Investigating The Neural Mechanisms of Motivational Responses to Food Cues in Female Rats

by

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DEDICATION

To my family: Mami Lucy, Papi Neo, Nina, Jani, Lucía y Lucas

Porque son mi pasado, presente y futuro.

Con ustedes todo y sin ustedes nada. ¡Los amo!

And

To my favorite person: Arif Hamid, por ser el mejor compañero de vida.

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ABSTRACT

Obesity is a complex and multifactorial disease in which an individual's genetic background can interact with environmental factors in ways that promote unhealthy feeding behaviors. The nutrients found in the food that we consume, as well as the amount of food consumed are important for healthy energy balance and proper development of the body. Importantly, overconsumption of sugary-fatty diets can lead to neuroadaptations that promote weight gain, although knowledge in this area is still developing.

The mesocorticolimbic system regulates the appetitive and consummatory aspects of feeding. Activity in this network in anticipation of food is enhanced in obese compared to lean people. Specifically, overweight and obese individuals have greater activation in these brain areas in response to the smells or sights of food. Interestingly, in women, neural activations in response to these cues, and cue-triggered food cravings fluctuate across the menstrual cycle. However, few preclinical studies have examined pre-existing differences in motivational responses to food cues (conditioned approach) and the neural mechanisms involved in female rats. In addition, little is known about the role and effects of the cycle in conditioned approach or in the brain areas involved.

In Chapter 2, I examined differences in food intake, motivation to work for food and conditioned approach between obesity-prone and obesity-resistant female rats. Additionally, I determined the effects of the estrous cycle and ovarian hormones on these behaviors. Here, I measured daily food intake, used progressive ratio testing to measure motivation to work for food and Pavlovian conditioning to measure conditioned approach. I found that obesity-prone females eat more and have enhanced conditioned approach compared to obesity-resistant. Moreover, the cycle modulates food intake and motivation to work for food in both groups, but only affected conditioned approach in obesity-prone females. Next, I determined the role of estradiol and progesterone in conditioned approach. I found that estradiol and progesterone synergistically decreased conditioned

approach in ovariectomized obesity-prone and outbred rats. Furthermore, I determined effects of cycle in intrinsic excitability of medium spiny neurons (MSN) in the nucleus accumbens (NAc) core of obesity-prone and obesity-resistant females. I found that excitability is enhanced in obesity-prone compared to obesity-resistant only during metestrus/diestrus.

In Chapter 3, I determined how sugary, fatty “junk-food” diet (JF) affect calcium-permeable AMPA receptor (CP-AMPAR) expression and function in obesity-prone and obesity-resistant male and female rats. I used BS^3-crosslink to examine the effects of JF and JF-deprivation in GluA1 surface expression in the NAc of obesity-prone and obesity-resistant male and female rats. I found that JF increased GluA1 surface expression only in obesity-prone males. Next, patch clamp electrophysiology was used to determine JF-deprivation effects on CP-AMPAR function and silent synapses generation in the NAc core of obesity-prone males. I found that JF generates silent synapses, but JF-deprivation decreased silent synapses and increased CP-AMPAR function. Together, this suggest synaptic insertion of CP-AMPARs, and possible maturation of silent synapses. Surprisingly, no effects of JF on CP-AMPAR function were found in obesity-prone females.

Together, these data suggest that the mechanisms by which ovarian hormones influence motivational responses to food cues differs from those that influence motivation for food itself and that there are sex differences in diet-induced glutamatergic plasticity within the NAc. Thus, to properly address the obesity epidemic, studies of the neurobiology of motivation and feeding in females (both lean and obese models) are needed.

CHAPTER 1

General Introduction

Obesity is a major public health concern and a worldwide epidemic with the number of obese individuals increasing at alarming rates. Almost 40% of the adult population in the United States are obese (Hales et al., 2015). It is well-known that obesity is a major risk factor for heart disease, metabolic dysregulation, type-2 diabetes and some types of cancer (Cavalera et al., 2014; Dixon, 2010; Gade et al., 2010; Lashinger et al., 2014). When divided by sex, 41.1% of women vs. 37.9% of men are obese in the United States (Hales et al., 2015). In addition, the prevalence of morbid obesity and eating disorders is higher in women than in men (Hu, 2003; Kyrou et al., 2018).

It is well-established that sights, sounds, and smells that are associated with food (i.e., external food cues) can influence feeding behaviors that lead to obesity. Human studies have shown that behavioral and neural responses to external food cues differ dramatically in obese vs. lean individuals (Polivy & Herman, 2017; Stice et al., 2015; Tang et al., 2012; Wardle, 1990). For example, obese people report stronger food craving and eat larger portions when primed with food cues (Ferriday & Brunstrom, 2011; Tetley et al., 2009). In addition, the magnitude of activations in the brain reward system in response to food cues are stronger in overweight and obese, compared to healthy weight people (Baicy et al., 2007; Frankort et al., 2012; Ho et al., 2012). These include activations in nuclei that play crucial roles in the mesocorticolimbic brain reward system, for example ventral tegmental area (VTA), prefrontal cortex (PFC), amygdala (Amy), and the nucleus accumbens (NAc; Cardinal & Everitt, 2004; Ikemoto & Panksepp, 1999; Kalivas et al., 2005; Martin et al., 2010; Self, 2004). This has led to the idea that enhanced motivational responses to food cues contribute to obesity.

The neural mechanisms underlying cue-triggered motivation have been studied extensively in food restricted rodent models and lean humans, and rely in large part on activity within the mesocorticolimbic reward system. It has been previously reported that the magnitude of increases in the fMRI BOLD signal in the NAc triggered by food-cues predicts future weight gain in normal weight people and future inability to lose weight after obesity (Demos et al., 2012; Murdaugh et al., 2012; Yokum et al., 2011). These findings suggest that enhanced responsivity of the brain reward system may be a cause, rather than consequence of obesity.

It is known that diets rich in sugars and fat can alter the neurochemical and neurophysiological properties of the brain (Gómez-Pinilla, 2008; Levine et al., 2003), including producing alterations in NAc function (Brown et al., 2015; Darling et al., 2016; Dingess et al., 2017; Oginsky et al., 2016a; Oginsky et al., 2016b). Little is known about the effects these diets have on cue-triggered motivation. Studies suggests that diet-induced changes in cue-triggered motivation could be due to weight gain (and accompanying metabolic changes) or to the effects of the high-fat, high-sugar diet before the onset of obesity and that obesity produces alterations in brain regions responsible for motivational responses to food cues that likely hamper weight loss. Thus, animal models of predisposition to obesity are a great tool to understand the interactions between diet, food cues and obesity-susceptibility (Gorski, 2006; Gorski, et al., 2007; Levin, 2007; Levin et al., 1997).

Rodent models of individual susceptibility to obesity:

There is a substantial animal literature on the neurobiology of conditioned approach (i.e., cue-triggered motivation), its role in food-seeking, and the influence of hunger and satiety on these behaviors (for review see Berridge et al., 2010; Berthoud, 2012; Ferrario et al., 2016; Fulton, 2010). However, the literature examining effects of calorie dense foods and obesity on these behaviors and how these experiences interact with susceptibility to obesity is only beginning to emerge. One way to model individual susceptibility to obesity in rodents is to conduct initial behavioral testing and then subsequently identify obesity susceptible rats based on weight gain when given access to a moderately fatty diet (~19-25%). Under these conditions, some rats rapidly gain a substantial amount of weight and

fat mass, while others stay on a similar weight gain trajectory as animals given standard lab chow (Levin et al., 1997). Behavioral data collected prior to diet manipulation can then be examined post hoc to determine relationships between food intake, weight gain, and baseline behavior. Using this approach, our lab has found that the magnitude of cue-triggered motivation, measured by approach elicited by a food cue, is greater in male rats that were subsequently identified as susceptible to weight gain (i.e. "gainers" Robinson et al., 2015). This is consistent with human studies described above and supports the idea that there are basal differences in neural systems mediating incentive motivation in obesity susceptible vs. resistant populations. However, using weight gain to identify sub-populations as described above precludes examination of basal neural differences.

In humans, weight is a heritable trait (Wardle & Carnell, 2009), and obesity is polygenic and influenced by early life experiences (see Albuquerque et al., 2015 for review). Similarly, in rats, obesity can be influenced by genetic background and environmental factors (Gorski, 2006; Madsen et al., 2010). For example, variation in weight gain and fat accumulation have been reported in outbred Sprague-Dawley rats fed a high fat diet (Chang et al., 1990; Mickelsen et al., 1955; Schemmel et al., 1970). Furthermore, selectively breeding of outbred Sprague-Dawley rats by their susceptibility or resistance to obesity after a high energy diet, results in two different strains: obesity-prone and obesity-resistant rats (Levin et al., 1997). In these rats, the obesity phenotype is not spontaneous, it requires the consumption of highly caloric diet in order for the obesity-prone rats to become obese, whereas the obesity-resistant rats adjust their body weight to the caloric density of the diet to maintain a stable weight (Levin & Dunn-Meynell, 2000, 2002; Levin & Keesey, 1998). Additionally, male obesity-prone rats avidly defend their body weight and adipose set-points against caloric restriction. For example, exposure to a high energy diet induces weight gain and increases adiposity in obesity-prone rats. Next, obesity-prone are food restricted and forced to lose weight, followed this restriction they are fed a chow diet. It was observed that obesity-prone rats did not maintain a stable weight on chow. Instead, they re-gain the weight reached when fed the high-energy diet. To re-gain that weight, obesity-prone rats overeat the chow diet, thus defending their new “set point” for weight gained while on a high energy diet (Levin & Keesey, 1998; Levin et al., 2000). Most of the studies using the obesity-prone/obesity-resistant rat model have

focused on examining pre-existing differences in hypothalamic control of feeding behaviors, metabolism, and body weight (Bouret et al., 2008; Levin & Dunn-Meynell, 2002; Levin et al., 2004). For example, obesity-prone rats are born with hypothalamic resistance to the anorectic and thermogenic effects of leptin (Bouret et al., 2008; Levin et al., 2004 Gorski et al., 2007; Irani et al., 2007). Some of these studies were conducted in both male and female rats, however it is unclear if there are sex differences from these data in obesity-prone and resistant rats. Additionally, as described before, the mesocorticolimbic reward system is a key player in regulating food seeking behaviors. However, previous studies comparing between obesity-prone and resistant rats did not address potential differences in motivation or mesocorticolimbic function. Examining pre-existing differences in this brain system can shed light on the neurobehavioral mechanisms of cue-triggered motivation and overeating, main factors that can lead to obesity.

We have found evidence for pre-existing differences in cue-triggered motivation between obesity-prone and obesity-resistant rats. For example, obesity-prone males show stronger Pavlovian to instrumental transfer (PIT), a classic measure of incentive motivation, compared to obesity-resistant rats (Derman & Ferrario, 2018). Similarly, outbred Sprague-Dawley rats subsequently identified as obesity-susceptible or resistant also show stronger cue-triggered motivation in the form of sign-tracking and goal-tracking (Robinson et al., 2015). However, all these studies were done in male rats. Thus, studies presented in this thesis examine potential differences in cue-triggered motivation between obesity-prone and resistant females and potential roles for ovarian hormones in the regulation of these behaviors and of activity within the NAc.

Effects of ovarian hormones on food intake:

In women, caloric intake fluctuates across the menstrual cycle (Asarian & Geary, 2006; Buffenstein et al., 1995). For example, during the periovulatory phase, when estradiol reaches its peak, food intake decreases (Asarian & Geary, 2006; Gong et al., 1989). In contrast, food intake increases during the follicular phase, when estradiol is low (Barr et al., 1995; Lissner et al., 1988; Palmer & Clegg, 2015). Ovarian hormones also have important effects on food intake in female rodents (Asarian & Geary, 2013; Tarttelin &

Gorski, 1971; Wade, 1975). For example, estradiol seems to be the principal hormone affecting body weight by decreasing food intake during the estrus phase of the estrous cycle in female rats (Blaustein & Wade, 1976; Tarttelin & Gorski, 1971).

Estradiol acts on estrogen receptors in key hypothalamic nuclei that mediate food intake and adiposity. For example, previous studies have shown that estradiol implants into the ventral medial hypothalamus (VMH) and the paraventricular nucleus (PVN) results in decreased food intake in rodents (Beatty et al., 1975; Butera & Beikirch, 1989; Butera & Czaja, 1984; Palmer & Gray, 1986; Wade & Zucker, 1970). Furthermore, estradiol interacts with both orexigenic and anorexigenic peptides to modulate food intake (see Brown & Clegg, 2010; Rivera & Stincic, 2018 for review). It has been suggested that estrogen receptor alpha (ERα) is the main receptor responsible for the modulation of food intake and body weight (Geary et al., 2001; Heine et al., 2000). Specifically, selective deletion of ERα in the VMH results in obesity and metabolic dysfunction in female rats (Musatov et al., 2007).

However, how the reproductive cycle and ovarian hormones affect cue-triggered motivation in females is poorly understood. There is evidence that in overweight women, stimuli that predict food availability (i.e., food cues) elicit stronger activations in brain regions that influence motivation including the NAc (Stoeckel et al., 2008) compared to lean women. Furthermore, the magnitude of NAc activations in response to food cues predicts future weight gain in healthy weight females and future inability to lose weight after obesity (Demos et al., 2012; Murdaugh et al., 2012). Thus, in the first set of studies presented in this thesis I examined basal differences in food intake, cue-triggered motivation and motivation to obtain food between obesity-prone and obesity-resistant female rats. Furthermore, I determined the role of the estrous cycle in modulating these behaviors, in addition to examining the effects of estradiol and progesterone treatment on cue-triggered motivation in female obesity-prone and outbred rats (Chapter 2).

Sex differences in gonadal hormone regulation of food intake:

Both estradiol and testosterone can influence food intake and body weight, but their roles are different between males and females. For example, ovariectomized rats have an increased in daily food intake and body weight by increasing their meal size. When these

rats were treated with estradiol, their food intake, body weight and meal size normalized. The role of progesterone has been also studied, but progesterone treatment alone is not sufficient to attenuate ovariectomy-induced hyperphagia and it fails to alter estradiol's anorexigenic effect in ovariectomized rats. However, in males, it was shown that castration in male rats decreased daily food intake and body weight by decreasing meal frequency, and testosterone treatment was able to normalize all three (reviewed in Butera, 2010).

Another example of sexual dimorphism in gastrointestinal function is in gastric emptying. For example, ovariectomized female rats that received a treatment of estradiol or a combination of estradiol and progesterone, had a significant inhibition in gastric emptying compared to the vehicle and intact group. In contrast, there was no difference in gastric emptying among intact males, castrated males treated with testosterone or castrated males treated with vehicle. These findings support the notion that female gonadal hormones rather than androgens (testosterone) are the ones contributing to the sex difference in gastric emptying. In terms of body composition, the distribution of fat in the body differs between male and female sexes. The difference in fat distribution is associated with the relative secretion of both leptin and ghrelin (Clegg et al., 2003). More importantly, the brains of male and female rats are differentially sensitive to the catabolic actions of small doses of these two hormones. Insulin secretion is highly correlated with visceral fat content as well as with the risk for developing complications of obesity, whereas leptin secretion correlates better with subcutaneous fat and is therefore less of a risk factor for complications of obesity. Both leptin and insulin selectively decrease meal size in rats, although this has been demonstrated in female rats only in the case of leptin (Eckel & Krauss, 1998).

Leptin also has other effects that make it an attractive candidate for sexually differentiated control of eating: it plays a critical role in the control of pubertal development in both sexes (Clegg et al 2003). For example, leptin levels are directly correlated with estrogen levels in women. It has not been reported to be influenced by the estrous cycle but are decreased with ovariectomy. The brains of female rats are relatively more sensitive to the catabolic actions of leptin, whereas the brains of male rats are more sensitive to the catabolic actions of insulin (Clegg et al 2003). In males, the effects of testosterone are

mediated by changes in body weight, and do not support a direct inhibitory effect of testosterone on leptin secretion. Furthermore, testosterone administration to ovariectomized, but not to orchidectomized rats, led to a decrease in serum leptin levels (Pinilla et al., 1999). Although basis of the interaction between estrogen and leptin is unknown, there are reports that leptin receptors and estrogen receptors are colocalized on the same hypothalamic neurons, suggesting that one point of interaction may be in individual hypothalamic neurons.

Neural mechanisms of incentive motivation in response to food cues:

External cues that have been associated with a given experience can directly activate complex emotional and motivational states. Incentive motivation is described as psychological process of intense cognitive desires or cravings (cue-triggered "wanting") directed towards rewards and triggered by cues paired with these rewards. This cue-triggered "wanting" is a form of Pavlovian conditioned motivation mediated by the mesocorticolimbic brain circuit (Berridge, 2007; Berridge & Robinson, 1998).

The mesocorticolimbic system is comprised of VTA, PFC, Amy and the NAc. Importantly, within this system, the NAc integrates dopaminergic signals from the VTA with glutamatergic inputs from several regions including the PFC, hippocampus, Amy and thalamus (Fig.1; Everitt et al., 1991; Groenewegen et al., 1999; Sesack et al., 1989; Swanson & Cowan, 1975). This circuitry is critically involved in generating motivated behaviors, including cue-triggered "wanting" for drugs and food (Castro et al., 2015; Kelley et al., 2005; Wise, 2006). In addition, glutamatergic excitatory drive within the NAc also plays critical roles in cue-triggered motivation (Di Ciano et al., 2001; Corbit & Balleine, 2011, 2016; Di Ciano & Everitt, 2001). Both infralimbic- and prelimbic-PFC to NAc glutamatergic projections are important for the initiation and inhibition of cue-triggered reward-

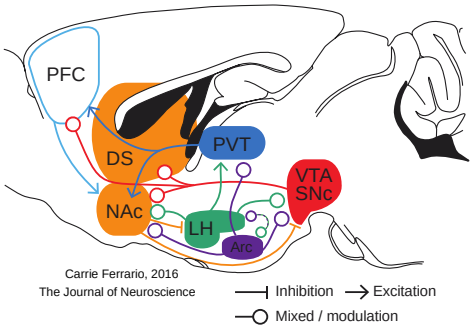

Figure 1. Schematic of mesocorticolimbic brain reward system. The NAc integrates dopaminergic signals from the VTA with glutamatergic inputs from the PFC, hippocampus, Amy and thalamus.

seeking behaviors. For example, activity in prelimbic and infralimbic regions of the PFC influence cue-triggered motivation (Moorman & Aston-Jones, 2015; Petrovich & Gallagher, 2007; Petrovich et al., 2007). Furthermore, glutamatergic afferents from the PFC innervating the NAc can control other motivated behaviors, such as drug cravings (Ma et al., 2014). For example, cue-induced cocaine seeking can be reduced by inducing long term depression (LTD) in the prelimbic-mPFC to NAc core projection or increased by inducing LTD in the infralimbic-mPFC to Nac shell projection (Ma et al., 2014). Thus, opposite effects can be observed for the same behaviors depending on the neuronal input from PFC (infralimbic vs. prelimbic) to NAc (core vs. shell). AMPAR-mediated transmission has been most tightly linked to cue-triggered motivation in core vs. the NAc shell (Bäckström & Hyytiä, 2007; Di Ciano & Everitt, 2001; Conrad et; al., 2008). Therefore, in studies below we focused on glutamatergic transmission within the NAc core.

NAc Medium spiny neurons and intrinsic excitability:

The NAc is comprised of several different cell populations including fast-spiking interneurons, cholinergic interneurons, and medium spiny neurons (MSN). MSNs are GABAergic projection neurons that comprise ~95% of all neurons within the NAc. MSNs are the critical players in many aspects of motivation, integrating dopaminergic and glutamatergic inputs to ultimately drive motor output to obtain rewards like food, drug, and sex. Intrinsic excitability of MSNs influences the integration of dopamine and glutamate inputs and ultimately the behavioral responses like food, sex, and drugs (Berridge & Robinson, 1998b; Schultz, 1997, 2013; Wolf, 2002; Wolf et al., 2003). Intrinsic excitability is determined by the number and distribution of ion channels and receptors that contribute to the electrical properties and membrane depolarization of a neuron (Schulz, 2006).

Dopamine-mediated transmission strongly influences NAc MSNs intrinsic excitability (Azdad et al., 2009; Perezet al., 2006; Podda et al., 2010). For example, dopamine receptor activation modulates intrinsic excitability by altering the gating of Ca^{2+}, Na^{+} and K^{+} channels (Surmeieret al., 2007, for review). Our lab found pre-existing differences in NAc core MSN intrinsic excitability in obesity-prone compared to obesity-resistant males. Specifically, obesity-prone male rats have enhanced intrinsic excitability compared to

obesity-resistant rats prior to obesity or diet manipulation (Oginsky et al., 2016a). Additionally, junk-food diet decreases excitability in obesity-prone males (Oginsky & Ferrario, 2019). Potential differences in females had not been examined. Thus, in Chapter 2, I determined basal differences in intrinsic excitability between obesity-prone and obesity-resistant female rats. Additionally, there is evidence that NAc core MSN intrinsic excitability is enhanced in diestrus, in comparison to proestrus/estrus in outbred female rats (Proaño et al., 2018). Thus, I also examined the effect of the estrous cycle on intrinsic excitability in obesity-prone and obesity-resistant female rats.

AMPA-type glutamate receptors:

Glutamatergic transmission provides the main excitatory drive to MSNs via the activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs); (Hu & White, 1996; Pennartz et al., 1994; Wolf, 2010). AMPARs are heterodimers composed of GluA1-4 subunits (Collingridge et al., 2009; Wenthold et al., 1996). Subunit composition is important because it determines how AMPARs are trafficked. For example, AMPARs comprised of GluA2 and GluA3 subunits (short C-terminus) cycle constitutively in and out of synapses, whereas AMPAR containing GluA1 or GluA4 (long C-terminus; GluA4 mostly predominates during development) are inserted into synapses in an activity-dependent manner (Malinow, 2003; Wolf, 2010). Additionally, most of the GluA1-containing AMPARs in the striatum also contain GluA2 (Bernard et al., 1997; Gold et al., 1997; Wolf, 2010).

The activity-dependent insertion of GluA1/2 AMPARs requires a two-step process involving externalization onto the cell surface at extrasynaptic sites followed by translocation into the synapse (see Shepherd & Huganir, 2007 for review). Externalization onto the cell surface is accelerated by protein kinase A (PKA) via phosphorylation of GluA1 at serine 845 (Lin et al., 2009; Wolf, 2010). On the other hand, translocation into the synapse requires NMDAR stimulation and CAMKII activation (Lisman et al., 2002). Additionally, because MSNs receive both dopaminergic and glutamatergic inputs, dopamine receptors are well-positioned to modulate externalization of GluA1-containing AMPARs into the cell surface (Sun et al., 2008; Wolf, 2010). For example, stimulation of D1 receptors increases externalization of GluA1-containing AMPARs into extrasynaptic

sites, through a mechanism involving PKA activation (Gao et al., 2006; Sun et al., 2008; Sun et al., 2005).

In addition to their roles in trafficking, AMPAR subunit composition also determines the biophysical properties of these receptors (Dingledine et al., 1999; Greger & Esteban, 2007; Palmer et al., 2005). For example, absence of the GluA2 subunit results in an increase in Ca^{2+} permeability of the AMPAR (which normally conduct Na^{+} ions). These receptors are called GluA2-lacking Ca^{2+} permeable AMPARs or CP-AMPARs. The expression of these receptors is very low in NAc synapses in drug naïve rats (Conrad et al., 2008; Reimers et al., 2011). Below I discuss the role of CP-AMPAR role on cue-triggered motivation as well as experience-dependent upregulation of CP-AMPARs in the NAc. However, the specific mechanisms for CP-AMPAR upregulation and synaptic insertion are not well understood.

Role of NAc AMPARs in food vs. drug-seeking:

Excitatory transmission in the core and shell sub-regions of the NAc play different roles in consummatory vs. food-seeking behavior (Maldonado-Irizarry et al., 1995; Reynolds & Berridge, 2003). For example, antagonism of AMPARs in rostral NAc shell, but not core, increases food consumption outside the home cage (Maldonado-Irizarry et al., 1995; Reynolds & Berridge, 2003). In contrast, AMPAR transmission in the NAc core has been tightly linked to cue-triggered motivation for both food and cocaine (Bäckström & Hyytiä, 2007; Di Ciano et al., 2001; Conrad et al., 2008; Derman & Ferrario, 2018). For example, blockade of AMPARs in the NAc blocks cocaine seeking under second-order schedules of reinforcement (Di Ciano et al., 2001; Di Ciano & Everitt, 2004). Thus, not only are AMPARs important for food-seeking behaviors, but specific subpopulations of AMPARs are essential in modulating incentive motivation for food.

Experience-dependent plasticity of NAc CP-AMPARs:

Most of the studies on CP-AMPARs expression and function can be found in the addiction literature. Biochemically, it has been shown that CP-AMPARs accumulate in the NAc core during withdrawal from long access cocaine self-administration (Ferrario et al., 2016; Wolf, 2016). Furthermore, these receptors are required for enhanced cocaine-seeking behaviors after withdrawal (“incubation of cocaine craving”; Conrad et al., 2008; Loweth,

Tseng, & Wolf, 2014; Wolf, 2016). Incubation of craving refers to the withdrawal dependent enhancement of cocaine-seeking. In this procedure, rats are trained to self-administer intravenous cocaine and each infusion is paired with the presentation of a discrete cue. Following training, rats go through a withdrawal period in which they remain in their home cages and no longer receive drug. Next, rats are tested using conditions in which responding results in cue presentation but no drug delivery (Counotte et al., 2014; Grimm et al., 2001). This protocol results in enhanced cue-triggered drug seeking, determined by the increased in rates of lever presses for cue presentation. After cocaine self-administration there is a progressive increased in cue-induced cocaine-seeking behaviors after a few weeks to months of withdrawal (Conrad et al., 2008; Grimm et al., 2001). This phenomenon has been termed “incubation of craving” (Grimm et al., 2001; Lu et al., 2004a; Lu et al., 2004b; Neisewander et al., 2000; Sorge & Stewart, 2005).

The upregulation of AMPAR GluA1 and GluA2 subunits vary with the cocaine administration procedure that is used. For example, between day 1 and 21 of withdrawal of non-contingent intraperitoneal cocaine exposure, GluA1 and GluA2 surface expression (Boudreau et al., 2009; Boudreau et al., 2007; Boudreau & Wolf, 2005; Ferrario et al., 2010), as well as AMPAR-mediated transmission, increased (Kourrich et al., 2007). However, no increases in CP-AMPARs in the NAc are observed following experimenter administered (non-contingent) cocaine. On the other hand, during early withdrawal of long access cocaine self-administration, there are increases in intracellular GluA1 in the NAc that are suggested to mediate synaptic scale down in response to high levels of synaptic activity during self-administration period (Loweth et al., 2014). Moreover, after a longer period of withdrawal from long access self-administration (~45 days; cocaine-seeking incubation), rats show an increase in NAc surface expression of GluA1, with no changes in GluA2 (Conrad et al., 2008). Thus, these data suggest increases in CP-AMPAR accumulation following prolonged withdrawal from long-access cocaine-self-administration. Additionally, incubation effects after long access self-administration can be prevented by blocking CP-AMPARs in the NAc core (Conrad et al., 2008; Loweth et al., 2014; Ma et al., 2014; Wolf, 2016; Wolf & Ferrario, 2010).

Interestingly, consumption of foods high in sugar, fat, or a combination of the two, regardless of obesity, is sufficient to enhance NAc core AMPAR-mediated transmission

and expression in outbred male Sprague Dawley rats (Dingess et al., 2017; Oginsky et al., 2016b; Tukey et al., 2013). Furthermore, in obesity-prone rats, 10 days of junk-food exposure followed by junk-food deprivation (24-48 hours or 2 weeks) results in accumulation and synaptic insertion of CP-AMPARs in the NAc core of obesity-prone male rats (Oginsky et al., 2016b). However, the effects of junk-food on CP-AMPAR expression and synaptic transmission in females (following junk-food food or cocaine exposure) are unknown.

Experience generation of silent synapses:

Non-contingent cocaine exposure generates de novo silent excitatory synapses in the NAc (Brown et al., 2011; Huang et al., 2009; Koya et al., 2012). Silent synapses are synapses that express stable NMDARs and lack AMPARs (Isaac et al., 1997; Liao et al.,1995). In these synapses, only NMDAR-mediated currents can be reliably detected. Cocaine-induced silent synapses can be observed during or shortly after (1-2 days of withdrawal) repeated non-contingent exposure to cocaine (Huang et al., 2009). Additionally, it has been shown that after 1-day of withdrawal from repeated non-contingent cocaine injections there is a decrease in AMPAR/NMDAR ratio in NAc MSNs (Kourrich et al., 2007; Lee & Dong, 2011). Decreases in silent synapses are also observed during the ~42-day-period of withdrawal from cocaine self-administration (Lee et al., 2013; Ma et al., 2014). The synaptic insertion of CP-AMPARs concurrent with the decrease in silent synapses has been interpreted as a maturation of silent synapses into un-silenced synapses due to the recruitment of CP-AMPARs (Dong et al., 2017; Huang et al., 2015).

Cocaine-induced generation of silent synapses is accompanied by synaptic insertion of GluN2B-containing NMDARs (Huang et al., 2009; Brown et al., 2011) and increases in thin spine density that declined to basal levels following the maturation of the silent synapses (Graziane et al., 2016). Additionally, density of mushroom-type spines increased after cocaine injections (Graziane et al., 2016). Thin spines are transient spines that emerge and disappear over a few days, whereas mushroom spines can persist for months (Holtmaat et al., 2005; Bourne & Harris, 2007). Thus, mushroom-type spines are considered to be mature synapses that arise in parallel with long-term potentiation

(Matsuzaki et al., 2004; Toni, et al., 1999; Yuste & Bonhoeffer, 2001). It has been observed that mushroom-type spines contain more AMPARs than thin spines (Holtmaat & Svoboda, 2009; Takumi et al., 1999). These results combined with the CP-AMPAR synaptic insertion suggest that cocaine-generated silent synapses are formed *de novo* in the NAc (Dong & Nestler, 2014; Huang et al., 2015).

There is also evidence that suggests that deprivation of high-fat diet increases mushroom spines, whereas deprivation of chow is associated with an increase of thin and mushroom spines in the NAc (Dingess et al., 2017). Increases in surface GluA1 and decreases in GluA2 have also been observed following training for PIT in obesity-prone male rats (Derman & Ferrario, 2018). High fat/high sugar diets and experience to PIT for palatable foods increase CP-AMPARs in obesity-prone rats. This is similar to what has been observed following withdrawal to long access self-administration of cocaine. However, to date there are no published studies on how cocaine or food affect CP-AMPAR expression and transmission in female rats. Thus, in Chapter 3, I examined the effects of junk-food on glutamatergic plasticity in the NAc core of female rats.

Female studies:

Historically, the lack of inclusion of female subjects in preclinical research has led to a gap in knowledge about female physiology and behavior. Although we are doing a better job examining sex differences in basic science studies, there is still a lot to discover and learn. A significant amount of literature on sex differences exists in the addiction field but not much is known about food-induced plasticity or plasticity of reward circuitry and motivation between male and female rats. For example, it is known that males and females differ in their propensity to initiate cocaine-seeking behavior following a withdrawal period (Anker & Carroll, 2010; Kerstetter et al., 2008; Nicolas et al., 2019; Pickens et al., 2011). Additionally, female rats have enhanced incubation of cocaine craving compared to male rats during the estrus phase of the estrous cycle (Kerstetter et al., 2008; Nicolas et al., 2019). Although this literature exists for drugs of abuse, studies examining sex differences solely in incubation of craving for food or cue-triggered food seeking are limited.

As mentioned above, during incubation of cocaine craving, increased CP-AMPAR synaptic insertion has been observed. Given the enhanced incubation of craving in females compared to males, it is surprising that most of the CP-AMPAR mechanisms involving this addiction process has been conducted mainly in males. There is, however, one paper, that examines insertion of CP-AMPARs in both male and female mice. They showed that synaptic insertion of CP-AMPARs occurs in D2-MSNs receiving inputs from the basolateral amygdala in both males and females (Terrier et al., 2016).

A handful of studies have shown sex differences in glutamatergic transmission and dendritic spine density in rats. In addition, it has been shown that correlations exist between dendritic spine size and postsynaptic density (PSD; Harris' & Stevens, 1989), and between PSD size and AMPAR density at the synapse (Hanley, 2008; Takumi et al., 1999). For example, female rats have greater basal MSN dendritic spine density compared to male rats (Forlano & Woolley, 2010). Additionally, female rats have cocaine-induced increases in spine density in their NAc core compared to male rats (Wissman et al., 2012). Furthermore, sex differences in MSN synaptic physiology have also been observed. For example, female rats had greater miniature EPSC frequency in NAc core compared to male rats. This suggests that females might have enhanced spine density and glutamatergic transmission in response to food rewards compared to male rats.

As stated above, little is known about the effects of junk-food diets on CP-AMPAR mediated glutamatergic transmission in female rats. Moreover, there are no studies examining the interactions of junk-food diets with obesity-susceptibility and how they affect glutamatergic transmission in female rats. Thus, in Chapter 3, I examined the effects of junk-food diets on CP-AMPAR expression and function in obesity-prone and obesity-resistant males and females. Finally, I studied the effects of AMPA/NMDA receptor ratio in obesity-prone female rats.

Overview of studies:

The role of the mesocorticolimbic pathway in cue-triggered motivational processes have been extensively studied. However, recent work has begun to examine the potential contribution of plasticity in these circuits to obesity. Despite the inclusion of women in human obesity studies examining neurobehavioral alterations in cue-triggered motivation, preclinical studies have focused mainly on male subjects. This lack of female subjects in preclinical research had led to a gap in the basic understanding of the neural mechanisms underlying over-eating in females.

The goal of this dissertation was to examine how ovarian hormones and individual susceptibility to obesity influence cue-triggered food-seeking, MSNs intrinsic properties and AMPAR-mediated transmission in female rats. Additionally, I explored sex differences in the effects of a junk-food diet on CP-AMPAR mediated transmission and silent synapses in the NAc core of obesity-prone and obesity-resistant rats.

In Chapter 2, I examined basal differences in intrinsic excitability of NAc MSNs of obesity-prone compared to obesity-resistant females across the estrous cycle. Here, I found that obesity-prone female rats have enhanced MSN core intrinsic excitability compared to obesity-resistant rats but only during the metestrus/diestrus phase of the estrous cycle. Furthermore, in Chapter 2, we determined how motivational responses to Pavlovian food cues and motivation for food vary between obesity-prone and obesity-resistant rats. Additionally, we look at the role of the estrous cycle in modulating both responses to Pavlovian cues and motivation for food. I found that obesity-prone female rats have enhanced motivational responses to Pavlovian food cues compared to obesity-resistant. Interestingly, this enhanced motivation response to Pavlovian food cues is modulated by the estrous cycle only in obesity-prone rats. Motivation for food was similar between obesity-prone and obesity-resistant rats but was modulated by the estrous cycle in both groups. Thus, these data suggest a dissociation between the ability of the estrous cycle to influence motivation for food but not motivation for Pavlovian cues in obesity-resistant rats. Finally, we examine the effects of estradiol and progesterone treatments on the modulation of Pavlovian food cues in obesity-prone and obesity-resistant rats. Here, I found that repeated treatment decreases responses to Pavlovian food cues in both

obesity-prone and obesity-resistant rats. This chapter collects data that demonstrates cycle- and hormone-dependent effects on motivational responses to Pavlovian food cues. It also demonstrates that individual susceptibility influences NAc excitability and responses to Pavlovian cues in an estrous-cycle-dependent manner in female rats. Collectively, the data from this chapter suggests that obesity-prone female rats have enhanced motivational responses to food cues and hyper-responsivity of NAc MSNs. Importantly, both the motivational responses to food cues and NAc excitability are modulated by the estrous cycle in obesity-prone female rats. Thus, fluctuations of ovarian hormones throughout the estrous cycle can alter both the behavioral responses to food cues as well as the brain areas mediating these responses.

In Chapter 3, we first examined the effects of 10 days of junk-food followed by 2 weeks of deprivation in CP-AMPAR accumulation in the NAc of obesity-prone and obesity-resistant male rats. We found that junk-food increased CP-AMPAR accumulation in the NAc of obesity-prone but not obesity-resistant male rats. When this experiment was done in female obesity-prone and obesity-resistant rats, we did not find an effect of junk-food in either group. Next, we examined the effects of 10 days of junk-food without deprivation or 10 days of junk-food with 24-hour deprivation on CP-AMPAR accumulation in the NAc of obesity-prone and obesity-resistant male rats. We found that junk-food with and without deprivation resulted in an increase of CP-AMPAR accumulation in the NAc of obesity-prone rats. To determine the effects of junk-food on synaptic insertion of CP-AMPARs, we examined if 10 days of junk-food with and without a deprivation period resulted in synaptic insertion of CP-AMPARs. We found that junk-food, without a deprivation period did not resulted in synaptic insertion of CP-AMPARs in the NAc core of obesity-prone male rats. However, 24-hour of junk-food deprivation resulted in CP-AMPAR synaptic insertion in the NAc core of obesity-prone male rats.

In addition, we also conducted an experiment examining the effects of 10 days of junk-food followed by 2-weeks of withdrawal on synaptic insertion of CP-AMPAR in female obesity-prone rats. Surprisingly, we did not find evidence for synaptic insertion of CP-AMPARs in female obesity-prone rats. Moreover, we also determined the effects of 10 days of junk-food with and without deprivation (24-48 hours) on silent synapses on obesity-prone male rats. We found that 10 days of junk-food without deprivation resulted

in an increase of silent synapses in the NAc core. However, silent synapses decreased after deprivation in obesity-prone male rats. Thus, synaptic insertion of CP-AMPARs after junk-food deprivation, suggests maturation of silent synapses in obesity-prone male rats in a similar mechanism observed with cocaine. Additional data in this chapter suggests, that obesity-prone male rats may have a greater capacity for synaptic plasticity compared to obesity-resistant rats. In sum, the data in Chapter 3 suggests that eating a junk-food diet alter AMPAR expression and function in obesity-prone male but not in obesity-prone female rats.

