by the CS in presentation. Trials presenting CS3 were designed to capture General PIT.

Analyses of lever response data revealed that rats showed substantial SS PIT, preferential responding on the lever that produced the Same outcome than on the lever that produced a Different outcome than that predicted by the CS in presentation (Fig 3.2D: One-way RM ANOVA: main effect of time: $F_{(1.62,\ 61.54)}$=5.67, p<0.01; HS multiple comparisons, Same v Different, $t_{(38)}$=2.83, p=0.02). General transfer elicited by CS3 was not significantly different then Same or Different transfer, indicating that the mean rate of CS3 elicited General transfer falls in between Same and Different transfer (Fig 3.2D: HS multiple comparisons, Same vs General, $t_{(38)}$=1.65, p=0.11; Different vs General, $t_{(38)}$=2.25, p=0.06). However, these comparisons do not provide a direct measure of General PIT. Therefore, to determine if rats exhibited CS3 elicited General PIT, we compared the rate of lever responding in the pre-CS versus the CS periods during presentation of the General CS3. Rats exhibited this form of General PIT as is evident by the increase in the rate of lever responding during General CS3 presentation over pre-CS responding (Fig 3.2E: Paired t test, Pre v CS, $t_{(39)}$=2.78, p<0.01). Together these data confirm the expression of both SS and General PIT.

In addition to measuring PIT, we also recorded food cup responding during PIT testing. Because food cup approach can compete with lever responding, we sought to determine whether food cup response rates differed between the SS (CS1 and CS2) and General (CS3) CSs, to provide an indication as to whether response competition between the food cup and levers was different between these CS types. Presentation of both types of CS evoked substantial food cup approach with no differences in magnitude of

responding elicited by the SS and General CSs (Fig 3.2F: Two-way RM ANOVA: main effect of phase: $F_{(1,15)}$=151, p<0.01; no effect of CS type, p=0.54; no phase x CS type interaction, p=0.74). These data provide evidence against differences in response competition between SS and General CSs.

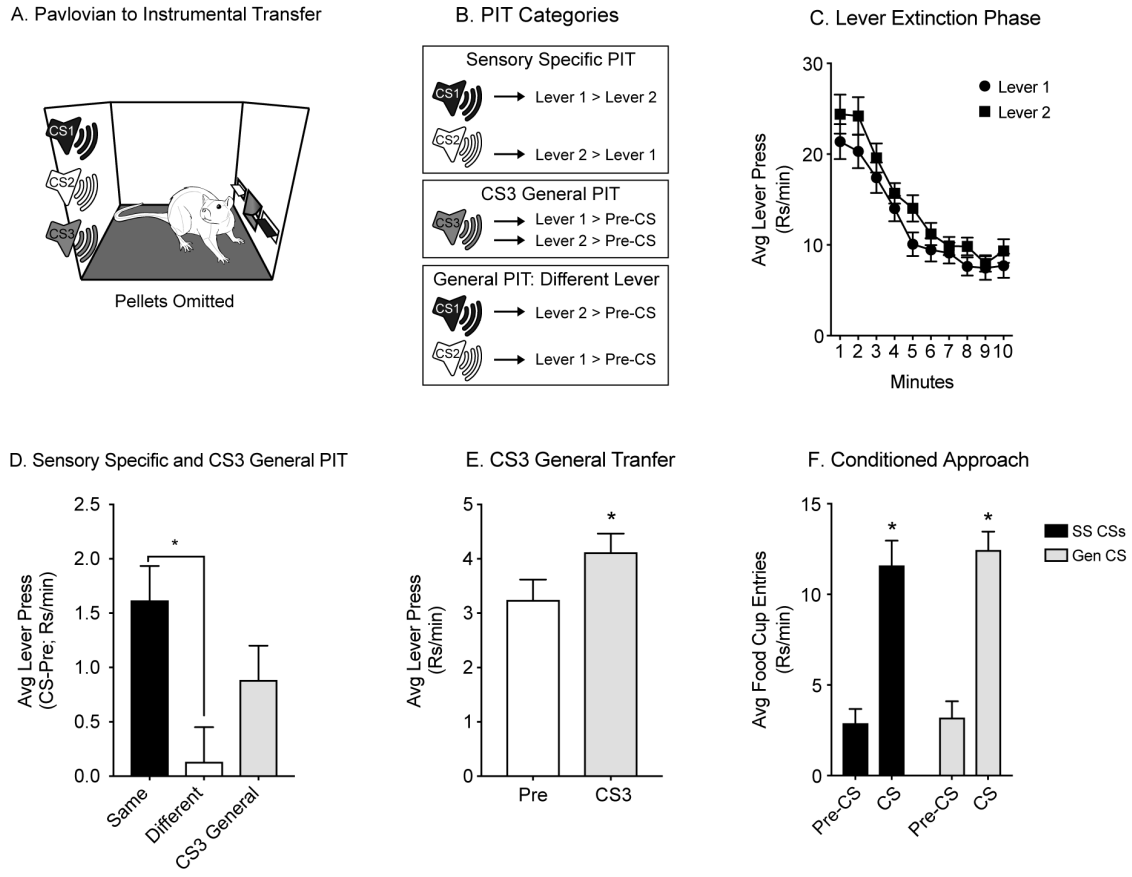

Figure 3.2: PIT testing prior to identification of individual obesity susceptibility. **A)** Schematic of PIT testing. **B)** Categories of PIT: Sensory Specific PIT is seen when CS preferentially invigorates lever responding on the lever that shares an outcome with the CS. General transfer arises when responding for an outcome not predicted by the CS augments responding above pre-CS levels. This can be seen as CS3 General transfer to either lever or as transfer to the levers predicting a different outcome on CS1 and CS2 trials. **C)** Lever pressing decreases in the first 10 min of testing during the lever extinction phase prior to CS presentation. **D)** As a group, outbred rats exhibited SS PIT, where lever pressing was greater on the Same lever than on the Different Lever. **E)** General PIT to the CS3 was apparent as lever pressing increases above pre-CS levels. F) Conditioned food cup approach is similar between SS and General CSs. (All data are shown as averages ±SEM; *=p<0.05).

3.4: Post-training JF consumption and weight gain

Following training and testing rats were relieved from food restriction and placed on to an *ad libitum* diet of JF mash (see methods for macronutrient composition). The purpose of this treatment was to allow us to identify the obesity vulnerability of rats so that we could determine whether the PIT magnitudes from testing correlated with this vulnerability. On this diet, all rats gained significant weight across the weeks (Fig 3.3A: One-way RM ANOVA: main effect of week: $F_{(1.35,49.99)}=252$, $p<0.01$). In the 5th and final week of *ad libitum* JF access, the average weight was 394g (SEM: 4.2g) with a range of 103g. For a subset of rats, body composition data was collected using nuclear magnetic resonance analysis (NMR) before and after 35 days of JF consumption. Across all rats, JF consumption increased body fat percentage, decreased lean mass percentage and had no effect on fluid mass percentage (data not shown: Two-way RM ANOVA: main effect of timepoint: $F_{(1,18)}=15.20$, $p<0.01$; main effect of mass type: $F_{(1.04,18.69)}=30504$, $p<0.01$; timepoint x mass type interaction, $F_{(1.04,18.68)}=103.5$, $p<0.01$). Body fat percentages increased dramatically, whereas lean tissue and fluid percentages were actually decreased during this period (Fig 3.3B: One-way RM ANOVA: main effect of tissue type: $F_{(1.01,18.21)}=91.71$, $p<0.01$; HS multiple comparisons: Fat v Lean, $t_{(18)}=9.53$, $p<0.01$; Fat v Fluid, $t_{(18)}=9.67$, $p<0.01$). Collectively these data demonstrate that JF induced weight gain and increased bodyfat percentages in all rats, with a wide range in the magnitude of these changes.

3.5: Correlations between JF induced weight gain and PIT magnitude

In order to assess whether susceptibility to weight gain was associated with a tendency to exhibit sensory specific or affective motivation, we conducted correlational analyses between the bodyweight in the final week of JF consumption and SS and General PIT magnitudes from before JF exposure. The sensory specific nature of SS PIT is defined by the differential influence of SS CSs on instrumental responding for the Same versus a Different outcome. Accordingly, the difference in transfer between the Same and Different levers provides a classic measure of the sensory specificity of PIT. Therefore, to determine if the tendency to exhibit sensory specific motivation was associated with subsequent vulnerability to diet induced weight gain, we compared week 5 weights and

SS PIT magnitudes in a correlational analysis. SS PIT magnitude was significantly inversely correlated with subsequent weight gain (Fig 3.3C: Pearson correlational analysis: r=-0.37, p=0.02, n=38). However, given that SS PIT magnitude is comprised of two variables, 'Same' transfer and 'Different' transfer, we determined the contribution of each of these transfer effects to this correlation. Thus, we directly examined correlations between week 5 weights and response rates on the Same lever or response rates on the Different lever in separate analyses. These revealed that response rates on the Same lever were not significantly correlated with week 5 weights (Fig 3.3D: Pearson correlational analysis: r=-0.06, p=0.70, n=38). In contrast, we observed a moderate positive correlation with response rates on the Different lever and week 5 weights (Fig 3.3E: Pearson correlational analysis: r=0.53, p<0.01, n=38).

The finding that weight correlates positively with Different transfer, but not with Same transfer has important implications as to how we interpret the inverse correlation between SS PIT magnitude and weight gain. Critically, if responding on the Different lever is substantially greater than pre-CS responding, then this form of transfer falls within the domain of General PIT. This is due to the fact that General PIT is classically defined as the ability for a CS to augment instrumental responding for an outcome that is not directly predicted by the CS in presentation. The two critical features in this operational definition are the that: 1) instrumental responding must be increased above baseline levels and that 2) the instrumental response must be for an outcome other than that predicted by the CS. Thus, in addition to General PIT captured by responding elicited by CS3, General PIT is also captured when presentation of CS1 or CS2 elevates responding on the different lever (as in Holland, 2004). In defense of this concept, we found a moderate positive correlation between different lever transfer and transfer during CS3 presentations (Data not shown: Pearson correlational analysis: r=0.45, p<0.01, n=38).