References

Albuquerque, D., Stice, E., Rodríguez-López, R., Manco, L., & Nóbrega, C. (2015). Current review of genetics of human obesity: from molecular mechanisms to an evolutionary perspective. *Molecular Genetics and Genomics*. https://doi.org/10.1007/s00438-015-1015-9

Anker, J. J., & Carroll, M. E. (2010). Females Are More Vulnerable to Drug Abuse than Males: Evidence from Preclinical Studies and the Role of Ovarian Hormones (pp. 73–96). https://doi.org/10.1007/7854_2010_93

Asarian, L., & Geary, N. (2006). Modulation of appetite by gonadal steroid hormones. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1471), 1251–1263. https://doi.org/10.1098/rstb.2006.1860

Asarian, L., & Geary, N. (2013). Sex differences in the physiology of eating. *AJP: Regulatory, Integrative and Comparative Physiology*, *305*(11), R1215–R1267. https://doi.org/10.1152/ajpregu.00446.2012

Azdad, K., Chàvez, M., Bischop, P. D., Wetzelaer, P., Marescau, B., De Deyn, P. P., … Schiffmann, S. N. (2009). Homeostatic plasticity of striatal neurons intrinsic excitability following dopamine depletion. *PLoS ONE*, *4*(9). https://doi.org/10.1371/journal.pone.0006908

Bäckström, P., & Hyytiä, P. (2007). Involvement of AMPA/kainate, NMDA, and mGlu5 receptors in the nucleus accumbens core in cue-induced reinstatement of cocaine seeking in rats. *Psychopharmacology*, *192*(4), 571–580. https://doi.org/10.1007/s00213-007-0753-8

Baicy, K., London, E. D., Monterosso, J., Wong, M. L., Delibasi, T., Sharma, A., & Licinio, J. (2007). Leptin replacement alters brain response to food cues in genetically leptin-

deficient adults. *Proc Natl Acad Sci U S A*, *104*(46), 18276–18279. https://doi.org/10.1073/pnas.0706481104

Barr, S. I., Janelle, C., & Prior, J. C. (1995). *Energy intakes are higher during the luteal phase of ovulatory menstrual cycles13*. *Am J Cliii Nuin* (Vol. 61). Retrieved from https://academic.oup.com/ajcn/article-abstract/61/1/39/4651565

Beatty, W. W., O'Briant, D. A., & Vilberg, T. R. (1975). Effects of ovariectomy and estradiol injections on food intake and body weight in rats with ventromedial hypothalamic lesions. *Pharmacology, Biochemistry and Behavior*, *3*(4), 539–544. https://doi.org/10.1016/0091-3057(75)90169-0

Bernard, V., Somogyi, P., & Bolam, J. P. (1997). Cellular, subcellular, and subsynaptic distribution of AMPA-type glutamate receptor subunits in the neostriatum of the rat. *Journal of Neuroscience*, *17*(2), 819–833. https://doi.org/10.1523/JNEUROSCI.17-18-07180.1997

Berridge, K. C. (2007). The debate over dopamine's role in reward: The case for incentive salience. *Psychopharmacology*. https://doi.org/10.1007/s00213-006-0578-x

Berridge, K. C., Ho, C. Y., Richard, J. M., & Difeliceantonio, A. G. (2010). The tempted brain eats: Pleasure and desire circuits in obesity and eating disorders. *Brain Research*. NIH Public Access. https://doi.org/10.1016/j.brainres.2010.04.003

Berridge, K. C., & Robinson, T. E. (1998a). What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*. https://doi.org/10.1016/S0165-0173(98)00019-8

Berridge, K. C., & Robinson, T. E. (1998b). What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*. https://doi.org/10.1016/S0165-0173(98)00019-8

Berthoud, H. R. (2012). The neurobiology of food intake in an obesogenic environment. In *Proceedings of the Nutrition Society* (Vol. 71, pp. 478–487). https://doi.org/10.1017/S0029665112000602

Blaustein, J. D., & Wade, G. N. (1976). Ovarian influences on the meal patterns of female rats. *Physiology and Behavior*, *17*(2), 201–208. https://doi.org/10.1016/0031-

9384(76)90064-0

Boudreau, A. C., Ferrario, C. R., Glucksman, M. J., & Wolf, M. E. (2009). Signaling pathway adaptations and novel protein kinase A substrates related to behavioral sensitization to cocaine. *Journal of Neurochemistry*, *110*(1), 363–377. https://doi.org/10.1111/j.1471-4159.2009.06140.x

Boudreau, A. C., Reimers, J. M., Milovanovic, M., & Wolf, M. E. (2007). Cell surface AMPA receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize after cocaine challenge in association with altered activation of mitogen-activated protein kinases. *Journal of Neuroscience*, *27*(39), 10621–10635. https://doi.org/10.1523/JNEUROSCI.2163-07.2007

Boudreau, A. C., & Wolf, M. E. (2005). Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *Journal of Neuroscience*, *25*(40), 9144–9151. https://doi.org/10.1523/JNEUROSCI.2252-05.2005

Bouret, S. G., Gorski, J. N., Patterson, C. M., Chen, S., Levin, B. E., & Simerly, R. B. (2008). Hypothalamic Neural Projections Are Permanently Disrupted in Diet-Induced Obese Rats. *Cell Metabolism*, *7*(2), 179–185. https://doi.org/10.1016/j.cmet.2007.12.001

Brown, L. M., & Clegg, D. J. (2010). Central effects of estradiol in the regulation of food intake, body weight, and adiposity. *The Journal of Steroid Biochemistry and Molecular Biology*, *122*(1–3), 65–73. https://doi.org/10.1016/j.jsbmb.2009.12.005

Brown, R. M., Kupchik, Y. M., Spencer, S., Garcia-Keller, C., Spanswick, D. C., Lawrence, A. J., … Kalivas, P. W. (2015). Addiction-like Synaptic Impairments in Diet-Induced Obesity. *Biological Psychiatry*, *81*(9), 797–806. https://doi.org/10.1016/j.biopsych.2015.11.019

Brown, T. E., Lee, B. R., Mu, P., Ferguson, D., Dietz, D., Ohnishi, Y. N., … Schlüüter, O. M. (2011). A silent synapse-based mechanism for cocaine-induced locomotor sensitization. *Journal of Neuroscience*, *31*(22), 8163–8174. https://doi.org/10.1523/JNEUROSCI.0016-11.2011

Buffenstein, R., Poppitt, S. D., McDevitt, R. M., & Prentice, A. M. (1995). Food intake and the menstrual cycle: A retrospective analysis, with implications for appetite research. *Physiology and Behavior*, *58*(6), 1067–1077. https://doi.org/10.1016/0031-9384(95)02003-9

Butera, P. C. (2010). Estradiol and the control of food intake. *Physiology and Behavior*, *99*(2), 175–180. https://doi.org/10.1016/j.physbeh.2009.06.010

Butera, P. C., & Beikirch, R. J. (1989). Central implants of diluted estradiol: independent effects on ingestive and reproductive behaviors of ovariectomized rats. *Brain Research*, *491*(2), 266–273. https://doi.org/10.1016/0006-8993(89)90062-0

Butera, P. C., & Czaja, J. A. (1984). Intracranial estradiol in ovariectomized guinea pigs: Effects on ingestive behaviors and body weight. *Brain Research*, *322*(1), 41–48. https://doi.org/10.1016/0006-8993(84)91178-8

Cardinal, R. N., & Everitt, B. J. (2004). Neural and psychological mechanisms underlying appetitive learning: links to drug addiction. *Curr Opin Neurobiol*, *14*(2), 156–162. https://doi.org/10.1016/j.conb.2004.03.004

Castro, D. C., Cole, S. L., & Berridge, K. C. (2015). Lateral hypothalamus, nucleus accumbens, and ventral pallidum roles in eating and hunger: interactions between homeostatic and reward circuitry. *Frontiers in Systems Neuroscience*, *9*, 90. https://doi.org/10.3389/fnsys.2015.00090

Cavalera, M., Wang, J., & Frangogiannis, N. G. (2014, October 1). Obesity, metabolic dysfunction, and cardiac fibrosis: Pathophysiological pathways, molecular mechanisms, and therapeutic opportunities. *Translational Research*. Mosby Inc. https://doi.org/10.1016/j.trsl.2014.05.001

Chang, S., Graham, B., Yakubu, F., Lin, D., Peters, J. C., Hill, J. 0, & Metabolic, J. 0 H. (1990). *Metabolic differences between obesity-prone and obesity-resistant rats*. Retrieved from www.physiology.org/journal/ajpregu

Ciano, P. Di, Cardinal, R. N., Cowell, R. A., Little, S. J., & Everitt, B. J. (2001). *Differential Involvement of NMDA, AMPA/Kainate, and Dopamine Receptors in the Nucleus Accumbens Core in the Acquisition and Performance of Pavlovian Approach*

Behavior.

Clegg, D. J., Riedy, C. A., Smith, K. A. B., Benoit, S. C., & Woods, S. C. (2003). Differential Sensitivity to Central Leptin and Insulin in Male and Female Rats. *Diabetes*, *52*(3), 682–687. https://doi.org/10.2337/diabetes.52.3.682

Collingridge, G. L., Olsen, R. W., Peters, J., & Spedding, M. (2009). A nomenclature for ligand-gated ion channels. *Neuropharmacology*, *56*(1), 2–5. https://doi.org/10.1016/j.neuropharm.2008.06.063

Conrad, K. L., Tseng, K. Y., Uejima, J. L., Reimers, J. M., Heng, L. J., Shaham, Y., … Wolf, M. E. (2008). Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature*, *454*(7200), 118–121. https://doi.org/10.1038/nature06995

Corbit, L. H., & Balleine, B. W. (2011). The General and Outcome-Specific Forms of Pavlovian-Instrumental Transfer Are Differentially Mediated by the Nucleus Accumbens Core and Shell. *Journal of Neuroscience*, *31*(33), 11786–11794. https://doi.org/10.1523/JNEUROSCI.2711-11.2011

Corbit, L. H., & Balleine, B. W. (2016). Learning and motivational processes contributing to pavlovian–instrumental transfer and their neural bases: Dopamine and beyond. *Current Topics in Behavioral Neurosciences*, *27*, 259–289. https://doi.org/10.1007/7854_2015_388

Counotte, D. S., Schiefer, C., Shaham, Y., & O'Donnell, P. (2014). Time-dependent decreases in nucleus accumbens AMPA/NMDA ratio and incubation of sucrose craving in adolescent and adult rats. *Psychopharmacology*, *231*(8), 1675–1684. https://doi.org/10.1007/s00213-013-3294-3

Darling, R. A., Dingess, P. M., Schlidt, K. C., Smith, E. M., & Brown, T. E. (2016). Incubation of food craving is independent of macronutrient composition. *Scientific Reports*, *6*, 30900. https://doi.org/10.1038/srep30900

Demos, K. E., Heatherton, T. F., & Kelley, W. M. (2012). Individual Differences in Nucleus Accumbens Activity to Food and Sexual Images Predict Weight Gain and Sexual Behavior. *Journal of Neuroscience*, *32*(16), 5549–5552.

https://doi.org/10.1523/JNEUROSCI.5958-11.2012

Derman, R. C., & Ferrario, C. R. (2018). Enhanced incentive motivation in obesity-prone rats is mediated by NAc core CP-AMPARs. *Neuropharmacology*, *131*, 326–336. https://doi.org/10.1016/j.neuropharm.2017.12.039

Di Ciano, P., & Everitt, B. J. (2001). Dissociable Effects of Antagonism of NMDA and AMPA/KA Receptors in the Nucleus Accumbens Core and Shell on Cocaine-seeking Behavior. *Neuropsychopharmacology*, *25*(3), 341–360. https://doi.org/10.1016/S0893-133X(01)00235-4

Di Ciano, P., & Everitt, B. J. (2004). Direct interactions between the basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(32), 7167–7173. https://doi.org/10.1523/JNEUROSCI.1581-04.2004

Dingess, P. M., Darling, R. A., Derman, R. C., Wulff, S. S., Hunter, M. L., Ferrario, C. R., & Brown, T. E. (2017). Structural and Functional Plasticity within the Nucleus Accumbens and Prefrontal Cortex Associated with Time-Dependent Increases in Food Cue-Seeking Behavior. *Neuropsychopharmacology*, *42*(12), 2354–2364. https://doi.org/10.1038/npp.2017.57

Dingess, P. M., Darling, R. A., Kurt Dolence, E., Culver, B. W., & Brown, T. E. (2017). Exposure to a diet high in fat attenuates dendritic spine density in the medial prefrontal cortex. *Brain Struct Funct*, *222*(2), 1077–1085. https://doi.org/10.1007/s00429-016-1208-y

Dingledine, R., Borges, K., Bowie, D., & Traynelis, S. (1999). The Glutamate Receptor Ion Channels. *The Journal of Pharmacology and Experimental Therapeutics*, *51*(1), 7–62.

Dixon, J. B. (2010, March 25). The effect of obesity on health outcomes. *Molecular and Cellular Endocrinology*. https://doi.org/10.1016/j.mce.2009.07.008

Dong, Y., & Nestler, E. J. (2014). The neural rejuvenation hypothesis of cocaine addiction. *Trends in Pharmacological Sciences*. Elsevier Ltd. https://doi.org/10.1016/j.tips.2014.05.005

Dong, Y., Taylor, J. R., Wolf, M. E., & Shaham, Y. (2017). Circuit and synaptic plasticity mechanisms of drug relapse. *Journal of Neuroscience*, *37*(45), 10867–10876. https://doi.org/10.1523/JNEUROSCI.1821-17.2017

Eckel, R. H., & Krauss, R. M. (1998). *American Heart Association Call to Action: Obesity as a Major Risk Factor for Coronary Heart Disease*. Retrieved from http://ahajournals.org

Everitt, B. J., Morris, K. A., O'Brien, A., & Robbins, T. W. (1991). The basolateral amygdala-ventral striatal system and conditioned place preference: Further evidence of limbic-striatal interactions underlying reward-related processes. *Neuroscience*, *42*(1), 1–18. https://doi.org/10.1016/0306-4522(91)90145-E

Ferrario, C. R., Labouèbe, G., Liu, S., Nieh, E. H., Routh, V. H., Xu, S., & O'Connor, E. C. (2016). Homeostasis Meets Motivation in the Battle to Control Food Intake. *The Journal of Neuroscience*, *36*(45), 11469–11481. https://doi.org/10.1523/JNEUROSCI.2338-16.2016

Ferrario, C. R., Li, X., Wang, X., Reimers, J. M., Uejima, J. L., & Wolf, M. E. (2010). The role of glutamate receptor redistribution in locomotor sensitization to cocaine. *Neuropsychopharmacology*, *35*(3), 818–833. https://doi.org/10.1038/npp.2009.190

Ferriday, D., & Brunstrom, J. M. (2011). I just can't help myself: Effects of food-cue exposure in overweight and lean individuals. *International Journal of Obesity*, *35*(1), 142–149. https://doi.org/10.1038/ijo.2010.117

Forlano, P. M., & Woolley, C. S. (2010). Quantitative Analysis of Pre-and Postsynaptic Sex Differences in the Nucleus Accumbens. *J. Comp. Neurol*, *518*, 1330–1348. https://doi.org/10.1002/cne.22279

Frankort, A., Roefs, A., Siep, N., Roebroeck, A., Havermans, R., & Jansen, A. (2012). Reward activity in satiated overweight women is decreased during unbiased viewing but increased when imagining taste: an event-related fMRI study. *Int J Obes (Lond)*, *36*(5), 627–637. https://doi.org/10.1038/ijo.2011.213

Fulton, S. (2010, January). Appetite and reward. *Frontiers in Neuroendocrinology*. https://doi.org/10.1016/j.yfrne.2009.10.003

Gade, W., Schmit, J., Collins, M., Gade, J., Reaven, G., Kaplan, N., … Fried-man, J. (2010). *Beyond Obesity: The Diagnosis and Pathophysiology of Metabolic Syndrome*. *FOCUS: OBESITY AND METABOLIC SYNDROME* (Vol. 23).

Gao, L., Blair, L. A. C., & Marshall, J. (2006). CaMKII-independent effects of KN93 and its inactive analog KN92: Reversible inhibition of L-type calcium channels. *Biochemical and Biophysical Research Communications*, *345*(4), 1606–1610. https://doi.org/10.1016/j.bbrc.2006.05.066

Geary, N., Asarian, L., Korach, K. S., Pfaff, D. W., & Ogawa, S. (2001). Deficits in E2-dependent control of feeding, weight gain, and cholecystokinin satiation in ER-α null mice. *Endocrinology*, *142*(11), 4751–4757. https://doi.org/10.1210/endo.142.11.8504

Gold, S. J., Ambros-Ingerson, J., Horowitz, J. R., Lynch, G., & Gall, C. M. (1997). Stoichiometries of AMPA receptor subunit mRNAs in rat brain fall into discrete categories. *Journal of Comparative Neurology*, *385*(4), 491–502. https://doi.org/10.1002/(SICI)1096-9861(19970908)385:4<491::AID-CNE1>3.0.CO;2-Z

Gómez-Pinilla, F. (2008). Brain foods: The effects of nutrients on brain function. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn2421

Gong, E. J., Garrel, D., & Calloway, D. H. (1989). Menstrual cycle and voluntary food intake. *American Journal of Clinical Nutrition*, *49*(2), 252–258. https://doi.org/10.1093/ajcn/49.2.252

Gorski, J. N. (2006). Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance. *AJP: Regulatory, Integrative and Comparative Physiology*, *291*(3), R768–R778. https://doi.org/10.1152/ajpregu.00138.2006

Gorski, J. N., Dunn-Meynell, A. A., & Levin, B. E. (2007). Maternal obesity increases hypothalamic leptin receptor expression and sensitivity in juvenile obesity-prone rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *292*(5), R1782–R1791. https://doi.org/10.1152/ajpregu.00749.2006

Greger, I. H., & Esteban, J. A. (2007). AMPA receptor biogenesis and trafficking. *Current Opinion in Neurobiology*, *17*(3), 289–297. https://doi.org/10.1016/j.conb.2007.04.007

Grimm, J. W., Hope, B. T., Wise, R. A., & Shaham, Y. (2001). Neuroadaptation. Incubation of cocaine craving after withdrawal. *Nature*, *412*(6843), 141–142. https://doi.org/10.1038/35084134

Groenewegen, H., Mulder, A. B., Beijer, A. V. J., Wright, C. I., Wpes, F. H., Silva, D. A., & Pennartz, C. M. A. (1999). *Hippocampal and amygdaloid interactions in the nucleus accumbens*. *Psychobiology* (Vol. 27).

Hales, C. M., Carroll, M. D., Fryar, C. D., & Ogden, C. L. (2015). *Prevalence of Obesity Among Adults and Youth: United States, 2015-2016 Key findings Data from the National Health and Nutrition Examination Survey*. Retrieved from https://www.cdc.gov/nchs/data/databriefs/db288_table.pdf#1.

Hanley, J. G. (2008, April). PICK1: A multi-talented modulator of AMPA receptor trafficking. *Pharmacology and Therapeutics*. https://doi.org/10.1016/j.pharmthera.2008.02.002

Harris', K. M., & Stevens, J. K. (1989). *Dendritic Spines of CA1 Pyramidal Cells in the Rat Hippocampus: Serial Electron Microscopy with Reference to Their Biophysical Characteristics*. *The Journal of Neuroscience*.

Heine, P. A., Taylor, J. A., Iwamoto, G. A., Lubahn, D. B., & Cooke, P. S. (2000). Increased adipose tissue in male and female estrogen receptor-α knockout mice. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(23), 12729–12734. https://doi.org/10.1073/pnas.97.23.12729

Ho, A., Kennedy, J., & Dimitropoulos, A. (2012). Neural correlates to food-related behavior in normal-weight and overweight/obese participants. *PLoS One*, *7*(9), e45403. https://doi.org/10.1371/journal.pone.0045403

Holtmaat, A., & Svoboda, K. (2009, September). Experience-dependent structural synaptic plasticity in the mammalian brain. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn2699

Hu, F. B. (2003). Overweight and obesity in women: health risks and consequences.

Journal of Women's Health (15409996), *12*(2), 163–172. Retrieved from https://www.liebertpub.com/doi/pdf/10.1089/154099903321576565

Hu, X. T., & White, F. J. (1996). Glutamate receptor regulation of rat nucleus accumbens neurons in vivo. *Synapse*, *23*(3), 208–218. https://doi.org/10.1002/(SICI)1098-2396(199607)23:3<208::AID-SYN10>3.0.CO;2-V

Huang, Y. H., Lin, Y., Mu, P., Lee, B. R., Brown, T. E., Wayman, G., … Dong, Y. (2009). In vivo cocaine experience generates silent synapses. *Neuron*, *63*(1), 40–47. https://doi.org/10.1016/j.neuron.2009.06.007

Huang, Y. H., Schlüter, O. M., & Dong, Y. (2015, October 30). Silent Synapses Speak Up: Updates of the Neural Rejuvenation Hypothesis of Drug Addiction. *Neuroscientist*. SAGE Publications Inc. https://doi.org/10.1177/1073858415579405

Ikemoto, S., & Panksepp, J. (1999). The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Res Brain Res Rev*, *31*(1), 6–41. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10611493

Irani, B. G., Dunn-Meynell, A. A., & Levin, B. E. (2007). Altered hypothalamic leptin, insulin, and melanocortin binding associated with moderate-fat diet and predisposition to obesity. *Endocrinology*, *148*(1), 310–316. https://doi.org/10.1210/en.2006-1126

Isaac, J. T. R., Crair, M. C., Nicoll, R. A., & Malenka, R. C. (1997). Silent synapses during development of thalamocortical inputs. *Neuron*, *18*(2), 269–280. https://doi.org/10.1016/S0896-6273(00)80267-6

Kalivas, P. W., Volkow, N., & Seamans, J. (2005). Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. *Neuron*, *45*(5), 647–650. https://doi.org/10.1016/j.neuron.2005.02.005

Kelley, A. E., Baldo, B. A., Pratt, W. E., & Will, M. J. (2005). Corticostriatal-hypothalamic circuitry and food motivation: Integration of energy, action and reward. In *Physiology and Behavior* (Vol. 86, pp. 773–795). https://doi.org/10.1016/j.physbeh.2005.08.066

Kerstetter, K. A., Aguilar, V. R., Parrish, A. B., & Kippin, T. E. (2008). Protracted time-

dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. *Psychopharmacology*, *198*(1), 63–75. https://doi.org/10.1007/s00213-008-1089-8

Kourrich, S., Rothwell, P. E., Klug, J. R., & Thomas, M. J. (2007). Cocaine Experience Controls Bidirectional Synaptic Plasticity in the Nucleus Accumbens. *Journal of Neuroscience*, *27*(30), 7921–7928. https://doi.org/10.1523/JNEUROSCI.1859-07.2007

Koya, E., Cruz, F. C., Ator, R., Golden, S. A., Hoffman, A. F., Lupica, C. R., & Hope, B. T. (2012). Silent synapses in selectively activated nucleus accumbens neurons following cocaine sensitization. *Nature Neuroscience*, *15*. https://doi.org/10.1038/nn.3232

Kyrou, I., Randeva, H. S., Tsigos, C., Kaltsas, G., & Weickert, M. O. (2000). *Clinical Problems Caused by Obesity*. *Endotext*. MDText.com, Inc. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/25905207

Lashinger, L. M., Rossi, E. L., & Hursting, S. D. (2014). Obesity and resistance to cancer chemotherapy: Interacting roles of inflammation and metabolic dysregulation. *Clinical Pharmacology and Therapeutics*, *96*(4), 458–463. https://doi.org/10.1038/clpt.2014.136

Lee, B. R., & Dong, Y. (2011). Cocaine-induced metaplasticity in the nucleus accumbens: Silent synapse and beyond. *Neuropharmacology*. https://doi.org/10.1016/j.neuropharm.2010.12.033

Lee, B. R., Ma, Y. Y., Huang, Y. H., Wang, X., Otaka, M., Ishikawa, M., … Dong, Y. (2013). Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving. *Nature Neuroscience*, *16*(11), 1644–1651. https://doi.org/10.1038/nn.3533

Levin, B. E. (2007). Why some of us get fat and what we can do about it. *The Journal of Physiology*, *583*(Pt 2), 425–430. https://doi.org/10.1113/jphysiol.2007.135434

Levin, B. E., & Dunn-Meynell, A. A. (2000). Defense of body weight against chronic caloric restriction in obesity- prone and -resistant rats. *American Journal of Physiology* -

Regulatory Integrative and Comparative Physiology, *278*(1 47-1), R231–R237. https://doi.org/10.1152/ajpregu.2000.278.1.R231

Levin, B. E., & Dunn-Meynell, A. A. (2002). Defense of body weight depends on dietary composition and palatability in rats with diet-induced obesity. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *282*(1 51-1), R46–R54. https://doi.org/10.1152/ajpregu.2002.282.1.R46

Levin, B. E., Dunn-Meynell, A. A., Balkan, B., & Keesey, R. E. (1997). Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *273*(2), R725–R730. https://doi.org/10.1152/ajpregu.1997.273.2.R725

Levin, B. E., Dunn-Meynell, A. A., & Banks, W. A. (2004). Obesity-prone rats have normal blood-brain barrier transport but defective central leptin signaling before obesity onset. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *286*(1 55-1), R143–R150. https://doi.org/10.1152/ajpregu.00393.2003

Levin, B. E., & Keesey, R. E. (1998). Defense of differfing body weight set points in diet-induced obese and resistant rats. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, *274*(2 43-2), R412–R419. https://doi.org/10.1152/ajpregu.1998.274.2.R412

Levine, A. S., Kotz, C. M., & Gosnell, B. A. (2003). Sugar and Fat — From Genes to Culture Sugars and Fats : The Neurobiology of Preference 1. *Jounal of Nutrition*, (9), 831–834. Retrieved from https://academic.oup.com/jn/article-abstract/133/3/831S/4688013/

Liao, D., Hessler, N. A., & Malinow, R. (1995). Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature*, *375*(6530), 400–404. https://doi.org/10.1038/375400a0

Lin, D. T., Makino, Y., Sharma, K., Hayashi, T., Neve, R., Takamiya, K., & Huganir, R. L. (2009). Regulation of AMPA receptor extrasynaptic insertion by 4.1N, phosphorylation and palmitoylation. *Nature Neuroscience*, *12*(7), 879–887. https://doi.org/10.1038/nn.2351

Lisman, J., Schulman, H., & Cline, H. (2002). The molecular basis of CaMKII function in synaptic and behavioural memory. *Nature Reviews Neuroscience*, *3*(3), 175–190. https://doi.org/10.1038/nrn753

Lissner, L., Stevens, J., Levitsky, D., Rasmussen, K. M., & Strupp, B. J. (1988). *implications for food-intake research13*. Retrieved from https://academic.oup.com/ajcn/article-abstract/48/4/956/4716197

Loweth, J. A., Tseng, K. Y., & Wolf, M. E. (2014). Adaptations in AMPA receptor transmission in the nucleus accumbens contributing to incubation of cocaine craving. *Neuropharmacology*. https://doi.org/10.1016/j.neuropharm.2013.04.061

Lu, L., Grimm, J. W., Dempsey, J., & Shaham, Y. (2004). Cocaine seeking over extended withdrawal periods in rats: Different time courses of responding induced by cocaine cues versus cocaine priming over the first 6 months. *Psychopharmacology*, *176*(1), 101–108. https://doi.org/10.1007/s00213-004-1860-4

Lu, L., Grimm, J. W., Hope, B. T., & Shaham, Y. (2004). Incubation of cocaine craving after withdrawal: A review of preclinical data. *Neuropharmacology*, *47*(SUPPL. 1), 214–226. https://doi.org/10.1016/j.neuropharm.2004.06.027

Ma, Y. Y., Lee, B. R., Wang, X., Guo, C., Liu, L., Cui, R., … Dong, Y. (2014). Bidirectional modulation of incubation of cocaine craving by silent synapse-based remodeling of prefrontal cortex to accumbens projections. *Neuron*, *83*(6), 1453–1467. https://doi.org/10.1016/j.neuron.2014.08.023

Madsen, A. N., Hansen, G., Paulsen, S. J., Lykkegaard, K., Tang-Christensen, M., Hansen, H. S., … Vrang, N. (2010). Long-term characterization of the diet-induced obese and diet-resistant rat model: A polygenetic rat model mimicking the human obesity syndrome. *Journal of Endocrinology*, *206*(3), 287–296. https://doi.org/10.1677/JOE-10-0004

Maldonado-Irizarry, C. S., Swanson, C. J., & Kelley, A. E. (1995). Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *15*(10), 6779–6788. Retrieved from

http://www.jneurosci.org/content/jneuro/15/10/6779.full.pdf

Malinow, R. (2003, April 29). AMPA receptor trafficking and long-term potentiation. *Philosophical Transactions of the Royal Society B: Biological Sciences*. Royal Society. https://doi.org/10.1098/rstb.2002.1233

Martin, L. E., Holsen, L. M., Chambers, R. J., Bruce, A. S., Brooks, W. M., Zarcone, J. R., … Savage, C. R. (2010). Neural mechanisms associated with food motivation in obese and healthy weight adults. *Obesity (Silver Spring)*, *18*(2), 254–260. https://doi.org/10.1038/oby.2009.220

Matsuzaki, M., Honkura, N., Ellis-Davies, G. C., & Kasai, H. (2004). Structural basis of long-term potentiation in single dendritic spines. *Nature*, *429*(6993), 761–766. https://doi.org/10.1038/nature02617

McCutcheon, J. E., Loweth, J. A., Ford, K. A., Marinelli, M., Wolf, M. E., & Tseng, K. Y. (2011). Group I mGlur activation reverses cocaine-induced accumulation of calcium-permeable AMPA receptors in nucleus accumbens synapses via a protein kinase C-dependent mechanism. *Journal of Neuroscience*, *31*(41), 14536–14541. https://doi.org/10.1523/JNEUROSCI.3625-11.2011

McCutcheon, J. E., Wang, X., Tseng, K. Y., Wolf, M. E., & Marinelli, M. (2011). Calcium-permeable AMPA receptors are present in nucleus accumbens synapses after prolonged withdrawal from cocaine self-administration but not experimenter-administered cocaine. *Journal of Neuroscience*, *31*(15), 5737–5743. https://doi.org/10.1523/JNEUROSCI.0350-11.2011

Mickelsen, O., Takahashi, S., & Craig, C. (1955). Experimental obesity. I. Production of obesity in rats by feeding high-fat diets. *The Journal of Nutrition*, *57*(4), 541–554. https://doi.org/10.1093/jn/57.4.541

Moorman, D. E., & Aston-Jones, G. (2015). Prefrontal neurons encode context-based response execution and inhibition in reward seeking and extinction. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(30), 9472–9477. https://doi.org/10.1073/pnas.1507611112

Murdaugh, D. L., Cox, J. E., Cook 3rd, E. W., & Weller, R. E. (2012). fMRI reactivity to

high-calorie food pictures predicts short- and long-term outcome in a weight-loss program. *Neuroimage*, *59*(3), 2709–2721. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22332246

Musatov, S., Chen, W., Pfaff, D. W., Mobbs, C. V., Yang, X. J., Clegg, D. J., … Ogawa, S. (2007). Silencing of estrogen receptor α in the ventromedial nucleus of hypothalamus leads to metabolic syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(7), 2501–2506. https://doi.org/10.1073/pnas.0610787104

Neisewander, J. L., Baker, D. A., Fuchs, R. A., Tran-Nguyen, L. T. L., Palmer, A., & Marshall, J. F. (2000). Fos Protein Expression and Cocaine-Seeking Behavior in Rats after Exposure to a Cocaine Self-Administration Environment. *The Journal of Neuroscience*, *20*(2), 798–805. https://doi.org/10.1523/JNEUROSCI.20-02-00798.2000

Nicolas, C., Russell, T. I., Pierce, A. F., Maldera, S., Holley, A., You, Z. B., … Ikemoto, S. (2019). Incubation of Cocaine Craving After Intermittent-Access Self-administration: Sex Differences and Estrous Cycle. *Biological Psychiatry*, *85*(11), 915–924. https://doi.org/10.1016/j.biopsych.2019.01.015

Oginsky, M. F., & Ferrario, C. R. (2019). Eating “junk-food” has opposite effects on intrinsic excitability of nucleus accumbens core neurons in obesity-susceptible vs. -resistant rats. *Journal of Neurophysiology*, *122*, 1264–1273. https://doi.org/10.1152/jn.00361.2019

Oginsky, M. F., Goforth, P. B., Nobile, C. W., Lopez-Santiago, L. F., & Ferrario, C. R. (2016). Eating “Junk-Food” Produces Rapid and Long-Lasting Increases in NAc CP-AMPA Receptors: Implications for Enhanced Cue-Induced Motivation and Food Addiction. *Neuropsychopharmacology*, *41*(13), 2977–2986. https://doi.org/10.1038/npp.2016.111

Oginsky, M. F., Maust, J. D., Corthell, J. T., & Ferrario, C. R. (2016). Enhanced cocaine-induced locomotor sensitization and intrinsic excitability of NAc medium spiny neurons in adult but not in adolescent rats susceptible to diet-induced obesity.

Psychopharmacology, *233*(5), 773–784. https://doi.org/10.1007/s00213-015-4157-x

Palmer, B. F., & Clegg, D. J. (2015, February 15). The sexual dimorphism of obesity. *Molecular and Cellular Endocrinology*. NIH Public Access. https://doi.org/10.1016/j.mce.2014.11.029

Palmer, C. L., Cotton, L., & Henley, J. M. (2005, June). The molecular pharmacology and cell biology of α-amino-3-hydroxy-5- methyl-4-isoxazolepropionic acid receptors. *Pharmacological Reviews*. https://doi.org/10.1124/pr.57.2.7

Palmer, K., & Gray, J. M. (1986). Central vs. peripheral effects of estrogen on food intake and lipoprotein lipase activity in ovariectomized rats. *Physiology and Behavior*, *37*(1), 187–189. https://doi.org/10.1016/0031-9384(86)90404-X

Pennartz, C. M. A., Groenewegen, H. J., & Lopes da Silva, F. H. (1994). The nucleus accumbens as a complex of functionally distinct neuronal ensembles: An integration of behavioural, electrophysiological and anatomical data. *Progress in Neurobiology*. https://doi.org/10.1016/0301-0082(94)90025-6

Perez, M. F., White, F. J., & Hu, X.-T. (2006). Dopamine D 2 Receptor Modulation of K 2 Channel Activity Regulates Excitability of Nucleus Accumbens Neurons at Different Membrane Potentials. *J Neu-Rophysiol*, *96*(2), 2217–2228. https://doi.org/10.1152/jn.00254.2006

Petrovich, G. D., & Gallagher, M. (2007). Control of food consumption by learned cues: A forebrain-hypothalamic network. *Physiology and Behavior*, *91*(4), 397–403. https://doi.org/10.1016/j.physbeh.2007.04.014

Petrovich, G. D., Ross, C. A., Holland, P. C., & Gallagher, M. (2007). Medial prefrontal cortex is necessary for an appetitive contextual conditioned stimulus to promote eating in sated rats. *Journal of Neuroscience*, *27*(24), 6436–6441. https://doi.org/10.1523/JNEUROSCI.5001-06.2007

Pickens, C. L., Airavaara, M., Theberge, F., Fanous, S., Hope, B. T., & Shaham, Y. (2011, August). Neurobiology of the incubation of drug craving. *Trends in Neurosciences*. https://doi.org/10.1016/j.tins.2011.06.001

Pinilla, L., Seoane, L. M., Gonzalez, L., Carro, E., Aguilar, E., Casanueva, F. F., &

Dieguez, C. (1999). Regulation of serum leptin levels by gonadal function in rats. *European Journal of Endocrinology*, *140*(5), 468–473. https://doi.org/10.1530/eje.0.1400468

Podda, M. V., Riccardi, E., D'Ascenzo, M., Azzena, G. B., & Grassi, C. (2010). Dopamine D1-like receptor activation depolarizes medium spiny neurons of the mouse nucleus accumbens by inhibiting inwardly rectifying K+ currents through a cAMP-dependent protein kinase A-independent mechanism. *Neuroscience*, *167*(3), 678–690. https://doi.org/10.1016/j.neuroscience.2010.02.075

Polivy, J., & Herman, C. P. (2017). Restrained Eating and Food Cues: Recent Findings and Conclusions. *Current Obesity Reports*. https://doi.org/10.1007/s13679-017-0243-1

Proaño, S. B., Morris, H. J., Kunz, L. M., Dorris, D. M., & Meitzen, J. (2018). Estrous cycle-induced sex differences in medium spiny neuron excitatory synaptic transmission and intrinsic excitability in adult rat nucleus accumbens core. *Journal of Neurophysiology*, *120*(3), 1356–1373. https://doi.org/10.1152/jn.00263.2018

Reimers, J. M., Milovanovic, M., & Wolf, M. E. (2011). Quantitative analysis of AMPA receptor subunit composition in addiction-related brain regions. *Brain Research*, *1367*, 223–233. https://doi.org/10.1016/j.brainres.2010.10.016

Reynolds, S. M., & Berridge, K. C. (2003). Glutamate motivational ensembles in nucleus accumbens: Rostrocaudal shell gradients of fear and feeding. *European Journal of Neuroscience*, *17*(10), 2187–2200. https://doi.org/10.1046/j.1460-9568.2003.02642.x

Rivera, H., & Stincic, T. (2018). Estradiol and the control of feeding behavior. *Steroids*, *133*, 44–52. https://doi.org/10.1016/j.steroids.2017.11.011

Robinson, M. J. F., Burghardt, P. R., Patterson, C. M., Nobile, C. W., Akil, H., Watson, S. J., … Ferrario, C. R. (2015). Individual Differences in Cue-Induced Motivation and Striatal Systems in Rats Susceptible to Diet-Induced Obesity. *Neuropsychopharmacology*, *40*(9), 2113–2123. https://doi.org/10.1038/npp.2015.71

Schemmel, R., Mickelsen, O., & Gill, J. L. (1970). Dietary Obesity in Rats: Body Weight

and Body Fat Accretion in Seven Strains of Rats. *The Journal of Nutrition*, *100*(9), 1041–1048. https://doi.org/10.1093/jn/100.9.1041

Schultz, W. (1997). Dopamine neurons and their role in reward mechanisms Abbreviations GABA y-aminobutyric acid NMDA N-methyl-o-aspartate TD models temporal difference models. *Current Opinion in Neurobiology*, *7*, 191–197.

Schultz, W. (2013). Updating dopamine reward signals. *Current Opinion in Neurobiology*, *23*, 229–238. https://doi.org/10.1016/j.conb.2012.11.012

Schulz, D. J. (2006). Plasticity and stability in neuronal output via changes in intrinsic excitability: It's what's inside that counts. *Journal of Experimental Biology*. https://doi.org/10.1242/jeb.02567

Self, D. W. (2004). Regulation of drug-taking and -seeking behaviors by neuroadaptations in the mesolimbic dopamine system. *Neuropharmacology*, *47 Suppl 1*, 242–255. https://doi.org/10.1016/j.neuropharm.2004.07.005

Sesack, S. R., Deutch, A. Y., Roth, R. H., & Bunney, B. S. (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: An anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *Journal of Comparative Neurology*, *290*(2), 213–242. https://doi.org/10.1002/cne.902900205

Shepherd, J. D., & Huganir, R. L. (2007). The Cell Biology of Synaptic Plasticity: AMPA Receptor Trafficking AMPAR: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor. https://doi.org/10.1146/annurev.cellbio.23.090506.123516

Sorge, R. E., & Stewart, J. (2005). The contribution of drug history and time since termination of drug taking to footshock stress-induced cocaine seeking in rats. *Psychopharmacology*, *183*(2), 210–217. https://doi.org/10.1007/s00213-005-0160-y

Stice, E., Burger, K. S., & Yokum, S. (2015). Reward Region Responsivity Predicts Future Weight Gain and Moderating Effects of the TaqIA Allele. *Journal of Neuroscience*, *35*(28), 10316–10324. https://doi.org/10.1523/JNEUROSCI.3607-14.2015

Stoeckel, L. E., Weller, R. E., Cook 3rd, E. W., Twieg, D. B., Knowlton, R. C., & Cox, J. E. (2008). Widespread reward-system activation in obese women in response to pictures of high-calorie foods. *Neuroimage*, *41*(2), 636–647.

https://doi.org/10.1016/j.neuroimage.2008.02.031

Sun, X., Milovanovic, M., Zhao, Y., & Wolf, M. E. (2008). Acute and chronic dopamine receptor stimulation modulates AMPA receptor trafficking in nucleus accumbens neurons cocultured with prefrontal cortex neurons. *Journal of Neuroscience*, *28*(16), 4216–4230. https://doi.org/10.1523/JNEUROSCI.0258-08.2008

Sun, X., Zhao, Y., & Wolf, M. E. (2005). Dopamine receptor stimulation modulates AMPA receptor synaptic insertion in prefrontal cortex neurons. *Journal of Neuroscience*, *25*(32), 7342–7351. https://doi.org/10.1523/JNEUROSCI.4603-04.2005

Surmeier, D. J., Ding, J., Day, M., Wang, Z., & Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends in Neurosciences*. https://doi.org/10.1016/j.tins.2007.03.008

Swanson, L. W., & Cowan, W. M. (1975). Hippocampo-hypothalamic connections: Origin in subicular cortex, not Ammon's horn. *Science*, *189*(4199), 303–304. https://doi.org/10.1126/science.49928

Takumi, Y., Ramírez-León, V., Laake, P., Rinvik, E., & Ottersen, O. P. (1999). Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. *Nature Neuroscience*, *2*(7), 618–624. https://doi.org/10.1038/10172

Tang, D. W., Fellows, L. K., Small, D. M., & Dagher, A. (2012). Food and drug cues activate similar brain regions: A meta-analysis of functional MRI studies. *Physiology and Behavior*, *106*(3), 317–324. https://doi.org/10.1016/j.physbeh.2012.03.009

Tarttelin, M. F., & Gorski, R. A. (1971). Variations in food and water intake in the normal and acyclic female rat. *Physiology and Behavior*, *7*(6), 847–852. https://doi.org/10.1016/0031-9384(71)90050-3

Terrier, J., Lüscher, C., & Pascoli, V. (2016). Cell-Type Specific Insertion of GluA2-Lacking AMPARs with Cocaine Exposure Leading to Sensitization, Cue-Induced Seeking, and Incubation of Craving. *Neuropsychopharmacology*, *41*(7), 1779–1789. https://doi.org/10.1038/npp.2015.345

Tetley, A., Brunstrom, J., & Griffiths, P. (2009). Individual differences in food-cue reactivity. The role of BMI and everyday portion-size selections. *Appetite*, *52*(3), 614–

620. https://doi.org/10.1016/j.appet.2009.02.005

Toni, N., Buchs, P. A., Nikonenko, I., Bron, C. R., & Muller, D. (1999). LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. *Nature*, *402*(6760), 421–425. https://doi.org/10.1038/46574

Wade, G. N. (1975). Some effects of ovarian hormones on food intake and body weight in female rats. *Journal of Comparative and Physiological Psychology*, *88*(1), 183–193. https://doi.org/10.1037/h0076186

Wade, G. N., & Zucker, I. (1970). Modulation of food intake and locomotor activity in female rats by diencephalic hormone implants. *Journal of Comparative and Physiological Psychology*, *72*(2), 328–336. https://doi.org/10.1037/h0029461

Wardle, J. (1990). Conditioning processes and cue exposure in the modification of excessive eating. *Addictive Behaviors*, *15*(4), 387–393. https://doi.org/10.1016/0306-4603(90)90047-2

Wardle, J., & Carnell, S. (2009). Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. *American Journal of Clinical Nutrition*, *87*(2), 398–404. https://doi.org/10.1007/s12160-009-9116-5

Wenthold, R., Petralia, R., Blahos J, I., & Niedzielski, A. (1996). Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. *The Journal of Neuroscience*, *16*(6), 1982–1989. https://doi.org/10.1523/JNEUROSCI.16-06-01982.1996

Wise, R. A. (2006). Role of brain dopamine in food reward and reinforcement. *Philosophical Transactions of the Royal Society B: Biological Sciences*. Royal Society. https://doi.org/10.1098/rstb.2006.1854

Wissman, A. M., May, R. M., & Woolley, C. S. (2012). Ultrastructural analysis of sex differences in nucleus accumbens synaptic connectivity. *Brain Structure & Function*, *217*(2), 181–190. https://doi.org/10.1007/s00429-011-0353-6

Wolf, M. E. (2002). Addiction: Making the Connection Between Behavioral Changes and Neuronal Plasticity in Specific Pathways. *Molecular Interventions*, *2*(3), 146–157. https://doi.org/10.1124/mi.2.3.146

Wolf, Marina E., Mangiavacchi, S., & Sun, X. (2003). Mechanisms by which Dopamine Receptors May Influence Synaptic Plasticity. In *Annals of the New York Academy of Sciences*. https://doi.org/10.1196/annals.1300.015

Wolf, Marina E. (2010). Regulation of AMPA receptor trafficking in the nucleus accumbens by dopamine and cocaine. *Neurotoxicity Research*, *18*(3–4), 393–409. https://doi.org/10.1007/s12640-010-9176-0

Wolf, Marina E. (2016). Synaptic mechanisms underlying persistent cocaine craving. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn.2016.39

Wolf, Marina E, & Ferrario, C. R. (2010). AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2010.01.013

Yokum, S., Ng, J., & Stice, E. (2011). Attentional bias to food images associated with elevated weight and future weight gain: An fMRI study. *Obesity*, *19*(9), 1775–1783. https://doi.org/10.1038/oby.2011.168

Yuste, R., & Bonhoeffer, T. (2001). Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annu Rev Neurosci*, *24*, 1071–1089. https://doi.org/10.1146/annurev.neuro.24.1.1071

CHAPTER 2

Effects of The Estrous Cycle and Ovarian Hormones on Cue-Triggered Motivation and Intrinsic Excitability of Medium Spiny Neurons in The Nucleus Accumbens Core of Female Rats.

Abstract

Naturally occurring alterations in estradiol influence food intake in females. However, how motivational responses to food cues are affected by the estrous cycle or ovarian hormones is unknown. In addition, while individual susceptibility to obesity is accompanied by enhanced incentive motivational responses to food cues and increased NAc intrinsic excitability in males, studies in females are absent. Therefore, we examined basal differences in intrinsic NAc excitability of obesity-prone vs. obesity-resistant females and determined how conditioned approach (a measure of cue-triggered motivation), food intake, and motivation for food vary with the cycle in naturally cycling female obesity-prone, obesity-resistant, and outbred Sprague-Dawley rats. Finally, we used ovariectomy followed by hormone treatment to determine the role of ovarian hormones in cue-triggered motivation in selectively bred and outbred female rats. We found that intrinsic excitability of NAc MSNs and conditioned approach are enhanced in female obesity-prone vs. obesity-resistant rats. These effects were driven by greater MSN excitability and conditioned approach behavior during metestrus/diestrus vs. proestrus/estrus in obesity-prone but not obesity-resistant rats, despite similar regulation of food intake and food motivation by the cycle in these groups. Furthermore, estradiol and progesterone treatment reduced conditioned approach behavior in obesity-prone and outbred Sprague-Dawley females. To our knowledge, these data are the first to demonstrate cycle- and hormone-dependent effects on the motivational response to a food cue, and the only studies to date to determine how individual susceptibility to obesity influences NAc excitability, cue-triggered food-seeking, and differences in the regulation of these neurobehavioral responses by the estrous cycle.

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Introduction

Naturally occurring fluctuations in ovarian hormones influence food consumption and food preference in both humans and rodents (Eckel, 2011; Hirschberg, 2012). For example, in rats, food intake is lower during estrus compared to metestrus and diestrus (Tarttelin & Gorski, 1971). This effect of the cycle on food intake is mediated in part by central actions of estrogens (see Asarian and Geary, 2013 for review). Consistent with this, the removal of ovarian hormones via ovariectomy produces significant increases in food intake that are accompanied by weight gain (Blaustein & Wade, 1976; Yu et al., 2011). This increase can be normalized by treating the ovariectomized rats with estradiol (Tarttelin & Gorski, 1971; Wade, 1972; Asarian et al., 2002, 2006; Yu et al., 2011). Thus, ovarian hormones influence food intake.

In overweight women, stimuli that predict food availability (i.e., food cues) elicit stronger activations in brain regions that influence motivation including the Nucleus Accumbens (NAc; Stoeckel et al., 2008) compared to lean women. Furthermore, the magnitude of food cue elicited NAc activations predicts future weight gain in healthy weight females and future inability to lose weight after obesity (Murdaugh et. al 2012, Demos et al. 2012). These human studies suggest that enhanced neurobehavioral responses to food cues may promote the development of obesity. However, identifying pre-existing differences is not feasible in human studies and there is limited understanding of how ovarian hormones influence brain mechanisms governing cue-triggered food seeking (see Alonso-Caraballo et al., 2018 for additional discussion).

Consistent with a role for pre-existing differences, preclinical studies from our lab have shown that motivational responses to food cues are stronger in obesity-prone compared to obesity-resistant male rats (Derman and Ferrario, 2018; Robinson et al., 2015). These behavioral differences in males are mediated by the NAc (Derman and Ferrario, 2018) and intrinsic excitability of medium spiny neurons (MSNs) in the NAc is enhanced in obesity-prone vs. obesity-resistant males (Oginsky, et al., 2016). The risk for obesity is greater in females compared to males, and ovarian hormones influence NAc activity and plasticity (Cyr et al., 2001; Le Saux et al., 2006; Peterson et al., 2015; Cao et al., 2018). However, it is not known whether similar enhancements in motivational responses to food

cues are also found in female obesity-prone vs. obesity-resistant rats. Furthermore, neural activations in response to monetary rewards or to visual food cues vary with the menstrual cycle in women, with higher activations observed in corticolimbic areas during the luteal phase compared to the follicular phase (Dreher et al., 2007; Frank et al., 2010; Arnoni-Bauer et al., 2017). This suggests that motivational responses to food cues may be influenced by ovarian hormones; however, no studies to date have directly examined this possibility.

In the current study, we first examined basal differences in intrinsic excitability of NAc MSNs of obesity-prone vs. obesity-resistant females across the cycle. Next, we determined how motivational responses to Pavlovian food cues and motivation for food itself vary with the cycle in naturally cycling female obesity-prone and obesity-resistant rats. Finally, we used ovariectomy followed by hormone treatment to determine the role of ovarian hormones in cue-triggered motivation in selectively bred and outbred female rats.

Materials and Methods

Subjects:

Female selectively-bred obesity-prone (OP) and obesity-resistant (OR) rat lines were originally developed by Barry Levin (Levin et al., 1997) and were bred in house. Female outbred Sprague-Dawley rats were purchased from Charles River Breeding Labs (Portage, MI). Rats were housed on a reverse 12-h light/dark cycle, had free access to food and water throughout, and were group housed unless otherwise noted. For studies involving ovariectomy, estrogen-free bedding (7090 Teklad sani-chips) and estrogen-free chow (Harlan 2916: 3 kcal/g; 4% fat; 16.4% protein; 48.5% protein; % of caloric content) were used. Rats were weighed 3-4 times per week unless otherwise specified. Procedures were approved by The University of Michigan Committee on the Use and Care of Animals in accordance with AAALAC and AVMA guidelines. See also https://sites.google.com/a/umich.edu/ferrario-lab-public-protocols/ for additional details.

Monitoring the Estrous Cycle:

Estrous cycle phase was determined by daily observations of vaginal epithelial cell cytology, precopulatory, and copulatory behaviors (Marcondes et al., 2002). Epithelial cells were collected daily by vaginal lavage (during the dark phase) and visualized using an inverted light microscope (Olympus CKX53) under bright-field. Estrous cycle cell pictures were taken at 20x magnification with an EVOS XL Cell Imaging System (Thermo Fisher Scientific). Cell morphology was then used to determine cycle phase with *metestrus* characterized by a mix of lymphocytes, cornified cells and epithelial nucleated cells, *diestrus* by lymphocytes and a little to any epithelial nucleated cells, *proestrus* by nucleated cells that form sheets, and *estrus* by masses of large cornified cells that lack nuclei (see Fig. 2A). Body weight, food intake, precopulatory and copulatory behaviors (such as ear wiggling, darting, and lordosis) were also used to further verify the estrous cycle phase.

Behavioral training and testing:

For all behavioral studies, rats were trained and tested hungry (chow removed from cages, 5-6 hrs prior to training/testing and replaced following training/testing). All training

and testing occurred in standard operant boxes (Med Associates, St. Albans City, VT) housed within sound attenuating chambers. Testing occurred at the same time each day, approximately 6-8 hours after the start of the dark cycle.

Instrumental procedures: To evaluate motivation for food in obesity-prone and obesity-resistant females, we used instrumental training followed by progressive ratio testing. First, rats underwent two magazine training sessions in which 20 sucrose pellets (45 mg TestDiet; cat. #1811251) were delivered into the food cup on a variable interval of 60 seconds (VI 60). Next, they were trained to press one lever (active) that resulted in the delivery of one sucrose pellet. Pressing a second lever (inactive) had not consequences but was recorded. No discrete cues were paired with pellet delivery. Left/right position of the active and inactive levers relative to the food cup was counterbalanced. Rats were trained in three sessions in which each response on the active lever resulted in delivery of a food pellet (i.e., fixed ratio 1 [FR1], 60min/session or until 50 pellets were earned). This was followed by three sessions in which five responses on the active lever were required to receive one food pellet (FR5, 60min/session or until 50 pellets were earned). Progressive ratio (PR) testing was then used to determine motivation to obtain food. During PR testing the number of lever presses required to obtain each subsequent sucrose pellet increased exponentially ($5e^{(0.2*\text{delivery}+1)}-5$; adapted from (Richardson and Roberts, 1996). The PR session ended when rats did not meet the next ratio requirement within 60 minutes (i.e., breakpoint). The number of active and inactive lever presses, pellets earned, and final break point achieved were recorded.

Pavlovian procedures: First rats underwent two magazine training sessions in which 20 sucrose pellets were delivered into the food cup on a VI 60 schedule. During Pavlovian conditioning sessions, one auditory cue (CS+; 2min) was paired with the delivery of 4 sucrose pellets (US), whereas a second auditory cue (CS-; 2min) was never paired with sucrose (CS+/CS- tone or white noise counterbalanced). Sucrose pellets were delivered on a VI of 20 seconds during CS+ presentation. A total of 4 CS+ and 4 CS- trials separated by a variable 5-min inter-trial-interval (ITI; range 2-7 min) were given per session (5-8 sessions, 60 min per session, 1 session/day). Subsequent testing was identical to initial conditioning except that no sucrose was given. Food cup entries during the ITI, CS+ and CS- periods were recorded throughout.

Electrophysiology:

Whole-cell patch clamp recordings of MSNs in the NAc core were conducted in naturally cycling obesity-prone and obesity-resistant rats (P70-P80) during the metestrus/diestrus or proestrus/estrus phases using established procedures (e.g., Oginsky et al., 2016). Briefly, rats were anesthetized with chloral hydrate (400mg/kg, i.p.) prior to slice preparation, brains were rapidly removed and placed in ice-cold oxygenated (95% O2 – 5% CO2) aCSF containing (in mM): 125 NaCl, 25 $NaHCO_3$, 12.5 glucose, 1.25 NaH_2PO_4, 3.5 KCl, 1 L-ascorbic acid, 0.5 $CaCl_2$, 3 $MgCl_2$, pH 7.4, 305 mOsm. A vibratome (Leica Biosystems, Buffalo Grove, IL, USA) was used to make 300 µm coronal slices containing the NAc. Before recording, slices were allowed to rest in oxygenated aCSF for at least 40 minutes at 35°C, followed by a 10-minute recovery time at room temperature. For the recording aCSF, $CaCl_2$ was increased to 2.5 mM and $MgCl_2$, was decreased to 1mM. Patch pipettes were pulled from 1.5 mm borosilicate glass capillaries (WPI, Sarasota, FL; 4-7 MΩ resistance) with a horizontal puller (model P97, Sutter Instruments, Novato, CA, USA) and filled with a solution containing (in mM): 130 K-gluconate, 10 KCl, 1 EGTA, 2 Mg^{2+}-ATP, 0.6 Na^{+}-GTP and 10 HEPES, pH 7.45, 285mOsm. Recordings were conducted in the presence of the $GABA_A$ receptor antagonist, picrotoxin (50 µM). MSNs in the NAc core were identified based on resting membrane potential and action potential firing in response to current injection (-200 to +200pA, 25pA increments, 500ms). For data analysis, only cells with a leak of less than 50pA and access resistance of less than 30pA were used. These cell parameters were recorded before at the start and end of data collection and only cells with less than 20% change between the start and end of recordings were included in analyses.

I/V relationships were determined by calculating the difference between the baseline voltage and the voltage 200ms after initial current injections. Input resistance was determined by the change in voltage from -50pA to +50pA current injections. The number of action potentials elicited by each depolarizing current injection were used to determine neuronal excitability. Rheobase was defined as the minimum amount of current injection to elicit an action potential. The maximum second derivative method (Sekerli et al., 2004) was used to determine the action potential threshold.

Ovariectomy and hormone treatment:

Rats were bilaterally ovariectomized under isoflurane anesthesia (2.5-5%, inhalation) via a single back incision. Before surgery rats were given the analgesic Carprofen (3mg/kg, s.c.; Rimadyl®, Henry Schein). The incision was closed using dissolvable sutures internally (Reli Redi Gut Chromic Suture), and 9 mm wound clips externally (Reflex 9 mm wound clips, VWR international). In addition, carprofen was given again 48 hours after surgery. Rats were allowed to recover for at least 10 days prior to any behavioral testing. Vaginal lavages were taken beginning 7 days after surgery and throughout the remainder of the study.

The effect of three different hormone treatments was evaluated. Hormone treatment consisted of subcutaneous injections of 17β-estradiol benzoate (estradiol; Sigma-Aldrich, E8515) for two consecutive days (5 µg in 0.1ml of peanut oil, 24 hrs apart), followed by a single progesterone injection on the third day (500mg in 0.1ml of peanut oil, Sigma-Aldrich, P0130). Doses and treatment regimen were based on (Cummings & Becker, 2012 and Yu et. al., 2011). For repeated hormone treatment this cycle was repeated 3 or 4 times (see additional details below). Controls were given the same number of vehicle injections (0.1ml of peanut oil, s.c.). For studies of estradiol or progesterone alone, the same total number of injections were given, with progesterone or estradiol injection replaced by vehicle as appropriate. Finally, for all studies involving progesterone, the final progesterone injection was given 4-6 hrs prior to testing (see also timelines in Figs. 6A and 7A, D).

Statistics and data analysis:

Two-tailed t-tests, two-way or three-way ANOVAs and Sidak's post-hoc multiple comparisons were used (Prism 8, GraphPad, San Diego, CA). Electrophysiology data were analyzed using both Clampfit 10.4 (Molecular Devices) and MATLAB (R2018b, Mathworks Software).

Experimental Design

Experiment 1: Verification of female obesity-prone and obesity-resistant phenotypes and effects of the cycle on food intake and motivation for food.

We first verified differences in body weight, adiposity and food intake between obesity-prone (OP) and obesity-resistant (OR) females and evaluated the effects of the cycle on food intake and motivation for food (Levin & Govek, 1998). Rats were maintained on either standard lab chow (Lab Diet 5001: 4kcal/g; 4.5% fat, 23% protein, 48.7% carbohydrates; % of caloric content) or a "junk-food" diet (JF) made in house (Robinson et al., 2015; Oginsky et al., 2016). The junk-food diet was a mash of Ruffle potato chips (40g), Chips Ahoy (130g), Nesquik (130g), Jiff peanut butter (130g), Lab Diet 5001 (200g) and 180ml of water (19.6% fat, 14% protein, and 58% carbohydrates; 4.5 kcal/g). Before junk-food diet exposure, body composition was determined by nuclear magnetic resonance spectroscopy (NMR; Minispec LF90II, Bruker Optics) conducted by the University of Michigan Animal Phenotyping Core in female obesity-prone (n = 16) and obesity-resistant (n = 16) rats. The rats were then assigned to chow or junk-food groups (counter balanced by initial weight and fat mass within obesity-prone and obesity-resistant groups) in order to verify weight-gain phenotypes (n = 8 per group: OP-chow, OP-JF, OR-chow, and OR-JF). Food intake and body weight were monitored throughout the 4 weeks of junk-food vs. chow consumption and NMR measures were made again at the end of this period.

In a separate set of rats, we determined how food intake varied across the estrous cycle in naturally-cycling obesity-prone and obesity-resistant rats. Home cage food intake, body weight, and estrous cycle phase were measured for 13 days in singly-housed naturally cycling obesity-prone (n = 6) and obesity-resistant (n = 6) rats and estrous cycle phase was determined as described above. Finally, we also determined estrous cycle effects on motivation to work for food using progressive ratio testing. Naturally-cycling obesity-prone (n = 10) and obesity-resistant (n = 10) rats were trained to lever press for a sucrose pellet and motivation to obtain this food was then determined using the progressive ratio procedure described above. In order to determine potential differences in break point across the cycle, all rats were given 8 PR test sessions and data were analyzed according

to cycle phase during testing. This allowed us to make within subject comparisons of motivation to work for food during metestrus/diestrus vs. proestrus/estrus phases.

Experiment 2: Differences in NAc core MSN intrinsic excitability between obesity-prone and obesity-resistant female rats.

Potential differences in intrinsic excitability of MSNs in the NAc core between obesity-prone and obesity-resistant rats were determined in naturally cycling females. Estrous cycle phase was monitored for 5-7 days as described above and reconfirmed 1 hour before slice preparation and electrophysiological recordings (OP-M+D n = 6 rats, 22 cells; OP-P+E n = 5 rats, 13 cells; OR-M+D n = 5 rats, 13 cells; OR-P+E n = 5 rats, 15 cells).

Experiment 3: Modulation of cue-triggered motivation by the estrous cycle in obesity-prone and obesity-resistant rats.

We determined whether there were differences in cue-triggered motivation (as measured by conditioned approach) between obesity-prone and obesity-resistant rats and the role of the estrous cycle in modulating this behavior. Naturally-cycling obesity-prone (n = 31) and obesity-resistant (n = 31) rats underwent 5 sessions of Pavlovian training followed by a single session of testing in extinction conditions. Vaginal lavages were taken after the end of each session to confirm cycle phase on the day of testing.

Experiment 4: Effects of single and repeated estradiol-progesterone treatment on cue-triggered motivation in obesity-prone female rats.

Here we determined the effect of estradiol and progesterone treatment on cue-triggered motivation in obesity-prone rats (obesity-resistant rats were not included because there were no effects of the cycle on cue-triggered motivation in this group, see results below). Naturally cycling obesity-prone rats (n = 20) underwent 8 sessions of Pavlovian training followed by ovariectomy and 10 days of recovery as described above. Some rats were then given one cycle of estradiol and progesterone treatment (see above) followed by a single test in extinction conditions (n = 10), while others received vehicle treatment prior to the extinction test. Next, rats in the vehicle group were treated with 3 cycles of hormone treatment while the rats that initially received one cycle of treatment were given repeated vehicle injections before undergoing an additional test in extinction conditions.

Experiment 5: Effects of repeated hormone treatment on cue-triggered motivation in outbred female rats.

Here we determined whether repeated hormone treatment with both estradiol and progesterone, or repeated treatment with each hormone alone were sufficient to alter cue-triggered motivation in outbred Sprague Dawley female rats. To examine the effects of dual hormone treatment, naturally cycling outbred rats (n = 20) were trained and ovariectomized as described above. After recovery from surgery, they were assigned to vehicle (n = 10) or repeated hormone treatment (n = 10) groups, counterbalanced by post-operative weight. They received 4 consecutive cycles of either vehicle or hormone treatment followed by a single extinction test (see Fig. 7A).

To assess the effects of each hormone on its own, a separate set of naturally-cycling outbred rats (n = 40) underwent 8 sessions of Pavlovian training followed by ovariectomy and recovery as described above; 2 rats were excluded from studies due to complications during ovariectomy surgery. Rats were then assigned to vehicle (Veh; n = 9), estradiol alone (E; n = 10), progesterone alone (P; n = 10) or estradiol and progesterone treated groups (E+P; n = 9), counterbalanced by post-operative weight. Body weight and food intake were measured throughout the hormone injection cycles.

Results

Verification of female obesity-prone and obesity-resistant phenotypes.

Figure 2.1 shows weight, adiposity and food intake of obesity-prone and obesity-resistant female rats at baseline (Fig. 2.1A-C) and after 4 weeks of junk-food exposure (Fig. 2.1D-F). Although body weight was similar between groups (Fig. 2.1A), fat mass was significantly greater in female obesity-prone vs. obesity-resistant groups at baseline (Fig. 2.1B: $t_{30} = 3.593$, $p < 0.01$). This was accompanied by significantly lower lean mass in obesity-prone vs. obesity-resistant groups (Fig. 2.1C: $t_{30} = 3.642$, $p < 0.01$). After 4 weeks of free access to junk-food or chow, obesity-prone rats remained heavier than obesity-resistant rats (Fig. 2.1D: Two-way ANOVA main effect of group: $F_{(1,28)} = 9.05$, $p < 0.01$, no diet x group interaction). Measurements of adiposity revealed that junk-food consumption significantly increased fat mass in both groups (Fig. 2.1E: Two-way ANOVA main effect of diet: $F_{(1, 28)} = 18.09$, $p < 0.001$) and reduced lean mass (Fig. 2.1F: Two-way ANOVA main effect of diet: $F_{(1, 28)} = 9.78$, $p < 0.01$) compared to chow-fed groups. However, as expected, the effect of junk-food on fat mass was more pronounced in obesity-prone groups (Fig. 2.1E: Average change from chow OP: 16.3 ± 4.2; OR: 9.4 ± 1.8). Figure 2.1G shows food intake during the 4-week diet manipulation. As expected, junk-food consumption was greater than chow consumption in both groups, with obesity-prone rats consuming more junk-food than obesity-resistant rats (Fig. 2.1G: Three-way ANOVA main effect of time: $F_{(3,36)} = 54.07$, $p < 0.0001$; main effect of diet: $F_{(1,12)} = 22.5$, $p = 0.0005$; main effect of group: $F_{(1,12)} = 4.33$, $p = 0.06$; and group x diet x time interaction: $F_{(3, 36)} = 3.31$, $p = 0.03$). In addition, chow consumption was similar between obesity-prone and obesity-resistant (Fig. 2.1G, open symbols) even though obesity-prone rats were heavier and had more fat mass than obesity-resistant rats. These data are consistent with previous reports in males (Vollbrecht et al., 2015) and confirm obesity-prone and obesity-resistant phenotypes in females.

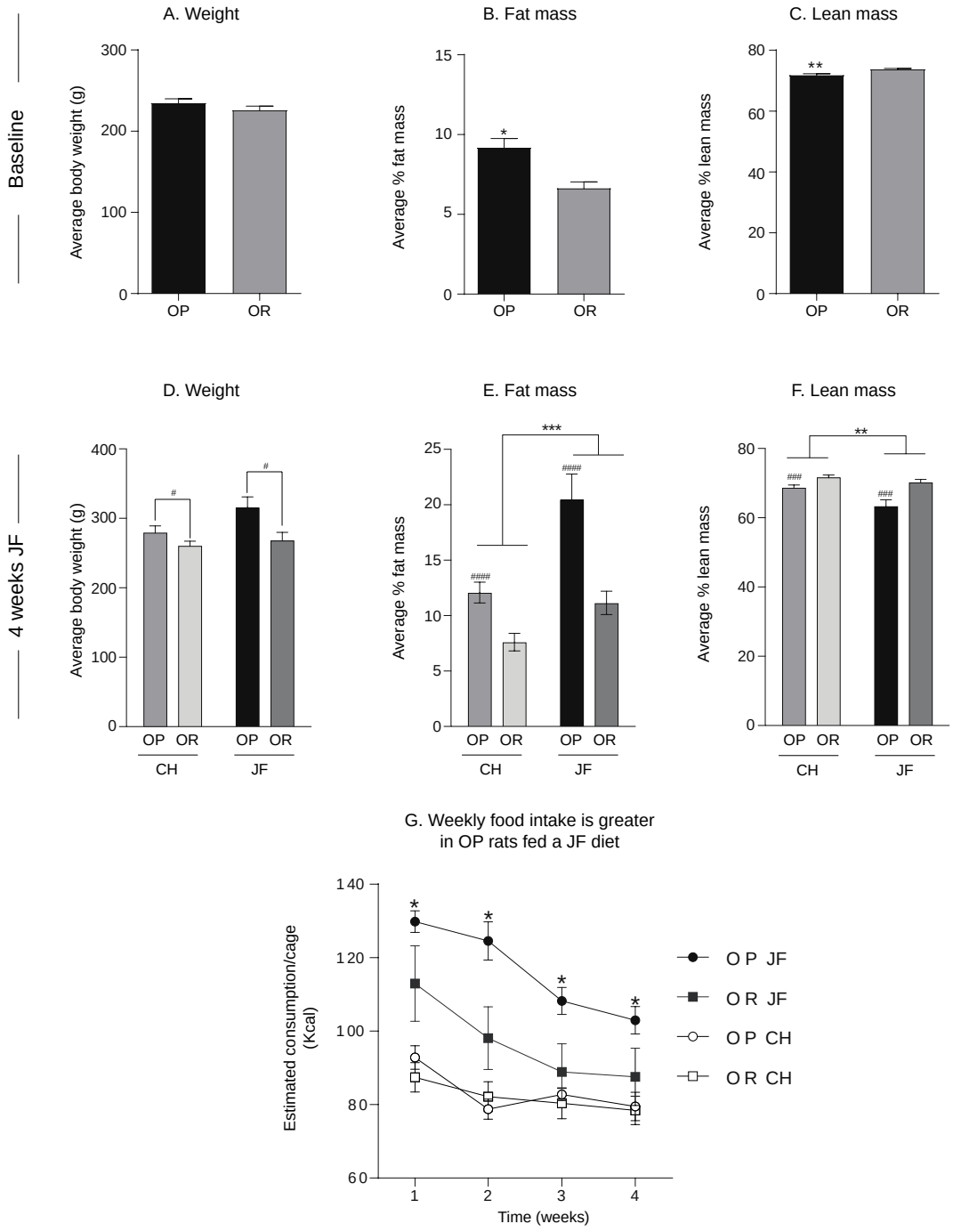


Figure 2.1. Verification of female obesity-prone and obesity-resistant phenotype. A-C) Weight and adiposity measures at baseline: Although body weight is similar between groups, obesity-prone rats (OP) have greater fat mass and lower lean mass vs. obesity-resistant (OR) rats. **D-F)** Weight and adiposity measures after 4 weeks of junk-food diet (JF) or chow (CH) consumption: Obesity-prone rats remain heavier than obesity-resistant rats, and have greater fat mass and lower lean mass vs. obesity-resistant rats. Junk-food increases fat mass and reduces lean mass in both groups, but the magnitude of this effect is stronger in obesity-prone rats. **G)** Home cage food intake during 4 weeks of junk-food or chow consumption: Junk-food consumption was greater than chow consumption in both groups, with obesity-prone rats consuming more junk-food than obesity-resistant rats. All data are shown as mean ±SEM unless otherwise noted. # = differences between obesity-prone and obesity-resistant rats, * = differences between chow and junk-food, $p < 0.05$.

Food intake and motivation to obtain sucrose are reduced during estrus in obesity-prone and obesity-resistant females

Home cage food intake and responding during progressive ratio testing were examined in each phase of the cycle in naturally cycling females. Figure 2.2A shows example images capturing cell cytology distinctive of each phase of the cycle (20x magnification). Consistent with the existing literature, home cage food intake was reduced during the estrus phase compared to other phases of the cycle in both groups (Fig. 2.2B: Two-way RM ANOVA main effect of estrous phase $F_{(3,30)} = 8.34$, $p < 0.001$; planned Sidak's multiple comparisons proestrus vs. estrus, $p < 0.05$). In addition, food intake was again greater in obesity-prone vs. obesity-resistant rats, regardless of estrous phase (Fig. 2.2B: Two-way RM ANOVA main effect of group: $F_{(1,10)} = 28.40$, $p = 0.0003$). Importantly, there were no differences in number of cycles completed between obesity-prone vs. obesity-resistant groups across 13 days (data not shown).

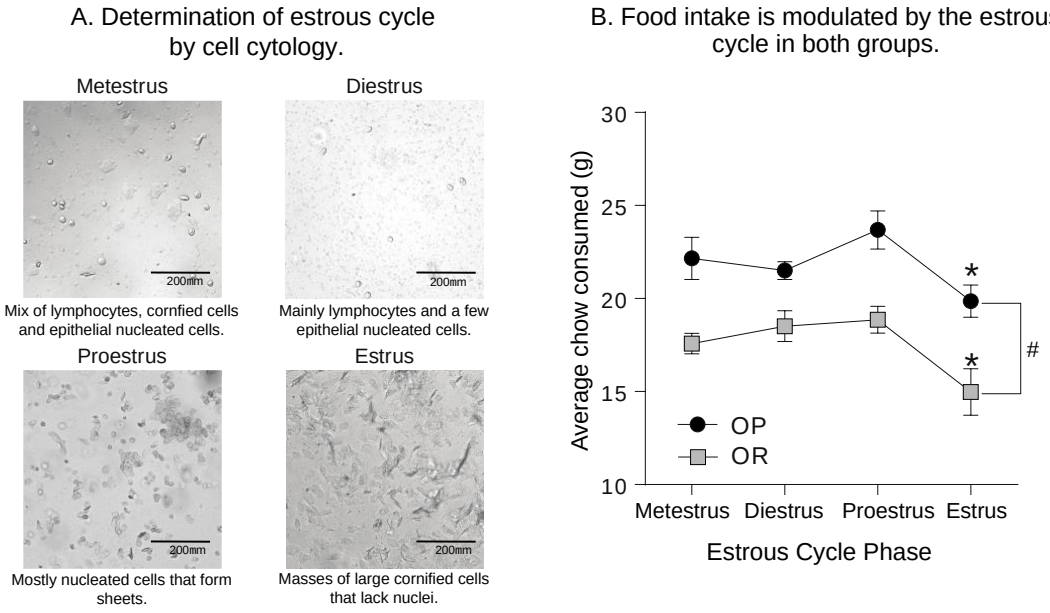


Figure 2.2. Home cage food intake decreases during estrus in both obesity-prone and obesity-resistant female rats. A) Representative pictures from vaginal lavages at each phase of the estrous cycle in female rats. **B)** Home cage chow consumption across the cycle. Home-cage chow consumption was greater in obesity-prone (OP) vs. obesity-resistant (OR) rats, and was reduced during estrus in both groups. * = planned post-hoc comparisons, # = obesity-prone vs. obesity-resistant rats, $p < 0.05$.

We next determined how motivation to obtain sucrose changes across the cycle. Figure 2.3 shows behavior during initial fixed ratio training (Fig. 2.3A, B) and subsequent progressive ratio testing (Fig. 2.3C). Acquisition of instrumental responding for sucrose was similar between groups, with all rats preferentially responding on the active vs. inactive lever during FR1 (Fig. 2.3A: Three-way ANOVA main effect of lever $F_{(1,108)} = 0.08$, $p < 0.0001$) and FR5 training (Fig. 2.3B: Three-way ANOVA main effect of lever $F_{(1,108)} = 137.9$, $p < 0.0001$). In addition, the magnitude of responding was similar between obesity-prone and obesity-resistant groups. Progressive ratio testing was conducted across 8 consecutive days and analyzed by averaging breakpoints within subjects (OP n = 10; OR n = 10) tested during metestrus/diestrus and proestrus/estrus. Break point was significantly lower during proestrus/estrus vs. metestrus/diestrus in both obesity-prone and obesity-resistant groups (Fig. 2.3C: Two-way RM ANOVA main effect of estrous cycle phase: $F_{(1,18)} = 12.05$, $p < 0.01$). Although some visual trends were present, no significant differences in average breakpoint between obesity-prone and obesity-resistant groups were observed (Fig. 2.3C: Two-way RM ANOVA effect of group: $F_{(1,18)} = 2.0$, $p = 0.2$). These data show that despite the differences in home cage food intake between obesity-prone and obesity-resistant rats, motivation to obtain sucrose pellets is similar between groups. Additionally, the estrous cycle affects motivation to obtain food and food intake similarly in both groups.

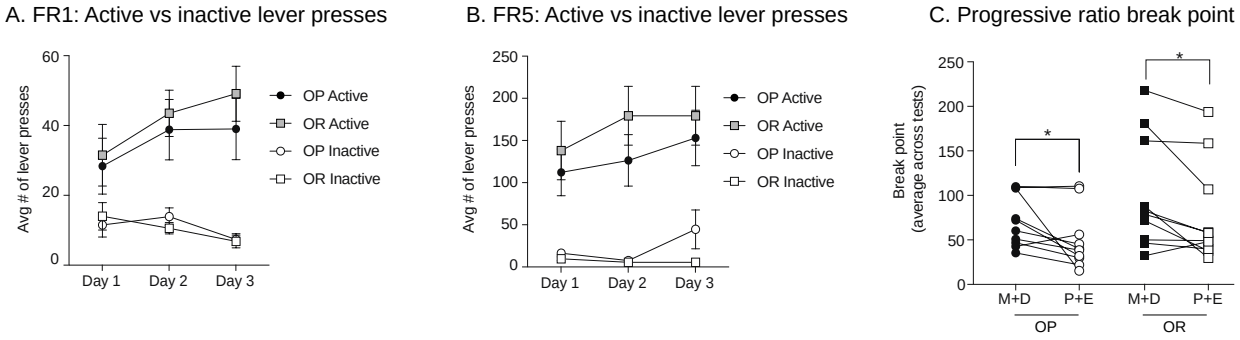
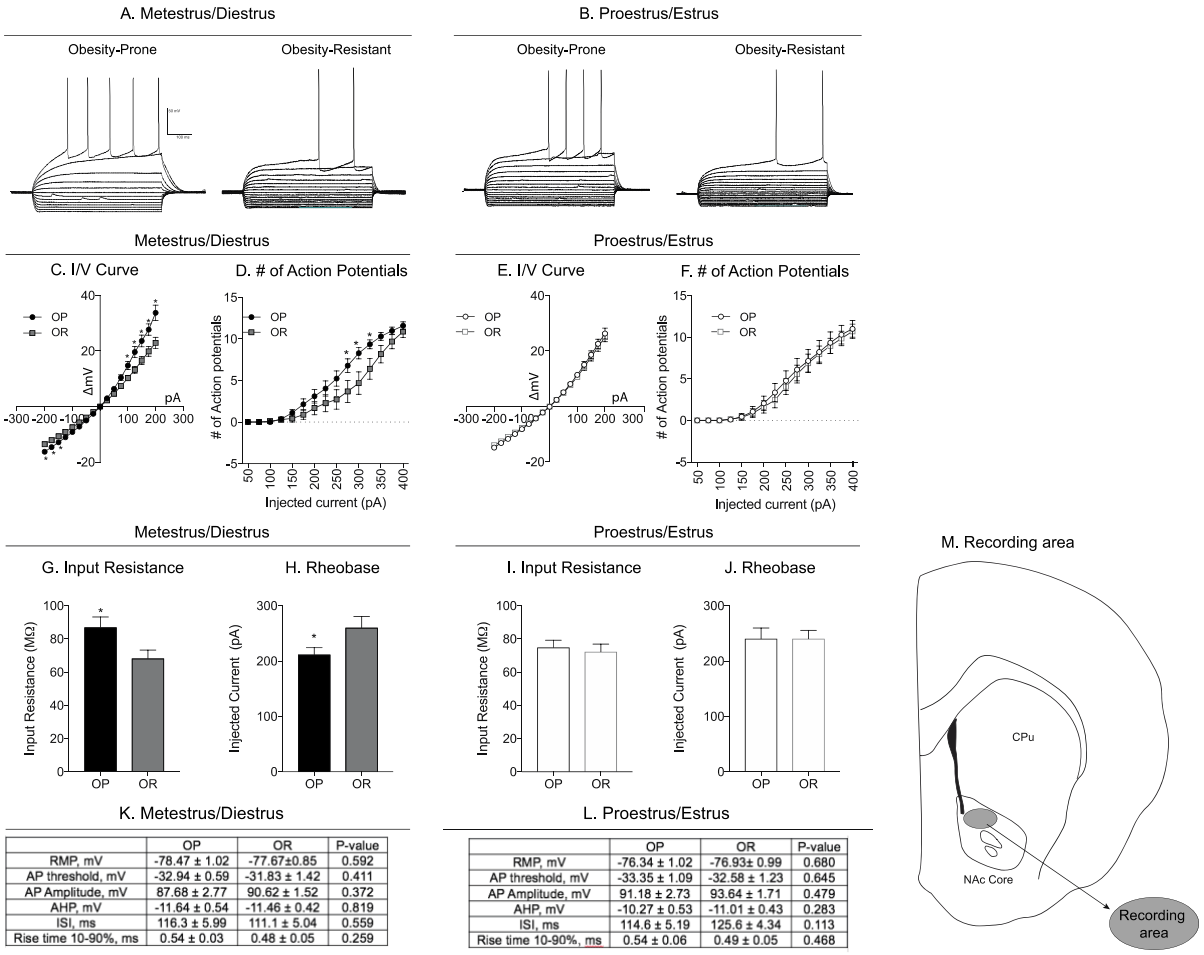


Figure 2.3. Motivation to work for a sucrose pellet decreases during proestrus/estrus in both obesity-prone and obesity-resistant rats. A, B) Average number of active and inactive lever presses across training. Acquisition of instrumental responding for sucrose was similar between groups. All rats preferentially responded on the active vs. inactive lever during fixed ratio 1 (FR1) and fixed ratio 5 (FR5) sessions. **C)** Average break point reached during progressive ratio testing. Break point was significantly lower during proestrus/estrus (P+E) vs. metestrus/diestrus (M+D) in both groups. No differences between obesity-prone (OP) and obesity-resistant (OR) groups were observed; * = $p < 0.05$.