Therefore, the positive correlation between week 5 weight and transfer to the Different lever observed here suggests that weight gain is correlated with General transfer, but not SS transfer. Consistent with this, and with the moderate correlation between our two measures of General transfer, there is also a moderate positive correlation between week 5 weight and CS3 elicited General PIT (Fig 3.3F: Pearson correlational analysis: r=0.41, p<0.01, n=38). Finally, considering the conceptual and

empirical similarity between General transfer measured by Different lever responding or CS3-elicited responding, we collapsed across these data to obtain a broad measure General PIT (i.e., “Broad General PIT). We then determined if weight correlated with this Broad General PIT. Indeed, we found an even stronger correlation between Broad General PIT and weight than considering relationships to Different lever and CS3 responding alone (Fig 3.3G: Pearson correlational analysis: $r=0.56$, $p<0.01$, $n=38$). Critically the same correlational analyses performed using pre-training weights were not significantly correlated with SS PIT magnitude, same lever responding, different lever responding, or General PIT (Data not shown: Pearson correlational analysis: SS PIT magnitude, $r=0.04$, $p=0.80$, $n=38$; Same transfer, $r=0.05$, $p=0.73$, $n=38$; Different transfer, $r<0.01$, $p=0.96$, $n=38$; CS3 General transfer, $r=0.06$, $p=0.71$, $n=38$; CS3 Broad General transfer, $r=0.03$, $p=0.86$, $n=38$). Thus, it is not simply that the heaviest rats show the strongest magnitude of PIT. Collectively these data demonstrate that in outbred rats the expression of General PIT, but not SS PIT, strongly correlates with subsequent junk-food diet induced weight gain.

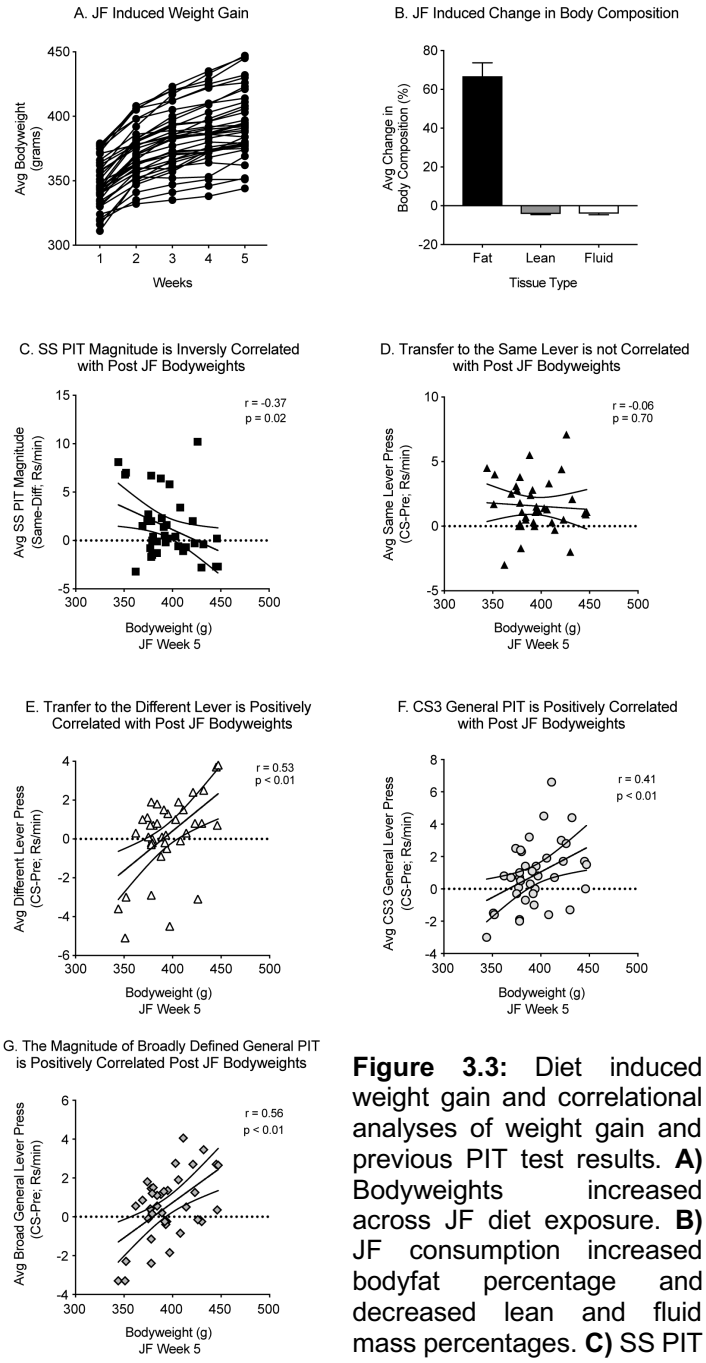

Figure 3.3: Diet induced weight gain and correlational analyses of weight gain and previous PIT test results. **A)** Bodyweights increased across JF diet exposure. **B)** JF consumption increased bodyfat percentage and decreased lean and fluid mass percentages. **C)** SS PIT magnitude as defined by the difference between transfer to the Same and Different levers is negatively correlated with weight. **D)** However, transfer to the same lever did not correlated to subsequent weight gain. **E)** Whereas General transfer to the different lever was strongly correlated with subsequent weight gain**. F)** Consistently, General transfer elicited by CS3 was also strongly correlated with subsequent weight gain. G) Broad General PIT including CS3 and Different transfer was also strongly correlated with subsequent weight gain.

4. Results Experiment 2: Differences in selectively bred rats.

4.1: Instrumental training

As in experiment 1, following food cup training, rats were trained to press one lever to earn one outcome and another lever to earn a different outcome. First rats were trained on a CRF schedule until reaching the criterion of earning 50 consecutive outcome deliveries in under 40 min. Obesity-resistant and obesity-prone rats acquired this criterion in a similar amount of time on both levers (Fig 3.4A: Two-way RM ANOVA: no effect of group, p=0.57; no effect of lever, p=0.20; no group x lever interaction, p=0.33). The average time to reach the acquisition criterion on both levers was 36.15 min (SEM: ±4.37). Rats were then transitioned to VI schedules. During this phase, the rate of lever pressing increased as the VI lengths expanded across training and did not differ between levers (Fig 3.4B: Three-way RM ANOVA: main effect of VI schedule, $F_{(3,84)}$=80.92, p<0.01; no effect of lever, p=0.65; no lever x schedule interaction, p=0.14). Across VI training, lever responding in obesity-prone increased relative to obesity-resistant rats (Fig 3.4B: Three-way RM ANOVA: main effect of group, $F_{(1,28)}$=4.78, p=0.03; group x schedule interaction, $F_{(3,84)}$=3.09, p=0.03; no schedule x group x lever interaction, p=0.17). In contrast to rates of lever responding, the number of pellets earned decreased across the VI schedules, as expected by the thinning reinforcement schedules (Fig 3.4C: Three-way RM ANOVA: main effect of schedule, $F_{(3,84)}$=69.88, p<0.01). Moreover, despite the apparent group differences in lever response rates, both groups earned similar amounts of pellets across training on both levers similarly schedules (Fig 3.4C: Three-way RM ANOVA: no effect of group, p=0.97; no effect of lever, p=0.96; no group x lever interaction, p=0.41; no group x lever x schedule interaction, p=0.12). In sum, both obesity-prone and obesity-resistant rats acquire instrumental lever responding on two separate levers and, notwithstanding differences in lever response rate, both groups earned similar number of outcomes.

4.2: Pavlovian conditioning

Following instrumental training, rats were conditioned in 9 sessions to associate three distinct CSs with distinct outcomes, as above. Anticipatory responding within the first 10 seconds of CS presentation, during which no pellets were present, increased

across training in both groups (Fig 3.4D: Two-way RM ANOVA: main effect of session, OP: $F_{(8,112)}$=5.55, p<0.01; OR: $F_{(8,112)}$=3.70, p<0.01). Moreover, anticipatory responding did not differ across CSs in either group (Fig 3.4D: Two-way RM ANOVA: no effect of CS, OP: p=0.96; OR: p=0.68; no CS x session interaction, OP: p=0.47; OR, p=0.48). Additionally, rates of responding were similar between obesity-prone and obesity-resistant groups (Fig 3.4D: Two-way RM ANOVA: no effect of group, CS1: p=0.28; CS2: p=0.62; CS3: p=0.64; no group x session interaction, CS1: p=0.63; CS2: p=0.27; CS3: p=0.99). As a second measure of conditioning, we compared the latencies to enter the food cup following CS onset versus offset. For ease of presentation we collapsed the latencies across CSs given that no CS effects were observed (data not shown: Two-way RM ANOVA: no effect of CS, Onset: OP: p=0.47; OR: p=0.77; Offset: OP: p=0.80; OR: p=0.11). Rats displayed rapid food cup approach following CS onset, and as conditioning progressed approach following CS offset slowed (Fig 3.4E: Three-way RM ANOVA: main effect of session, $F_{(8,224)}$=5.58, p<0.01; main effect of phase, $F_{(1,28)}$=69.98, p<0.01; session x phase interaction, $F_{(8,224)}$=6.46, p<0.01). Furthermore, these response latencies were similar in obesity-prone and obesity-resistant rats (Fig 3.4E: Three-way RM ANOVA: no effect of group, p=0.07; no group x phase interaction, p=13; no group x phase x session interaction, p=0.97). Lastly, in addition to measuring anticipatory approach, we also evaluated approach during the entire CS during which pellets are variably delivered. While this is not a clean measure of conditioned responding, it does provide an ecologically relevant measure of food-seeking. These data revealed a modest trend for greater approach during the entire CS than in obesity-prone versus obesity-resistant rats (Fig 1F: Two-way RM ANOVA: main effect of group: $F_{(1,28)}$=20.54, p=0.08). These data confirm that obesity-prone and obesity-resistant rats acquired these three CS-O associations without any notable differences in acquisition between the groups.

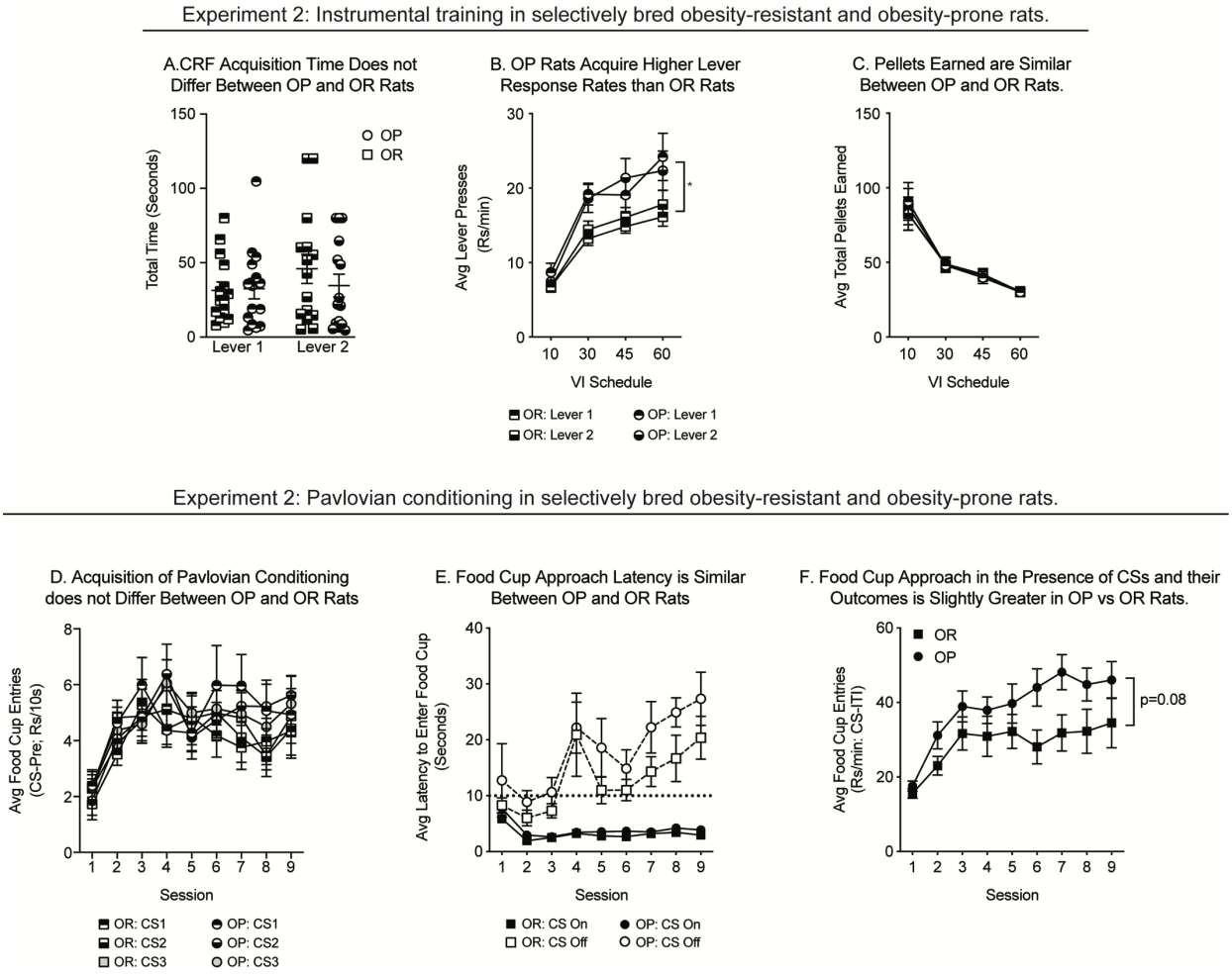

Figure 3.4: Instrumental and Pavlovian conditioning in selectively bred rats. **A)** Total time to reach the criteria during continual reinforcement training was similar between groups. **B)** The average rate of lever responding during variable interval instrumental training increased as VI lengths increase and is greater in obesity-prone rats. **C)** The average number of pellets earned decreased across VI training, as the schedule became leaner and was similar between groups. **D)** Pavlovian anticipatory responding during the first 10 seconds of CS presentation increased between sessions 1-3 and remained stable thereafter. Approach was similar between groups and CSs. **E)** The latency to enter the food cup following CS onset grew faster between sessions 1 and 2 and remained stable across the remaining sessions and did not differ between groups. In contrast, latency to approach following CS offset slowed across sessions and did not differ between groups. **F)** Food cup approach during the entire CS during which pellets were variably delivered is modestly greater in obesity-prone versus obesity-resistant rats. (All data are shown as averages $\pm$SEM, unless otherwise noted; *=$p<0.05$).

4.3: Pavlovian-to-instrumental transfer testing

As in experiment 1, rats were tested for PIT following training. In the first 10 min of testing, lever responding declined as expected, and lever response rates did not differ between groups (Fig 3.5A: Three-way RM ANOVA: main effect of time, $F_{(9,252)}$=7,12, p<0.01; no effect of group, p=0.41). After this period, CSs were presented intermittently to enable testing for PIT. CS presentation evoked significantly more lever responding in obesity-prone rats than in obesity-resistant rats regardless of transfer type (Fig 3.5B: Two-way RM ANOVA: main effect of group, $F_{(1,28)}$=8.33, p<0.01; no group x transfer interaction, p=0.23). In this experiment we found substantial general transfer to the Different lever which somewhat masked SS PIT (Holland, 2004). Specifically, while transfer to the Same lever was substantially greater than CS3-elicited General transfer, it did not significantly differ from transfer expressed by responding on the Different lever, the classic definition of SS PIT (Fig 3.5B: Two-way RM ANOVA: main effect of transfer, $F_{(2,56)}$=3.86, p=0.03; HS multiple comparisons: Same v Diff, p=0.21). Thus, SS PIT as classically defined by the difference in Same and Different transfer was not plainly apparent in this experiment. Next, we compared the expression of Broad General PIT between groups and found that obesity-prone rats exhibited greater Broad General PIT than obesity-resistant rats (Fig 3.5C: Two-way RM ANOVA: main effect of phase, $F_{(1,28)}$=94.79, p<0.01; main effect of group, $F_{(1,28)}$=6.41, p=0.01; phase x group interaction, $F_{(1,28)}$=5,45, p=0.02; HS multiple comparisons: Pre v CS: OR $t_{(28)}$=5.23, p<0.01; OP $t_{(28)}$=8.53, p<0.01; OR v OP: Pre-CS, p=0.13; CS: $t_{(56)}$=3.19, p<0.01).

Lastly, in addition to measuring PIT during testing, we also recorded food cup approach during testing. These data provide information regarding response competition between lever responding and food cup approach. This is important because the group differences observed in PIT could theoretically arise via stronger magnetism of the food cup during CS presentation between the groups. However, analysis of this behavior revealed that obesity-prone rats expressed significantly higher rats of conditioned approach than obesity-resistant rats despite the fact that no differences in conditioned approach had been observed during conditioning (Fig 3.5D: Two-way RM ANOVA: main effect of group, $F_{(1,28)}$=16.71, p<0.01). In addition, we did not observe substantial

differences in conditioned approach during presentation of SS versus General CSs (Fig 3.5E: Two-way RM ANOVA: no effect of CS type, p=0.22). These data argue against response competition effects driving group differences in PIT. Collectively these data show that obesity-prone rats exhibit significantly greater PIT than obesity-resistant rats, with no apparent differences in the magnitude of SS PIT, though notably in this cohort General PIT effects obscured observation of robust SS PIT. Moreover, not only do obesity-prone rats exhibit stronger PIT, but they also show markedly more robust conditioned approach than obesity-resistant rats during PIT testing.

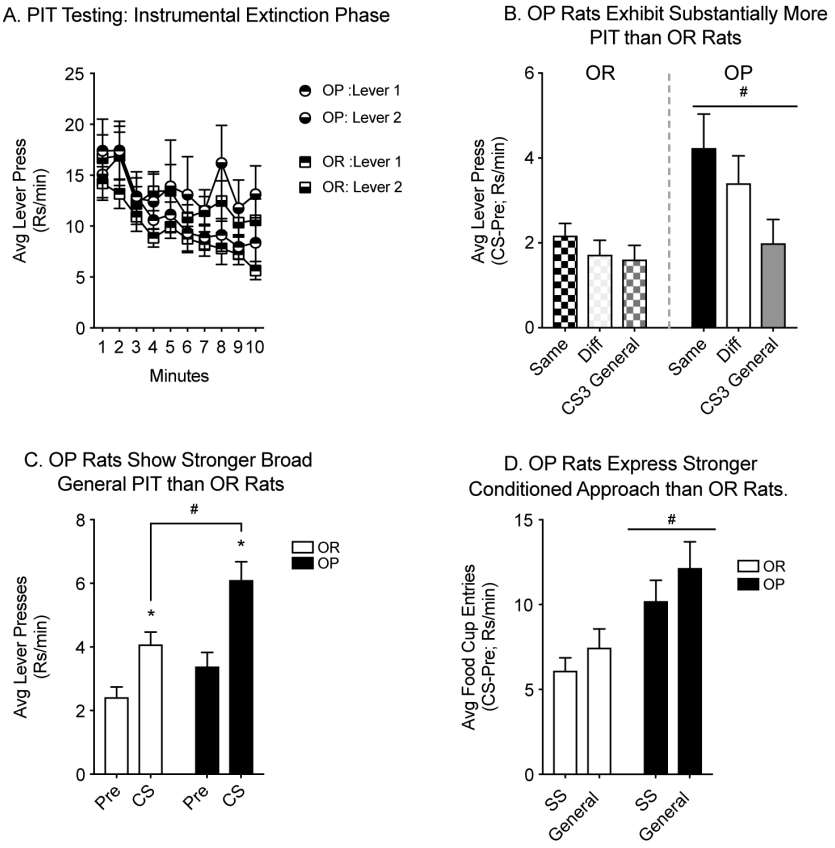

Figure 3.5: PIT testing in selectively bred rats. **A)** Lever pressing decreased in the first 10 minutes of testing, prior to CS presentation, and did not differ between groups. **B)** PIT overall was greater in obesity-prone rats and both groups showed substantial General transfer to the Different lever, masking Sensory Specific PIT. **C)** Both groups exhibited Broad General PIT, but this effect was stronger in obesity-prone rats. **D)** Conditioned food cup approach was greater in obesity-prone rats and did not differ between SS and General CSs in either group. (All data are shown as averages ±SEM; *=p<0.05; #=p<0.05).