NAc core MSN intrinsic excitability is enhanced in obesity-prone vs. obesity-resistant rats during metestrus/diestrus.

Recordings were made in both obesity-prone and obesity-resistant rats in metestrus/diestrus (Fig. 2.4 left graphs: OP n = 22 cells from 6 rats; OR n = 13 from 5 rats) or proestrus/estrus (Fig. 2.4 right graphs: OP n = 13 cells from 5 rats; OR n = 15 cells from 5 rats) to examine differences in MSN intrinsic excitability in the NAc core between groups and across the estrous cycle. Consistent with our previous results in males (Oginsky et al., 2016), the I/V curved showed a greater change in membrane potential in response to positive and negative current injections in MSNs from obesity-prone vs. obesity-resistant groups (Fig. 2.4C: Two-way RM ANOVA group x current injection interaction: $F_{(16,528)} = 6.348$, $p < 0.0001$; main effect of group $F_{(1,33)} = 5.683$, $p = 0.02$; main effect of current injection $F_{(16,528)} = 263.9$, $p < 0.0001$; Sidak's multiple comparison $p < 0.05$). Similarly, during metestrus/diestrus, the number of action potentials elicited across current injections was greater in obesity-prone vs. obesity-resistant groups (Fig. 2.4D: Two-way RM ANOVA group x current injection interaction $F_{(14,462)} = 3.04$, $p = 0.0002$; main effect of current injection $F_{(14,462)} = 129.1$, $p < 0.0001$; main effect of group $F_{(1,33)} = 3.67$, $p = 0.06$; Sidak's multiple comparison $p < 0.05$). In contrast, when comparisons were made during the proestrus/estrus phases, I/V relationships and the number of action potentials elicited by current injection were similar between groups (Fig. 2.4E, F). Consistent with group differences found during metestrus/diestrus, input resistance was greater in obesity-prone rats (Fig. 2.4G: Two-tailed unpaired t-test, $t_{(33)} = 2.09$, $p = 0.04$) and rheobase was reduced (Fig. 2.4H: Two-tailed unpaired t-test, $t_{(33)} = 2.15$, $p = 0.04$) compared to obesity-resistant rats during metestrus/diestrus. However, these group differences were absent when comparison were made during the proestrus/estrus phases (Fig. 2.4I, J). We did not observe differences in basal cell parameters including resting membrane potential (RMP), action potential threshold, amplitude, rise time (10-90%), after-hyperpolarization (AHP) amplitude, or duration of the first inter-spike interval between recordings made during metestrus/diestrus in obesity-prone and obesity-resistant rats (Fig. 2.4K) or proestrus/estrus (Fig. 2.4L) phases. In sum, these data show that similar to male obesity-prone rats, basal intrinsic excitability is enhanced in female obesity-prone vs. obesity-

resistant rats, but these differences are only apparent when recordings are made during the metestrus/diestrus phase of the estrous cycle.



K. Metestrus/Diestrus

	OP	OR	P-value
RMP, mV	-78.47 ± 1.02	-77.67±0.85	0.592
AP threshold, mV	-32.94 ± 0.59	-31.83 ± 1.42	0.411
AP Amplitude, mV	87.68 ± 2.77	90.62 ± 1.52	0.372
AHP, mV	-11.64 ± 0.54	-11.46 ± 0.42	0.819
ISI, ms	116.3 ± 5.99	111.1 ± 5.04	0.559
Rise time 10-90%, ms	0.54 ± 0.03	0.48 ± 0.05	0.259

L. Proestrus/Estrus

	OP	OR	P-value
RMP, mV	-76.34 ± 1.02	-76.93± 0.99	0.680
AP threshold, mV	-33.35 ± 1.09	-32.58 ± 1.23	0.645
AP Amplitude, mV	91.18 ± 2.73	93.64 ± 1.71	0.479
AHP, mV	-10.27 ± 0.53	-11.01 ± 0.43	0.283
ISI, ms	114.6 ± 5.19	125.6 ± 4.34	0.113
Rise time 10-90%, ms	0.54 ± 0.06	0.49 ± 0.05	0.468

Figure 2.4. NAc core MSN intrinsic excitability is enhanced in obesity-prone vs. obesity-resistant rats during metestrus/diestrus. A) Example traces of current-clamp recordings from MSNs in slices from obesity-prone (OP) and obesity-resistant (OR) rats prepared during metestrus/diestrus. **B)** Example traces of current-clamp recordings from MSNs in slices from obesity-prone and obesity-resistant rats prepared during proestrus/estrus. **C, D)** Current/voltage (I/V) relationship and number of action potentials in obesity-prone and obesity-resistant from slices made during metestrus/diestrus. I/V relationships are shifted to the right in in obesity-prone vs. obesity-resistant rats and the same current injection intensity elicits more action potentials in MSNs from obesity-prone vs. obesity-resistant rats. **E, F)** Current/voltage (I/V) relationship and number of action potentials in obesity-prone and obesity-resistant from slices made during proestrus/estrus. I/V relationships and the number of action potentials fired are similar between obesity-prone and obesity-resistant rats in proestrus/estrus. **G, H)** Input resistance and rheobase: Obesity-prone rats have a higher input resistance and lower rheobase vs. obesity-resistant rats. **I, J)** Input resistance and rheobase during proestrus/estrus: No differences were observed in input resistance or rheobase between obesity-prone and obesity-resistant rats. **K, L)** Cell parameters from recordings conducted in slices prepared during metestrus/diestrus and proestrus/estrus. No differences in resting membrane potential (RMP), action potential threshold, action potential amplitude (AP), afterhyperpolarization amplitude (AHP), inter-spike interval (ISI) or rise time were observed between groups or cycle phase metestrus/diestrus or proestrus/estrus; * = $p < 0.05$. **M)** Map of NAc core MSN recording area.

Cue-triggered motivation is modulated by the estrous cycle in obesity-prone, but not obesity-resistant rats.

Here, we determined how cue-triggered motivation in the form of conditioned approach varies across the cycle in naturally cycling obesity-prone and obesity-resistant rats. Figure 2.5 shows behavior during initial conditioning (Fig. 2.5A-C) and subsequent testing in extinction conditions (Fig. 2.5D). Behavior during initial Pavlovian conditioning was similar between groups, with both obesity-prone and obesity-resistant rats acquiring a similar magnitude of discrimination between the CS+ and CS- (Fig. 2.5A OP: Two-way RM ANOVA: main effect of CS $F_{(1,30)} = 9.79$ $p < 0.01$; Fig. 2.5B OR: Two-way RM ANOVA: main effect of CS $F_{(1,30)} = p < 0.01$; Fig. 2.5C OP vs. OR: main effect of session: $F_{(4,240)} = 4.98$, $p < 0.001$; no effect of group $F_{(1,60)} = 0.17$, $p = 0.7$; no group x session interaction $F_{(4,120)} = 1.78$, $p = 0.14$). Rats were then tested for conditioned approach in extinction conditions and data were analyzed according to which phase of the cycle rats were in during testing (Fig. 2.5D). The CS+ elicited strong conditioned approach in all groups (Fig. 2.5D: Two-way RM ANOVA period (ITI, CS+,CS-) x group interaction $F_{(6,116)} = 3.1$, $p = 0.007$; main effect of group $F_{(3,58)} = 3.85$, $p = 0.01$; main effect of period $F_{(2,116)} = 222.7$, $p < 0.0001$; Sidak's multiple comparison: CS+ vs. ITI and CS+ vs. CS-, $P < 0.0001$). However, the magnitude of conditioned approach in response to the CS+ was significantly greater in obesity-prone rats tested during metestrus/diestrus compared to all other groups (Fig. 2.5D; Sidak's multiple comparison, $p < 0.05$). Furthermore, differences in CS+ responding between obesity-prone and obesity-resistant groups were driven entirely by enhanced approach behavior during metestrus/diestrus vs. proestrus/estrus in obesity-prone, but not obesity-resistant rats (Fig. 2.5D: Sidak's multiple comparison CS+: OP-M+D vs. OP-P+E, $p = 0.0001$; CS+: OR-M+D vs. OR- P+E, $p = 0.73$). No significant differences in responding during the ITI or CS- were found between groups or across cycle phase. Thus, in obesity-prone, but not obesity resistant rats, cue-triggered motivation varied across the cycle and was stronger in obesity-prone vs. obesity-resistant rats tested in metestrus/diestrus phases. Taken with results from progressive ratio testing above, data show a dissociation between the ability of the cycle to influence motivation for food (Fig. 2.3) but not motivation triggered by a food cue in obesity-resistant rats (Fig. 2.5D).

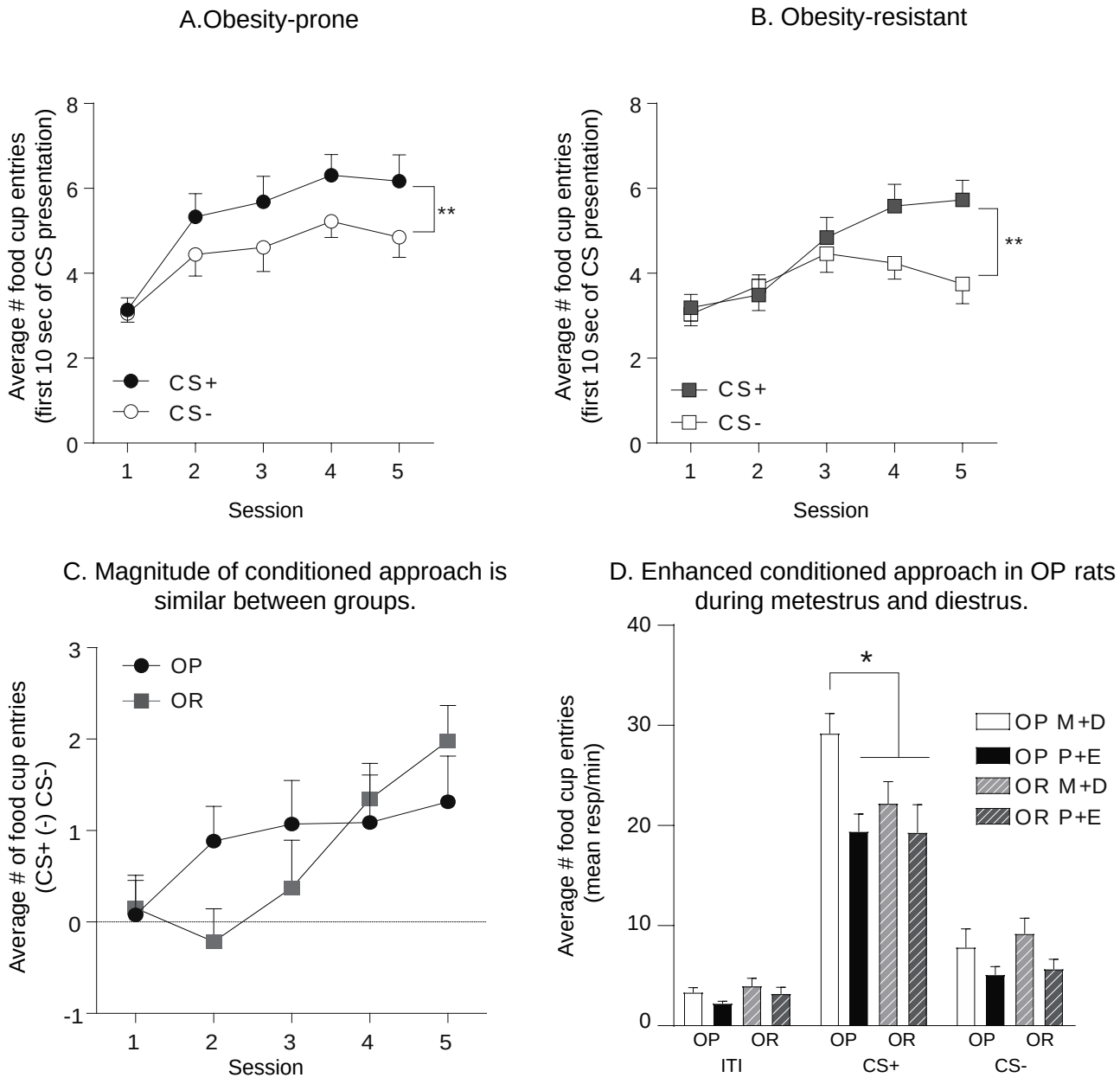


Figure 2.5. Cue-triggered motivation is modulated by the estrous cycle in obesity-prone but not in obesity-resistant rats. A) Average number of food cup entries during the first 10 seconds of CS presentation in obesity-prone rats (OP). Obesity-prone rats discriminate between the CS+ and the CS- during initial Pavlovian conditioning. **B)** Average number of food cup entries during the first 10 seconds of CS presentation in obesity-resistant rats (OR). Obesity-resistant rats discriminate between the CS+ and the CS- during initial Pavlovian conditioning. **C)** Average difference in the number of food cup entries during the first 10 sec of CS+ vs. CS- presentation. No difference in magnitude of conditioned approach between obesity-prone and obesity-resistant rats were observed. **D)** Extinction Test: Average number of food cup entries during the CS+, CS- and inter-trial interval (ITI) in obesity-prone and obesity-resistant rats tested in metestrus/diestrus (M+D) and proestrus/estrus (P+E). Conditioned approach is stronger during metestrus/diestrus vs proestrus/estrus in obesity-prone, but not obesity-resistant rats. In addition, the magnitude of conditioned approach is greater in obesity-prone vs. obesity-resistant rats tested during the metestrus/diestrus phase. * = $p < 0.05$, ** = $p < 0.01$.

Repeated treatment with estradiol and progesterone is sufficient to decrease cue-triggered motivation in obesity-prone rats.

Given that the estrous cycle modulates cue-triggered motivation in obesity-prone but not obesity-resistant rats (Exp. 3), we next determined whether single vs. repeated treatment with estradiol and progesterone in ovariectomized rats is sufficient to replicate this effect in obesity-prone rats (Fig. 2.6A: experimental timeline). As expected, obesity-prone rats learned to discriminate between the CS+ and the CS- during Pavlovian training (Fig. 2.6B: Two-way RM ANOVA main effect of CS $F_{(1,19)} = 25.6$, $p < 0.0001$; significant session x CS interaction $F_{(7,133)} = 3.74$, $p = 0.001$; Sidak's post-test $p < 0.05$). A single cycle of estradiol and progesterone treatment was not sufficient to alter conditioned approach behavior compared to vehicle treated controls (Fig. 2.6C). However, repeated estradiol and progesterone treatment (repeated E+P), decreased conditioned approach compared to controls (Fig. 2.6D: Two-way RM ANOVA; main effect of treatment, $p < 0.05$; significant period (ITI, CS+ and CS-) x treatment interaction, $p < 0.05$; Sidak's multiple comparisons CS+: vehicle vs. hormone treatment, $p = 0.002$). It should be noted that the same rats were tested twice (see timeline Fig. 2.6A). Thus, it is not surprising that the magnitude of conditioned approach was lower overall during the second test conducted under extinction conditions (Fig. 2.6D vs. C). Nonetheless, these data show that single hormone treatment does not affect conditioned approach, whereas repeated hormone treatment reduces it in obesity-prone rats.

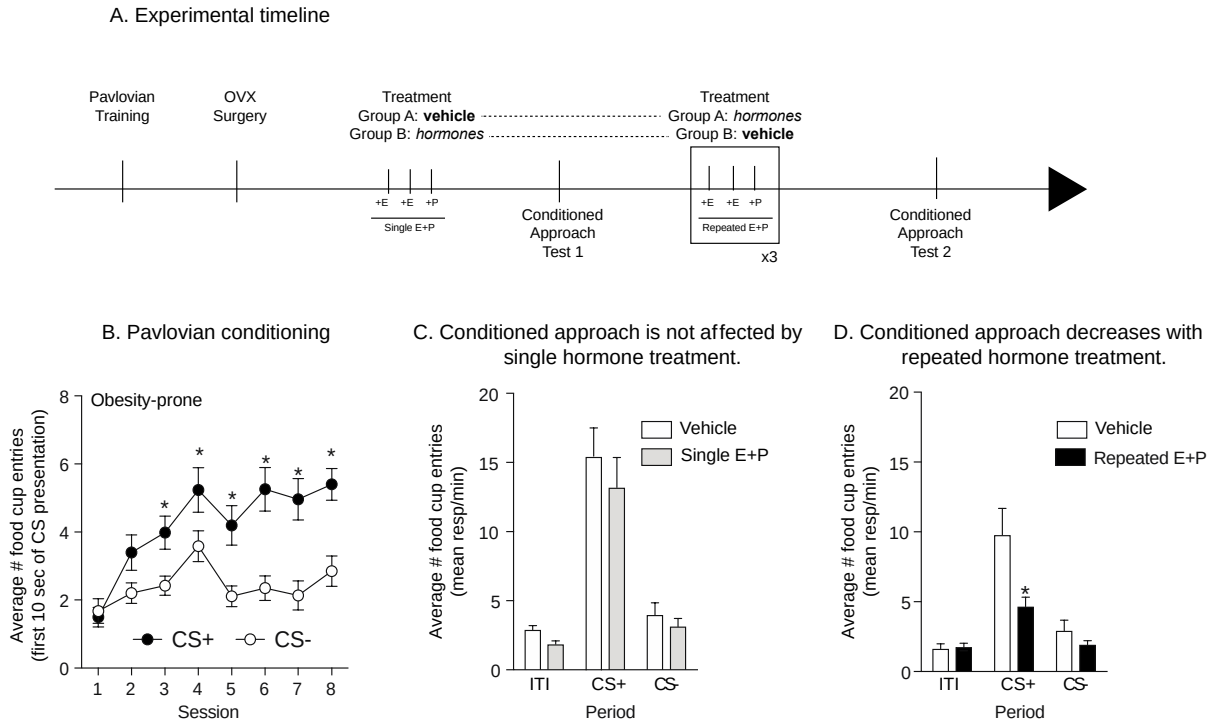


Figure 2.6. Repeated treatment with estradiol and progesterone decreased conditioned approach in obesity-prone females. A) Schematic of experimental timeline. Rats received 8 sessions of Pavlovian conditioning, followed by ovariectomy surgery (OVX) and 10 days of recovery before being treated with estradiol (+E) and progesterone (+P) or vehicle. 4-6 hours after the last progesterone injection rats were tested in extinction conditions. **B)** Average number of food cup entries during the first 10 seconds of CS presentation in obesity-prone rats (OP). Obesity-prone rats learned to discriminate between the CS+ and CS-. **C)** Average number of food cup entries during the CS+, CS- and inter-trial interval (ITI) in vehicle and hormone treated groups. A single cycle of hormone treatment is not sufficient to decrease conditioned approach. **D)** Average number of food cup entries during the CS+, CS- and inter-trial interval (ITI) in vehicle and hormone treated groups. Repeated estradiol and progesterone treatment decreased conditioned approach compared to vehicle treated controls, although repeated testing reduced the magnitude of conditioned approach in both treatment groups. * = $p < 0.05$.

Repeated treatment with estradiol and progesterone is sufficient to decrease cue-triggered motivation in outbred rats.

Here, we determined if the decrease in cue-triggered motivation with repeated E+P observed in ovariectomized obesity-prone rats also extend to ovariectomized outbred Sprague-Dawley females (see Fig. 2.7A for experiment timeline). As expected, outbred females learned to discriminate between the CS+ and the CS- during Pavlovian conditioning (Fig. 2.7B: Two-way RM ANOVA: main effect of CS $F_{(1,19)} = 13.83$, $p = 0.002$; significant session x CS interaction $F_{(7,133)} = 7.78$, $p < 0.0001$, Sidak's multiple comparisons $p < 0.05$). Consistent with effects in selectively-bred rats, repeated estradiol and progesterone treatment resulted in a decrease in conditioned approach behavior to

the CS+ (Fig. 2.7C: Two-way RM ANOVA main effect of treatment $p < 0.05$; significant period x treatment interaction $p = 0.05$; Sidak's multiple comparisons CS+: vehicle vs. hormone treated, $p < 0.05$). There was a slight effect of hormone treatment on food cup entries during the ITI (Fig. 2.7C; vehicle vs. hormone treated $p = 0.056$) but not during the CS- (Fig. 2.7C vehicle vs. hormone treated $p = 0.83$). However, when comparing across all three separate experiments (see below), there do not appear to be consistent effects of hormone replacement on responding during the ITI. In sum, data from outbred females replicate the primary effect of hormone treatment found in obesity-prone rats, supporting a role for ovarian hormones in reductions in conditioned approach behavior during metestrus/diestrus.

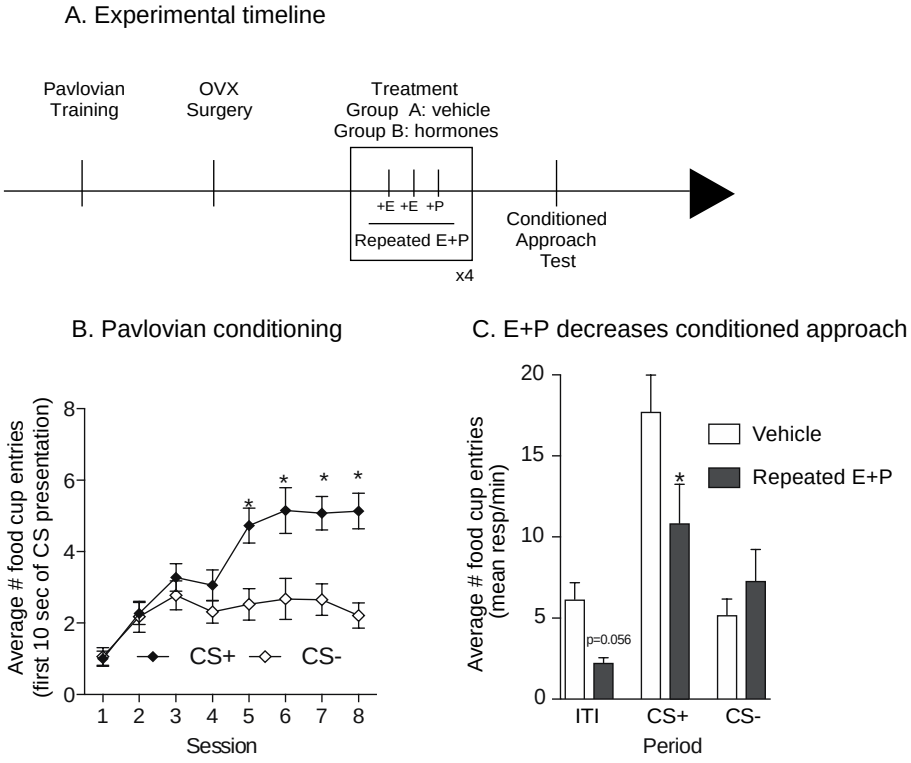


Figure 2.7. Repeated treatment with estradiol and progesterone decreased conditioned approach in outbred females. A) Schematic of experimental timeline. Rats received 8 sessions of Pavlovian conditioning, followed by ovariectomy surgery (OVX) and 10 days of recovery before being treated with estradiol (E) and progesterone (P) or vehicle. 4-6 hours after the last progesterone injection rats were tested in extinction conditions. **B)** Average number of food cup entries during the first 10 seconds of CS presentation. Outbred rats learned to discriminate between the CS+ and the CS-. **C)** Average number of food cup entries during the CS+, CS- and inter-trial interval (ITI) in vehicle and hormone treated groups. Four cycles of estradiol and progesterone treatment decreased conditioned approach in outbred rats. * = $p < 0.05$.

Estradiol and progesterone act synergistically to decrease cue-triggered motivation in outbred rats.

Above we demonstrate that repeated estradiol and progesterone treatment decreases cue-triggered motivation in both obesity-prone and outbred females. Here we evaluated the ability of repeated estradiol or progesterone alone to reduce cue-triggered motivation in outbred rats (see Fig. 2.8A for experimental timeline.). We also included an additional experimental group that was given repeated treatment with both hormones in order to replicate effects above in a separate group of rats. After acquisition of Pavlovian discrimination (Fig. 2.8B: main effect of CS $F_{(1,38)} = 26.9$, $p < 0.0001$; significant session x CS interaction $F_{(7,266)} = 6.29$, $p < 0.0001$, Sidak's multiple comparisons, $p < 0.05$), rats were separated into 4 treatment groups [vehicle (veh), estradiol and progesterone (E+P), estradiol alone (E) and progesterone alone (P)], counterbalanced for behavior during the last Pavlovian training session.

Throughout hormone treatment we measured body weight and food intake. We found that body weight and food intake were reduced in both estradiol treated groups (E and E+P; Fig. 2.8C body weight: Two-way RM ANOVA: main effect of injection cycle $F_{(4,144)} = 45.63$, $p < 0.0001$; main effect of group $F_{(3,36)} = 8.43$, $p = 0.0002$; significant interaction injection cycle x group $F_{(12,144)} = 45.21$, Sidak's multiple comparisons $p < 0.05$;Fig. 2.8D food intake: Two-way RM ANOVA: main effect of injection cycle $F_{(4,64)} = 70.14$, $p = <0.0001$; main effect of group $F_{(3,16)} = 17.88$, $p < 0.0001$; significant injection cycle x group $F_{(12,64)} = 3.73$, $p = 0.0003$, Sidak's multiple comparison, $p < 0.05$). Consistent with experiment 5a above, repeated E+P treatment significantly decreased conditioned approach to the CS+ (Fig. 2.8E: Two-way RM ANOVA significant period x treatment interaction $F_{(6,68)} = 2.6$; main effect of period $F_{(2,68)} = 62.5$, $p < 0.0001$; main effect of treatment $F_{(3,34)} = 5.4$, $p = 0.004$; Planned Sidak's multiple comparisons CS+: vehicle vs. E+P, $p = 0.0008$), with no effect of treatment on responding during the CS- or ITI (vehicle vs. E+P; p=0.84, 0.46 respectively). However, treatment with estradiol or progesterone alone were not sufficient to decrease conditioned approach in outbred rats (Fig. 2.8E: Sidak's multiple comparisons CS+: Veh vs. E, $p = 0.54$; Fig. 2.8E: Sidak's multiple comparisons CS+: Veh vs. P, $p = 0.84$). Together these data suggest a synergistic effect of repeated estradiol

and progesterone treatment on cue-triggered motivation, even though estradiol is sufficient to decrease home-cage food intake and body weight.

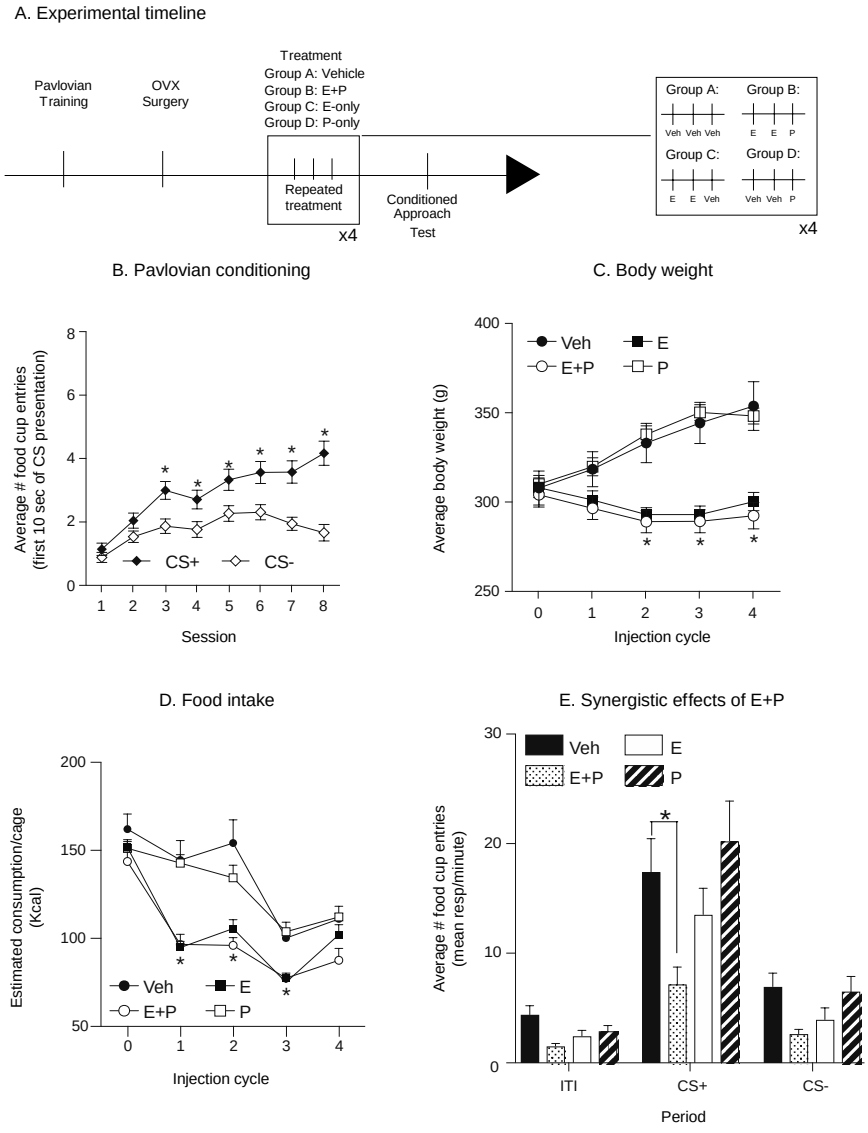


Figure 2.8. Estradiol and progesterone act synergistically to decrease cue-triggered motivation in outbred rats. A) Schematic of experimental timeline. Outbred rats received 8 sessions of Pavlovian conditioning, followed by ovariectomy surgery (OVX) and 10 days of recovery. Rats were the counterbalanced by weight and divided into 4 groups: vehicle, estradiol and progesterone (E+P), estradiol alone (E) and progesterone alone (P). **B)** Average number of food cup entries during the first 10 seconds of CS presentation. Outbred rats learned to discriminate between the CS+ and the CS-. **C, D)** Average body weight and food intake across the treatment period. Estradiol treatment (E or E+P) reduced body weight and food intake compared to the vehicle (Veh) and progesterone (P) groups. **E)** Average number of food cup entries during the CS+, CS- and inter-trial interval (ITI) in vehicle and hormone treated groups. Estradiol or progesterone alone did not affect conditioned approach. However, repeated estradiol and progesterone treatment decreased conditioned approach in outbred rats. Thus, although estradiol is sufficient to reduce weight and food intake, both estradiol and progesterone are needed to reduce conditioned approach. * = $p < 0.05$.

Discussion

Naturally occurring alterations in estradiol influence food intake in females. However, how motivational responses to food cues are affected by the estrous cycle or elevations in ovarian hormones is unknown. In addition, while individual susceptibility to obesity is accompanied by enhanced incentive motivational responses to food cues and increased intrinsic excitability of MSNs within the NAc of males, studies in females are lacking (see Introduction and Alonso-Caraballo et al., 2018). Here, we show that intrinsic excitability of NAc MSNs and conditioned approach behavior are enhanced in female obesity-prone vs. obesity-resistant rats when measured during metestrus/diestrus. However, neural and behavioral responses between these groups were similar when measured during proestrus/estrus. The emergence of group differences during metestrus/diestrus were due to the effects of the cycle on NAc excitability and cue-triggered motivation within obesity-prone, but not obesity-resistant rats. Additionally, we found that estradiol and progesterone treatment in ovariectomized females reduced conditioned approach behavior in obesity-prone and outbred Sprague-Dawley rats. To our knowledge, these data are the first to demonstrate cycle- and hormone-dependent effects on the motivational response to a food cue, and the only studies to date to determine how individual susceptibility to obesity influences NAc excitability, cue-triggered food-seeking, and differences in the regulation of these neurobehavioral responses by the cycle.

Effects of the cycle on weight, food intake, and motivation for sucrose in obesity-prone and obesity-resistant females:

We began by verifying obesity-prone and obesity-resistant phenotypes in females. As expected, female obesity-prone rats were heavier, ate more, and had greater fat mass and less lean mass than obesity-resistant females (Fig. 2.1). In addition, weight gain induced by a junk-food diet was more pronounced in obesity-prone vs. obesity-resistant females. Instrumental responding for a sucrose pellet during FR1, FR5 or progressive ratio testing was similar between groups (Fig. 2.3), despite differences in home cage food consumption. In males break point is modestly elevated in obesity-prone vs. obesity-resistant rats working for sucrose (Vollbrecht et. al., 2015). In the current study, pressing on the active lever resulted in the delivery of a sucrose pellet but no discrete cue.

However, in our previous study of males, active lever presses resulted in the presentation of an auditory cue in addition to the delivery of the sucrose pellet. Thus, it's possible that inclusion of a discrete cue added salience to the motivation to work for food in our previous study using males, and that the absence of cue presentation in the current study contributed to similar break points in female obesity-prone and resistant groups. However, the absence of strong differences in break point between obesity-prone and obesity-resistant females here did not impede our ability to observe the effects of the cycle on this behavior, discussed further below. Overall, obesity-prone and -resistant phenotypes of females are similar to those previously reported for males of these selectively bred lines (Vollbrecht et al., 2015, 2016). Additionally, within naturally cycling obesity-prone and obesity-resistant females, food intake and body weight were reduced during estrus (Fig. 2.2B). Furthermore, motivation to obtain sucrose was lower when rats were tested during proestrus/estrus vs. metestrus/diestrus (Fig. 2.3C), regardless of susceptibility to obesity. These changes in motivation to obtain sucrose in naturally cycling females are consistent with variations in home cage food intake across the cycle found here and with established roles for ovarian hormones in the regulation of food intake (Tarttelin & Gorski, 1971; Asarian and Geary, 2013).

Differences in NAc MSN intrinsic excitability in obesity-prone vs. obesity-resistant rats:

The NAc is comprised predominantly of MSNs which integrate both dopaminergic and glutamatergic inputs to ultimately influence behavioral responses to reinforces like food, sex, and drugs as well as cues paired with them (Schultz, 1997; Berridge and Robinson, 1998; Schultz, 2013; Wolf et al., 2002; Wolf, 2003). One factor that strongly influences the output of the NAc is the intrinsic excitability of MSNs, i.e., how readily they can be depolarized and fire action potentials (Nicola et al., 2000; Hu, 2007). We found that excitability of MSNs in the NAc core is enhanced in obesity-prone vs. obesity-resistant females when recordings are made during metestrus/diestrus, but that this group difference is not apparent when recordings are made during proestrus/estrus (Fig. 2.4). This was due to cycle effects on MSN excitability in obesity-prone, but not obesity-resistant rats. Specifically, in obesity-prone rats, changes in voltage at positive current injections were significantly greater during metestrus/diestrus vs. proestrus/estrus. This

is similar to effects of the cycle on NAc excitability in outbred Sprague-Dawley females (Proaño et al., 2018). To our knowledge this is the only other study to examine effects of the estrous cycle on MSN excitability, and specific mechanisms by which ovarian hormones influence NAc excitability have not been determined, although there is evidence for sex differences (Cao et al., 2018).

MSN excitability is largely determined by the number and distribution of voltage-gated potassium channels, with inwardly-rectifying K+ currents (I_{Kir}) dominating at negative membrane potentials (< -90mV) and A-type K+ currents (I_A) dominating at positive membrane potentials (> -40mV; Nisenbaum and Wilson 1995; Perez et al. 2006). Differences during metestrus/diestrus between obesity-prone and obesity-resistant females were present across a wide range of current injections, but were most pronounced at positive potentials. This, in combination with a lower rheobase and absence of differences in resting membrane potential, suggests that group differences may be due to lower I_A in obesity-prone vs. obesity-resistant females. These basal group differences in excitability during metestrus/diestrus are similar to those seen in male obesity-prone vs. obesity-resistant rats where differences were found across the I/V curve and were accompanied by a lower rheobase and an absence of differences in resting membrane potential (Oginsky et al., 2016).

The reduction in excitability during proestrus/estrus vs. metestrus/diestrus within obesity-prone rats is consistent with the ability of estradiol to enhance I_A, thereby reducing excitability in cultured hippocampal neurons (Zhang et al., 2015). Progesterone also increases during the proestrus phase, and progesterone can block voltage-gated sodium and calcium channels, resulting in decreased neuronal excitability (Kelley & Mermelstein, 2011). However, we did not observed changes in the threshold for action potential firing or action potential rise time, which are mediated by Na^+ currents, nor did we find changes in after hyperpolarization amplitude, which are mainly influenced by Ca^+ channels. Thus, it seems less likely that alterations in excitability observed across the cycle in obesity-prone rats are mediated by progesterone. Of course, many hormones change across the cycle, and effects observed here could be either direct, or indirect. Nonetheless, these data demonstrate that in the NAc core MSN excitability is enhanced in obesity-prone rats

during phases of the cycle when motivational responses to food cues are also elevated (see below for additional discussion of this relationship).

Basal differences in cue-triggered motivation in obesity-prone vs. obesity-resistant females:

Food intake is not only regulated by homeostatic feedback, but external signals including food cues also influence the motivation to eat independent of hunger state (Derman & Ferrario, 2018; Holland, 1977). These cue-triggered urges to seek out and consume food contribute to opportunistic eating that drives obesity (Ferrario et al., 2016, Ferrario, 2017, Stice et al., 2013) and are more pronounced in male obesity-susceptible vs. -resistant populations (Robinson et al., 2015, Derman & Ferrario, 2018, Alonso-Caraballo et al., 2018). Conditioned approach behavior was stronger in female obesity-prone vs. obesity-resistant rats (Fig. 2.5D). This is consistent with data from males, where the magnitude of Pavlovian conditioned approach, conditioned reinforcement, and Pavlovian to instrumental transfer are greater in obesity-susceptible vs. –resistant populations (Robinson et al., 2015; Derman & Ferrario, 2018). Taken as a whole, these data support the idea enhanced responsivity to the motivational properties of food cues is likely a phenotypic, neurobehavioral difference in those individuals that are more vs. less susceptible to diet-induced weight gain.