5: Discussion:

Obesity is caused by chronic consumption of hypercaloric diets (Akiyama et al., 1996; Horton et al., 1995; Nascimento et al., 2008). Moreover, genetic factors contribute significantly to bodyweight and BMI (Ajslev et al., 2012; Dawson et al., 2013; Hur, 2003; Jacobson et al., 2007). Thus, identifying individual trait differences that lead to overeating is a critical for understanding the etiology of obesity. In the current study, we examined whether differences in the mechanisms of Pavlovian motivation are associated with obesity vulnerability. In particular we asked whether obesity vulnerability was associated with differences in the propensity to exhibit cue-triggered food-seeking via dissociable sensory specific versus affective mechanisms, using a sophisticated PIT paradigm. We found that in outbred populations of rats, individuals subsequently identified as vulnerable to diet induced weight gain exhibited stronger affective Pavlovian motivation in the form of General PIT. Consistent with these data, we also found that in rats selectively bred for susceptibility to diet induced obesity General PIT was significantly greater than in rats selectively bred for resistance to diet induced obesity.

5.1: Obesity vulnerability in outbred rats is associated with enhanced affective motivation:

Obesity vulnerability is associated with enhanced brain and behavioral responsivity to food related stimuli, the latter of which may be one of the key behavioral traits that renders this vulnerability (Boswell and Kober, 2016). However, the process by which Pavlovian stimuli come to exert control over behaviors, particularly instrumental behaviors, can arise via at least two distinct mechanisms, a sensory specific process and a general affective process (Corbit and Balleine, 2005; Corbit and Balleine, 2011; Corbit et al., 2007). In Experiment 1, we determined the relationship between the magnitudes of SS and General PIT prior to diet manipulation and subsequent diet induced weight gain. Rats were first taught two instrumental associations (R1-O1; R2-O2) and then in a separate phase three Pavlovian associations (CS1-O1; CS2-O2; CS3-O3). Following this training, they were tested for SS and General PIT, by presenting the Pavlovian CSs in the presence of both levers (under extinction conditions). As expected, rats displayed both SS and General PIT (Fig 3.2D-E). To our knowledge, this is the first time this procedure

has been used successfully outside the laboratory of group that originally developed it (Corbit and Balleine, 2005). Following testing, rats were placed on junk-food diet for 35 days to induce weight gain. This moderately fatty junk-food diet (19.6% fat) was designed to allow us to detect individual differences in weight gain as diets with higher fat content (40-60%) tend to induce robust obesity in the majority of subjects. In addition, we have previously found that ~30 days of exposure to this diet is needed to reliably identify individual differences (Oginsky et al., 2016a; Robinson et al., 2015). Thus, bodyweight at the end of this diet manipulation was used to assess the relationships between SS PIT and General PIT magnitudes and obesity-susceptibility.

Classically, SS PIT is defined by the differential influence on CS presentation on instrumental responding for the same versus a different outcome than that predicted by the CS (Same>Diff; Colwill and Motzkin, 1994). General PIT, on the other hand, is more broadly defined as an increase in instrumental responding on a lever that does not share an outcome with the CS in presentation. Thus, in our procedure responding on either lever during presentation of CS3 (which does not share an outcome with either lever) or responding on the different lever (i.e., CS1-Lever2 or CS2-Lever1) both capture General PIT (here termed Broad PIT; Holland, 2004). This is substantiated by a significant positive correlation between responding on the different lever and CS3-elicited lever responding (Section 3.5).

Examination of relationships between weight and SS PIT revealed a significant *negative* correlation between SS PIT magnitude and weight (Fig 3.3C). However, when relationships between weight and responding on the Same and the Different levers were examined separately we found that transfer to the Same lever was not correlated with weight (Fig 3.3D), but that transfer to the Different lever was moderately positively correlated to weight (Fig 3.3D-E). Thus, the initial negative correlation when considering the SS PIT difference score, arises because subtraction of a positive relationship (Different responding) from the absence of any relationship (Same responding) results in the appearance of a negative correlation. This also indicates that the main driver of these relationships is responding on the Different lever. In terms of behavioral interpretation, these data suggest that obesity-susceptibility is associated with stronger general affective incentive motivation, but is not strongly related to sensory specific incentive motivation.

Consistent with this, there is also a moderate positive correlation between weight and General PIT as measured by CS3 evoked responding (Fig 3.3F). When CS3-elicited and different lever responding are considered together as one metric of Broad General PIT, this positive relationship between weight and general PIT magnitude becomes even stronger (Fig 3.3G). Together these findings indicate that susceptibility to diet induced weight gain is accompanied by enhanced affective motivation.

The stronger affective motivation in obesity susceptible populations found here is consistent with a previous study in outbred rats that linked obesity susceptibility to enhancements in the expression of conditioned approach (Robinson et al., 2015). However, our data expand this finding to demonstrate that the range of this enhanced Pavlovian motivation extends to include transfer of control over instrumental associations. One point of difference in our findings here is that while we found enhanced General PIT in susceptible individuals, we did not observe a relationship between conditioned approach and obesity vulnerability. This is not entirely surprising, considering the numerous procedural differences between these studies. Robinson et al (2015), trained rats with a single 8-sec lever-CS and measured approach to either the CS or the food cup, following training, rats were placed on junk-food diet to identify susceptible individuals. Retroactive analysis of conditioned approach during conditioning, showed that obesity susceptible rats had exhibited greater conditioned approach across conditioning. In our current study, rats were trained with three 2-min auditory CS, with relatively lean CS-O densities (4 pellets per 120” of CS), and the only conditioned response measured was food cup approach. CS duration, CS-O density and the conditioned responses measured all impact the magnitude of conditioned responding, such that shorter denser CSs generally supporting greater magnitudes of responding (Silva and Timberlake, 1997). Nevertheless, both studies independently demonstrate that Pavlovian motivation is enhanced in obesity vulnerable individuals from outbred populations prior to the manifestation of this vulnerability.

5.2: Selectively bred obesity-prone rats exhibit enhanced affective motivation

Studies in outbred populations are highly ecologically relevant. However, use of outbred population to study basal mechanism or pre-existing neuronal differences that

render individuals vulnerable, can be exceedingly challenging and in some cases impossible. Consequently, the development of selectively bred lines can enable this type of research by identifying, a priori, individuals who are susceptible through heritability of a given trait. The use of selectively bred obesity-prone and obesity-resistant rats has proven to be extremely valuable in the exploration of pre-existing differences and basal mechanisms that drive this highly heritable vulnerability (Levin and Dunn-Meynell, 2000; Levin et al., 2003; Levin et al., 1998). However, it is valuable to determine if effects found in outbred populations are carried through via selective breeding. Furthermore, previous work from our lab has demonstrated that obesity-prone rats exhibit stronger single outcome PIT than obesity-resistant rats (Derman and Ferrario, 2018). This variant of PIT does not distinguish between sensory specific and affective mechanisms of control, hence the underlying psychological mechanism of this effect remained to be elucidated. Thus, in Experiment 2 we sought to determine if parallel enhancements in affective Pavlovian motivation would be observed in selectively bred obesity-prone and obesity-resistant rats.

Rats were trained and tested identically to the outbred rats from Experiment 1. In testing, obesity-prone rats exhibited much stronger transfer effects than obesity-resistant rats on all three transfer measures (Same, Different, and General; Fig 3.5B). Moreover, Broad General PIT including both transfer during CS3 and transfer to the Different lever on CS1 and CS2 trials, was stronger in obesity-prone rats (Fig 3.5C). This is consistent with our previous study demonstrating that obesity-prone rats exhibit stronger single outcome PIT, but provides clarification on this effect by pointing to the affective Pavlovian motivation as the primary driver of this effect. Our finding here in selectively bred obesity-prone rats is also consistent with our observation in Experiment 1, that obesity susceptibility in outbred rats is passively correlated with enhanced General PIT. Therefore, identifying enhanced Pavlovian affective motivation as a common mechanism mediating enhanced food-seeking in selectively bred and outbred obesity susceptible populations.

Interestingly, we also found that during testing, conditioned approach was stronger in obesity-prone rats (Fig 3.5D). Considering that conditioned approach responses compete with transfer effects, the finding that obesity-prone rats show both enhanced PIT

and simultaneously enhanced approach underscores the magnitude of motivational control of Pavlovian stimuli in these rats (see below for additional discussion of conditioned approach and obesity susceptibility).

One outstanding consideration of the data in Experiment 2, is that the observation of robust General transfer to the Different lever masked our ability to measure SS PIT by the classically defined comparison between Same and Different responding. While the data here do not provide strong evidence for notable differences in SS PIT (Same [-] Different) between obesity-prone and resistant rats, neither do these data rule out the possibility that differences do exist. To better answer this question future studies can utilize experimental procedures designed to maximize observation of SS PIT.

One feature of the current experiment that may have promoted the expression of General transfer, was that the same modality was used for all three outcomes. Specifically, CSs were paired with one of three differently flavored food pellets, where flavor and scent were the primary distinguishing sensory properties. The use of more distinct outcomes, for instance liquid versus pellet reinforcers, is likely to promote sensory specific encoding and better capture SS PIT. Procedural modifications such as this one may maximize the ability to observe differences in SS PIT between prone and resistant individuals in future studies.

5.3: Conditioned approach is enhanced in selectively bred obesity-prone rats

In Experiment 2, selectively bred obesity-prone rats showed considerably greater conditioned food cup approach during PIT testing than obesity-resistant rats (Fig 5D). This effect was especially notable considering that during conditioning, anticipatory conditioned approach rates were indistinguishable between the groups (Fig 4D). Furthermore, in our previous study comparing single outcome PIT in obesity-prone and obesity-resistant rats, we did not find significant differences in approach during training or subsequent PIT testing (Derman and Ferrario, 2018). This discrepancy is likely the result of differences in the training paradigms between these studies. Notably, to assess single outcome PIT rats were conditioned using a Pavlovian discrimination task, where a CS+ was paired with pellets contrasted with a CS- that was never paired with pellets, whereas in the current training protocol rats were trained with 3 distinct CSs each paired with

pellets. These conditioning paradigms differ by two relevant aspects. Discrimination conditioning entails some degree of inhibitory learning as rats learn to withhold conditioned approach to CS- presentations. The engagement of inhibitory processes in this procedure may have dampened the expression of enhanced conditioned approach in obesity-prone rats. Another related distinguishing feature between the current and previous study is that the density of outcomes within the conditioning session was significantly leaner in the previous study, where in 60-minute sessions rats were presented with 16 total pellets across four CS+ trials. In contrast, to the current experiment where 16 total pellets were presented in each 30-minute session and rats underwent 90 minutes of training in 3 sessions per day. Consequently, the total number of rewards experienced and the density of outcomes per session was much richer in the current experiment. It is likely that the richness of training in the current experiment enhanced the attribution of incentive salience of the CSs which was most pronounced in obesity-prone rats due to their sensitivity to Pavlovian motivation. This finding is particularly interesting to consider in terms of ecological relevance of these effects, because it suggests that in environment replete with rewarding food experiences Pavlovian stimuli can exert differential motivational effects in vulnerable versus resistant individuals.

5.4: Comparisons between obesity vulnerable individuals in outbred versus selectively bred populations

Experiment 1 and 2 each independently demonstrate that susceptibility to obesity is strongly associated with enhancements in affective motivational control by Pavlovian stimuli. This finding provides unique insights as to the underlying psychological mechanisms that drive food-seeking and ultimately overeating in susceptible individuals. Critically these data may help explain how difficult prevention of weight gain is in vulnerable individuals. Specifically, affective motivational control is unique in its breadth of control over a wide range of instrumental behaviors. This is in contrast to sensory specific motivation, which triggers responding directly associated with unique outcomes. Thus, considering that modern environments are suffused with food related stimuli and that susceptible individuals are more sensitive to the broad reaching variant of

motivational control, it is likely that his manifests in stronger food-seeking and consumption in vulnerable individuals in real world settings. However, as disheartening as this realization is, one unique feature of General PIT is that it is a particularly labile form of transfer, which is sensitive to shifts in appetite, such that satiation abolishes General PIT (Balleine, 1994; Corbit et al., 2007). This suggests that prevention plans centered on maintaining the sensation of satiation may help to curb expression of enhanced incentive motivation in vulnerable individuals. Concomitantly, efforts to reduce the range of instrumental behaviors available to an individual during states of hunger may also serve to blunt the influence of this behavioral sensitivity on food-seeking. This may include learning behavioral modifications, such as avoiding stimulus rich environments and situations while in a state of hunger (e.g., shopping on a full stomach).

The consistency between Experiment 1 and 2 in demonstrating that susceptible individuals show enhancements in affective motivational control by Pavlovian stimuli in both outbred and selectively bred rats, points to the heritability of this trait. While it is well established that bodyweight and BMI are highly heritable particularly in humans, the co-heritability of underlying behavioral traits is less established (Ajslev et al., 2012; Dawson et al., 2013; Hur, 2003; Jacobson et al., 2007). Our data here, demonstrate that selective breeding for body phenotype carries with it, the trait of behavioral sensitivity to Pavlovian stimuli. The heritability of this behavioral trait opens the potential for identifying specific genetic factors that promote incentive motivational processes, particularly in the affective domain. Additionally, the fact that selecting for obesity vulnerability indirectly selected for enhanced incentive sensitivity, presents the interesting inverse possibility that artificially selecting for enhanced affective motivation may indirectly select for obesity susceptibility. This could be tested by breeding the top tail end of PIT responders together. This could serve the utility of enabling the identification of specific factors driving the cooccurrence of these two traits by genomic comparisons of offspring selectively bred for obesity vulnerability against those bred for enhanced General PIT. Specifically, these comparisons may help triangulate critical genes mediating the comorbidity of these traits.

Finally, one noteworthy difference in our results from outbred versus selectivity bred rats, was that selectively bred obesity-prone rats showed stronger conditioned approach (Fig 3.5D), whereas we did not observe any correlations between obesity

vulnerability and conditioned approach in outbred rats. This discrepancy suggests that selective breeding for obesity susceptibility magnified the intensity of Pavlovian motivational control above and beyond that seen in obesity vulnerable outbred rats. This observation is highly relevant when we consider that in humans, assortative mating by BMI has been demonstrated, is stronger within higher BMI groups, and this latter finding has intensified over the decades; consequently, this assortative mating has been suggested to play a small, but important role in the obesity epidemic (Ajslev et al., 2012; Dawson et al., 2013; Hur, 2003; Jacobson et al., 2007). Our finding that in rats, selective breeding for obesity susceptibility magnifies the enhanced Pavlovian motivation inherent in vulnerable individuals suggest that naturally occurring sexual selection for similarity in BMI in humans may produce a similar amplification effect. This may in turn contribute to the continually growing obesity epidemic, posing an even greater vulnerability to offspring of vulnerable individuals.

5.5: Implications for neuronal underpinnings of obesity vulnerability

In the current study, we have shown that Pavlovian affective motivation is stronger in obesity vulnerable individuals either through naturally occurring variance in obesity vulnerability (Experiment 1) or via selective breeding for this vulnerability (Experiment 2). The underlying neurocircuitry mediating General PIT has been partially revealed, though much of the circuitry remains to be revealed. Lesion and inactivation studies have revealed that General PIT is mediated by the ventral tegmental area (VTA), the Central nucleus of the amygdala (CN), and the NAc Core (Corbit and Balleine, 2005; Corbit and Balleine, 2011; Corbit et al., 2007). Beyond these studies little else is known regarding the underlying cell populations or the connectivity between these structures that mediate General PIT. However, considering our understanding of the nuclei involved, our behavioral data in this study suggests that differences within these nuclei or their connectivity may exist between obesity susceptible and non-susceptible individuals and may underlie the behavioral effects observed here. Consistent with this possibility, imaging studies in humans have shown that susceptibility to weight gain is associated with enhanced activity in the amygdala and NAc elicited by food cues (Demos et al., 2012; Yokum et al., 2014). Moreover, we have recently shown that within the NAc Core,

calcium-permeable AMPAR receptors (CP-AMPARs) mediate single outcome PIT in obesity-prone, but not obesity-resistant rats, pointing to mechanistic difference in the function of these structures in Pavlovian motivational processes (Derman and Ferrario, 2018). Given that single outcome PIT does not discretely fall into either SS or General PIT and may arise via either mechanism or an amalgam of the two, it is not clear whether this whether NAc Core CP-AMPARs also mediate General PIT in selectively bred obesity-prone individuals or whether this is also true in outbred obesity vulnerable individuals remains to be determined.

With respect to the involvement of the CN in this enhanced Pavlovian affective motivation, given the known anatomy mediating General PIT, it seems likely that mechanistic differences in this nucleus may contribute to the enhanced General PIT observed in susceptible individuals. What this mechanism might be is unclear. The CN is a GABAergic structure that receives inputs from the insular cortex, hypothalamus, midbrain, pons, and medulla; many of these are asymmetric synapses suggesting they are glutamatergic (Sah et al., 2003). Consequently, difference in experience induced glutamatergic plasticity at these afferent sites may promote enhanced incentive motivation as has been observed in the NAc Core (Conrad et al., 2008; Lee et al., 2013; Loweth et al., 2014; Ma et al., 2014; Wolf, 2016). In addition, in contrast to other amygdalar regions, the CN receives dense dopamine inputs, and antagonism of D1 receptors in the CN has been shown to potentiate cocaine self-administration (Freedman and Cassell, 1994; McGregor and Roberts, 1993). Thus, alterations in dopaminergic transmission in the CN may also play a role in mediating enhanced affective incentive motivation observed in susceptible individuals. Relatedly, our lab has previously shown that sensitization to cocaine induced locomotion is enhanced in obesity-prone rats (Oginsky et al., 2016b). This finding suggests that dopamine (DA) transmission may be enhanced more dramatically by experiences with reward in obesity-prone versus obesity-resistant rats. Given that the CN receives substantial DA inputs these enhancements may impact the function of the CN more strongly in obesity susceptible individuals. This is an open question that needs to be explored.

5.6: Concluding thoughts:

Obesity remains a major health concern of modern times and identifying factors driving obesity is critical. Here, we identify a psychological trait associated with obesity vulnerability, namely enhanced sensitivity to affective Pavlovian motivation. This extends existing work that had previously identified enhanced incentive sensitization more broadly as a trait psychological associated with obesity vulnerability. We did not observe relationships between obesity susceptibility and differences in sensory specific incentive motivation. This suggests that incentive mechanisms contributing to obesity vulnerability arise via enhancements in the control of affective aspects of experience.

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Chapter 4: Outbred Obesity Susceptibility is Associated with Nucleus Accumbens Calcium-Permeable AMPA Receptors Mediation of Pavlovian Motivation

Abstract:

Efforts to enrich our understanding of contributing factors to the obesity epidemic have begun to focus on the role of Pavlovian motivational processes in food-seeking and feeding behaviors. In humans, food related stimuli trigger craving and activation of reward related nuclei, including the Nucleus Accumbens (NAc). Moreover, this brain reactivity is stronger among individuals who subsequently gain more weight in the following year. Consistent with this, in selectively bred obesity-prone rats in the absence of obesity, Pavlovian-to-instrumental transfer (PIT) is stronger than in obesity-resistant rats. These data support the idea that enhanced Pavlovian motivation precedes weight gain in susceptible individuals. Furthermore, in obesity-prone rats, calcium-permeable AMPA receptors in the NAc mediate the expression of single outcome PIT, but not in obesity-resistant rats. This reveals a mechanistic difference in the expression of Pavlovian motivation between selectively bred obesity-prone and obesity-resistant rats. However, whether a similar difference exists in outbred populations is unknown. Therefore, the goal of the current study was to determine whether the magnitude of single outcome PIT or its sensitivity to NAc CP-AMPAR blockade correlate with subsequent weight gain. Outbred rats were tested for PIT following Vehicle or NASPM NAc infusions and then subsequently placed on a “Junk-Food” diet to determine individual obesity susceptibility. We found a strong correlation between weight and the degree to which CP-AMPAR blockade attenuated PIT and conditioned approach. These data point to a common role of NAc CP-AMPARs in the expression of Pavlovian motivation that is unique to obesity susceptible individuals.