Difference in conditioned approach between obesity-prone and obesity-resistant females was apparent when naturally cycling rats were tested during the metestrus/diestrus phases of the cycle, but the magnitude of conditioned approach was similar across groups when tested during proestrus/estrus. Specifically, in obesity-prone rats conditioned approach was elevated during metestrus/diestrus compared to proestrus/estrus (Fig. 2.5), consistent with shifts in home cage food intake and break point across the cycle. In contrast, in obesity-resistant females, conditioned approach behavior was stable across the cycle, even though motivation for food itself and food consumption rose and fell with the cycle in a manner comparable to that found in obesity-prone rats (Fig. 2.5). This dissociation between motivation for food and motivational responses triggered by a food cue does not appear to be due to deficits in Pavlovian learning in obesity-resistant rats, as behavior during acquisition and the degree of discrimination between the CS+ and CS-

were similar to that seen in the obesity-prone group (Fig. 2.5). Indeed, obesity-resistant rats show quite reliable conditioned approach and discrimination between the CS+ and CS- during testing. Thus, the lack of cycle dependent shifts in conditioned approach, despite shifts in food intake and break point, suggest a potential mechanistic dissociation between appetitive and consummatory behaviors (see Berridge et al., 2010 for review). Although speculative, given the role of the NAc core in motivational responses to food cues (Kelley et al., 2005; Aitken et al., 2016) it's possible that the cycle-dependent fluctuations in MSN excitability in obesity-prone rats discussed above, could contribute to differences in the modulation of conditioned approach across the cycle in obesity-prone but not obesity-resistant rats. This should be examined in future studies.

Ovarian hormones modulate cue-triggered motivation in obesity-prone and outbred females:

Given that the magnitude of conditioned approach varied across the cycle in intact obesity-prone rats, we also determined the degree to which estradiol and progesterone treatment of ovariectomized rats reduces conditioned approach behavior. A single cycle of estradiol and progesterone treatment did not alter the expression of conditioned approach behavior (Fig. 2.6C), whereas repeated cycles of this treatment were sufficient to reduce conditioned approach in three separate experiments (Figs. 2.6D, 2.7C, 2.8E). Importantly, this effect was found in both obesity-prone and outbred Sprague Dawley females; thus, it is not unique to our selectively bred rat strain. Similarities across obesity-prone and outbred rats are not surprising given that obesity-susceptible rats can be identified in the outbred population (e.g., Levin et al., 1997; Robinson et al., 2015; Madsen et al., 2010).

The fact that repeated hormone treatment was needed in order to recapitulate effects seen in naturally cycling rats may in part be due to the effects of ovariectomy. Following Pavlovian conditioning rats were ovariectomized and hormone replacement did not begin until rats failed to enter estrus within an 8-10 day period. The removal of the ovaries could itself produce neuroadaptations, such as changes in progesterone and estradiol receptor expression. Thus, repeated cycles of estradiol and progesterone may have been needed in order to compensate for adaptations induced by ovariectomy itself. Nonetheless, these

data demonstrate that ovarian hormones strongly influence conditioned approach elicited by a food cue. Furthermore, repeated treatment with estradiol or progesterone alone were not sufficient to reduce conditioned approach behavior (Fig. 2.5). In contrast, repeated treatment with estradiol was sufficient to decrease home cage food intake and body weight, consistent with previous reports (Blaustein & Wade, 1975). However, it was only when estradiol and progesterone were co-administered that a decrease in conditioned approach was observed. This again points to dissociable mechanisms through which ovarian hormones regulate food intake vs. food-seeking.

To our knowledge, this is the first demonstration that ovarian hormones influence cue-triggered food-seeking. There have been many studies examining how ovarian hormones influence motivation for primary reinforcers like food, sex, and potentially addictive substances like cocaine (Yoest et al., 2018; Yoest et al., 2014; Rivera & Stincic, 2018; Frye, 2007). Although the mechanisms underlying these behaviors are not well understood, ovarian hormones do influence both dopaminergic and glutamatergic transmission within the NAc core in ways that are consistent with the behavioral effects found here (Micevych & Meisel, 2017, Tonn Eisinger et al., 2018, Yoest et al., 2018). In addition to the effects of dopamine on NAc intrinsic excitability (discussed above), estradiol treatment also decreases dendritic spine density (an indirect measure of excitatory synapses) in the NAc (Peterson et al., 2015), and reduces AMPA receptor specific binding (Cyr et al., 2001) and GluA2 AMPAR subunit mRNA in the NAc (Le Saux et al., 2006). Thus, reductions in the motivational responses to food cues may be related to the effects of estradiol on excitatory transmission in the NAc. However, it's important to keep in mind that effects on conditioned approach required both estradiol and progesterone treatment, suggesting a more complex mechanism.

Conclusions & Future Directions:

In sum, we find that in females individual susceptibly to obesity is associated with enhanced motivational responses to food cues and increased intrinsic excitability of NAc core MSNs, consistent with overall patterns found in males. In addition, both estradiol and progesterone are needed to reduce conditioned approach behavior in obesity-prone rats, whereas estradiol treatment alone is sufficient to reduce food intake and motivation for food. These data suggest dissociations between hormonal regulation of food-seeking vs. consumption. Furthermore, interactions between individual susceptibility to obesity and the regulation of incentive motivation by ovarian hormones likely represent phenotypic differences that may be mediated by altered NAc responsivity. Future studies of the precise mechanisms involved, as well as the effects of diet-induced obesity and consumption of sugary, fatty foods are needed in order to understand the fundamental neurobiological mechanisms of food-seeking in the non-obese and obese state. Finally, effects of ovarian hormones on conditioned approach were similar in obesity-prone and outbred females, suggesting that neurobehavioral effects of ovarian hormones observed here extend to outbred populations.

References

Aitken, T. J., Greenfield, V. Y., & Wassum, K. M. (2016). Nucleus accumbens core dopamine signaling tracks the need-based motivational value of food-paired cues. *Journal of Neurochemistry*, *136*(5), 1026–1036. https://doi.org/10.1111/jnc.13494

Alonso-Caraballo, Y., Jorgensen, E. T., Brown, T. E., & Ferrario, C. R. (2018). Functional and structural plasticity contributing to obesity: roles for sex, diet, and individual susceptibility. *Current Opinion in Behavioral Sciences*, *23*, 160–170. https://doi.org/10.1016/j.cobeha.2018.06.014

Arnoni-Bauer, Y., Bick, A., Raz, N., Imbar, T., Amos, S., Agmon, O., Marco L., Levin N. and Weiss, R. (2017). Is it me or my hormones? neuroendocrine activation profiles to visual food stimuli across the menstrual cycle. *Journal of Clinical Endocrinology and Metabolism*, *102*(9), 3406–3414. https://doi.org/10.1210/jc.2016-3921

Asarian, L., & Geary, N. (2002). Cyclic estradiol treatment normalizes body weight and restores physiological patterns of spontaneous feeding and sexual receptivity in ovariectomized rats. *Hormones and Behavior*, *42*(4), 461–471. https://doi.org/10.1006/hbeh.2002.1835

Asarian, L., & Geary, N. (2006). Modulation of appetite by gonadal steroid hormones. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *361*(1471), 1251–63. https://doi.org/10.1098/rstb.2006.1860

Asarian, L., & Geary, N. (2013). Sex differences in the physiology of eating. *AJP: Regulatory, Integrative and Comparative Physiology*, *305*(11), R1215–R1267. https://doi.org/10.1152/ajpregu.00446.2012

Berridge, K. C., Ho, C. Y., Richard, J. M., & Difeliceantonio, A. G. (2010). The tempted brain eats: Pleasure and desire circuits in obesity and eating disorders. *Brain Research*. NIH Public Access. https://doi.org/10.1016/j.brainres.2010.04.003

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*. https://doi.org/10.1016/S0165-0173(98)00019-8

Blaustein, J. D., & Wade, G. N. (1976). Ovarian influences on the meal patterns of female rats. *Physiology and Behavior*, *17*(2), 201–208. https://doi.org/10.1016/0031-9384(76)90064-0

Cao, J., Willett, J. A., Dorris, D. M., & Meitzen, J. (2018). Sex differences in medium spiny neuron excitability and glutamatergic synaptic input: Heterogeneity across striatal regions and evidence for estradiol-dependent sexual differentiation. *Frontiers in Endocrinology*. https://doi.org/10.3389/fendo.2018.00173

Cummings, J. A., & Becker, J. B. (2012). Quantitative assessment of female sexual motivation in the rat: Hormonal control of motivation. *Journal of Neuroscience Methods*, *204*(2), 227–233. https://doi.org/10.1016/j.jneumeth.2011.11.017

Cyr, M., Ghribi, O., Thibault, C., Morissette, M., Landry, M., & Di Paolo, T. (2001). Ovarian steroids and selective estrogen receptor modulators activity on rat brain NMDA and AMPA receptors. In *Brain Research Reviews* (Vol. 37, pp. 153–161). https://doi.org/10.1016/S0165-0173(01)00115-1

Demos, K. E., Heatherton, T. F., & Kelley, W. M. (2012). Individual Differences in Nucleus Accumbens Activity to Food and Sexual Images Predict Weight Gain and Sexual Behavior. *Journal of Neuroscience*, *32*(16), 5549–5552. https://doi.org/10.1523/JNEUROSCI.5958-11.2012

Derman, R. C., & Ferrario, C. R. (2018). Enhanced incentive motivation in obesity-prone rats is mediated by NAc core CP-AMPARs. *Neuropharmacology*, *131*, 326–336. https://doi.org/10.1016/j.neuropharm.2017.12.039

Derman, R. C., & Ferrario, C. R. (2018). 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. *Physiology and Behavior*, 192, 145–157. https://doi.org/10.1016/j.physbeh.2018.03.012

Dreher, J.-C., Schmidt, P. J., Kohn, P., Furman, D., Rubinow, D., & Berman, K. F. (2007). Menstrual cycle phase modulates reward-related neural function in women. *Proceedings of the National Academy of Sciences*, *104*(7), 2465–2470. https://doi.org/10.1073/pnas.0605569104

Eckel, L. A. (2011). The ovarian hormone estradiol plays a crucial role in the control of food intake in females. *Physiology & Behavior*, *104*(4), 517–24. https://doi.org/10.1016/j.physbeh.2011.04.014

Ferrario, C. R. (2017). Food Addiction and Obesity, *42*. https://doi.org/10.1038/npp.2016.228

Ferrario, C. R., Labouèbe, G., Liu, S., Nieh, E. H., Routh, V. H., Xu, S., & O'Connor, E.C. (2016). Homeostasis Meets Motivation in the Battle to Control Food Intake. *The Journal of Neuroscience*, *36*(45), 11469–11481. https://doi.org/10.1523/JNEUROSCI.2338-16.2016

Frank, T. C., Kim, G. L., Krzemien, A., & Van Vugt, D. A. (2010). Effect of menstrual cycle phase on corticolimbic brain activation by visual food cues. *Brain Research*, *1363*, 81–92. https://doi.org/10.1016/j.brainres.2010.09.071

Frye, C. A. (2007). Progestins influence motivation, reward, conditioning, stress, and/or response to drugs of abuse. *Pharmacology Biochemistry and Behavior*. https://doi.org/10.1016/j.pbb.2006.07.033

Hirschberg, A. L. (2012). Sex hormones, appetite and eating behaviour in women. *Maturitas*. https://doi.org/10.1016/j.maturitas.2011.12.016

Holland, P. C. (1977). Conditioned stimulus as a determinant of the form of the Pavlovian conditioned response. *Journal of Experimental Psychology: Animal Behavior Processes*, *3*(1), 77–104. https://doi.org/10.1037/0097-7403.3.1.77

Hu, X. T. (2007). Cocaine withdrawal and neuro-adaptations in ion channel function. *Molecular Neurobiology*, *35*(1), 95–112. https://doi.org/10.1007/BF02700626

Kelley, Ann E, Brian A Baldo, Wayne E Pratt, and Matthew J Will. 2005. "Corticostriatal-Hypothalamic Circuitry and Food Motivation: Integration of Energy, Action and Reward." In *Physiology and Behavior*, 86:773–95. https://doi.org/10.1016/j.physbeh.2005.08.066.

Kelley, B. G., & Mermelstein, P. G. (2011). Progesterone blocks multiple routes of ion flux. *Molecular and Cellular Neurosciences*, *48*(2), 137–41. https://doi.org/10.1016/j.mcn.2011.07.002

Le Saux, M., Estrada-Camarena, E., & Di Paolo, T. (2006). Selective estrogen receptor-alpha but not -beta agonist treatment modulates brain alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. *Journal of Neuroscience Research*, *84*(5), 1076–1084. https://doi.org/10.1002/jnr.21007

Levin, B. E., Dunn-Meynell, A., Balkan, B., & Keesey, R. E. (1997). Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. *The American Journal of Physiology*, *273*, R725–R730. Retrieved from http://www.physiology.org/doi/pdf/10.1152/ajpregu.1997.273.2.R725

Levin, B. E., & Govek, E. (1998). Gestational obesity accentuates obesity in obesity-prone progeny. *The American Journal of Physiology*, *275*(4 Pt 2), R1374-9. Retrieved from http://ajpregu.physiology.org/content/ajpregu/275/4/R1374.full.pdf

Madsen, A. N., Hansen, G., Paulsen, S. J., Lykkegaard, K., Tang-Christensen, M., Hansen, H. S., … Vrang, N. (2010). Long-term characterization of the diet-induced obese and diet-resistant rat model: A polygenetic rat model mimicking the human obesity syndrome. *Journal of Endocrinology*, *206*(3), 287–296. https://doi.org/10.1677/JOE-10-0004

Marcondes, F. K., Miguel, K. J., Melo, L. L., & Spadari-Bratfisch, R. C. (2002). Estrous cycle influences the response of female rats in the elevated plus-maze test. *Physiology and Behavior*, *74*(4–5), 435–440. https://doi.org/10.1016/S0031-9384(01)00593-5

Micevych, P. E., & Meisel, R. L. (2017). Integrating Neural Circuits Controlling Female Sexual Behavior. *Frontiers in Systems Neuroscience*, *11*, 42. https://doi.org/10.3389/fnsys.2017.00042

Murdaugh, D. L., Cox, J. E., Cook 3rd, E. W., & Weller, R. E. (2012). fMRI reactivity to high-calorie food pictures predicts short- and long-term outcome in a weight-loss program. *Neuroimage*, *59*(3), 2709–2721. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22332246

Nicola, S. M., Surmeier, D. J., & Malenka, R. C. (2000). Dopaminergic Modulation of Neuronal Excitability in the Striatum and Nucleus Accumbens. *Annual Review of Neuroscience*, *23*(1), 185–215. https://doi.org/10.1146/annurev.neuro.23.1.185

Nisenbaum, E. S., & Wilson, C. J. (1995). Potassium currents responsible for inward and outward rectification in rat neostriatal spiny projection neurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *15*(6), 4449–4463. Retrieved from http://www.jneurosci.org/content/jneuro/15/6/4449.full.pdf

Oginsky, M. F., Goforth, P. B., Nobile, C. W., Lopez-Santiago, L. F., & Ferrario, C. R. (2016). Eating "Junk-Food" Produces Rapid and Long-Lasting Increases in NAc CP-AMPA Receptors: Implications for Enhanced Cue-Induced Motivation and Food Addiction. *Neuropsychopharmacology*, *41*(13), 2977–2986. https://doi.org/10.1038/npp.2016.111

Oginsky, M. F., Maust, J. D., Corthell, J. T., & Ferrario, C. R. (2016). Enhanced cocaine-induced locomotor sensitization and intrinsic excitability of NAc medium spiny neurons in adult but not in adolescent rats susceptible to diet-induced obesity. *Psychopharmacology*, *233*(5), 773–784. https://doi.org/10.1007/s00213-015-4157-x

Perez, M. F., White, F. J., & Hu, X.T. (2006). Dopamine D 2 Receptor Modulation of K + Channel Activity Regulates Excitability of Nucleus Accumbens Neurons at Different Membrane Potentials. *Journal of Neurophysiology*, *96*(5), 2217–2228. https://doi.org/10.1152/jn.00254.2006

Peterson, B. M., Mermelstein, P. G., & Meisel, R. L. (2015). Estradiol mediates dendritic spine plasticity in the nucleus accumbens core through activation of mGluR5. *Brain Structure and Function*, *220*(4), 2415–2422. https://doi.org/10.1007/s00429-014-0794-9

Proaño, S. B., Morris, H. J., Kunz, L. M., Dorris, D. M., & Meitzen, J. (2018). Estrous cycle-induced sex differences in medium spiny neuron excitatory synaptic transmission and intrinsic excitability in adult rat nucleus accumbens core. *Journal of Neurophysiology*, *120*(3), 1356–1373. https://doi.org/10.1152/jn.00263.2018

Richardson, N. R., & Roberts, D. C. S. (1996). Progressive ratio schedules in drug self-administration studies in rats: A method to evaluate reinforcing efficacy. *Journal of Neuroscience Methods*. https://doi.org/10.1016/0165-0270(95)00153-0

Rivera, H., & Stincic, T. (2018). Estradiol and the control of feeding behavior. *Steroids*, *133*, 44–52. https://doi.org/10.1016/j.steroids.2017.11.011

Robinson, M. J. F., Burghardt, P. R., Patterson, C. M., Nobile, C. W., Akil, H., Watson, S. J., … Ferrario, C. R. (2015). Individual Differences in Cue-Induced Motivation and Striatal Systems in Rats Susceptible to Diet-Induced Obesity. *Neuropsychopharmacology*, *40*(9), 2113–2123. https://doi.org/10.1038/npp.2015.71

Schultz, W. (1997). Dopamine neurons and their role in reward mechanisms. *Current Opinion in Neurobiology*, *7*(2), 191–197. https://doi.org/10.1016/S0959-4388(97)80007-4

Schultz, W. (2013). Updating dopamine reward signals. *Current Opinion in Neurobiology*, *23*, 229–238. https://doi.org/10.1016/j.conb.2012.11.012

Sekerli, M., Del Negro, C. A., Lee, R. H., & Butera, R. J. (2004). Estimating action potential thresholds from neuronal time-series: New metrics and evaluation of methodologies. *IEEE Transactions on Biomedical Engineering*, *51*(9), 1665–1672. https://doi.org/10.1109/TBME.2004.827531

Stice, E., Figlewicz, D. P., Gosnell, B. A., Levine, A. S., & Pratt, W. E. (2013). The contribution of brain reward circuits to the obesity epidemic. *Neuroscience and Biobehavioral Reviews*, *37*(9 Pt A), 2047–58. https://doi.org/10.1016/j.neubiorev.2012.12.001

Stoeckel, L. E., Weller, R. E., Cook, E. W., Twieg, D. B., Knowlton, R. C., & Cox, J. E. (2008). Widespread reward-system activation in obese women in response to pictures of high-calorie foods. *NeuroImage*, *41*(2), 636–647. https://doi.org/10.1016/j.neuroimage.2008.02.031

Tarttelin, M. F., & Gorski, R. A. (1971). Variations in food and water intake in the normal and acyclic female rat. *Physiology & Behavior*, *7*(6), 847–852. https://doi.org/10.1016/0031-9384(71)90050-3

Tonn Eisinger, K. R., Larson, E. B., Boulware, M. I., Thomas, M. J., & Mermelstein, P. G. (2018). Membrane estrogen receptor signaling impacts the reward circuitry of the female brain to influence motivated behaviors. *Steroids*, *133*, 53–59. https://doi.org/10.1016/j.steroids.2017.11.013

Vollbrecht, P. J., Mabrouk, O. S., Nelson, A. D., Kennedy, R. T., & Ferrario, C. R. (2016). Pre-existing differences and diet-induced alterations in striatal dopamine systems of obesity-prone rats. *Obesity*, *24*(3), 670–677. https://doi.org/10.1002/oby.21411

Vollbrecht, P. J., Nobile, C. W., Chadderdon, A. M., Jutkiewicz, E. M., & Ferrario, C. R. (2015). Pre-existing differences in motivation for food and sensitivity to cocaine-induced locomotion in obesity-prone rats. *Physiology and Behavior*, *152*(Pt A), 151–160. https://doi.org/10.1016/j.physbeh.2015.09.022

Wade, G. N. (1972). Gonadal hormones and behavioral regulation of body weight. *Physiology and Behavior*. https://doi.org/10.1016/0031-9384(72)90340-X

Wolf, M. E. (2002). Addiction: Making the Connection Between Behavioral Changes and Neuronal Plasticity in Specific Pathways. *Molecular Interventions*, *2*(3), 146–157. https://doi.org/10.1124/mi.2.3.146

Wolf, M. E., Mangiavacchi, S., & Sun, X. (2003). Mechanisms by which Dopamine Receptors May Influence Synaptic Plasticity. In *Annals of the New York Academy of Sciences*. https://doi.org/10.1196/annals.1300.015

Yoest, K. E., Cummings, J. A., & Becker, J. B. (2014). Estradiol, Dopamine and Motivation. *Cent Nerv Syst Agents Med Chem. Cent Nerv Syst Agents Med Chem*, *14*(2), 83–89. https://doi.org/10.14440/jbm.2015.54.A

Yoest, K. E., Quigley, J. A., & Becker, J. B. (2018). Rapid effects of ovarian hormones in dorsal striatum and nucleus accumbens. *Hormones and Behavior*. https://doi.org/10.1016/j.yhbeh.2018.04.002

Yu, Z., Geary, N., & Corwin, R. L. (2011). Individual effects of estradiol and progesterone on food intake and body weight in ovariectomized binge rats. *Physiol Behav*, *104*(5), 687–693. https://doi.org/10.1016/j.physbeh.2011.07.017

Zhang, Y., Huang, Y., Liu, X., Wang, G., Wang, X., & Wang, Y. (2015). Estrogen suppresses epileptiform activity by enhancing Kv4.2-mediated transient outward potassium currents in primary hippocampal neurons. *International Journal of Molecular Medicine*, *36*(3), 865–872. https://doi.org/10.3892/ijmm.2015.2287

CHAPTER 3

Sex Specific Effects Of Junk-Food Diet On Calcium Permeable AMPA Receptors And Silent Synapses In The Nucleus Accumbens Core.

Abstract

The nucleus accumbens (NAc) plays critical roles in motivated behaviors, including food-seeking in response to Pavlovian cues. In particular, increases in the activity of calcium-permeable AMPAR receptors (CP-AMPARs) within the NAc mediate enhanced cue-triggered food- and cocaine-seeking behaviors. We previously found that consumption of a sugary, fatty junk-food diet followed by a junk-food free period results in an increase in CP-AMPAR expression and function in the NAc of male rats. However, whether a junk-food deprivation period is necessary is unknown. Furthermore, CP-AMPAR upregulation following cocaine exposure in males is associated with metaplasticity of silent synapses in the NAc, but potential effects of junk-food on this phenomenon have not been examined. Finally, no studies have examined effects of food or cocaine on NAc CP-AMPAR expression or transmission in females. Therefore, here we used biochemical and electrophysiological approaches to determine the effects of junk-food on NAc AMPAR protein expression and synaptic transmission in male and female rats. We found that junk-food consumption increases CP-AMPAR surface expression and generates silent synapses in the NAc of male rats. In addition, a brief period of junk-food deprivation is needed for the synaptic insertion of CP-AMPARs and the maturation of silent synapses in males. In contrast junk-food did not induce AMPAR plasticity in females. Thus, these studies reveal sex differences in the effects of junk-food on NAc synaptic plasticity. In addition, they provide novel insights into how essential food rewards alter NAc function.

Introduction:

Activity within the nucleus accumbens core (NAc) mediates complex motivational processes including cue-triggered seeking of food and addictive substances like cocaine (Chaudhri et al., 2010; Counotte et al., 2014; Ito et al., 2004; Wolf & Tseng, 2012). In particular, glutamatergic transmission within the NAc mediates these cue-triggered reward seeking behaviors (Corbit & Balleine, 2011, 2016; Di Ciano et al., 2001; Patricia Di Ciano & Everitt, 2001). For example, cue-triggered food-seeking is mediated by activation of high conductance calcium permeable-AMPA receptors (CP-AMPARs) within the NAc core (Derman & Ferrario, 2018). Furthermore, consumption of a sugary, fatty, junk-food diet enhances NAc core CP-AMPAR-mediated transmission and expression and potentiates cue-triggered food-seeking (Derman and Ferrario, 2018). These data are consistent with increases in NAc CP-AMPARs that mediate the incubation of cocaine craving (Wolf, 2016), and the incubation of craving for food (Darling et al., 2016). However, it's unclear whether glutamatergic plasticity following food compared to drug consumption rely on similar mechanisms.

Increases in CP-AMPAR synaptic transmission associated with the incubation of cocaine craving are persistent (lasting months) and require a relatively long drug free period (~3 weeks). During this time, there is thought to be an extrasynaptic accumulation of CP-AMPARs that is followed by their synaptic recruitment and retention (Conrad et al., 2008; Ferrario et al., 2010; Wolf, 2016; Wolf & Ferrario, 2010). Furthermore, the synaptic insertion of CP-AMPARs is preceded by increases the number of silent synapses in the NAc (i.e., immature synapses containing NMDARs, but lacking AMPARs), which are thought to then mature via insertion of CP-AMPARs (Dong et al., 2017; Huang et al., 2009). Thus, following cocaine withdrawal and the development of incubation of craving there is an increase in functional mature synapses and an increase in CP-AMPAR insertion (Dong et al., 2017; Huang et al., 2015). Somewhat surprisingly, consumption of junk-food increases NAc CP-AMPAR synaptic transmission in males after just one day of junk-food deprivation, an effect that persists for at least one month (Oginsky et al., 2016a). However, whether a junk-food free period is necessary for CP-AMPAR up-regulation, or whether metaplasticity of silent synapses precedes the insertion of CP-AMPARs following

junk-food consumption is unknown. Furthermore, effects of junk-food on NAc glutamatergic transmission and AMPAR expression in females has not been determined.

Studies below determined the effects of junk-food on NAc surface expression of GluA1 and GluA2 AMPAR subunits and NAc synaptic transmission in males and females. Overall, we found sex specific effects, with junk-food enhancing CP-AMPAR transmission and inducing metaplasticity of silent synapses in males, but not females. These results are discussed in light of established roles for CP-AMPARs in cue-triggered craving and enhanced motivation.

Materials and methods:

Subjects: Male and female selectively bred obesity-prone and obesity-resistant rats were used. The original selection was conducted by Barry Levin from outbred Sprague Dawley rats (Levin et al., 1997). Rats used in this study were bred in house and housed on a reverse 12-h light/dark cycle, had free access to food and water throughout, and were group housed unless otherwise noted. Rats were 60-70 days old and were weighed 3-4 times per week unless otherwise specified. Procedures were approved by The University of Michigan and The University of Wyoming Institutional Animal Care and Use Committee (IACUC) in accordance with AAALAC and AVMA guidelines. See also https://sites.google.com/a/umich.edu/ferrario-lab-public-protocols/ for additional details.

Junk-food and chow diet: Control groups were given free access to standard lab chow (Lab Diet 5001: 4kcal/g; 4.5% fat, 23% protein, 48.7% carbohydrates; % of caloric content) in their home cage, whereas experimental groups were given free access to a **junk-food** diet (JF) made in house (Oginsky et al., 2016a; Robinson et al., 2015). The junk-food was a mash of Ruffle potato chips (40g), Chips Ahoy (130g), Nesquik (130g), Jiff peanut butter (130g), Lab Diet 5001 (200g) and 180ml of water (19.6% fat, 14% protein, and 58% carbohydrates; 4.5 kcal/g). For all studies, rats were assigned to junk-food or chow groups counterbalanced for initial weight within OP and OR groups.

Effects of junk-food on GluA1 and GluA2 surface expression in the NAc of male rats.

We first determined the effect of 10 days of junk-food followed by two weeks of junk-food deprivation on AMPAR subunit surface expression. During the deprivation period rats in the junk-food group were given free access to standard lab chow and junk-food was no longer available. Next, we used an established BS_3-crosslinking procedure followed by western blotting to determine the surface and intracellular expression of GluA1 and GluA2 AMPAR subunits (Derman & Ferrario, 2018; Dingess et al., 2017; Oginsky et al., 2016a). Briefly, NAc tissue was extracted, chopped (400μm) and incubated in ACSF containing BS_3 (5mM) for 30 min (4 °C). Glycine (100mM) was added to stop the crosslinking reaction (10 min, 4 °C). Samples were centrifuged (2 min; 14,000 RPM; 4 °C and the pellet was re-suspended in ice cold lysis buffer containing (in mM): 25 HEPES, 500 NaCl,

2 EDTA, 1 DTT, 1 PMSF 20 NaF; 1:100 EDTA-free Protease Inhibitor Cocktail (Sigma-Aldridge; 11836170001); and 0.1% Nonidet P-40 [v/v]; pH 7.4) and homogenized by sonication. Samples were then stored at −80 °C. For SDS-PAGE and Western blotting samples were heated (70°C, 10 min) in Laemmli sample treatment buffer with 5% β-mercaptoethanol, loaded (20 µg protein) and electrophoresed on 6% gels made in house. Proteins were transferred onto PVDF membranes (GE Healthcare Amersham™ Hyperfilm™ Fisher Scientific; 45-001-505). Membranes were rinsed, blocked (1hr, RT, 5% [w/v] nonfat dry milk in TBS-Tween 20 [TBS-T; 0.05% Tween 20, v/v]), and incubated overnight (4°C) with primary antibodies to GluA1 (Thermo Scientific; PA1-37776; 1:1000 in TBS) or GluA2 (EMD Millipore; AB1768-I; 1:2000 in TBS-T and 5% milk). Membranes were then washed in TBS-T, incubated with HRP-conjugated secondary (Invitrogen, Carlsbad, CA; 1hr, RT), washed, and immersed in chemiluminescence detecting substrate (Thermo Scientific; Pierce™ ECL Western Blotting Substrate Cat. No 32106). Images were acquired on film and Ponceau S (Sigma-Aldrich) was used to determine total protein in each land. Bands of interest were then quantified using Image J (NIH).

To determine the effects of junk-food deprivation on GluA1 and GluA2 surface expression, a separate cohort of obesity-prone (n = 26) and obesity-resistant (n = 23) male rats were separated into 3 groups: chow-fed, junk-food no deprivation and junk-food 24-hr deprivation. The junk-food deprivation and no deprivation groups received junk-food for 10 days, while the chow group remain on chow throughout the study. The junk-food deprivation group received chow diet for 24 hours before tissue collection and BS_3-crosslink, whereas the junk-food no deprivation remained on junk-food throughout. GluA1 and GluA2 surface expression were determined as described above.

Effects of junk-food on synaptic transmission in NAc core of males:

CP-AMPAR mediated transmission and silent synapses were evaluated in obesity-prone male rats. Recordings were made from chow controls, and on day 10 of junk-food exposure or after 24-48 hours of junk-food deprivation. This timing was chosen to improve feasibility of recordings, rather than timing all recordings to 24 hours. Recordings from chow controls and junk-food groups were interspersed.

Established whole-cell patch clamping approaches were used (Oginsky et al., 2016a; Slaker et al., 2015). Briefly, coronal slices (300 µm) containing the NAc were made using a vibratory microtome (Leica Biosystems, Buffalo Grove, IL, USA) and allowed to recover in oxygenated aCSF prior to recording. Patch pipettes were pulled from 1.5 mm borosilicate glass capillaries (WPI, Sarasota, FL; 3–7 MΩ resistance). Evoked EPSCs (eEPSCs) were elicited by local stimulation (0.05 to 0.30 mA square pulses, 0.1 ms, delivered every 20s) using a bipolar electrode placed ~300 µm lateral to recorded neurons. The minimum amount of current needed to elicit a synaptic response with <15% variability in amplitude was used. If > 0.30 mA was required, the neuron was discarded. Pipettes were filled with a solution containing (in mM): 140 CsCl, 10 HEPES, 2 MgCl2, 5 Na+-ATP, 0.6 Na+-ATP, 2 QX314, pH 7.3, 285 mOsm. eEPSCs were recorded at -70 mV before and after application of the CP-AMPAR selective antagonist Naspm (200 µM; as in Conrad et al., 2008; Oginsky et al., 2016a) in the presence of the $GABA_A$ receptor antagonist picrotoxin (50 µM). For measures of the AMPA/NMDA ratio, eEPSCs were recorded at +40 mV and -70 mV before and after bath application of NMDA receptor antagonist APV as previously described (50 µM; Kourrich et al., 2007; Kreitzer & Malenka, 2008).

For silent synapse measures, recording conditions and solutions were as previously described (Brown et al., 2011; Huang et al., 2009; Slaker et al., 2018). Pipettes were filled with cesium chloride (CsCl) internal solution containing: (in mM) 117 CsCl, 2.8 NaCl, 5 MgCl2, 20 HEPES, 2 Mg2+ATP, 0.3 Na2+GTP, 0.6 EGTA, 0.1 spermine, and sucrose to bring osmolarity to 275-280 mOsm and pH to ~7.25. Minimal stimulation protocols were performed as previously described (Brown et al., 2011; Huang et al., 2009). Briefly, the frequency of presynaptic stimulation was set at 0.33 Hz. After obtaining small (<40 pA) eEPSCs at -70 mV, stimulation intensity was reduced in small increments to the point where failures (no response) versus successes (visible eEPSC) were clearly and visually distinctive. Stimulation intensity and frequency were kept constant for the remaining duration of the experiment. Following 50 sweeps at -70 mV, cells were gradually raised to +45 mV for 50 sweeps and then back down for a second set of 50 sweeps at -70 mV. Failures versus successes were defined visually. The percentages of silent synapses were calculated using the equation: (1-Ln(F-70)/Ln(F+45))*100, in which F-70 was the

failure rate at -70 mV and F+45 was the failure rate at +45 mV. All drugs and reagents were obtained from Sigma-Aldrich (St Louis, MO).

Effects of junk-food on GluA1 and GluA2 surface expression and synaptic transmission in the NAc of female rats.

Studies in females were conducted alongside those in males but are described separately for the sake of clarity. We first determined the effects of 10 or 30 days of junk-food diet consumption followed by 2 weeks of junk-food deprivation on GluA1 and GluA2 surface expression using the BS_3-crosslinking procedure described above. Separate cohorts with their own chow control groups were used for 10-days and 30-days studies. These durations and time points were chosen because they produce increases in GluA1 surface expression in males (Oginsky et al., 2016a, and results below).

Separate cohorts of obesity-prone female rats were used to examine potential effects of 10 days of junk-food followed by 2 weeks of junk-food deprivation on CP-AMPARs and AMPA/NMDA ratio using the electrophysiological approaches described above. All recordings were conducted from slices prepared when females were in the metestrus/diestrus phase of the cycle. This phase was chosen because this is when motivation for food, food intake, and cue-triggered food-seeking are highest in females (Alonso-Caraballo & Ferrario, 2019; Asarian & Geary, 2006; Palmer & Clegg, 2015). Estrous cycle phase was determined by daily observations of vaginal epithelial cell cytology, precopulatory, and copulatory behaviors as previously described (Alonso-Caraballo & Ferrario, 2019; Marcondes et al., 2002). Briefly, epithelial cells were collected at the same time each day by vaginal lavage during the dark phase and cell morphology was then used to determine cycle phase (Olympus CKX53, bright field). Body weight, food intake, precopulatory and copulatory behaviors (such as ear wiggling, darting, and lordosis) were also used to further verify the estrous cycle phase.

Statistics and data analysis: Two-tailed t-tests, one-way or two-way ANOVAs and Sidak's post-hoc multiple comparisons were used (Prism 8, GraphPad, San Diego, CA). Electrophysiology data was analyzed using Clampfit 10.4 (Molecular Devices). Comparisons were made between data collected within the same cohort of animals (i.e., given chow or junk-food side by side).

Results:

Food intake and body weight:

As expected, male obesity-prone rats are generally heavier than obesity-resistant rats, regardless of diet manipulation (Table 1A). In addition, obesity-prone male rats fed a junk-food diet were heavier than obesity-prone males fed a chow diet (Table 1B). In contrast, junk-food does not produce differential weight gain in obesity-resistant males (Table 1A: Two-way ANOVA group (OP vs. OR) x diet (CH vs. JF) interaction, $p = 0.02$; Sidak's multiple comparisons OR-CH vs. OR-JF, $p = 0.1$). Additionally, obesity-prone males ate more than obesity-resistant males and all males fed a junk-food diet ate more than the chow groups (Table 2A, B).

Similar to the males, female obesity-prone rats are generally heavier than obesity-resistant (Table 1A). Obesity-prone female rats fed a junk-food diet did not gain more weight than obesity-prone females fed a chow diet (Table 1A, B). Furthermore, female obesity-prone ate more junk-food than obesity-resistant (Table 2A: Two-way ANOVA group x diet interaction, $p = 0.02$; Sidak's multiple comparisons OP-JF vs OR-JF $p = 0.03$), chow intake between obesity-prone and obesity-resistant females was similar (Table 2A: Sidak's multiple comparisons OP-CH vs. OR-CH, $p = 0.61$). All female rats fed a junk-food diet ate more than the chow groups (Table 2A, B).

A. Biochemistry:
Effects of junk-food on GluA1/GluA2 surface expression in OP and OR rats.

					Cumulative weight gained (grams)				
						Two-way ANOVA			
Experiment	*Sex*	*Strain*	*Diet*	*N*	*Mean ± SE*	*Interaction*	*OP vs. OR*	*CH vs. JF*	*post-test*
"Junk-food" increases NAc GluA1 surface expression only in obesity-prone male rats.	M	OP	CH	10	102 ± 7.3	p = 0.3	p = 0.1	p = 0.4	no group x diet interaction
			JF	10	101 ± 7				
		OR	CH	8	79 ± 1.6				
			JF	12	96 ± 11				
"Junk-food" deprivation is not necessary for increased GluA1 surface expression in obesity-prone male rats.	M	OP	CH	10	91 ± 4	*p = 0.02	*p < 0.0001	p = 0.7	OP CH vs. OP JF: $t_{(56)}$ = 1.4, p = 0.3
			JF	20	98 ± 3.5				
		OR	CH	10	77 ± 5.2				
			JF	20	67 ± 1.6				
Effects of "junk-food" diet on AMPAR protein expression in female rats.	F	OP	CH	10	41 ± 7.3	p = 0.3	*p = 0.005	p = 0.5	no group x diet interaction
			JF	10	51 ± 7.2				
		OR	CH	8	30 ± 2.4				
			JF	12	27 ± 3.5				

B. Electrophysiology:
Effects of junk-food on CP-AMPAR function and silent synapses in OP rats.

						Unpaired t-test		
Experiment	*Sex*	*Strain*	*Diet*	*N*	*Mean ± SE*	*t*	*df*	*p-value*
"Junk-food" deprivation is required for increased synaptic CP-AMPAR mediated transmission in obesity-prone male rats.	M	OP	CH	6	38 ± 12	4.7	17	*p = 0.0002
			JF	13	89 ± 4.9			
Generation of silent synapses after "junk-food" exposure	M	OP	CH	6	37 ± 6.4	2.8	13	*p = 0.02
			JF	9	60.9 ± 5.5			
Effects of "junk-food" diet on AMPAR synaptic transmission in females.	F	OP	CH	5	93 ± 15	0.2	8	p = 0.83
			JF	5	89 ± 9			

Table 1. Cumulative weight gained across experiments. Weight was monitored daily across all experiments. **A)** Cumulative weight gained during the biochemistry experiments. **B)** Cumulative weight gained during the electrophysiology experiments.