1. Introduction

Selectively bred obesity-prone rats often exhibit stronger Pavlovian motivation than obesity-resistant rat, particularly in the ability for Pavlovian stimuli to control instrumental behaviors, a phenomenon known as Pavlovian-to-instrumental Transfer (PIT). Moreover, calcium-permeable AMPA receptors (CP-AMPARs) within the Nucleus Accumbens (NAc) Core mediated single outcome PIT in obesity-prone, but not obesity-resistant rats (Chapter 2; Derman and Ferrario, 2018). While these data suggest that obesity vulnerability in selectively bred rats is associated with heighted Pavlovian motivation and with a distinct role for NAc Core CP-AMPARs in the mediation of this behavior, it is unclear whether similar neurobehavioral distinctions are present in outbred obesity susceptible populations.

As discussed in the introduction, there is naturally occurring variance in the degree of weight gain within outbred Sprague Dawley rats (Levin et al., 1997; Levin and Keesey, 1998). This was exploited through selective breeding based on weight gain to establish obesity-prone and obesity-resistant lines used for studies in Chapter 2-3. However, selective breeding carries with it the risk of inadvertent selection of traits that are unrelated though perhaps seem intuitively linked to obesity, but are in fact unconnected. Therefore, determining whether outbred obesity susceptibility is also associated with similar neurobehavioral traits provides a means to corroborate the reliability and validity of the effects observed in selectively bred rats. To this end, the current study examined whether expression of single outcome PIT and sensitivity of this behavior to NAc Core CP-AMPAR blockade were correlated with subsequent weight gain in outbred rats.

2. Methods

Procedures here were identical to those in Chapter 2 unless otherwise specified. A brief overview of methods follows.

2.1: Subjects

Thirteen outbred male Sprague Dawley rats were purchased from Envigo. Rats were housed in groups of two or three and remained in these pairs throughout the study, and were maintained on a reverse light-dark circadian cycle (12/12). Training and testing

were conducted during the dark phase of this cycle. All procedures were approved by the University of Michigan Institutional Animal Care and Use Committee.

2.2: Experimental overview

Rats were acclimated to our vivarium for one week before surgeries. Rats were then implanted with cannulae targeting the NAc Core. Following a one-week recovery period, rats were trained and subsequently tested for SO PIT following intracranial infusions. After testing, rats were provided with unrestricted access to a junk-food (JF) mash diet for 5 weeks to reveal individual variance obesity susceptibility.

2.3: Stereotaxic surgery

Surgical procedures and stereotaxic coordinates were identical to those described in Chapter 2. However, note that in this study, cannulae were implanted prior to training, whereas in Chapter 2, implantation was conducted following training.

2.4: Behavioral training and testing

Procedures for training were identical to those described in Chapter 2. Briefly, rats were trained on an instrumental discrimination task (active and inactive levers), followed by Pavlovian conditioning with a simple discrimination task (CS+ and CS-). Rats were then tested for the expression of SO PIT following intracranial infusion of NASPM or Vehicle; infusion and testing procedures were identical to those described in Chapter 2.

2.5: Post-testing junk-food diet

Rats were relieved from food restriction following testing and then placed on a JF diet in order to identify individual propensity to weight gain (as in Robinson et al., 2015). This diet was a mash comprised of Chips Ahoy! chocolate chip cookies (16% w/w; 260g), Frito Lays potato chips (5% w/w; 80g), Jif peanut butter (16% w/w; 260g), Nestle Nesquik chocolate powder (16% w/w; 260g), Test Diet, 5001 (25% w/w; 400g) and water (22% w/w; 355ml). Food intake and bodyweights were recorded at least 5 days a week.

2.6: Statistical analysis

Data were organized using Microsoft Excel and statistical analyses were conducted using GraphPad Prism. Differences between groups and treatments were determined using student's t-tests, One-way and Two-way repeated measures ANOVAs (RM ANOVAs) as appropriate. Holmes-Sidak's tests were used post-hoc planned and unplanned comparisons. Correlations were determined with Pearson product-moment correlation coefficients.

3: Results

3.1: Instrumental and Pavlovian training

During instrumental training, rats learned to press an active lever to earn Biosrv pellets and to ignore a simultaneously available inactive lever. During continual reinforcement training, rats reached the acquisition criterion (50 pellets in less than 40 minute) in an average time of 23.99 min (SEM: 7.17). Rats were then transitioned to a variable interval (VI) reinforcement schedule, which was made leaner across sessions. Active lever pressing increased across training and was significantly greater than inactive pressing (Fig 4.1A: Two-way RM ANOVA: main effect of lever: $F_{(1,12)}$=205.4, p<0.01; main effect of session: $F_{(7, 84)}$=23.43, p<0.01; session x lever interaction, $F_{(7,84)}$=21.24, p<0.01). Next, rats were conditioned to associate a CS+ with pellet delivery; a CS- was presented an equal number of times, but never paired with pellets. Anticipatory conditioned food cup approach during the CS+ grew across training and was greater than approach during CS- presentations (Fig 4.1B: Two-way RM ANOVA:

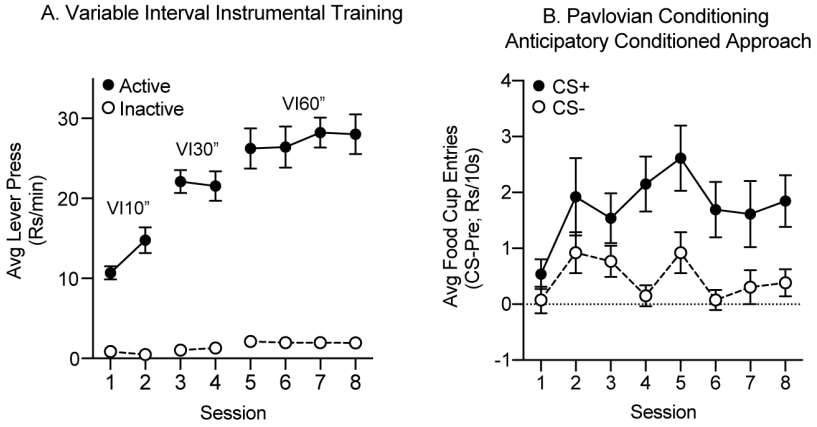

Figure 4.1: Instrumental and Pavlovian training data. **A)** During variable interval instrumental training, active lever pressing increased across sessions as the schedules of reinforcement thinned. Inactive lever pressing was significantly lower than active lever pressing and did not change across sessions. **B)** Anticipatory conditioned food cup approach during the first 10 seconds following CS onset was greater for CS+ versus CS- trials. (All data are shown as averages ±SEM).

main effect of CS: $F_{(1,12)}$=14.48, p<0.01; main effect of session: $F_{(7, 84)}$=2.25, p=0.04; no session x lever interaction, p=0.22).

3.2: Histology

Cannula placements and injections sites were verified at the end of the experiment, using established landmarks to determine the boundaries of the NAc Core (Paxinos and Watson, 2007). No exclusions were necessary given that placements were on target in all 13 rats and no excessive scarring or lesions were found. During testing, bilateral infusions of Vehicle were confirmed for all 13 rats; during intracranial infusion of NASPM, bilateral infusion was confirmed for 9 rats, but for 4 rats only unilateral infusion could be confirmed by tracking fluid movement through the infusion lines. However, behavior did not differ between unilateral and bilaterally NASPM infused rats (Data not show: Two-way RM ANOVA: PIT: main effect of lever: $F_{(1,11)}$=5.81, p=0.04; no effect of laterality: p=0.31; no lever x laterality, p=0.72; Conditioned Approach: main effect of CS: $F_{(1,11)}$=8.80, p=0.01; no effect of laterality: p=0.36; no lever x laterality, p=0.60). Therefore, all rats are included in analyses below.

3.3: PIT testing

PIT testing procedures were identical to those used in Chapter 2. Lever pressing in the first 10 min of testing when no CSs are presented was unaffected by NASPM infusion (Data not shown: Two-way RM ANOVA: main effect of lever: $F_{(1,12)}$=52.83, p<0.01; no effect of infusion, p=0.67; lever x infusion interaction, $F_{(1,12)}$=4.65, p=0.05; Post-hoc: Vehicle vs NASPM, Active Lever, p=0.14; Inactive Lever, p=0.30). When CSs were subsequently presented, rats showed SO PIT with CS+ elicited elevations in active lever pressing (Fig 4.2A: Two-way RM ANOVA: main effect of lever: $F_{(1,12)}$=6.63, p=0.02). As a group, the overall expression of SO PIT was not affected by NASPM infusion (Fig 4.2A: Two-way RM ANOVA: no effect of infusion: p=0.65; no lever x infusion interaction, p=0.52). However, qualitative analysis of the individual response of PIT to the effects of NASPM infusions shows a wide distribution of the effect, with some rats showing suppression by NASPM infusion (Fig 4.2B). In contrast, somewhat surprisingly, NASPM infusion significantly reduced conditioned approach during CS+ presentations (Fig 4.2C:

Two-way RM ANOVA: main effect of CS: $F_{(1,12)}$=15.24, p<0.01; main effect of infusion: $F_{(1,12)}$=4.51, p=0.06; no CS x infusion interaction, $F_{(1,12)}$=5.67, p=0.04). Thus, when considered as one population, intra NAc CP-AMPAR blockade does not disrupt the expression of SO PIT but does reduce conditioned approach in outbred male SD rats.

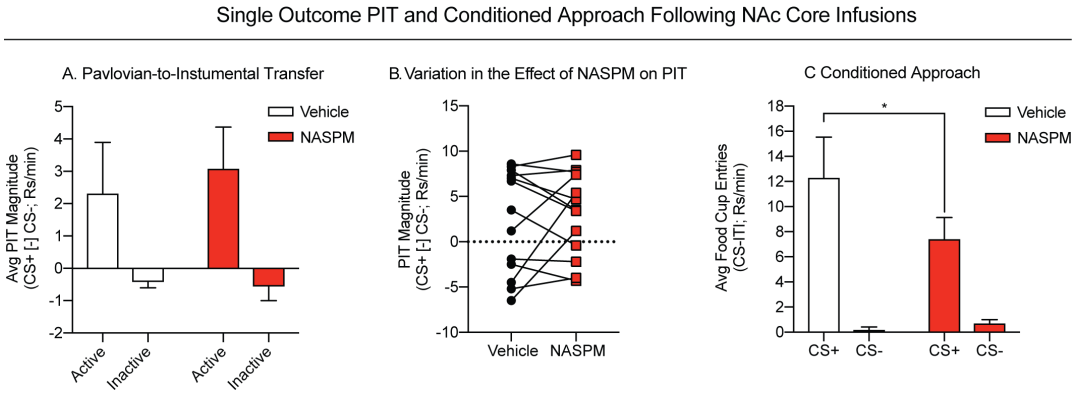

Figure 4.2: Effect of NASPM infusion on PIT prior to identification of obesity susceptibility. **A)** As a group, NASPM infusions did not disrupt the expression of single-outcome PIT. **B)** Individual variation in effect of NASPM infusion on PIT. **C)** NASPM infusion attenuated the expression of conditioned responding to the CS+, without abolishing conditioned discrimination. (All data are shown as averages ±SEM; *=p<0.05).

3.4: JF-Induced weight gain

Following testing, all rats were given unrestricted access to JF for 5 weeks. Across this period all rats gained a significant amount of weight and there was significant variance in weight across individuals (Fig 4.3: One-way RM ANOVA: main effect of week, $F_{(1.95,\ 23.36)}$=244.9, p<0.01; main effect of individual: $F_{(12,60)}$=56.26, p<0.01). The average weight in the final week of training was 414g, with a range of 79g.

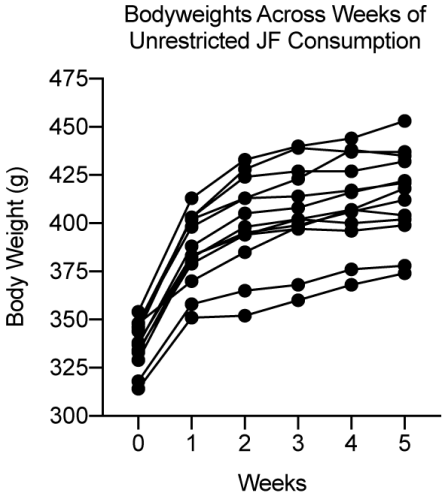

Figure 4.3: All rats gain weight during consumption of JF and there is significant variance in this distribution.

3.5: Correlational analyses

Correlational analyses were performed to assess relationships between behavior during the final testing session and subsequent JF-induced weight gain. For this analysis,

final weight was used, though similar results were obtained using weight gain (data not shown). First, we determined if the magnitude of SO PIT or conditioned approach to the CS+ following vehicle infusions correlated with subsequent weight. No correlations were found between week 5 weight and these behaviors (Fig 4.4A: Pearson correlational analysis: r=-0.23, p=0.45, n=13; Fig 4.4B: r=-0.01, p=0.97, n=13;). These data indicate sensitivity to obesity is not correlated with basal expression of SO PIT or conditioned approach in outbred rats.

Next, we determined the relationship between sensitivity to NAc Core CP-AMPAR blockade of SO PIT or conditioned approach and post JF bodyweight. To achieve this, we expressed each behavioral measure as a percentage of vehicle responding for that metric. These scores were then compared against weight. First, we examined correlations between weight and the effects of NASPM on active lever responding. We found a strong negative correlation between weight and the expression of PIT following NASPM infusions, as measured by CS+ driven active lever responding (Fig 4.4C: Pearson correlational analysis: r=-0.61, p=0.03, n=13). This effect was highly specific to the CS+ as no correlations were found between weight and the effects of NASPM on active lever responding during the CS- or the inter-trial-interval (data not shown: Pearson correlational analysis: CS-, r=0.12, p=0.68, n=13; ITI, r=0.20, p=0.51, n=13). Next, we examined correlations between weight and the effects of NASPM on food cup approach. As with PIT, weight was strongly negatively correlated with the expression of conditioned approach following NASPM infusion, as measured by CS+ elicited food cup approach (Fig 4.4D: Pearson correlational analysis: r=-0.60, p=0.03, n=13). In contrast we found no correlation between weight and the effects of NASPM on CS- elicited food cup approach (data not shown: Pearson correlational analysis: r=-0.16, p=0.62, n=13). We did however find a strong positive correlation between weight and food cup approach during the ITI following food cup approach (data not shown: Pearson correlational analysis: r=0.59, p=0.03, n=13).

Collectively, the correlational data here show that weight following JF was strongly correlated to the degree to which CP-AMPAR blockade suppressed CS+ evoked behaviors, both in the expression of PIT and conditioned approach. Critically, the absence

of correlations between weight and active lever responding during the CS- and ITI and food cup approach during the CS- demonstrates that the negative correlations observed between weight and CS+ evoked behaviors are not the result of general motor suppression by NASPM.

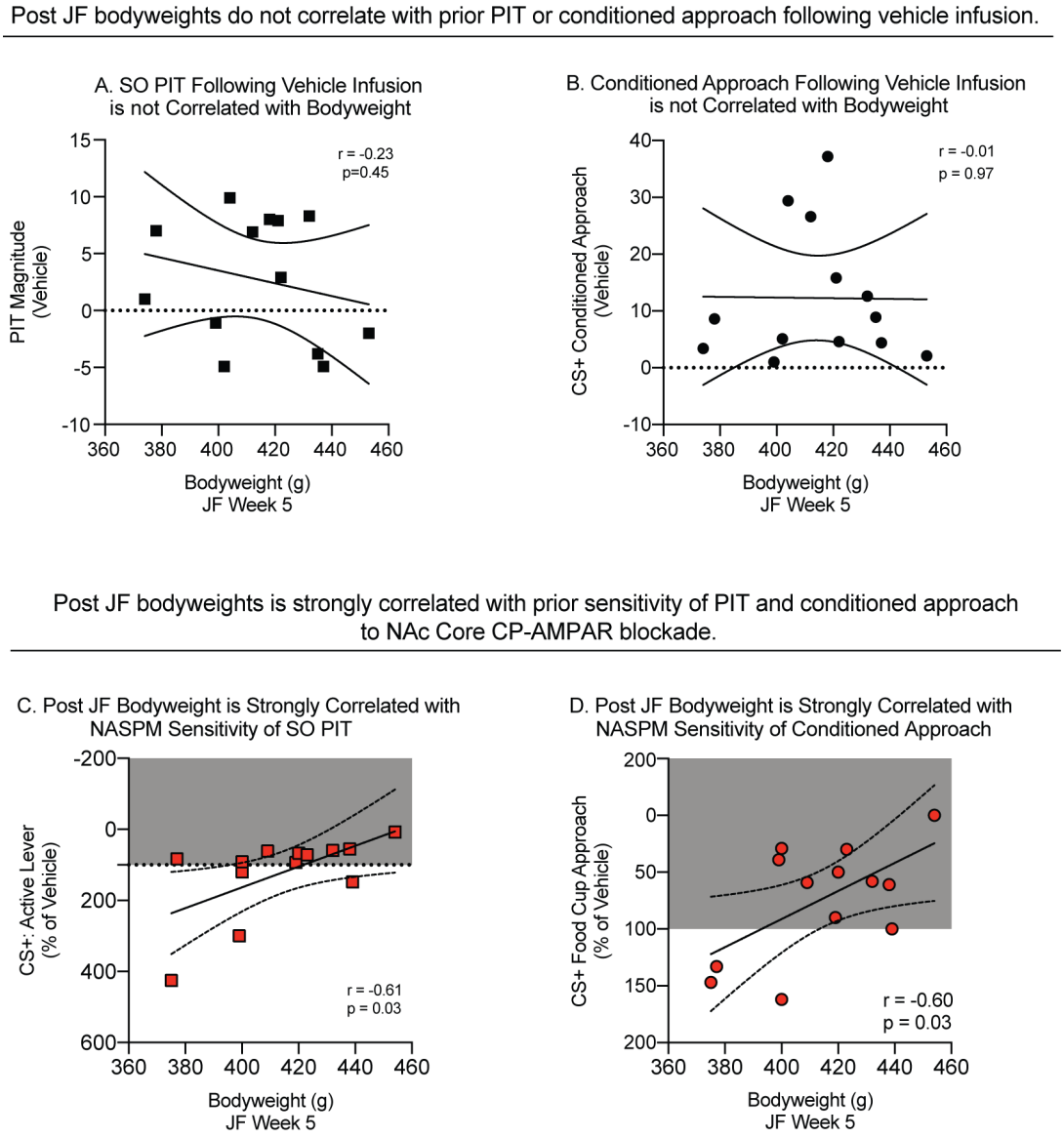

Figure 4.4: Correlational analyses of behavior during PIT testing and subsequent weight following 5 weeks of *ad libitum* junk-food consumption. **A)** SO PIT under Vehicle conditions did not correlate with subsequent weight. **B)** Conditioned approach under Vehicle conditions did not correlated with subsequent weight. **C)** The degree to which NASPM infusion attenuated PIT was strongly correlated subsequent weight. **D)** Similarly, the degree to which NASPM infusion attenuated conditioned approach is also strongly correlated with subsequent weight. Note that the y-axis is inverted on panels C and D to better illustrate these effects and the grey background indicates the percentage range that is less than 100% of Vehicle conditions.

4: Discussion

In Chapter 2, we demonstrated that selectively bred obesity-prone rats exhibit enhanced SO PIT that is mediated by NAc Core CP-AMPARs (Derman and Ferrario, 2018). In the current study, we sought to determine whether obesity susceptibility in outbred rats was associated with enhanced SO PIT and/or behavioral sensitivity to NAc Core NASPM. To achieve this, rats were initially trained and then tested for SO PIT following infusion of vehicle or NASPM into the NAc Core. After testing, rats were placed on an unrestricted diet of JF to identify obesity susceptible individuals. Correlational analyses were performed between weight in the final week of JF diet and behavior during PIT testing.