A. Biochemistry:
Effects of junk-food on GluA1/GluA2 surface expression in OP and OR rats.

					Average food intake (Kcal consumed)				
							Two-way ANOVA		
Experiment	*Sex*	*Strain*	*Diet*	*N*	*Mean ± SE*	*Interaction*	*OP vs. OR*	*CH vs. JF*	*post-test*
"Junk-food" increases NAc GluA1 surface expression only in obesity-prone male rats.	M	OP	CH	10	128 ± 1.4	p = 0.2	*p < 0.0001	*p < 0.0001	no group x diet interaction
			JF	10	147 ± 3.7				
		OR	CH	8	106 ± 1				
			JF	12	131 ± 2.4				
"Junk-food" deprivation is not necessary for increased GluA1 surface expression in obesity-prone male rats.	M	OP	CH	10	153 ± 4	p = 0.3	*p = 0.005	*p < 0.0001	no group x diet interaction
			JF	20	234 ± 5.4				
		OR	CH	10	128 ± 1.2				
			JF	20	222 ± 6.7				
Effects of "junk-food" diet on AMPAR protein expression in female rats.	F	OP	CH	10	76 ± 4.2	*p = 0.02	p = 0.29	*p < 0.0001	OP CH vs. OP JF: $t_{(36)}$ = 6.3, p < 0.0001
			JF	10	100 ± 2.1				
		OR	CH	8	79 ± 1.9				
			JF	12	90 ± 2.1				

B. Electrophysiology:
Effects of junk-food on CP-AMPAR function and silent synapses in OP rats.

					Unpaired t-test			
					Mean ± SE	*t*	*df*	*p-value*
"Junk-food" deprivation is required for increased synaptic CP-AMPAR mediated transmission in obesity-prone male rats.	M	OP	CH	6	130 ± 3	5.1	17	*p < 0.0001
			JF	13	184 ± 6.9			
Generation of silent synapses after "junk-food" exposure	M	OP	CH	6	131 ± 4	5.3	13	*p = 0.0002
			JF	9	167 ± 5			
Effects of "junk-food" diet on AMPAR synaptic transmission in females.	F	OP	CH	5	89 ± 4	2.4	6 see note	*p = 0.05
			JF	5	190 ± 42			

note: missing info for one rat per group

Table 2. Average food intake across experiments. Food intake was monitored daily across all experiments. **A)** Average food intake during the biochemistry experiments. **B)** Average food intake during the electrophysiology experiments.

Effects of junk-food diet on AMPAR subunit expression and synaptic transmission in males:

Junk-food increases NAc GluA1 surface expression only in obesity-prone male rats:

We first determined the effects of 10 days of junk-food diet followed by 2 weeks of withdrawal on GluA1 and GluA2 surface expression in obesity-prone and obesity-resistant male rats (see timeline Figure 1A, N = 10 rats per group). GluA1 surface expression was increased in obesity-prone but not obesity-resistant following 2 weeks of junk-food deprivation (Fig. 1B: Two-way ANOVA group x diet interaction: $F_{(1,36)} = 16.1$, $p = 0.0003$; main effect of group $F_{(1,36)} = 16.1$, $p = 0.0003$; Sidak's multiple comparisons, OP-CH vs. OP-JF, $p < 0.0001$). GluA2 surface was not altered by junk-food in either group compared to controls (Fig. 1C). This pattern is consistent with an increase in surface expression of CP-AMPARs and is consistent with previous results (Oginsky et al., 2016).

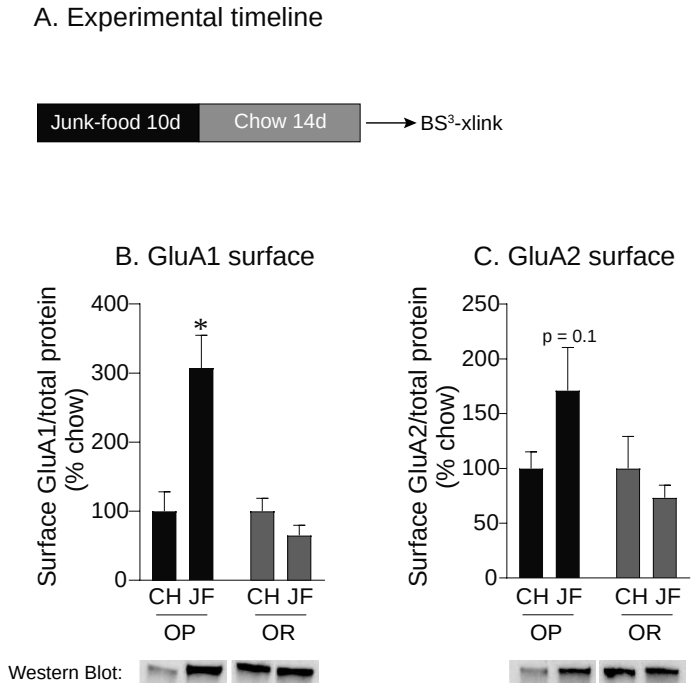


Figure 1. **Junk-food increases NAc GluA1 surface expression only in obesity-prone male rats. A)** Experimental timeline. **B)** Average GluA1 surface expression in obesity-prone and obesity-resistant rats. GluA1 surface expression was increased following 2 weeks of junk-food deprivation in obesity-prone, but not obesity resistant rats. **C)** Average GluA2 surface expression of GluA2 in obesity-prone and obesity-resistant rats. GluA2 surface expression was not altered by junk-food in either group. All data shown as mean ±SEM unless otherwise noted. * = $p < 0.05$ obesity-prone chow vs. junk-food $p < 0.05$.

Junk-food deprivation is not necessary for increased GluA1 surface expression in obesity-prone male rats:

Next, we determined if changes in surface expression of AMPAR subunits in the NAc require a period of junk-food deprivation (see timeline Figure 3.2A, obesity-prone: n = 26 and obesity-resistant: n = 23). We found increases in GluA1 surface expression in both junk-food no deprivation and 24-hr deprivation groups compared to controls (Fig. 3.2B: One-way ANOVA: $F_{(2,23)} = 6.4$, $p = 0.006$; Sidak's multiple comparison CH vs. JF no-dep, $p = 0.005$, CH vs. JF-dep, $p = 0.02$). In addition, junk-food did not affect GluA2 surface expression in either group (Fig. 3.2C). Thus, a period of junk-food deprivation is not needed for increased GluA1 surface expression in obesity-prone males. In contrast, junk-food did not affect GluA1 (Fig. 3.2D) or GluA2 (Fig. 3.2E) surface expression in obesity-resistant males.

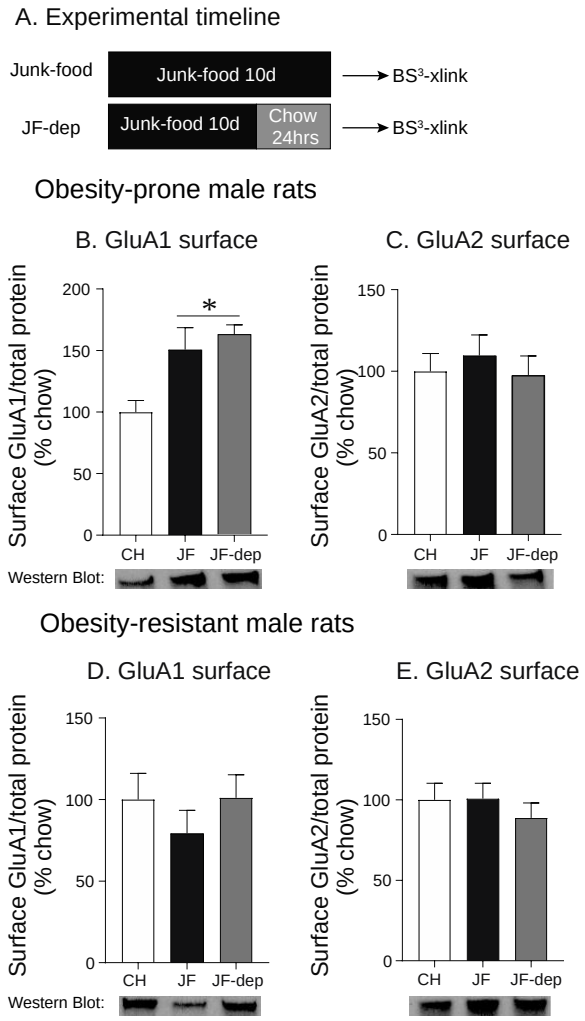


Figure 3.2. Junk-food deprivation is not necessary for increased GluA1 surface expression in obesity-prone male rats. A) Experimental timeline. **B)** Average surface expression of GluA1 in obesity-prone male rats. Similar increases in GluA1 surface expression were found following 10 days of junk-food consumption with and without junk-food deprivation. **C)** Average surface expression of GluA2 in obesity-prone male rats. GluA2 surface expression was not altered by junk-food or junk-food deprivation. **D)** Average surface expression of GluA1 in obesity-resistant male rats. No effects of junk-food or junk-food deprivation were found. **E)** Average surface expression of GluA2 in obesity-resistant male rats. No effects of junk-food or junk-food deprivation were found. * = $p < 0.05$ compared to chow.

Junk-food deprivation is required for increased synaptic CP-AMPAR mediated transmission in obesity-prone male rats.

Next, we used whole-cell patch clamping approaches to determine the contribution of CP-AMPARs to synaptic transmission in NAc MSNs following 10 days of junk-food with and without 24-hr junk-food deprivation (see timeline in Fig. 3.3A; chow: n = 11 cells, 6 rats; junk-food no deprivation: n = 9 cells, 5 rats; junk-food 24-hr deprivation: n = 7 cells; 5 rats). For these studies only obesity-prone rats were used, as we have yet to see effects of diet manipulation on AMPAR protein expression or synaptic transmission in obesity-resistant rats (current results and Oginsky et al., 2016; Derman and Ferrario, 2018). The CP-AMPAR selective antagonist, Naspm produced significant reduction in eEPSC relative amplitude in all groups (Fig. 3.3B: One-way ANOVA: $F_{(5,50)} = 19.5$, $p = 0.02$; Sidak's multiple comparisons: baseline vs. chow, $p < 0.001$, baseline vs. junk-food no dep, $p = 0.0004$, baseline vs. junk-food dep, $p < 0.0001$). However, Naspm produced a significantly larger reduction in eEPSC amplitude in the junk-food 24-hr deprivation group than either control or junk-food no deprivation groups (Fig. 3.3C: One-way ANOVA: $F_{(2,25)} = 4.4$, $p = 0.02$; Sidak's multiple comparisons: chow vs. junk-food no dep, $p = 0.99$; chow vs. junk-food dep, $p = 0.04$; junk-food no dep vs. junk-food dep, $p = 0.05$). Furthermore, the magnitude of Naspm sensitivity was comparable in chow controls and the junk-food no deprivation group (Fig. 3.3C, $p = 0.99$). Thus, although surface CP-AMPAR protein expression is increased in both junk-food exposed groups, a period of junk-food deprivation is necessary for increased CP-AMPAR synaptic transmission.

Generation of silent synapses after junk-food exposure:

The insertion of CP-AMPARs has been associated with metaplasticity of silent synapses in the NAc (Lee et al., 2013; Ma et al., 2014). Therefore, we determined the effects of junk-food with and without 24-hr deprivation on the generation of silent synapses in the NAc of obesity-prone rats (chow: n = 14 cells, 6 rats; junk-food no deprivation: n = 11 cells, 5 rats; junk-food 24-hr deprivation: n = 5 cells, 4 rats). There was a significant increase in the percentage of silent synapses, following 10 days of junk-food without deprivation compared to controls (Fig. 3.3E: One-way ANOVA: $F_{(2, 27)} = 13$, $p = 0.0001$, Sidak's multiple comparisons: chow vs junk-food no dep, $p < 0.05$). Furthermore, the

percentage of silent synapses following 24-hrs of junk-food deprivation was similar to that of chow fed controls (Fig. 3.3E: Sidak's multiple comparison: CH vs. junk-food dep, $p < 0.0001$; junk-food no dep vs. junk-food dep, $p = 0.02$; chow vs junk-food dep, $p = 0.8$). Taken with data above, results show that junk-food increases the number of silent synapses and suggest that the insertion of CP-AMPARs following junk-food deprivation may then lead to a reduction in silent synapses.

In order to provide clues as to why junk-food produces glutamatergic plasticity in obesity-prone, but not obesity-resistant males, we conducted additional analyses comparing GluA1 and GluA2 expression and excitatory transmission between obesity-prone (n = 8) and obesity-resistant (n = 9) male controls. Interestingly, GluA1 surface expression was lower in obesity-prone vs. obesity-resistant rats maintained on chow (Fig. 3.4B: Two-tailed unpaired t-test $t_{(15)} = 2.44$, $p = 0.03$) while GluA2 surface expression was similar between these groups (Fig 3.4C, $p = 0.13$). This may suggest that basal levels of CP-AMPARs are lower in obesity-prone rats, allowing for greater increases following junk-food diet. Furthermore, we also observed a smaller AMPA/NMDA ratio in obesity-prone (n = 11 cells, 8 rats) vs. obesity-resistant rats (n = 11 cells; 7 rats; Fig. 3.4D: Two-tailed unpaired t-test, $t_{(20)} = 2.67$, $p = 0.01$; Fig. 4E example traces), indicative of lower levels of excitatory synaptic transmission in obesity-prone groups. Thus, lower basal levels of excitatory transmission in obesity-prone rats may enhance their capacity for diet-induced plasticity (see discussion).

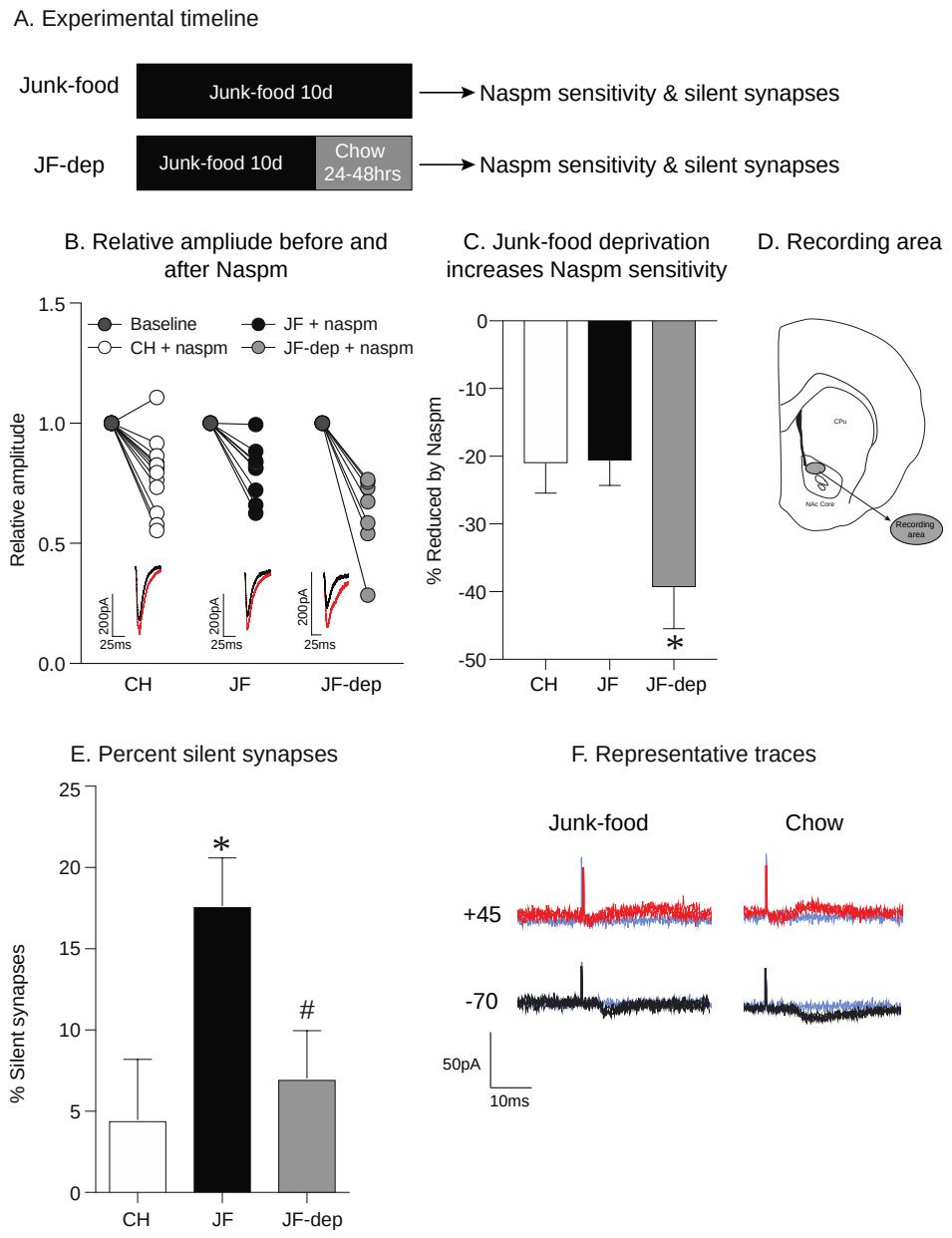


Figure 3.3. Effects of junk-food on CP-AMPAR mediated transmission and generation of silent synapses in obesity-prone male rats. A) Experimental timeline. CP-AMPAR mediated transmission was determined following 10 days of junk-food without junk-food deprivation, whereas measures of silent synapses were made following 10 days of junk-food with and without deprivation. **B)** Normalized eEPSC amplitude before and after Naspm (200 µM). Naspm produced similar decreases in eEPSC amplitude in obesity-prone chow and junk-food groups. However, junk-food deprivation enhanced Naspm sensitivity. The inset shows example traces from chow and junk-food groups before (black) and after bath application of Naspm (red). **C)** Average reduction in eEPSC amplitude in chow and junk-food groups. Junk-food deprivation resulted in enhanced Naspm sensitivity. **D)** Recording area in the NAc core. **E)** The percent of silent synapses in obesity-prone chow, junk-food and junk-food dep groups. The percentage of silent synapses was significantly increased in chow vs. junk-food groups, indicating that consumption of junk-food increases silent synapses in the NAc core of obesity-prone male rats. However, 24-48 hours of junk-food deprivation reduced the percentage of silent synapses back to levels comparable to chow-fed obesity-prone controls, resulting in significant reductions in silent synapses compared to the junk-food group. **F)** Representative traces of eEPSCs at +45 and -70mV for junk-food and chow groups. * = $p < 0.05$ chow vs junk-food, # = $p < 0.05$ junk-food vs. junk-food deprivation**.**

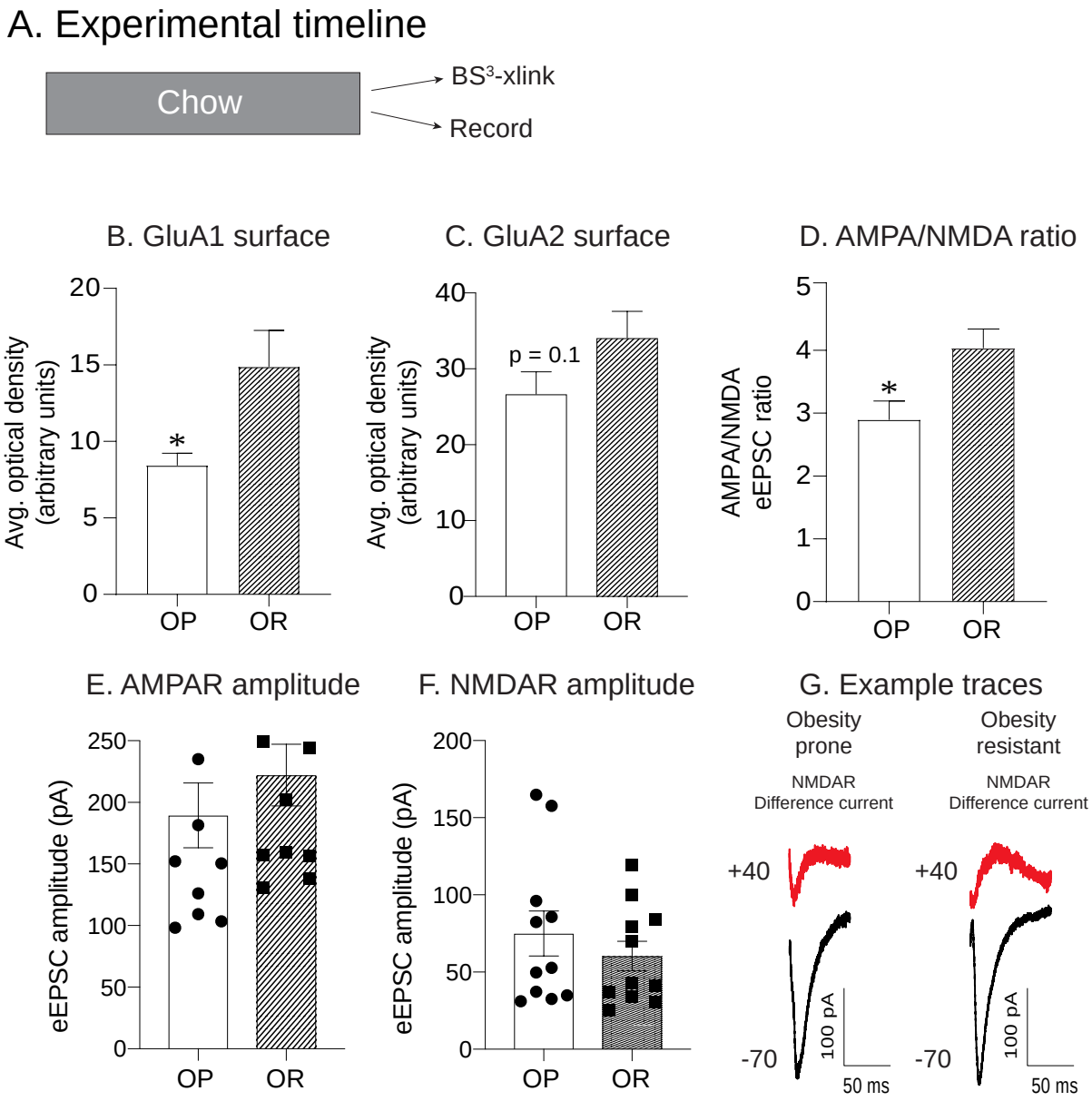


Figure 3.4. **Basal differences in GluA1 surface expression and AMPA/NMDA ratio in obesity-prone vs obesity-resistant male rats. A)** Experimental timeline. **B)** Average GluA1 surface expression in chow fed obesity-prone and obesity-resistant male rats. obesity-prone rats have lower GluA1 surface expression in the NAc compared to obesity-resistant rats. **C)** Average GluA2 surface expression in chow fed obesity-prone and obesity-resistant male rats. GluA2 surface expression was similar between obesity-prone and obesity-resistant groups. **D)** Average AMPA/NMDA ratio was smaller in obesity-prone vs. obesity-resistant male rats. **E)** Example traces for the AMPA/NMDA whole-cell patch clamp recordings. * = $p < 0.05$ OP vs OR.

Effects of junk-food diet on AMPAR protein expression and synaptic transmission in females:

Effects of junk-food diet on AMPAR subunit expression and excitatory synaptic transmission were also examined in obesity-prone (n = 19) and obesity-resistant (n = 17) female rats. For these studies we focused on the 2-week deprivation time point because we first wanted to determine if there are persistent effects of junk-food in females, and because this is when effects had been established in males (Oginsky et al., 2016).

Surprisingly, there was no effect of junk-food on GluA1 (Fig. 3.5B) or GluA2 (Fig. 3.5C) surface expression in females following 10 days of junk-food exposure and 2 weeks of deprivation. It's possible that 10 days of junk-food exposure was insufficient to trigger plasticity in females. Therefore, we also measured GluA1 and GluA2 surface expression following 30 days of junk-food diet exposure and 2 weeks of deprivation in obesity-prone females. However, extending the duration of junk-food exposure did not affect GluA1 or GluA2 surface expression in obesity-prone or obesity-resistant female rats (data not shown).

Given that effects on surface protein expression and synaptic transmission are not always parallel (e.g., results in males above), we also determined the contribution of CP-AMPARs to synaptic transmission following two weeks of junk-food deprivation in obesity-prone females (chow: 6 cells, 5; junk-food: 6 cells; 5 rats). Consistent with biochemical data, junk-food did not alter Naspm sensitivity in obesity-prone females (Fig. 3.5E: Two-tailed unpaired t-test, $t_{(10)} = 0.5$, $p = 0.6$). To determine effects on excitatory transmission more generally, we also measured the AMPA/NMDA ratio in MSNs from obesity-prone females (chow: n = 7 cells, 5 rats; junk-food: n = 8 cells, 5 rats). We found that an increase in the AMPA/NMDA ratio in in junk-food fed vs chow fed controls (Fig. 3.5G: Two-tailed unpaired t-test $t_{(13)} = 2.32$, $p = 0.04$). However, this effect was driven by *decreases* in NMDAR-mediated eEPSC amplitude following junk-food deprivation, and not overt alterations in AMPAR-mediated transmission (Fig. 3.5H: NMDA difference curve trace).

Thus, results in females differed dramatically from those in males, with no evidence of junk-food altering AMPAR-mediated transmission (CP-AMPARs or non-CP-AMPARs) or AMPAR subunit protein expression.

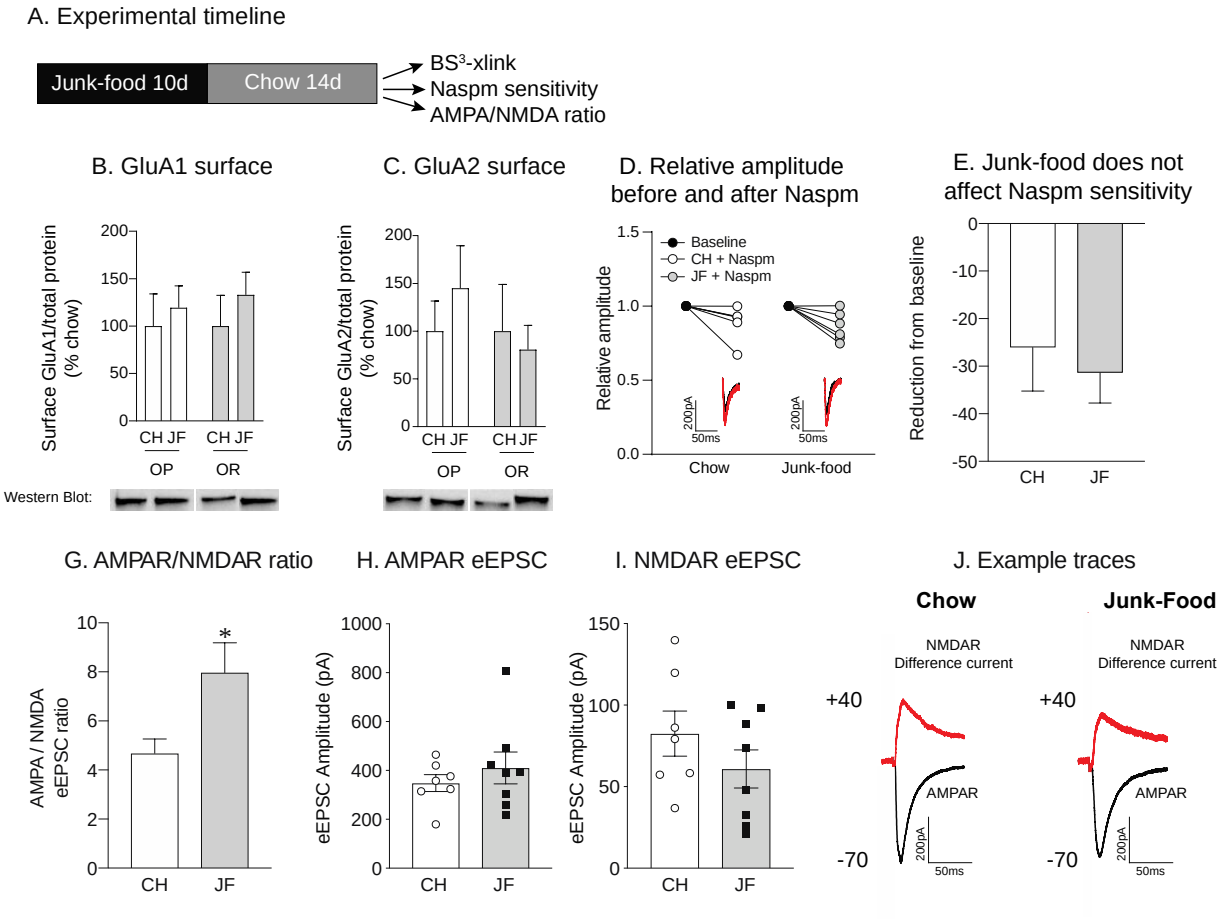


Figure 3.5. Junk-food does not alter CP-AMPARs expression or function in female rats. A) Experiment timeline. **B)** Average GluA1 surface expression was not affected by junk-food in obesity-prone or obesity-resistant female rats. **C)** Average GluA2 surface expression was not affected by junk-food in obesity-prone or obesity-resistant female rats. **D)** Normalized amplitude before and after Naspm (200 µM): Naspm decreases relative amplitude in both obesity-prone female rats fed a chow and junk-food groups. Inset: example eEPSC from chow and junk-food obesity-prone female groups before (black) and after Naspm (red). **E)** No differences in Naspm sensitivity were found between obesity-prone chow vs. junk-food groups.

Discussion

There are established roles for NAc CP-AMPARs in cue-triggered motivation for both food and drug (Wolf, 2017; Dingess et al., 2017; Derman and Ferrario, 2016). In addition, increases in NAc CP-AMPAR expression and function can be induced by both cocaine and junk-food (Wolf, 2016; Oginsky et al., 2016), with the former preceded by the induction of silent synapses (see introduction). However, the precise nature of these alterations and the degree to which they rely on the same underlying mechanisms is not well understood. This has important implications for understanding adaptive vs. maladaptive plasticity that drives food- and drug-seeking behaviors. Furthermore, studies to date have been conducted in males, leaving a large gap in our understanding of potential effects of junk-food in females. Therefore, here we determined the time course of CP-AMPAR up-regulation following junk-food consumption by measuring surface expression of AMPAR subunits and CP-AMPAR-mediated synaptic transmission within the NAc of males and females. In addition, we asked whether CP-AMPAR upregulation in males is accompanied by metaplasticity of NAc silent synapses.

Effects of junk-food in males:

We previously established that eating junk-food enhances NAc CP-AMPAR synaptic transmission after 1, 14 or 21 days of junk-food deprivation in males (Oginsky et al., 2016). However, potential effects on receptor expression at early time points and the necessity of a deprivation period had not been examined. In the current study we found that 10 days of junk-food consumption increased GluA1 but not GluA2 surface expression in obesity-prone males following one to 14 days of junk-food deprivation, and that this effect was similar with and without junk-food deprivation (Figs 3.1, 3.2). This pattern is consistent with an increase in the surface expression of CP-AMPARs (either GluA1/GluA1 or GluA1/GluA3 containing) and with previously observed increases in surface GluA1 but not GluA2 following 2-4 weeks of deprivation (Oginsky et al., 2016). Thus, it appears that increases in NAc CP-AMPAR surface protein expression occur during junk-food exposure (at least by day 11) and persist for relatively long periods after returning to a standard chow diet.

When the effects of junk-food on synaptic transmission were examined in males, we found an increase in the contribution of CP-AMPARs after 1 day of junk-food deprivation compared to chow fed controls (Fig 3.3), replicating previous results using this regimen (Oginsky et al.., 2016). However, CP-AMPAR-mediated transmission was similar between chow fed controls and the junk-food no deprivation group. Taken with biochemical results above, the data suggest that CP-AMPARs accumulate extrasynaptically during junk-food exposure and that junk-food deprivation is needed to then recruit them to the synapse. There is precedence for the extrasynaptic accumulation of CP-AMPARs following cocaine self-administration (Ferrario et al., 2010; see Wolf and Ferrario, 2010 for review). However, one notable difference is that CP-AMPAR accumulation following cocaine occurs gradually during withdrawal; it is absent 1 day after the last cocaine self-administration session and begins to appear ~ 14 days later (McCutcheon et al., 2011). Thus, junk-food consumption has a more rapid effect on CP-AMPARs than cocaine. This may not be entirely surprising, given that brain reward circuits evolved in part to be responsive to food and to direct behavior towards needs that are essential for survival.

It's unclear what triggers and maintains CP-AMPAR synaptic incorporation from a mechanistic standpoint; this is an important and outstanding question in the field (Dong, 2016; Loweth,et al., 2014; Wolf, 2016; Wolf & Ferrario, 2010). However, basal differences between obesity-prone vs. obesity-resistant males may provide some clues. Specifically, in chow fed controls, we found significantly lower basal GluA1 surface expression in obesity-prone vs. obesity-resistant rats (Fig 3.4B), without pronounced differences in surface GluA2. This replicates previously established differences in AMPAR subunit surface expression between these strains (see supplemental Fig 1 from Derman and Ferrario, 2018), and suggests that CP-AMPAR expression may be lower in obesity-prone vs. obesity-resistant males. Furthermore, basal intrinsic excitability of medium spiny neurons (i.e., how readily they fire an action potential) is enhanced in obesity-prone vs. obesity-resistant males (Oginsky & Ferrario, 2019; Oginsky et al., 2016b). Thus, the combination of a reduced firing threshold and relatively low basal CP-AMPAR surface expression may facilitate their up-regulation. While this may explain why CP-AMPAR

surface expression is altered following junk-food consumption, it does not address why deprivation is needed for their synaptic insertion.

As stated earlier, the literature on striatal CP-AMPAR plasticity has overwhelmingly focused on CP-AMPAR upregulation in the incubation of cocaine craving model, with relatively few studies of essential reinforcers (though see also below). In the case of cocaine, there is evidence for potential mechanistic links between increases in GluN3-containing NMDARs and CP-AMPAR synaptic incorporation (Dong et al., 2017 for review; Yuan et al., 2013). Although our AMPA/NMDA ratio data in males do not suggest strong alterations in overall NMDAR transmission, the NMDAR component was only evaluated at one holding potential (+40 mV). This could mask potential effects on GluN3- vs GluN2-containing NMDAR subtypes, given that GluN3-containing NMDARs (GluN3A, GluN3B) contribute more strongly at negative potentials (due to their low sensitivity to Mg^{2+}), while GluN2-containing NMDARs (GluN2B or GluN2A) dominate at positive potentials. Thus, potential effects of junk-food on NMDARs in males will be important to address in future studies.

Effects of junk-food on silent synapses in males:

Given the link between CP-AMPARs and metaplasticity of silent synapses (see introduction), we also determined the proportion of silent synapses in obesity-prone males following junk-food consumption. There was a marked increase in the proportion of silent synapses following 10 days of junk-food consumption compared to chow fed controls (Fig 3.3E). Furthermore, 1 day of junk-food deprivation resulted in a return of silent synapses to levels comparable to chow controls. This pattern of an increase followed by a return to baseline in the proportion of silent synapses is consistent with their maturation. In addition, CP-AMPAR insertion itself is associated with the maturation of silent synapses. Thus, these data in combination with CP-AMPAR measures discussed above suggest that junk-food consumption enhances the number of immature silent synapses, and that the subsequent synaptic insertion of CP-AMPARs following junk-food deprivation leads to their maturation. A similar pattern has been found in the NAc following cocaine withdrawal (see Dong et al., 2017), however on much longer time scales.

Increases in silent synapses can be due to the removal of AMPARs from existing synapses, or to the addition of new synapses lacking AMPARs (Brown et al., 2011; Lee et al., 2013; Graziane et al., 2016). Our results suggest that junk-food generates *de novo* silent synapses. Specifically, we see an increase in GluA1 surface expression in the NAc both with and without deprivation (see Fig. 3.1A and 3.2B) and no changes in GluA2 surface expression. If AMPARs were being removed to generate silent synapses from existing synapses, then may expect to find reductions in GluA1 and/or GluA2 surface protein expression following junk-food consumption without deprivation. However, this is not the case. In addition, we also saw no evidence for increases in intracellular protein expression, which would be expected to result from AMPAR internalization. Furthermore, high-fat diet consumption increases the density of mature mushroom shaped dendritic spines within the NAc (Dingess et al., 2017), an indirect indicator of increases in synaptic contacts. Although these spine measures were made after a period of junk-food deprivation, they are nonetheless consistent with the idea that consumption of calorie dense foods enhances number of synapses in the NAc.

Junk-food does not affect AMPAR expression or function in females:

When effects of junk-food were examined in females, we found no evidence for alterations in AMPAR expression or function. We began with biochemical studies in which we varied the duration of junk food exposure, collecting tissue after 10 days or one month of junk-food access and 2 weeks of deprivation. There were no differences in surface or intracellular GluA1 or GluA2 expression in any group. It's possible that junk-food could have produced transient effects that returned to levels comparable to that of chow controls within the 2-week deprivation period. However, we chose this time point in order to identify effects that persist for reasonably long periods following junk-food removal. Since we did not attempt to control for the cycle in biochemical studies, and ovarian hormones can affect NAc AMPAR levels (Le Saux et al., 2006), it's possible that variance across the cycle could have masked potentially small effects. Therefore, for electrophysiological studies, recordings were made from slices prepared when females were in metestrus/diestrus. This phase of the cycle was chosen because cue-triggered food seeking and food intake are greatest in this phase (Alonso-Caraballo et al., 2019). Despite controlling for the cycle, we still found no evidence for junk-food induced

alterations in the CP-AMPAR mediated transmission (Fig. 3.5E). Furthermore, although there was an increase in the AMPA/NMDA ratio following 10 days of junk-food exposure and 2 weeks of deprivation, this was actually due to a *reduction* in the NMDA receptor mediate component (Fig. 3.5G-J). Thus, junk-food induced plasticity differs quite dramatically in males and females.

To our knowledge this is the first examination of diet-induced NAc glutamatergic plasticity in females, and studies of CP-AMPARs following cocaine exposure have yet to be completed in females. In addition, there is surprisingly little information regarding NAc glutamatergic plasticity in females, although estradiol reduces NAc dendritic spine density (an indirect measure of excitatory synapses; Peterson, et al., 2015), AMPAR specific binding, and mRNA for the GluA2 subunit (Le Saux et al., 2006). There is of course a strong precedence for enhanced sensitivity of females to psychostimulant drugs including cocaine, as well as direct acting dopamine agonists (see Becker & Chartoff, 2019 and Yoest et al., 2014 for review), and established fluctuations in the preference for food, sex, or drugs across the cycle (Yoest et al., 2014; Alonso-Caraballo et al., 2019). It is worthwhile to note that repeated cocaine treatment produces more robust increases in dendritic spine density (Forlano & Woolley, 2010; Wissman et al., 2011) and miniature EPSC frequency (Wissman et al., 2012; Wissman et al., 2011) in the NAc of females vs. males. Thus, it seems that effects of sugary, fatty, foods vs. cocaine exposure on glutamatergic plasticity differs dramatically in females. This may not be entirely surprising given the strong influence of ovarian hormones on feeding, metabolism, and energy storage (Asarian & Geary, 2013; Palmer & Clegg, 2015) and certainly warrants further investigation.

Summary and additional considerations:

We found that junk-food consumption increases CP-AMPAR surface expression and generates silent synapses in the NAc of male rats. In addition, a brief period of junk-food deprivation is needed for the synaptic insertion of CP-AMPARs and the maturation of silent synapses in males. In contrast junk-food did not induce AMPAR plasticity in females, but may instead alter NMDAR-mediated transmission. Thus, these studies reveal sex differences in the effects of junk-food on NAc synaptic plasticity. In addition, they provide novel insights into how essential food rewards alter NAc function.

References:

Alonso-Caraballo, Y., & Ferrario, C. R. (2019). Effects of the estrous cycle and ovarian hormones on cue-triggered motivation and intrinsic excitability of medium spiny neurons in the Nucleus Accumbens core of female rats. *Hormones and Behavior*, *116*, 104583. https://doi.org/10.1016/j.yhbeh.2019.104583

Asarian, L., & Geary, N. (2006). Modulation of appetite by gonadal steroid hormones. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1471), 1251–1263. https://doi.org/10.1098/rstb.2006.1860

Asarian, L., & Geary, N. (2013). Sex differences in the physiology of eating. *AJP: Regulatory, Integrative and Comparative Physiology*, *305*(11), R1215–R1267. https://doi.org/10.1152/ajpregu.00446.2012

Becker, J. B., & Chartoff, E. (2019, January 1). Sex differences in neural mechanisms mediating reward and addiction. *Neuropsychopharmacology*. Nature Publishing Group. https://doi.org/10.1038/s41386-018-0125-6

Brown, T. E., Lee, B. R., Mu, P., Ferguson, D., Dietz, D., Ohnishi, Y. N., … Schlüüter, O. M. (2011). A silent synapse-based mechanism for cocaine-induced locomotor sensitization. *Journal of Neuroscience*, *31*(22), 8163–8174. https://doi.org/10.1523/JNEUROSCI.0016-11.2011

Chaudhri, N., Sahuque, L. L., Schairer, W. W., & Janak, P. H. (2010). Separable roles of the nucleus accumbens core and shell in context-and cue-induced alcohol-seeking. *Neuropsychopharmacology*, *35*(3), 783–791. https://doi.org/10.1038/npp.2009.187

Conrad, K. L., Tseng, K. Y., Uejima, J. L., Reimers, J. M., Heng, L. J., Shaham, Y., … Wolf, M. E. (2008). Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature*, *454*(7200), 118–121. https://doi.org/10.1038/nature06995

Corbit, L. H., & Balleine, B. W. (2011). The General and Outcome-Specific Forms of Pavlovian-Instrumental Transfer Are Differentially Mediated by the Nucleus Accumbens Core and Shell. *Journal of Neuroscience,* 31(33), 11786-11794.

Corbit, L. H., & Balleine, B. W. (2016). Learning and motivational processes contributing to pavlovian–instrumental transfer and their neural bases: Dopamine and beyond. *Current Topics in Behavioral Neurosciences*, *27*, 259–289. https://doi.org/10.1007/7854_2015_388

Counotte, D. S., Schiefer, C., Shaham, Y., & O'Donnell, P. (2014). Time-dependent decreases in nucleus accumbens AMPA/NMDA ratio and incubation of sucrose craving in adolescent and adult rats. *Psychopharmacology*, *231*(8), 1675–1684. https://doi.org/10.1007/s00213-013-3294-3

Darling, R. A., Dingess, P. M., Schlidt, K. C., Smith, E. M., & Brown, T. E. (2016). Incubation of food craving is independent of macronutrient composition. *Scientific Reports*, *6*, 30900. https://doi.org/10.1038/srep30900

Derman, R. C., & Ferrario, C. R. (2018). Enhanced incentive motivation in obesity-prone rats is mediated by NAc core CP-AMPARs. *Neuropharmacology*, *131*, 326–336. https://doi.org/10.1016/j.neuropharm.2017.12.039

Di Ciano, P., Cardinal, R. N., Cowell, R. A., Little, S. J., & Everitt, B. J. (2001). Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. *Journal of Neuroscience*, *21*(23), 9471–9477. https://doi.org/10.1523/jneurosci.21-23-09471.2001

Di Ciano, Patricia, & Everitt, B. J. (2001). Dissociable Effects of Antagonism of NMDA and AMPA/KA Receptors in the Nucleus Accumbens Core and Shell on Cocaine-seeking Behavior. *Neuropsychopharmacology*, *25*(3), 341–360. https://doi.org/10.1016/S0893-133X(01)00235-4

Dingess, P. M., Darling, R. A., Derman, R. C., Wulff, S. S., Hunter, M. L., Ferrario, C. R., & Brown, T. E. (2017). Structural and Functional Plasticity within the Nucleus Accumbens and Prefrontal Cortex Associated with Time-Dependent Increases in

Food Cue-Seeking Behavior. *Neuropsychopharmacology*, *42*(12), 2354–2364. https://doi.org/10.1038/npp.2017.57

Dong, Y. (2016). Silent synapse-based circuitry remodeling in drug addiction. *International Journal of Neuropsychopharmacology*. Oxford University Press. https://doi.org/10.1093/ijnp/pyv136

Dong, Y., Taylor, J. R., Wolf, M. E., & Shaham, Y. (2017). Circuit and synaptic plasticity mechanisms of drug relapse. *Journal of Neuroscience*, *37*(45), 10867–10876. https://doi.org/10.1523/JNEUROSCI.1821-17.2017

Ferrario, C. R., Li, X., Wang, X., Reimers, J. M., Uejima, J. L., & Wolf, M. E. (2010). The role of glutamate receptor redistribution in locomotor sensitization to cocaine. *Neuropsychopharmacology*, *35*(3), 818–833. https://doi.org/10.1038/npp.2009.190

Forlano, P. M., & Woolley, C. S. (2010). Quantitative analysis of pre- and postsynaptic sex differences in the nucleus accumbens. *The Journal of Comparative Neurology*, *518*(8), 1330–1348. https://doi.org/10.1002/cne.22279

Huang, Y. H., Lin, Y., Mu, P., Lee, B. R., Brown, T. E., Wayman, G., … Dong, Y. (2009). In Vivo Cocaine Experience Generates Silent Synapses. *Neuron*, *63*(1), 40–47. https://doi.org/10.1016/j.neuron.2009.06.007

Huang, Y. H., Schlüter, O. M., & Dong, Y. (2015). Silent Synapses Speak Up: Updates of the Neural Rejuvenation Hypothesis of Drug Addiction. *Neuroscientist*. https://doi.org/10.1177/1073858415579405

Ito, R., Robbins, T. W., & Everitt, B. J. (2004). Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. *Nature Neuroscience*, *7*(4), 389–397. https://doi.org/10.1038/nn1217

Kourrich, S., Rothwell, P. E., Klug, J. R., & Thomas, M. J. (2007). Cocaine Experience Controls Bidirectional Synaptic Plasticity in the Nucleus Accumbens. *Journal of Neuroscience*, *27*(30), 7921–7928. https://doi.org/10.1523/JNEUROSCI.1859-07.2007

Kreitzer, A. C., & Malenka, R. C. (2008, November 26). Striatal Plasticity and Basal Ganglia Circuit Function. *Neuron*. NIH Public Access.

https://doi.org/10.1016/j.neuron.2008.11.005

Le Saux, M., Estrada-Camarena, E., & Di Paolo, T. (2006). Selective estrogen receptor-alpha but not -beta agonist treatment modulates brain alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. *Journal of Neuroscience Research*, *84*(5), 1076–1084. https://doi.org/10.1002/jnr.21007

Lee, B. R., Ma, Y. Y., Huang, Y. H., Wang, X., Otaka, M., Ishikawa, M., … Dong, Y. (2013). Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving. *Nature Neuroscience*, *16*(11), 1644–1651. https://doi.org/10.1038/nn.3533

Levin, B. E., Dunn-Meynell, A., Balkan, B., & Keesey, R. E. (1997). Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. *The American Journal of Physiology*, *273*, R725–R730. Retrieved from http://www.physiology.org/doi/pdf/10.1152/ajpregu.1997.273.2.R725

Loweth, J. A., Tseng, K. Y., & Wolf, M. E. (2014). Adaptations in AMPA receptor transmission in the nucleus accumbens contributing to incubation of cocaine craving. *Neuropharmacology*. https://doi.org/10.1016/j.neuropharm.2013.04.061

Ma, Y.-Y., Lee, B. R., Wang, X., Guo, C., Liu, L., Cui, R., … Dong, Y. (2014). Bidirectional Modulation of Incubation of Cocaine Craving by Silent Synapse-Based Remodeling of Prefrontal Cortex to Accumbens Projections. *Neuron*, *83*(6), 1453–1467. https://doi.org/10.1016/j.neuron.2014.08.023

Marcondes, F. K., Bianchi, F. J., & Tanno, A. P. (2002). Determination of the estrous cycle phases of rats: some helpful considerations. *Brazilian Journal of Biology*, *62*(4a), 609–614. https://doi.org/10.1590/S1519-69842002000400008

Oginsky, M. F., & Ferrario, C. R. (2019). Eating junk-food has opposite effects on intrinsic excitability of nucleus accumbens core neurons in obesity-susceptible vs. -resistant rats. *Journal of Neurophysiology*, *122*, 1264–1273. https://doi.org/10.1152/jn.00361.2019

Oginsky, M. F., Goforth, P. B., Nobile, C. W., Lopez-Santiago, L. F., & Ferrario, C. R. (2016a). Eating junk-food Produces Rapid and Long-Lasting Increases in NAc CP-

AMPA Receptors: Implications for Enhanced Cue-Induced Motivation and Food Addiction. *Neuropsychopharmacology*, *41*(13), 2977–2986. https://doi.org/10.1038/npp.2016.111

Oginsky, M. F., Maust, J. D., Corthell, J. T., & Ferrario, C. R. (2016b). Enhanced cocaine-induced locomotor sensitization and intrinsic excitability of NAc medium spiny neurons in adult but not in adolescent rats susceptible to diet-induced obesity. *Psychopharmacology*, *233*(5), 773–784. https://doi.org/10.1007/s00213-015-4157-x

Palmer, B. F., & Clegg, D. J. (2015). The sexual dimorphism of obesity. *Molecular and Cellular Endocrinology*. NIH Public Access. https://doi.org/10.1016/j.mce.2014.11.029

Peterson, B. M., Mermelstein, P. G., & Meisel, R. L. (2015). Estradiol mediates dendritic spine plasticity in the nucleus accumbens core through activation of mGluR5. *Brain Structure and Function*, *220*(4), 2415–2422. https://doi.org/10.1007/s00429-014-0794-9

Robinson, M. J. F., Burghardt, P. R., Patterson, C. M., Nobile, C. W., Akil, H., Watson, S. J., … Ferrario, C. R. (2015). Individual Differences in Cue-Induced Motivation and Striatal Systems in Rats Susceptible to Diet-Induced Obesity. *Neuropsychopharmacology*, *40*(9), 2113–2123. https://doi.org/10.1038/npp.2015.71

Slaker, M., Churchill, L., Todd, R. P., Blacktop, J. M., Zuloaga, D. G., Raber, J., … Sorg, B. A. (2015). Removal of perineuronal nets in the medial prefrontal cortex impairs the acquisition and reconsolidation of a cocaine-induced conditioned place preference memory. *J Neurosci*, *35*(10), 4190–4202. https://doi.org/10.1523/JNEUROSCI.3592-14.2015

Slaker, M. L., Jorgensen, E. T., Hegarty, D. M., Liu, X., Kong, Y., Zhang, F., … Sorg, B. A. (2018). Cocaine exposure modulates perineuronal nets and synaptic excitability of fast-spiking interneurons in the medial prefrontal cortex. *ENeuro*, *5*(5). https://doi.org/10.1523/ENEURO.0221-18.2018

Wissman, A. M., May, R. M., & Woolley, C. S. (2012). Ultrastructural analysis of sex differences in nucleus accumbens synaptic connectivity. *Brain Structure & Function*,

217(2), 181–190. https://doi.org/10.1007/s00429-011-0353-6

Wissman, A. M., McCollum, A. F., Huang, G.-Z., Nikrodhanond, A. A., & Woolley, C. S. (2011). Sex differences and effects of cocaine on excitatory synapses in the nucleus accumbens. *Neuropharmacology*, *61*(1–2), 217–227. https://doi.org/10.1016/j.neuropharm.2011.04.002

Wolf, M. E. (2016). Synaptic mechanisms underlying persistent cocaine craving. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn.2016.39

Wolf, M. E., & Ferrario, C. R. (2010). AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/j.neubiorev.2010.01.013

Wolf, M. E., & Tseng, K. Y. (2012). Calcium-permeable AMPA receptors in the VTA and nucleus accumbens after cocaine exposure: When, how, and why? *Frontiers in Molecular Neuroscience*. https://doi.org/10.3389/fnmol.2012.00072

Yoest, K. E., Cummings, J. A., & Becker, J. B. (2014). Estradiol, Dopamine and Motivation. *Cent Nerv Syst Agents Med Chem. Cent Nerv Syst Agents Med Chem*, *14*(2), 83–89. https://doi.org/10.14440/jbm.2015.54.A

Yuan, T., Mameli, M., O'Connor, E. C., Dey, P. N., Verpelli, C., Sala, C., … Bellone, C. (2013). Expression of Cocaine-Evoked Synaptic Plasticity by GluN3A-Containing NMDA Receptors. *Neuron*, *80*(4), 1025–1038. https://doi.org/10.1016/j.neuron.2013.07.050

CHAPTER 4
General Discussion

Previous studies in our laboratory have found pre-existing differences in cue-triggered motivation, NAc MSN intrinsic excitability and AMPAR-mediated transmission in obesity-prone and obesity-resistant male rats. However, changes in the motivated behavior of female rats, or the accompanying changes in the brain were unknown. To help bridge this gap, my thesis focused on examining differences in cue-triggered motivation, NAc MSN intrinsic excitability and AMPAR-mediated transmission between obesity-prone and obesity-resistant *female* rats, and how ovarian hormones influence behavior.

Homeostatic regulation of food intake is controlled by hypothalamic brain circuits and interactions between peripheral hormonal signals and central nervous system processing of hunger and satiety (see Ferrario et al., 2016 for review). However, brain circuits other than those regulating homeostatic aspects of hunger and satiety are also involved in food consumption (Berthoud, 2012; Ferrario et al., 2016; Fulton, 2010; Kelley et al., 2005). For example, actions of metabolic signals, neuropeptides and neurotransmitters can modulate the mesolimbic and mesocorticolimbic reward systems to encode emotional and cognitive aspects of feeding (Fulton, 2010).

Based on studies in humans and male obesity-prone rats (discussed in the introduction), I examined differences in cue-triggered motivation between obesity-prone and -resistant females and roles for ovarian hormones in the regulation of these behaviors and of activity within the NAc. In addition, I determined how consumption of a sugary, fatty “junk-food” diet affects NAc glutamatergic transmission in male and female obesity-prone and resistant rats. Overall, these studies showed that obesity-prone rats have enhanced cue-triggered motivation and NAc core MSN intrinsic excitability. Additionally, both cue-triggered motivation and MSN intrinsic excitability are enhanced during metestrus/diestrus compared to proestrus/estrus in obesity-prone rats. Furthermore, effects of junk-food diet on glutamatergic transmission are mediated by AMPAR in

obesity-prone male rats, but obesity-prone female rats seem to have a different mechanism.

The discussion below focuses on effects of the estrous cycle on behavior and intrinsic excitability in female obesity-prone and obesity-resistant rats. I will discuss the differences between food intake, motivation to work for food and cue-triggered motivation in obesity-prone and obesity-resistant females and the role of the estrous cycle. Finally, differences in MSN core intrinsic excitability are also examined between obesity-prone and obesity-resistant female rats, as well as the role of the estrous cycle.

Food intake and motivation to work for food are modulated by the estrous cycle in obesity-prone and obesity-resistant female rats:

I first examined potential differences in food intake, weight and fat mass between obesity-prone and obesity-resistant female rats in order to verify phenotypes in these lines. Similar to males, obesity-prone female rats eat and weigh more than obesity-resistant rats (Fig. 2.1). Consistent with this, the percent of fat mass is also higher in obesity-prone compared to obesity-resistant females (Fig 2.1). Furthermore, when we determined food intake across the cycle in naturally cycling rats, I found that in both obesity-prone and obesity-resistant female rats food intake decreases during the proestrus/estrus phases compared to metestrus/diestrus (Fig 2.2). This is consistent with established effects of the cycle on food intake (see also Introduction section: ***Effects of ovarian hormones on food intake***). Additionally, I did not observe any differences in number of estrous cycles between obesity-prone and obesity-resistant rats (discussed in Chapter 2). Moreover, the number of cycles was comparable to those observed in outbred rats. These results are consistent with what has been previously shown in female outbred rats. Thus, there is no evidence for fundamental dysregulation of estrous cycle in obesity-prone and obesity-resistant female rats.

Next, I examined differences in motivation to work for sucrose between obesity-prone and obesity-resistant female rats. Here, I trained our rats to lever press in order to receive a sucrose pellet (instrumental conditioning that results in goal-directed actions). Then, I tested them under progressive ratio schedule of reinforcement to determine how motivated the rats are to work for sucrose pellet delivery. I found no differences in

motivation to work for a sucrose pellet between obesity-prone and obesity-resistant rats (i.e., they had similar break points). Additionally, I examined estrous cycle effects on break point and found that in both obesity-prone and obesity-resistant females the motivation to work for food was lower during the estrus/proestrus phases vs. metestrus/diestrus. This is similar to the observed estrous cycle effects on food intake in obesity-prone and obesity-resistant female rats, and to the existing literature (Hecht et al., 1999). Interestingly, motivation to work for food (i.e., break point) was similar between obesity-prone and obesity-resistant female rats. Although, visual trends were observed for obesity-resistant female rats to have higher break point compared to obesity-prone but it did not reach statistical significance.

This is the first study in our lab to determine differences in instrumental performance between obesity-prone and obesity-resistant female rats. Two additional studies from our lab have examined differences in instrumental responding between obesity-prone and obesity-resistant male rats. One study found that obesity-prone male rats had moderate increases in break point in progressive ratio testing compared to obesity-resistant rats (Vollbrecht et al., 2015). The other found that male obesity-resistant rats had greater rates of active lever responding on a variable interval schedule vs. obesity-prone (Derman & Ferrario, 2018). However, it is difficult to make direct comparisons between the three studies. This is because for each study there are differences in the experimental design that can impact the results. For example, in my female study, I used progressive ratio testing in which rats were mildly food restricted (food was removed 5-6 hours prior to testing), and lever presses resulted in food pellet delivery, but did not result in presentation of a discrete cue. In Derman & Ferrario, variable interval schedule was used, and rats were chronically food deprived across the experiment (food restricted to 85-90% of their free-feeding body weight), similarly lever presses did not result in the presentation of a discrete cue. However, in Vollbrecht et al., progressive ratio testing was used. The experimental design used by Vollbrecht et al., was different to the one used in the female study that I am discussing here. For example, in the male study (Vollbrecht), the rats were freely fed throughout the experiment, while the females in my study were food restricted. In addition, in the Vollbrecht study, active lever presses resulted in the presentation of an auditory cue in addition to the delivery of the food pellet, whereas in the female study,

pressing on either active or inactive levers did not result in presentation of any cues. In both the female study described here and in the male study by Derman & Ferrario, obesity-resistant rats tended to have greater active lever responses compared to obesity-prone rats (Derman & Ferrario, 2018). In contrast, in Vollbrecht et al., in obesity-prone male rats break point was modestly elevated vs. obesity-resistant rats (Vollbrecht et al., 2015). Thus, it is possible that presentation of the cue in the Vollbrecht study added salience to the task, resulting in enhanced motivation to work for food in obesity-prone vs. obesity-resistant male rats. In contrast, it is possible that the trend for obesity-resistant rats to have greater lever presses in the female study and Derman & Ferrario 2018, might be due to food deprivation. For example, it might be the case that obesity-resistant rats have low fat mass (thus, lower energy storage) and this makes them more motivated to work for food, so they can compensate for their energy loss during the deprivation period. Overall, differences in motivation to work for food between obesity-prone or obesity-resistant rats appear to depend on the conditions used in the experiment. For example, if obesity-resistant rats are food deprived they might work harder for food compared to obesity-prone rats. However, if a cue is associated with the food delivery obesity-prone rats may be more motivated to work for the food.

Cue-triggered motivation is enhanced in obesity-prone female rats:

It is well-established that sights, sounds, and smells that are associated with food (i.e., external food cues) can influence feeding behaviors that lead to obesity. This is because these cues can acquire incentive value and trigger food-seeking and ultimately food intake, even in the absence of hunger (Berridge, 2001; Cardinal et al., 2002; Milton & Everitt, 2010). However, previous studies have not addressed obesity or pre-existing differences in susceptibility to obesity. It has been shown that increased activation in the NAc triggered by food-cues predicts future weight gain in normal weight people and it is accompanied by future inability to lose weight after obesity (Demos et al., 2012; Kishinevsky et al., 2012; Yokum et al., 2011). This suggests that enhanced responsivity of the brain reward system may be a cause, rather than consequence of obesity. Therefore, I set out to test the hypothesis that obesity-prone female rats have enhanced cue-triggered motivation vs. obesity-resistant rats. In addition, given that motivation for

food itself is affected by the cycle in obesity-prone and obesity-resistant females (discussed above) I also determined the effects of the estrous cycle on cue-triggered motivation.

Rats were trained to discriminate between an auditory conditioned stimulus paired with sucrose pellet delivery (CS+) vs. an auditory CS that did not result in delivery of sucrose (CS-). Next, rats were tested under extinction conditions in which the CS+ or CS- were presented but no sucrose pellets were delivered. During this test, we measured the number of times the rats went to the food cup during the presentation of the CS but in the absence of sucrose delivery. We found that obesity-prone female rats have enhanced cue-triggered motivation vs. obesity-resistant female rats. Thus, both obesity-prone male and female rats have enhanced cue-triggered motivation. This is consistent with the enhanced incentive motivation found in obesity-susceptible male rats (Derman & Ferrario, 2018; Robinson et al., 2015). Although I found differences in the extinction test for cue-triggered motivation, I did not find differences in learning during training for Pavlovian conditioned approach between obesity-prone and obesity-resistant female rats. Additionally, conditioned approach behavior is mediated by the NAc (Berridge 2007, 2012; Berridge & Robinson, 1998; Robinson & Berridge, 1993). Thus, these results suggest that obesity-prone female rats might have enhanced activity in the NAc vs. obesity-resistant rats.

Cue-triggered motivation is modulated by the estrous cycle in obesity-prone but not obesity-resistant rats:

Additionally, I examined estrous cycle modulation of cue-triggered motivation. We found that this behavior was affected by the estrous cycle only in female obesity-prone rats, while it remained stable across the cycle in obesity-resistant rats (Fig 2.5). Specifically, in obesity-prone rats, conditioned approach was lower during proestrus/estrus phase of the estrous cycle than the metestrus/diestrus phase. It is well known that the estrous cycle influence motivation for a variety of rewards including food, sex, and drugs of addiction (Yoest et al., 2014). For example, during estrus, female rats are more motivated to work for cocaine (Hecht et al., 1999; Roberts et al., 1989) and more motivated to engage in sexual behaviors (Bermant, 1961; Cummings & Becker, 2012; Meyerson et al., 1973)

compared to other phases of the estrous cycle. However, during proestrus and estrus motivation to work for food is decreased relative to diestrus (Hecht et al., 1999; Yoest et al., 2014; Abbott et al., 2016; Pitchers et al., 2015; Richard et al., 2017). Hence, during estrus, motivation for sex and drug increases, whereas motivation for food decreases. Together these results suggest that the estrous cycle has opposing regulation of motivation for sex/drugs vs. food. However, these variables have been studied independently.

Overall, a competition in motivation for sex/drugs vs. food seems to be present during the estrus phase of the estrous cycle in female rats. For example, ovariectomized female rats exhibit *increased* sexual motivation after treatment with estradiol and progesterone (this treatment produces sexual receptivity similar to estrus; Cummings & Becker, 2012). Additionally, females in estrus form *stronger* associations with cues that predict cocaine reward (Calipari et al., 2017). Similarly, female rats that undergo Pavlovian conditioning during estrus have greater cocaine-seeking behaviors after a withdrawal period (Johnson et al., 2019). By contrast, we observed a decrease in motivational responses to food cues in obesity-prone female rats during the estrus phase of the estrous cycle. Thus, during the estrus phase cue-triggered motivation can be enhanced or reduced depending on the type of reward-paired cue that is presented.

Estrous cycle modulation of cue-triggered motivation was absent in obesity-resistant rats. Thus, in obesity-resistant female rats, there is a dissociation between the ability of the cycle to influence food intake and food-seeking behaviors but not motivational responses to food cues. Additionally, we also saw similar modulation across the cycle of NAc core MSN intrinsic excitability in obesity-prone but not obesity-resistant rats (discussed further below). Hence, there is the possibility that the brain areas that control only cue-triggered motivation in obesity-resistant rats are not sensitive to the fluctuations in ovarian hormones. However, this raises an interesting question. What if only brain areas that modulate cue-triggered motivation in obesity-resistant rats are not sensitive to estrous cycle fluctuations? Future studies could address this by examining Pavlovian-to-instrumental transfer (PIT) to determine estrous cycle effects on cue-triggered food-seeking in both obesity-prone and obesity-resistant rats. PIT incorporates Pavlovian

conditioning and instrumental learning, thus maybe by incorporating an instrumental task, we would be able to observe regulation of estrous cycle in obesity-resistant rats.

Repeated treatment with ovarian hormones decreased cue-triggered motivation in female obesity-prone and outbred rats:

To further understand the estrous cycle effects described above, we determined how ovarian hormones, specifically estradiol and progesterone, affect cue-triggered motivation in ovariectomized obesity-prone and outbred rats. We found that repeated treatment with estradiol and progesterone vs. vehicle control results in decreased cue-triggered motivation in obesity-prone and outbred rats. In contrast, a single treatment with estradiol and progesterone was not sufficient to decrease cue-triggered motivation. In these studies, we included ovariectomized rats that received repeated treatments with estradiol or progesterone alone. We found that repeated treatment with estradiol-alone or progesterone-alone did not significantly decrease cue-triggered motivation measured by conditioned approach. Together, our results suggest that estradiol and progesterone act synergistically to decrease cue-triggered motivation.

The repeated estradiol-progesterone treatment used in our study produces behavioral estrus and sexual receptivity in rats (see methods Chapter 2). When rats are sexually receptive, motivation shifts to favor mating over feeding (Fessler, 2003). However, the behavioral and neuronal mechanisms involved in these shifts are not well-understood. There is evidence suggesting that estradiol interacts with appetite regulating hormones such as GLP-1, leptin and insulin (Clegg, 2006; Vogel et al., 2017), but the role of progesterone is not thoroughly evaluated. Additionally, GLP-1, leptin and insulin have been shown to modulate motivation for food in rodents (Figlewicz & Benoit, 2009; Fulton, 2010; Richard et al., 2016).

Reductions in food intake following estradiol treatment in ovariectomized rats involve mechanisms that includes central actions of glucagon peptide-1 (GLP-1). GLP-1 is a peptide that can act both as a peripheral hormone or centrally as a neurotransmitter (Larsen & Holst, 2005). In the brain, GLP-1 receptor (GLP-1R) stimulation suppresses food intake (Williams et al., 2009), whereas blockade results in increases in food intake (Barrera et al., 2011; Turton et al., 1996). Additionally, there is evidence that suggests

that pharmacological blockade of GLP-1R in the VTA and NAc core increases food intake. However, stimulation of GLP-1R decreases food intake (Alhadeff et al., 2012). Alhadeff et al., 2012, suggested that the increase in food intake occurs endogenously via direct GLP-1 projections from the nucleus tractus solitarus to the VTA and NAc core. Interestingly, it has been shown that estradiol enhances responsiveness to GLP-1, resulting in decreased food intake (Maske et al., 2017). Thus, estradiol increases during proestrus/estrus could be enhancing the effects of GLP-1 on GLP1-Rs to decrease food intake. In our study, estradiol resulted in a reduction of food intake, but was not sufficient to decrease cue-triggered motivation. Instead, both estradiol and progesterone are required. A study by Outeiriño-Iglesias et al., showed that mRNA levels for the GLP-1R are higher in the hypothalamus during the night of proestrus (i.e., when the preovulatory gonadotropin surge occurs) when estradiol and progesterone act together to induce estrus (Outeiriño-Iglesias et al., 2015). It is possible that estradiol causes an increase in GLP-1, whereas progesterone increases GLP-1 receptor expression. Thus, both estradiol and progesterone may be acting synergistically to enhance GLP-1 signaling resulting in the decreased cue-triggered motivation observed here with the repeated estradiol and progesterone treatment.

Enhanced NAc core MSN intrinsic excitability in obesity-prone female rats:

The NAc is comprised predominantly of MSNs which integrate both dopaminergic and glutamatergic inputs to ultimately influence behavioral responses to reinforcers like food, sex, and drugs as well as cues paired with them (Berridge & Robinson, 1998; Schultz, 1997, 2013; Wolf, 2002; Wolf et al., 2003). Intrinsic excitability of MSNs influences the integration of dopamine and glutamate inputs and ultimately NAc output. There is evidence for dopamine-mediated transmission strongly influencing NAc MSNs intrinsic excitability (Azdad et al., 2009; Perez et al., 2006; Podda et al., 2010). Our lab has previously found that obesity-prone male rats have enhanced intrinsic excitability compared to obesity-resistant rats (Oginsky et al., 2016b). Additionally, junk-food diet decreases excitability in obesity-prone males (Oginsky & Ferrario, 2019). Here, I determined basal differences in intrinsic excitability between obesity-prone and obesity-resistant female rats. Additionally, the effect of the estrous cycle on intrinsic excitability

was also determined within obesity-prone and obesity-resistant female rats. There is evidence for estrous cycle modulation of intrinsic excitability in female outbred rats (Proaño et al., 2018), however this is the first study in examine cycle effects on MSN intrinsic excitability in obesity-prone and obesity-resistant female rats.

Female obesity-prone rats have decreased NAc core MSN intrinsic excitability during proestrus/estrus vs. metestrus/diestrus:

We found that excitability of MSNs in the NAc core is selectively enhanced in obesity-prone relative to obesity-resistant females during the metestrus/diestrus. This, group difference is not apparent when recordings are made during proestrus/estrus (Fig. 2.4). Specifically, in obesity-prone rats changes in voltage at positive current injections were significantly greater during metestrus/diestrus when compared to the proestrus/estrus phase. This is similar to effects of the estrous cycle on NAc excitability in outbred Sprague-Dawley females (Proaño et al., 2018). MSN excitability is largely determined by the number and distribution of voltage-gated potassium channels, with inwardly-rectifying K+ currents (I_{Kir}) dominating at negative membrane potentials (< -90mV) and A-type K+ currents (I_A) dominating at positive membrane potentials (> -40mV; Nisenbaum & Wilson, 1995; Perez et al., 2006). Differences during metestrus/diestrus between obesity-prone and obesity-resistant females were present across a wide range of current injections but were most pronounced at positive potentials. This, in combination with a lower rheobase and absence of differences in resting membrane potential, suggests that differences at the group level may be due to lower I_A in obesity-prone vs. obesity-resistant females. These group differences in excitability we observed during metestrus/diestrus in females are similar to those seen in male obesity-prone vs. obesity-resistant rats, where differences were found across the I/V curve and accompanied by a lower rheobase and an absence of differences in resting membrane potential (Oginsky et al., 2016b). The electrophysiological observations of decreased intrinsic excitability during proestrus/estrus correspond well with behavioral results of decreased cue-triggered motivation in obesity-prone rats, suggesting a correlation between MSN intrinsic excitability and behavior. Increases in NAc core activity promotes approach behaviors elicited by Pavlovian cues (McGinty et al., 2013).

Moreover, MSN activity can be shaped by local micro-circuits composed of interneurons, including fast-spiking and cholinergic interneurons (Kourrich et al., 2007). There is some evidence suggesting that estrogen receptor activation may directly regulate cholinergic activity (Towart et al., 2003) or indirectly by influencing inhibitory GABAergic interneurons in the hippocampus (Rudick, Gibbs, & Woolley, 2003; Rudick & Woolley, 2001). However, these effects within the NAc may be different from the mechanisms observed in the hippocampus. Additionally, it has been shown that progesterone and estradiol injections enhanced striatal release of dopamine and modulate the expression of dopamine receptors in the ventral striatum (Becker et al., 1984; Dluzen & Ramirez, 1984). It is possible that in obesity-prone female rats, during proestrus/estrus, estradiol and progesterone act synergistically to increase local circuit activity within the NAc, while decreasing MSN intrinsic excitability by acting indirectly via dopamine receptors.

MSN intrinsic excitability can be also modulated by cAMP response element-binding protein (CREB), NMDA type glutamate receptors (NMDAR) and metabotropic glutamate receptors (mGluRs). It has been shown that increases in CREB lead to increased MSN intrinsic excitability in the NAc, possibly by enhancing Na^+ conductance and inhibiting K^+ (Dong et al., 2006). There is also evidence that CREB can regulate the functional output of MSNs through NMDAR activation (Huang et al., 2007). For example, increases in CREB leads to increases in NMDAR function (Huang et al., 2007) and inhibition of NMDAR abolishes effects of CREB. In addition, mGluRs play a key role in CREB and NMDAR activity. Activation of type I mGluRs leads to increases in NMDAR activity (Lea et al., 2005; Skeberdis et al., 2001), whereas activation of type II and III mGluRs leads to attenuation of NMDAR activity (Martin et al., 1998). Interestingly, ERα and ERβ, can indirectly regulate NMDARs by activating type I and type II mGluRs (Grove-Strawser et al., 2010; Meitzen et al., 2018). ERα activates type I mGluRs, whereas ERβ activates type II mGluRs. Thus, it is possible that estradiol acts on ERβ, activating type II mGluRs decreasing NMDAR activity and subsequently decreasing CREB producing decreased intrinsic excitability in MSNs. However, not much is known about effects of progesterone on CREB or NMDAR activity. But, it is known that peripheral progesterone can be metabolized to allopregnanolone which has been shown to activate $GABA_A$ receptors (Hosie et al., 2006; Majewska et al., 1986; Micevych & Sinchak, 2011; Mitchell et al.,

2008; Puia et al., 1990), causing an overall increase in GABAergic transmission. Hence, estradiol might be indirectly decreasing MSN intrinsic excitability through mGluR type I activation of NMDAR, while progesterone could increase GABAergic transmission. The combination of both hormones' effects on MSN excitability and GABAergic transmission might then result in the decrease in cue-triggered motivation observed in these studies.

No effects of junk-food on AMPAR-mediated transmission in female obesity-prone rats:

Females show enhanced behavioral responses to the rewarding properties of food (Sinclair et al., 2017) and drugs compared to males (Becker & Hu, 2008). However, the mechanisms involved are poorly understood. In addition, female rats show enhanced incubation of cocaine craving vs. male rats when they are tested during the estrus phase (Kerstetter et al., 2008). Furthermore, female rats have increases in dendritic spine density (Forlano & Woolley, 2010; Wissman et al., 2011) and miniature EPSC frequency in NAc core MSNs (Wissman et al., 2012; Wissman et al., 2011) vs. male rats after cocaine exposure. In male rats, withdrawal from cocaine self-administration results in an increase in CP-AMPAR accumulation and synaptic insertion (Conrad et al., 2008; Dong & Nestler, 2014; Lee et al., 2013; Ma et al., 2014; Wolf, 2016). Similarly, in obesity-prone male rats, junk-food diet deprivation results in both the accumulation and synaptic insertion of CP-AMPARs in the NAc (Oginsky et al., 2016a). However, potential sex differences in CP-AMPAR expression and function following food or drug exposure have not been studied. Here, I examined the effects of junk-food on CP-AMPAR expression in both obesity-prone an obesity-resistant female rats. Additionally, I determined the effects of junk-food on CP-AMPAR mediated transmission and AMPA/NMDA ratio in obesity-prone female rats.

I did not observe any effects of junk-food on AMPAR subunit expression in obesity-prone female rats, even when junk-food exposure was increased (30 days instead of 10). However, our study did replicate previous work in males, showing that junk-food with and without deprivation increases GluA1 but not GluA2 surface expression (a classic indicator of CP-AMPAR increases) in obesity-prone rats. This is important because it shows that our null effects in females may be potentially due to a different mechanism and not an

experimental failure. Next, I determined the effects of 10 days of junk-food, followed by 2 weeks of withdrawal on CP-AMPAR mediated transmission in the NAc core of obesity-prone female rats. We previously have found that this type of exposure in male rats results in increases in CP-AMPAR mediated transmission. However, I did not find an effect of junk-food in female obesity-prone female rats.

In addition, while the electrophysiological recordings were controlled for estrous cycle phase, we did not determine estrous cycle in our biochemical studies. There is evidence that suggests that ovarian hormone treatment results in changes in expression in GluA1 and GluA2 AMPAR subunits. For example, in ovariectomized rats, treatment with estradiol and progesterone (treatment that produces similar behaviors to estrus phase) resulted in an increase in GluA1 immunoreactivity in the ventromedial hypothalamus, whereas GluA2 increased in the basolateral amygdala and central amygdala of female rats (Ferri et al., 2014). Although, these experiments were not conducted in the NAc and they are not specific to surface expression of AMPAR, they are informative in the sense that they show changes in GluA1 and GluA2 by ovarian hormones. Thus, we might be missing ovarian hormones effects on AMPAR subunit expression in female rats because we did not control for the cycle in our studies of protein expression.

AMPA/NMDA ratio and potential presynaptic effects:

I also determine the effects of 10 days of junk-food followed by 2 weeks of withdrawal on AMPAR/NMDAR eEPSC ratio in female obesity-prone rats. I found that junk-food *increases* AMPAR/NMDAR ratio in obesity-prone female rats. However, this increase was due to smaller NMDAR-mediated eEPSC amplitude and not AMPAR-mediated eEPSC amplitude in junk-food vs. chow groups. While it's difficult to directly compare the magnitude of eEPSC across cells, these data do suggest that NMDAR-mediated transmission function may decrease following junk-food consumption in females. It is possible that long exposure to junk-food diet followed by deprivation decreases NMDAR expression and function in a similar mechanism as described above; by increasing type I mGluRs which in turn decrease NMDAR activity. Our electrophysiological recordings were made in female rats in the metestrus/diestrus phase of the estrous cycle. Thus, it is possible that these effects vary depending the phase of the estrous cycle. For example,

obesity-prone fed a chow diet on proestrus/estrus may have a decrease in NMDAR-mediated transmission, similar to an obesity-prone fed a junk-food diet on metestrus/diestrus.

It is known that males and females have structural differences in synaptic connectivity and glutamatergic input in the NAc (Forlano & Woolley, 2009). For example, female rats have greater dendritic spine density (Forlano & Woolley, 2010; Wissman et al., 2011) and miniature EPSC frequency in MSNS of the NAc core compared to male rats, and these differences are enhanced after cocaine exposure (Wissman et al., 2012; Wissman et al., 2011). In addition, females have more glutamatergic input onto the distal dendrites of MSNs compared to males, regardless of estrous cycle phase (Forlano & Wolley, 2009). It is possible that these sex differences exist because of higher levels of estradiol in females vs. males, independent of estrous cycle phase. Estradiol also increases dendritic spine density in female rats (Forlano & Woolley, 2010). Thus, it suggests that effects of junk-food on glutamatergic transmission in females might be due to enhancement of dendritic spine density and synaptic connectivity and not by AMPAR-mediated transmission. However, this enhancement in dendritic spine density and glutamatergic transmission does not corroborate the idea of decreased in NMDAR activity discussed above. Since less NMDAR would suggest a decrease in transmission and dendritic spine density. Future studies examining differences in synaptic density synaptic connectivity and NMDAR-mediated mEPSC between groups should be conducted. Additional studies examining differences across the cycle could unmask the role of ovarian hormones on dendritic spine density and synaptic connectivity before and after junk-food exposure.

Differences in neuroadaptations after junk-food diet in obesity-prone males vs. females:

In Chapter 2, we found that obesity-prone female rats have enhanced cue-triggered motivation and NAc core MSN intrinsic excitability, similar to what we have observed in males. However, in Chapter 3, we found that consumption of junk-food diet does not result in the same neuroadaptations between male and female rats. Below, I discuss the differences in body fat storage and adiposity signals between males and females, that might lead to differences in neuroadaptations of the brain reward system.