Given that we previously found that PIT was stronger in selectively bred obesity-prone versus -resistant rats, we first sought to determine if vulnerability to obesity was associated with basal PIT expression in this outbred population. Performance during vehicle testing was used to address this. Here, we found no correlations between weight and magnitude of SO PIT. These data indicate that in outbred populations the magnitude of SO PIT is not significantly correlated with obesity susceptibility. The difference here between selectively bred and outbred obesity susceptible rats suggests that selective breeding for obesity susceptibility intensifies Pavlovian motivation above that seen in the outbred population. Another important consideration worth noting is that the neuropsychological mechanisms underlying PIT fall into at least two dissociable processes, a sensory specific versus an affective process, and importantly, SO PIT does not distinguish between these processes (Corbit and Balleine, 2005; Corbit and Balleine, 2011). Consequently, it is likely that the mechanism(s) driving SO PIT expression varies across individuals which could interfere with our ability to assess the relationship between obesity vulnerability and Pavlovian incentive motivation. Moreover, in Chapter 3 we demonstrate that it is the general affective process which is enhanced in susceptible individuals, but not the sensory specific process. However, we specifically chose to use SO PIT here to enable direct comparisons back to the findings in Derman and Ferrario (2018). Nevertheless, the current data illustrate the distinction between Pavlovian motivation driving SO PIT in selectively bred outbred obesity susceptible individuals.

Despite the lack of concordance between the enhanced magnitude of SO PIT observed in selectively bred obesity-prone rats versus outbred obesity susceptible individuals, the possibility remains that the neural mechanisms driving this behavior are similar in these populations. Indeed, correlational analyses of behavior during NASPM testing and post JF weight revealed that the degree to which NASPM attenuated SO PIT was strongly correlated with weight. In other words, obesity susceptibility was strongly associated with the ability of NAc Core CP-AMPAR blockade to blunt SO PIT. These data suggest that the dependence of SO PIT on NAc Core CP-AMPAR may be a common neural mechanism associated with obesity vulnerability in outbred and selectively bred individuals.

Importantly, identification of a common neural mechanism mediating expression of these behaviors suggests that the underlying neuropsychological process mediating SO PIT is similar between outbred and selectively bred obesity susceptible individuals. This brings us back to the idea that SO PIT may arise via dissociable processes. Results here suggest that this process overlaps in obesity susceptible outbred and selectively bred populations. Furthermore, the involvement of the NAc Core in this behavior suggests that SO PIT in these populations may depend on a General PIT process, as opposed to a Sensory Specific process which depends on the NAc Shell.

Likewise, the finding that obesity resistance was associated with insensitivity to this NAc Core CP-AMPAR blockade may be due to the fact that in resistant populations, SO PIT emerges via a Sensory Specific PIT process, in which case the NAc Shell would be more critical. This presents the interesting possibility that perhaps CP-AMPARs may play a role in SO PIT expression in obesity resistant populations too, but that it is the Shell rather than the Core that mediates this effect. However, in Chapter 2 we showed that the training leading up to SO PIT produced a change in AMPAR subunit expression in the whole NAc that were consistent with increased CP-AMPAR surface expression in obesity-prone, but not obesity-resistant rats. This suggests that glutamatergic plasticity induced by training experience in the whole NAc does not involve upregulation of CP-AMPARs in obesity-resistant rats. Thus, these data temper the prediction that CP-AMPARs may mediate SO PIT in the NAc Shell in resistant rats. Regardless of the specific receptors that may mediate SO PIT in resistant rats, it seems plausible that the NAc Shell is

differentially involved in this behavior. Future studies can explore this possibility by targeting NASPM infusion to the NAc Shell paralleling experiments in Chapter 2 and here. If SO PIT is expressed via a sensory specific process in resistant populations than disruption of the NAc Shell should selectively attenuate SO PIT expression in resistant, but not prone populations. If this turns out to be true, it might help explain why SO PIT magnitudes did not differ systematically with weight in the current study.

One interesting discrepancy between our study in selectively bred rats versus outbred rats here, was that NASPM attenuated conditioned approach here, but this effect had not been seen previously in selectively bred rats. Moreover, the magnitude of this suppression here was strongly correlated with post JF weight. One possible explanation for this could be that in obesity-prone rats the wider networks controlling Pavlovian conditioned approach is more robust and can therefore better withstand the loss of one receptor population contributing to its expression. Indeed, while the NAc Core has been shown to play a contributing role in conditioned food cup approach, it is not critical for its expression (Parkinson et al., 1999), thus other nuclei must also contribute to expression of this behavior, though it should be noted that there is a limited literature on this point. Moreover, in our hands, obesity-prone rats exhibit enhanced conditioned approach (discussed extensively in Chapter 7), which would suggest that the strength of the circuits driving this behavior are more robust and can likely withstand perturbations, especially those occurring in partially contributing nuclei.

Finally, these are the first data demonstrating that CP-AMPARs in the NAc Core contribute to conditioned food cup approach. This novel finding adds to a growing set of data pointing to the role NAc Core CP-AMPARs in appetitive processes associated with natural, rather than drug rewards. The pioneering work on the role of NAc CP-AMPARs in appetitive behaviors emerged in exploration of the underlying mechanisms driving the “incubation of drug craving” effects (Conrad et al., 2008; Scheyer et al., 2016; Wolf and Ferrario, 2010). These data lead to the proposal, that NAc Core CP-AMPARs represented a unique neural adaptation associated with forced abstinence from drug self-administration, reflecting a form aberrant plasticity arising from drug intake and subsequent withdrawal. However, in the years since, a few studies have emerged challenging this idea. For instance, a study from our group found that JF consumption

increased NAc Core CP-APARs in obesity susceptible rats. This was the first demonstration of an upregulation induced by natural reward (Oginsky et al., 2016). Subsequently, Dingess et al., (2017) demonstrated that incubation of craving for chow pellets also resulted in CP-AMPAR NAc Core upregulation in outbred rats. Then most recently, we demonstrated that SO PIT in obesity-prone rats relies on activity of NAc Core CP-AMPARs (Derman and Ferrario, 2018). In conjunction with our current study these collective findings establish a role for NAc Core CP-AMPARs in the experience of food rewards and in the expression of Pavlovian motivation associated with such rewards.

In sum, the data in this study demonstrate that outbred obesity susceptibility is associated with the mediation of Pavlovian motivation by NAc Core CP-AMPARs. These data complement our previous finding that NAc CP-AMPARs mediate PIT in selectively bred obesity prone rats. These data collectively suggest that NAc CP-AMPARs mediate the expression of Pavlovian motivation in obesity vulnerable populations, shedding light onto the potential neural mechanisms driving enhanced NAc reactivity to food stimuli observed in humans that are susceptible to weight gain.

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Chapter 5: Junk-food Enhances Conditioned Food Cup Approach to a Previously Established Food Cue, but Does Not Alter Cue Potentiated Feeding; Implications for the Effects of Palatable Diets on Incentive Motivation

Abstract:

Efforts to stem the global rise in obesity have been minimally effective, perhaps in part because our understanding of the psychological and behavioral drivers of obesity is limited. It is well established that stimuli that are paired with palatable foods can powerfully influence food-seeking and feeding behaviors. However, how consumption of sugary, fatty "junk-foods" affects these motivational responses to food cues is poorly understood. Here, we determined the effects of short- and long-term "junk-food" consumption on the expression of cue potentiated feeding and conditioned food cup approach to Pavlovian conditioned stimuli (CS). Further, to determine the degree to which effects of "junk-food" were selective to Pavlovian motivational processes, we varied the predictive validity of the CS by including training groups conditioned with unique CS-US contingencies ranging from −1.0 to +1.0. "Junk-food" did not enhance cue potentiated feeding in any group, but expression of this potentiation effect varied with the CS-US contingency independent of diet. In contrast, "junk-food" consistently enhanced conditioned approach to the food cup; this effect was dependent on the previously established CS-US contingency. That is, consumption of "junk-food" following training enhanced approach to the food cup only in response to CSs with previously positive CS-US contingencies. This was accompanied by reduced motivation for the US itself. Together these data show that "junk-food" consumption selectively enhances incentive motivational responses to previously established food CSs, without altering cue potentiated feeding induced by these same CSs, and in the absence of enhanced motivation for food itself.

Please note that the contents of this chapter have been published (Derman and Ferrario, 2018b)

1: Introduction

World-wide obesity rates have steadily increased over the past half century (W.H.O, 2017). Efforts to stem the tide of this trend have been minimally effective, perhaps in part because our understanding of the psychological and behavioral drivers of obesity is limited (Chan and Woo, 2010). One potentially important psychological contributor to the development of obesity is the influence that Pavlovian conditioned stimuli (CSs) exert on craving, food-seeking, and consummatory behaviors (Berridge et al., 2010; Chan and Woo, 2010; Dagher, 2009). Repeated pairings of an initially neutral CS with an unconditioned stimulus (US) food reward, results in the acquisition of an association between the CS and the food US (CS-US association). Once these associations are established, mere presentation of the CS elicits conditioned reflexes and behavioral responses appropriate to the nature of the initial US (Pavlov, 1927). Conditioned reflexes manifest as physiological changes, such as increased salivation, whereas conditioned responses manifest as changes in appetitive behaviors, such as increased approach toward the site of expected US delivery. In addition to acquiring predictive significance, CSs can also acquire strong motivational valence across conditioning. The motivational influence of CSs can trigger enhanced “cravings”, promote instrumental food-seeking behaviors (Pavlovian-to-instrumental transfer), and ultimately increase the amount of food consumed (Birch et al., 1989; Blechert et al., 2016; Pandit et al., 2012; Rogers and Hill, 1989; Weingarten, 1983).

Enhanced cue-triggered motivation for food has been implicated as a factor in the development of human obesity (Berridge et al., 2010; Dagher, 2009). For example, in humans the scent and sight of foods elicit increases in heart rate, blood pressure, skin conductance, salivation, and subjective ratings of craving and hunger. These responses occur in healthy weight individuals, but are enhanced in obese subjects and those identified as binge eaters (Nederkoorn et al., 2000; Udo et al., 2014; Wolz et al., 2017). Similarly, presentation of food CSs increases food consumption in healthy weight individuals, an effect that is also enhanced in obese subjects (Birch et al., 1989; Wolz et al., 2017). Furthermore, several brain imaging studies in healthy, overweight, and obese subjects have found that food cues elicit activations in corticolimbic regions, and that the magnitude of these effects is enhanced in overweight and obese populations (Bruce et