Males and females have different mechanisms for energy metabolism and fat storage. A striking difference is that females resist the loss of body energy stores, while males mobilize their energy stores quickly in response to increased energy demand. In addition, females store their fat in subcutaneous depots, whereas males mainly store their fat in visceral areas (Palmer & Clegg, 2015). This difference in adipose tissue storage is related to peripheral and central actions of leptin and insulin. Leptin is more associated to subcutaneous fat, insulin is associated to visceral fat (Clegg et al., 2003). In addition, the brain of male rats is relatively more sensitive to the catabolic action of insulin, whereas females are more sensitive to the catabolic actions of leptin. Thus, leptin better correlates with body fat in females, while insulin is a better correlation in males. Additional evidence suggests that estradiol via estrogen receptors act centrally to increase leptin sensitivity, decrease insulin sensitivity and favor subcutaneous vs. visceral fat accumulation in female rats (Clegg et al., 2006). For example, when estradiol levels decrease (i.e. during menopause) visceral fat accumulation increase in females, whereas subcutaneous accumulation of fat occurs when estradiol is high (i.e. reproductive years; Clegg, 2006; Clegg et al., 2003; Palmer & Clegg, 2015). Furthermore, ovariectomy results in an accumulation of visceral fat and treatment with estradiol restores the redistribution of body fat to subcutaneous areas, similar to naturally cycling females (Clegg et al., 2006). It is possible that consumption of high fat diets in females can lead to changes in central effects of estrogens, consequently resulting in insulin and leptin resistance leading to an accumulation of visceral fat in females. Thus, these sex differences in adipose tissue storage and central responses to leptin may lead to fundamentally different neuroadaptations between male and female rats in response to high fat, high sugar diets (i.e., junk-foods).

In addition, there is evidence that suggests that leptin can induce synaptic plasticity in the brain reward system. For example, leptin receptors are highly expressed on dopaminergic neurons within the VTA (Figlewicz et al., 2003) and modulate dopamine release. Thus, leptin may be important modulator of the reward system and consequently of food reward (Fulton et al., 2006). Additionally, leptin administration to the VTA caused decreased food intake (Hommel et al., 2006). However, most of these studies were conducted in males and there is not a lot information about leptin in the mesocorticolimbic system in female

rats. However, we could hypothesize that females may have a protective mechanism against junk-food effects on neuroadaptations in the NAc due to their increased sensitivity to leptin. Thus, it will be interesting to see if in ovariectomized rats, in the absence of circulating ovarian hormones, junk-food diet has the same effect as in male rats. This is different from our experimental design in which we recorded during metestrus/diestrus when hormones levels are low, but the system is still intact.

Overall conclusions:

In summary, the data presented here shows that obesity-prone female rats have enhanced cue-triggered motivation and NAc core MSN intrinsic excitability vs. obesity-resistant rats. These results are consisted with what we have previously found in males. Additionally, this is the first demonstration of cycle- and hormone-dependent effects on motivational responses to a food cue, and the only studies to date to determine how individual susceptibility to obesity influences NAc excitability, cue-triggered motivation, and differences in the regulation of these neurobehavioral responses by the estrous cycle. Interestingly, we show that neuroadaptations in response to junk-food diets are different in male and female obesity-prone rats. Future studies should focus on studying the effects of junk-food on the mesocorticolimbic system in females. The discussion presented above also points to possible basal and diet-induced differences on NMDAR transmission, dendritic spine density and leptin actions between females and males that could account for some of the differences observed in our studies.

References

Abbott, K. N., Morris, M. J., Westbrook, R. F., & Reichelt, A. C. (2016). Sex-specific effects of daily exposure to sucrose on spatial memory performance in male and female rats, and implications for estrous cycle stage. *Physiology and Behavior*, *162*, 52–60. https://doi.org/10.1016/j.physbeh.2016.01.036

Alhadeff, A. L., Rupprecht, L. E., & Hayes, M. R. (2012). GLP-1 neurons in the nucleus of the solitary tract project directly to the ventral tegmental area and nucleus accumbens to control for food intake. *Endocrinology*, *153*(2), 647–658. https://doi.org/10.1210/en.2011-1443

Azdad, K., Chàvez, M., Bischop, P. D., Wetzelaer, P., Marescau, B., De Deyn, P. P., … Schiffmann, S. N. (2009). Homeostatic plasticity of striatal neurons intrinsic excitability following dopamine depletion. *PLoS ONE*, *4*(9). https://doi.org/10.1371/journal.pone.0006908

Barrera, J. G., Sandoval, D. A., D'Alessio, D. A., & Seeley, R. J. (2011). GLP-1 and energy balance: An integrated model of short-term and long-term control. *Nature Reviews Endocrinology*. https://doi.org/10.1038/nrendo.2011.77

Becker, J. B., Beer, M. E., & Robinson, T. E. (1984). Striatal dopamine release stimulated by amphetamine or potassium: influence of ovarian hormones and the light-dark cycle. *Brain Research*, *311*(1), 157–160. https://doi.org/10.1016/0006-8993(84)91410-0

Becker, J. B., & Hu, M. (2008). Sex differences in drug abuse. *Frontiers in Neuroendocrinology*, *29*(1), 36–47. https://doi.org/10.1016/j.yfrne.2007.07.003

Bermant, G. (1961). Response latencies of female rats during sexual intercourse. *Science*, *133*(3466), 1771–1773. https://doi.org/10.1126/science.133.3466.1771

Berridge, K. C. (2001). *Reward Learning: Reinforcement, Incentives, and*

Expectations.

Berridge, K. C., & Robinson, T. E. (1998). *What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Research Reviews* (Vol. 28).

Berthoud, H. R. (2012). The neurobiology of food intake in an obesogenic environment. In *Proceedings of the Nutrition Society* (Vol. 71, pp. 478–487). https://doi.org/10.1017/S0029665112000602

Calipari, E. S., Juarez, B., Morel, C., Walker, D. M., Cahill, M. E., Ribeiro, E., … Nestler, E. J. (2017). ARTICLE Dopaminergic dynamics underlying sex-specific cocaine reward. https://doi.org/10.1038/ncomms13877

Cardinal, R. N., Parkinson, J. A., Hall, J., & Everitt, B. J. (2002). Emotion and motivation: The role of the amygdala, ventral striatum, and prefrontal cortex. *Neuroscience and Biobehavioral Reviews*. https://doi.org/10.1016/S0149-7634(02)00007-6

Clegg, D J. (2006). Gonadal hormones determine sensitivity to leptin and insulin. https://doi.org/10.2337/diabetes.55.04.06.db05-1339

Clegg, Deborah J., Riedy, C. A., Smith, K. A. B., Benoit, S. C., & Woods, S. C. (2003). Differential Sensitivity to Central Leptin and Insulin in Male and Female Rats. *Diabetes*, *52*(3), 682–687. https://doi.org/10.2337/diabetes.52.3.682

Conrad, K. L., Tseng, K. Y., Uejima, J. L., Reimers, J. M., Heng, L. J., Shaham, Y., … Wolf, M. E. (2008). Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature*, *454*(7200), 118–121. https://doi.org/10.1038/nature06995

Cummings, J. A., & Becker, J. B. (2012). Quantitative assessment of female sexual motivation in the rat: Hormonal control of motivation. *Journal of Neuroscience Methods*, *204*(2), 227–233. https://doi.org/10.1016/j.jneumeth.2011.11.017

Demos, K. E., Heatherton, T. F., & Kelley, W. M. (2012). Individual Differences in Nucleus Accumbens Activity to Food and Sexual Images Predict Weight Gain and Sexual Behavior. *Journal of Neuroscience*, *32*(16), 5549–5552.

https://doi.org/10.1523/JNEUROSCI.5958-11.2012

Derman, R. C., & Ferrario, C. R. (2018). Enhanced incentive motivation in obesity-prone rats is mediated by NAc core CP-AMPARs. *Neuropharmacology*, *131*, 326–336. https://doi.org/10.1016/j.neuropharm.2017.12.039

Dluzen, D. E., & Ramirez, V. D. (1984). Bimodal effect of progesterone on in vitro dopamine function of the rat corpus striatum. *Neuroendocrinology*, *39*, 149–155. https://doi.org/10.1159/000123971

Dong, Y., Green, T., Saal, D., Marie, H., Neve, R., Nestler, E. J., & Malenka, R. C. (2006). CREB modulates excitability of nucleus accumbens neurons. https://doi.org/10.1038/nn1661

Dong, Y., & Nestler, E. J. (2014). The neural rejuvenation hypothesis of cocaine addiction. *Trends in Pharmacological Sciences*. Elsevier Ltd. https://doi.org/10.1016/j.tips.2014.05.005

Ferrario, C. R., Labouèbe, G., Liu, S., Nieh, E. H., Routh, V. H., Xu, S., & O'Connor, E. C. (2016). Homeostasis Meets Motivation in the Battle to Control Food Intake. *The Journal of Neuroscience*, *36*(45), 11469–11481. https://doi.org/10.1523/JNEUROSCI.2338-16.2016

Ferri, S. L., Hildebrand, P. F., Way, S. E., & Flanagan-Cato, L. M. (2014). Estradiol regulates markers of synaptic plasticity in the hypothalamic ventromedial nucleus and amygdala of female rats. *Hormones and Behavior*, *66*(2), 409–420. https://doi.org/10.1016/j.yhbeh.2014.06.016

Fessler, D. M. T. (2003). No Time To Eat: An Adaptationist Account Of Periovulatory Behavioral Changes. *The Quarterly Review of Biology*, *78*(1), 3–21. https://doi.org/10.1086/367579

Figlewicz, D. ., Evans, S. ., Murphy, J., Hoen, M., & Baskin, D. . (2003). Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. *Brain Research*, *964*(1), 107–115. https://doi.org/10.1016/S0006-8993(02)04087-8

Figlewicz Lattemann, D. P., & Benoit, S. C. (2009, January). Insulin, leptin, and food

reward: Update 2008. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*. American Physiological Society. https://doi.org/10.1152/ajpregu.90725.2008

Forlano, P. M., & Woolley, C. S. (2010). Quantitative analysis of pre- and postsynaptic sex differences in the nucleus accumbens. *The Journal of Comparative Neurology*, *518*(8), 1330–1348. https://doi.org/10.1002/cne.22279

Fulton, S. (2010, January). Appetite and reward. *Frontiers in Neuroendocrinology*. https://doi.org/10.1016/j.yfrne.2009.10.003

Fulton, S., Pissios, P., Manchon, R. P., Stiles, L., Frank, L., Pothos, E. N., … Flier, J. S. (2006). Leptin Regulation of the Mesoaccumbens Dopamine Pathway. *Neuron*, *51*(6), 811–822. https://doi.org/10.1016/j.neuron.2006.09.006

Grove-Strawser, D., Boulware, M. I., & Mermelstein, P. G. (2010). Membrane estrogen receptors activate the metabotropic glutamate receptors mGluR5 and mGluR3 to bidirectionally regulate CREB phosphorylation in female rat striatal neurons. *Neuroscience*, *170*(4), 1045–1055. https://doi.org/10.1016/j.neuroscience.2010.08.012

Hecht, G. S., Spear, N. E., & Spear, L. P. (1999). Changes in progressive ratio responding for intravenous cocaine throughout the reproductive process in female rats. *Developmental Psychobiology*, *35*(2), 136–145.

Hommel, J. D., Trinko, R., Sears, R. M., Georgescu, D., Liu, Z. W., Gao, X. B., … DiLeone, R. J. (2006). Leptin Receptor Signaling in Midbrain Dopamine Neurons Regulates Feeding. *Neuron*, *51*(6), 801–810. https://doi.org/10.1016/j.neuron.2006.08.023

Hosie, A. M., Wilkins, M. E., Da Silva, H. M. A., & Smart, T. G. (2006). Endogenous neurosteroids regulate GABA A receptors through two discrete transmembrane sites, *444*. https://doi.org/10.1038/nature05324

Huang, Y. H., Lin, Y., Brown, T. E., Han, M.-H., Saal, D. B., Neve, R. L., … Dong, Y. (2007). CREB Modulates the Functional Output of Nucleus Accumbens Neurons A Critical Role Of N-Methyl-D-Aspartate Glutamate Receptor (Nmdar) Receptors *

Downloaded from. *The Journal Of Biological Chemistry*, *283*(5), 2751–2760. https://doi.org/10.1074/jbc.M706578200

Johnson, A. R., Thibeault, K. C., Lopez, A. J., Peck, E. G., Sands, L. P., Sanders, C. M., … Calipari, E. S. (2019). Cues play a critical role in estrous cycle-dependent enhancement of cocaine reinforcement. https://doi.org/10.1038/s41386-019-0320-0

Kelley, A. E., Baldo, B. A., Pratt, W. E., & Will, M. J. (2005). Corticostriatal-hypothalamic circuitry and food motivation: Integration of energy, action and reward. In *Physiology and Behavior* (Vol. 86, pp. 773–795). https://doi.org/10.1016/j.physbeh.2005.08.066

Kerstetter, K. A., Aguilar, V. R., Parrish, A. B., & Kippin, T. E. (2008). Protracted time-dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. *Psychopharmacology*, *198*(1), 63–75. https://doi.org/10.1007/s00213-008-1089-8

Kishinevsky, F. I., Cox, J. E., Murdaugh, D. L., Stoeckel, L. E., Cook, E. W., & Weller, R. E. (2012). FMRI reactivity on a delay discounting task predicts weight gain in obese women. *Appetite*, *58*(2), 582–592. https://doi.org/10.1016/j.appet.2011.11.029

Kourrich, S., Rothwell, P. E., Klug, J. R., & Thomas, M. J. (2007). Cocaine Experience Controls Bidirectional Synaptic Plasticity in the Nucleus Accumbens. *Journal of Neuroscience*, *27*(30), 7921–7928. https://doi.org/10.1523/JNEUROSCI.1859-07.2007

Larsen, P. J., & Holst, J. J. (2005, June 15). Glucagon-related peptide 1 (GLP-1): Hormone and neurotransmitter. *Regulatory Peptides*. Elsevier. https://doi.org/10.1016/j.regpep.2004.08.026

Lea IV, P. M., Movsesyan, V. A., & Faden, A. I. (2005). Neuroprotective activity of the mGluR5 antagonists MPEP and MTEP against acute excitotoxicity differs and does not reflect actions at mGluR5 receptors. *British Journal of Pharmacology*, *145*(4), 527–534. https://doi.org/10.1038/sj.bjp.0706219

Lee, B. R., Ma, Y. Y., Huang, Y. H., Wang, X., Otaka, M., Ishikawa, M., … Dong, Y. (2013). Maturation of silent synapses in amygdala-accumbens projection

contributes to incubation of cocaine craving. *Nature Neuroscience*, *16*(11), 1644–1651. https://doi.org/10.1038/nn.3533

Ma, Y. Y., Lee, B. R., Wang, X., Guo, C., Liu, L., Cui, R., … Dong, Y. (2014). Bidirectional modulation of incubation of cocaine craving by silent synapse-based remodeling of prefrontal cortex to accumbens projections. *Neuron*, *83*(6), 1453–1467. https://doi.org/10.1016/j.neuron.2014.08.023

Majewska, M. D., Harrison, N. L., Schwartz, R. D., Barker, J. L., & Paul, S. M. (1986). Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science*, *232*(4753), 1004–1007. https://doi.org/10.1126/science.2422758

Martin, G., Nie, Z., & Siggins, G. R. (1998). *Metabotropic Glutamate Receptors Regulate N-Methyl-D-Aspartate-Mediated Synaptic Transmission in Nucleus Accumbens*. Retrieved from www.physiology.org/journal/jn

Maske, C. B., Jackson, C. M., Terrill, S. J., Eckel, L. A., & Williams, D. L. (2017). Estradiol modulates the anorexic response to central glucagon-like peptide 1. *Hormones and Behavior*, *93*, 109–117. https://doi.org/10.1016/j.yhbeh.2017.05.012

McGinty, V. B., Lardeux, S., Taha, S. A., Kim, J. J., & Nicola, S. M. (2013). Invigoration of reward seeking by cue and proximity encoding in the nucleus accumbens. *Neuron*. https://doi.org/10.1016/j.neuron.2013.04.010

Meitzen, J., Meisel, R. L., & Mermelstein, P. G. (2018, October 1). Sex differences and the effects of estradiol on striatal function. *Current Opinion in Behavioral Sciences*. Elsevier Ltd. https://doi.org/10.1016/j.cobeha.2018.03.007

Meyerson, B. J., Lindström, L., Nordström, E. B., & Ågmo, A. (1973). Sexual motivation in the female rat after testosterone treatment. *Physiology and Behavior*, *11*(4), 421–428. https://doi.org/10.1016/0031-9384(73)90026-7

Micevych, P., & Sinchak, K. (2011). The neurosteroid progesterone underlies estrogen positive feedback of the LH surge. *Frontiers in Endocrinology*. https://doi.org/10.3389/fendo.2011.00090

Milton, A. L., & Everitt, B. J. (2010). The psychological and neurochemical mechanisms of drug memory reconsolidation: Implications for the treatment of addiction.

European Journal of Neuroscience. https://doi.org/10.1111/j.1460-9568.2010.07249.x

Mitchell, E. A., Herd, M. B., Gunn, B. G., Lambert, J. J., & Belelli, D. (2008). Neurosteroid modulation of GABAA receptors: Molecular determinants and significance in health and disease. *Neurochemistry International*, *52*(4–5), 588–595. https://doi.org/10.1016/j.neuint.2007.10.007

Nisenbaum, E. S., & Wilson, C. J. (1995). Potassium currents responsible for inward and outward rectification in rat neostriatal spiny projection neurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *15*(6), 4449–4463. Retrieved from http://www.jneurosci.org/content/jneuro/15/6/4449.full.pdf

Oginsky, M. F., & Ferrario, C. R. (2019). Eating “junk-food” has opposite effects on intrinsic excitability of nucleus accumbens core neurons in obesity-susceptible vs. -resistant rats. *Journal of Neurophysiology*, *122*, 1264–1273. https://doi.org/10.1152/jn.00361.2019

Oginsky, M. F., Goforth, P. B., Nobile, C. W., Lopez-Santiago, L. F., & Ferrario, C. R. (2016). Eating “Junk-Food” Produces Rapid and Long-Lasting Increases in NAc CP-AMPA Receptors: Implications for Enhanced Cue-Induced Motivation and Food Addiction. *Neuropsychopharmacology*, *41*(13), 2977–2986. https://doi.org/10.1038/npp.2016.111

Oginsky, M. F., Maust, J. D., Corthell, J. T., & Ferrario, C. R. (2016). Enhanced cocaine-induced locomotor sensitization and intrinsic excitability of NAc medium spiny neurons in adult but not in adolescent rats susceptible to diet-induced obesity. *Psychopharmacology*, *233*(5), 773–784. https://doi.org/10.1007/s00213-015-4157-x

Outeiriño-Iglesias, V., Romaní-Pérez, M., González-Matías, L. C., Vigo, E., & Mallo, F. (2015). GLP-1 Increases Preovulatory LH Source and the Number of Mature Follicles, As Well As Synchronizing the Onset of Puberty in Female Rats. https://doi.org/10.1210/en.2014-1978

Palmer, B. F., & Clegg, D. J. (2015, February 15). The sexual dimorphism of obesity. *Molecular and Cellular Endocrinology*. NIH Public Access.

https://doi.org/10.1016/j.mce.2014.11.029

Perez, M. F., White, F. J., & Hu, X.-T. (2006a). Dopamine D 2 Receptor Modulation of K + Channel Activity Regulates Excitability of Nucleus Accumbens Neurons at Different Membrane Potentials. *Journal of Neurophysiology*, *96*(5), 2217–2228. https://doi.org/10.1152/jn.00254.2006

Perez, M. F., White, F. J., & Hu, X.-T. (2006b). Dopamine D 2 Receptor Modulation of K 2 Channel Activity Regulates Excitability of Nucleus Accumbens Neurons at Different Membrane Potentials. *J Neu-Rophysiol*, *96*(2), 2217–2228. https://doi.org/10.1152/jn.00254.2006

Pitchers, K. K., Flagel, S. B., O'Donnell, E. G., Solberg Woods, L. C., Sarter, M., & Robinson, T. E. (2015). Individual variation in the propensity to attribute incentive salience to a food cue: Influence of sex. *Behavioural Brain Research*, *278*, 462–469. https://doi.org/10.1016/j.bbr.2014.10.036

Podda, M. V., Riccardi, E., D'Ascenzo, M., Azzena, G. B., & Grassi, C. (2010). Dopamine D1-like receptor activation depolarizes medium spiny neurons of the mouse nucleus accumbens by inhibiting inwardly rectifying K+ currents through a cAMP-dependent protein kinase A-independent mechanism. *Neuroscience*, *167*(3), 678–690. https://doi.org/10.1016/j.neuroscience.2010.02.075

Proaño, S. B., Morris, H. J., Kunz, L. M., Dorris, D. M., & Meitzen, J. (2018a). Estrous cycle-induced sex differences in medium spiny neuron excitatory synaptic transmission and intrinsic excitability in adult rat nucleus accumbens core. *J Neurophysiol*, *120*, 1356–1373. https://doi.org/10.1152/jn.00263.2018.-Naturally

Proaño, S. B., Morris, H. J., Kunz, L. M., Dorris, D. M., & Meitzen, J. (2018b). Estrous cycle-induced sex differences in medium spiny neuron excitatory synaptic transmission and intrinsic excitability in adult rat nucleus accumbens core. *Journal of Neurophysiology*, *120*(3), 1356–1373. https://doi.org/10.1152/jn.00263.2018

Puia, G., Santi, M. R., Vicini, S., Pritchett, D. B., Purdy, R. H., Paul, S. M., … Costa, E. (1990). Neurosteroids act on recombinant human GABAA receptors. *Neuron*, *4*(5), 759–765. https://doi.org/10.1016/0896-6273(90)90202-Q

Richard, J. E., Anderberg, R. H., López-Ferreras, L., Olandersson, K., & Skibicka, K. P. (2016). Sex and estrogens alter the action of glucagon-like peptide-1 on reward. *Biology of Sex Differences*, *7*(1), 6. https://doi.org/10.1186/s13293-016-0059-9

Richard, J. E., López-Ferreras, L., Anderberg, R. H., Olandersson, K., & Skibicka, K. P. (2017). Estradiol is a critical regulator of food-reward behavior. *Psychoneuroendocrinology*, *78*, 193–202. https://doi.org/10.1016/j.psyneuen.2017.01.014

Roberts, D. C. S., Bennett, S. A. L., & Vickers, G. J. (1989). The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. *Psychopharmacology*, *98*(3), 408–411. https://doi.org/10.1007/BF00451696

Robinson, M. J. F., Burghardt, P. R., Patterson, C. M., Nobile, C. W., Akil, H., Watson, S. J., … Ferrario, C. R. (2015). Individual Differences in Cue-Induced Motivation and Striatal Systems in Rats Susceptible to Diet-Induced Obesity. *Neuropsychopharmacology*, *40*(9), 2113–2123. https://doi.org/10.1038/npp.2015.71

Rudick, C. N., Gibbs, R. B., & Woolley, C. S. (2003). *A Role for the Basal Forebrain Cholinergic System in Estrogen-Induced Disinhibition of Hippocampal Pyramidal Cells*.

Rudick, C. N., & Woolley, C. S. (2001). *Estrogen Regulates Functional Inhibition of Hippocampal CA1 Pyramidal Cells in the Adult Female Rat*.

Schultz, W. (1997). Dopamine neurons and their role in reward mechanisms Abbreviations GABA y-aminobutyric acid NMDA N-methyl-o-aspartate TD models temporal difference models. *Current Opinion in Neurobiology*, *7*, 191–197.

Schultz, W. (2013). Updating dopamine reward signals. *Current Opinion in Neurobiology*, *23*, 229–238. https://doi.org/10.1016/j.conb.2012.11.012

Sinclair, E. B., Hildebrandt, B. A., Culbert, K. M., Klump, K. L., & Sisk, C. L. (2017). Preliminary evidence of sex differences in behavioral and neural responses to palatable food reward in rats. *Physiology & Behavior*, *176*, 165–173. https://doi.org/10.1016/j.physbeh.2017.03.042

Skeberdis, V. A., Lan, J. Y., Opitz, T., Zheng, X., Bennett, M. V. L., & Suzanne Zukin, R.

(2001). mGluR1-mediated potentiation of NMDA receptors involves a rise in intracellular calcium and activation of protein kinase C. *Neuropharmacology*, *40*(7), 856–865. https://doi.org/10.1016/S0028-3908(01)00005-3

Towart, L. A., Alves, S. E., Znamensky, V., Hayashi, S., Mcewen, B. S., & Milner, T. A. (2003). Subcellular Relationships between Cholinergic Terminals and Estrogen Receptor-in the Dorsal Hippocampus Indexing terms: sex steroids; acetylcholine; hippocampus; neurotransmitter release; rat; electron microscopy. *The Journal Of Comparative Neurology*, *463*, 390–401. https://doi.org/10.1002/cne.10753

Turton, M. D., O'Shea, D., Gunn, I., Beak, S. A., Edwards, C. M. B., Meeran, K., … Bloom, S. R. (1996). A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature*, *379*(6560), 69–72. https://doi.org/10.1038/379069a0

Vogel, H., Kraemer, M., Rabasa, C., Askevik, K., Adan, R. A. H., & Dickson, S. L. (2017). Genetic predisposition to obesity affects behavioral traits including food reward and anxiety-like behavior in rats. *Behavioural Brain Research*, *328*, 95–104. https://doi.org/10.1016/j.bbr.2017.02.037

Vollbrecht, P. J., Nobile, C. W., Chadderdon, A. M., Jutkiewicz, E. M., & Ferrario, C. R. (2015). Pre-existing differences in motivation for food and sensitivity to cocaine-induced locomotion in obesity-prone rats. *Physiology and Behavior*, *152*(Pt A), 151–160. https://doi.org/10.1016/j.physbeh.2015.09.022

Wissman, A. M., May, R. M., & Woolley, C. S. (2012). Ultrastructural analysis of sex differences in nucleus accumbens synaptic connectivity. *Brain Structure & Function*, *217*(2), 181–190. https://doi.org/10.1007/s00429-011-0353-6

Wissman, A. M., McCollum, A. F., Huang, G.-Z., Nikrodhanond, A. A., & Woolley, C. S. (2011). Sex differences and effects of cocaine on excitatory synapses in the nucleus accumbens. *Neuropharmacology*, *61*(1–2), 217–227. https://doi.org/10.1016/j.neuropharm.2011.04.002

Wolf, M. E. (2002). Addiction: Making the Connection Between Behavioral Changes and Neuronal Plasticity in Specific Pathways. *Molecular Interventions*, *2*(3), 146–157. https://doi.org/10.1124/mi.2.3.146

Wolf, Marina E., Mangiavacchi, S., & Sun, X. (2003). Mechanisms by which Dopamine Receptors May Influence Synaptic Plasticity. In *Annals of the New York Academy of Sciences*. https://doi.org/10.1196/annals.1300.015

Wolf, Marina E. (2016). Synaptic mechanisms underlying persistent cocaine craving. *Nature Reviews Neuroscience*. https://doi.org/10.1038/nrn.2016.39

Yoest, K. E., Cummings, J. A., & Becker, J. B. (2014). Estradiol, Dopamine and Motivation. *Cent Nerv Syst Agents Med Chem. Cent Nerv Syst Agents Med Chem*, *14*(2), 83–89. https://doi.org/10.14440/jbm.2015.54.A

Yokum, S., Ng, J., & Stice, E. (2011). Attentional bias to food images associated with elevated weight and future weight gain: An fMRI study. *Obesity*, *19*(9), 1775–1783. https://doi.org/10.1038/oby.2011.168

APPENDIX A

Enhanced Anxiety-Like Behavior Emerges with Weight Gain in Male and Female Obesity-Susceptible Rats.

Abstract

Epidemiological data suggest that body mass index and obesity are strong risk factors for depression and anxiety. However, it is difficult to separate cause from effect, as predisposition to obesity may enhance susceptibility to anxiety, or vice versa. Here, we examined the effect of diet and obesity on anxiety-like behaviors in male and female selectively bred obesity-prone and obesity-resistant rats, and outbred Sprague-Dawley rats. We found that when obesity-prone and obesity-resistant rats do not differ in weight or fat mass, measures of anxiety-like behavior in the elevated plus maze and open field are similar between the two groups. However, once weight and fat mass diverge, group differences emerge, with greater anxiety in obesity-prone relative to obesity-resistant rats. This same pattern was observed for males and females. In addition, anxiety-like behavior was greater during estrus and proestrus compared to metestrus and diestrus in females of both lines. Interestingly, even when obesity-resistant rats were “forced” to gain fat mass comparable to obesity prone rats (via prolonged access to 60% high-fat diet), anxiety-like behaviors did not differ from lean chow fed controls. In addition, a positive correlation between anxiety-like behaviors and adiposity were observed in male but not in female obesity-prone rats. Finally, diet-induced weight gain in and of itself was not sufficient to increase measures of anxiety in outbred male rats. Together, these data suggest that interactions between susceptibility to obesity and physiological alterations accompanying weight gain may contribute to the development of enhanced anxiety in obesity.

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Introduction

As the global obesity epidemic has progressed, examination of how diet and weight gain influence neural function to impact cognition, motivation, and emotion has come to the forefront of research. Human obesity is often comorbid with anxiety and depression, and recent studies suggest that this may be due in part to effects of diet, weight gain, and accompanying physiological alterations in brain regions that mediate affective processes (Daumit et al., 2003; Dickerson et al., 2006; Luppino et al., 2010). Consistent with mechanistic, rather than social underpinnings of this association, diet and obesity-induced alterations in gut microbiota can have pro-anxiety and pro-depressant effects in rodents (Bravo et al., 2011; Mackos et al., 2013; Ohland et al., 2013). For example, high fat diets can increase the risk of development of psychological disorders such as anxiety and depression (Souza et al., 2007, Sharma & Fulton, 2013). Highly palatable diets have been shown to be both anxiolytic as well as anxiogenic depending on a range of factors including the age of the animals and whether or not the animals develop obesity (Ulrich-Lai et al., 2011; de Lima Marcolin et al., 2012; Souza et al., 2007). In addition, consumption of sugary, fatty foods is sufficient to alter neural systems involved in cognition and anxiety including the hippocampus, nucleus accumbens, prefrontal cortex, and amygdala in ways that are expected to promote poor mental health (Avena et al., 2008; Bocarsly et al., 2015; Morganstern et al., 2012; Murphy & Mercer, 2013).

In humans, weight is a heritable trait (Wardle & Carnell, 2009), and obesity is polygenic and influenced by early life experiences (see Albuquerque et al., 2015 for review). This results in considerable individual differences in susceptibility to weight gain that may interact with experience to influence anxiety and depression (Zender and Olshansky, 2009; Zhao et al., 2009). Similar variation in susceptibility to weight gain exists in outbred Sprague- Dawley rats, with some readily gaining weight and becoming obese, and others remaining lean, even when given calorie dense diets (Alonso-Caraballo et al., 2018; Levin et al., 1997). This naturally occurring variance was amplified through selective breeding based on propensity or resistance to weight gain to generate obesity-prone and obesity-resistant rat lines (Levin et al., 1997). As in humans, obesity in this model is polygenic and influenced by early life experiences (Gorski, 2006; Madsen et al., 2010). In addition, the obesity-prone phenotype is associated with enhanced striatal function and plasticity

(Derman & Ferrario, 2017; Naneix et al., 2017; Oginsky et al., 2016; Vollbrecht et al., 2015), as well as alterations in hypothalamic and metabolic processes (see Levin, 2007 for review).

How obesity and palatable, calorie dense diets may affect anxiety-like behaviors in the obesity-prone/obesity-resistant model is poorly understood, and potential differences in females have not been examined. One recent study found mild elevations in anxiety-like behaviors in obesity-prone compared to obesity-resistant male rats prior to diet manipulation (Vogel et al., 2017). Therefore, in the current study we measured anxiety-like behaviors in both male and female obesity-prone and obesity-resistant rats before and after the development of spontaneous or diet-induced obesity. Additional behavioral studies were conducted in outbred Sprague-Dawley rats. Fasted plasma insulin and leptin levels, as well as weight and body composition were used to evaluate relationships between weight gain, alterations in peripheral hormones, and anxiety-like behaviors.

Materials and Methods

Subjects:

Selectively bred obesity-prone (OP) and obesity-resistant (OR) rats (Levin et al., 1997) were bred in house at the University of Michigan Breeding Core using an outbred rotational system within closed populations. Sprague-Dawley (SD) rats were purchased from Envigo (Haslett, MI). For all studies, rats were group or pair housed (according to AAALAC recommendations based on weight and housing size) and were approximately 65-70 days old at the start of the experiment. All rats housed at the University of Michigan (Experiments 1-4) were maintained on a reverse light-dark cycle (12/12; lights off 8AM) and behavioral tests were conducted between 2pm and 4pm. Rats housed at Hope College (Experiment 5) were maintained in 12h light-dark cycle with experiments performed between 3pm and 5pm. Animal facilities were maintained at 72 degrees F (+/- 2 degrees) and 30-70% humidity and conditions were monitored and recorded daily. All procedures were approved by The University of Michigan or Hope College Committee on the Use and Care of Animals.

Diet manipulations and measures of obesity:

For all diet manipulations, food was available ad libitum and rats were weighed at least 5 times per week. Standard chow diet (Lab Diet 5001: 4.07 kcal/g; 13.5% fat, 28.5% protein, 58% carbohydrates; % of caloric content), high-fat diet (HF; Open Source Diets D12492, 5.21 kcal/g, 60% fat), or a “junk-food” diet (JF) made in-house were used. The “junk-food” diet was a mash of Ruffles potato chips (40 g), Original Chips Ahoy (130 g), Nesquik (130 g), Jiff creamy peanut butter (130 g), powdered LabDiet 5001 (200 g) and 180 mL of water (19.6% fat, 14% protein, and 58% carbohydrates; 4.5 kcal/g). For all diet manipulations, only one type of food was available at a time, and rats were maintained on the given diet for the duration of the experiment (see also Figure 1). For studies of effects of high-fat diet, rats were given free access to this food for 4 or 8 weeks. For studies of junk-food, rats were given free access for 4 weeks. Age matched controls were given chow only throughout. Food intake and body weight for male obesity-prone and obesity-resistant rats were monitored throughout the experiment. In addition, nuclear magnetic resonance imaging (NMR; Minispec LF90II, Bruker Optics; conducted by the University of Michigan

Animal Phenotyping Core) was used to determine body composition in a subset of subjects.

After the completion of all testing, animals were fasted for 16 hours and blood samples were collected via tail nick into tubes containing EDTA (1.6 mg/ml, Sarstedt). The nick was covered with antibiotic ointment. Plasma was isolated by centrifugation (1000 x G, 4°C) and stored (-20°C) for analysis. Insulin levels were measured using a 125I-Human insulin tracer (Linco Research, St. Charles, MO), a rat insulin standard (Novo Nordisk, Plainsboro, NJ), a guinea pig anti-rat insulin first antibody (Linco Research), and a sheep anti-guinea pig gamma globulin-PEG second antibody (Michigan Diabetes Research Core). Plasma leptin concentrations were measured using a leptin ELISA kit (#90040, Crystal Chem Inc, Elk Grove Village, IL; Michigan Diabetes Research Core) and conducted according to insert instructions.

Measures of anxiety-like behaviors:

Two well-established measures of anxiety-like behavior were used, open field and elevated plus maze testing (Cryan & Sweeney, 2011). Both tests are based on intrinsic responses of rodents to avoid open areas in which they could be vulnerable to predation, and both tests have predictive validity for detecting anxiolytic and anxiogenic properties of drugs (Cryan & Sweeney, 2011). Open field and elevated plus maze testing occurred in the same testing room under the same lighting conditions. All behavior was video recorded during testing described in detail below, and videos were scored off-line by an observer that was blind to group and testing conditions.

Open field test:

Open field testing was done in a 70 x 70 x 31 cm Plexiglas arena placed in the center of a brightly-lit, white room. On the day of testing, rats were brought into the testing room at least 30 minutes before being placed randomly along an edge of the open field. Behavior was video recorded for 30 minutes, after which the animal was returned to its home cage. The duration of testing was based on previous studies (Walsh & Cummins, 1976). The open field was wiped clean with disinfectant prior to testing the next subject. The following behaviors were measured: traverses, defined as number of times the rat went from one side of the box to the opposite side or halfway across one side and returning back to the

same side, number of entrances into the center, defined as the number of times all four paws simultaneously entered the center region, as well as time spent in the center, and latency to first center entrance.

Elevated plus maze:

The maze consisted of 2 closed arms and 2 open arms; 50cm long x 10cm wide x 40cm tall and 50cm long x 10cm wide respectively, with a 10cm x 10cm square intersection in the middle that was not within either arm. On the day of testing, rats were brought into the testing room at least 30 minutes before being randomly placed in the maze and allowed to explore freely for 15 minutes (Experiments 2 and 4) or 10 minutes (Experiments 1, 3 and 5). The duration of testing was reduced in Experiments 1, 3 and 5 because rats tended to stop exploring the maze after ~ 10 minutes, thus this captured the majority of their behavior. The number of entrances to the open and closed arms as well as the time spent in each arm were determined by hand scoring each video. Entry into an arm was defined as the shoulders and both forepaw paws crossing into that arm. Time spent in the central intersection was not counted towards time in any arm.

Locomotor activity:

Animals were placed individually into a clean cage (41 x 25.4 x 20.3 cm) with fresh bedding. Locomotor activity was recorded for 45 minutes using an array of photocell beams to evaluate locomotor behavior (as in Oginsky et al., 2016; Vollbrecht et al., 2016). Crossovers were defined as the number of times the animal traveled from one end of the cage to the other, determined by breaking infrared beams at each end of the cage in succession.

Statistics:

Data were analyzed using Prism 6 and Prism 7 (GraphPad, San Diego, CA). Comparison between two groups were made with unpaired or paired two-tailed t-tests, as appropriate. For comparison of three or more groups, one-way and two-way-repeated measures ANOVAs were used, followed by Sidak's post-hoc multiple comparisons when appropriate. In addition, *a priori* comparisons of weight gain, fat, and lean mass were made between OP and OR groups within each diet condition. Power analyses were used

to determine that group sizes of 4-6 are sufficient to detect effect sizes of 40-50% with a power of 80%.

Experimental Design

Experiment 1: Differences in anxiety-like behavior in male OP vs. OR rats before and after 4 weeks of high-fat diet consumption.

We began by testing the same set of male obesity-prone and obesity-resistant rats both before and after 4 weeks of high-fat diet consumption. Pilot studies indicated that repeated open field or elevated plus maze testing was not feasible, as behavior in both groups changed across repeated testing. Thus, rats were first tested in the elevated plus maze at a time when there were no differences in body weight between OP and OR groups (OP N = 16, OR N = 15; one rat was excluded because it fell off the maze platform). Next, these same rats were divided into chow and high-fat groups, counterbalanced for initial weight and behavioral measures in the elevated plus maze, and allowed free access to their respective diets for 4 weeks (OP-Chow N = 8, OP-4wkHF N = 8, OR-Chow N = 8, OR-4wkHF N = 8). After this manipulation, anxiety-like behavior was evaluated in the open field and body composition was determined.

Experiment 2: Effect of 8 weeks of high-fat diet consumption on anxiety-like behavior in male OP and OR rats.

Although less pronounced than that of obesity-prone rats, increases in adiposity can be induced in obesity-resistant rats through prolonged exposure to high-fat diet. Thus, in order to determine whether anxiety-like behaviors can be enhanced by diet-induced obesity in obesity-resistant rats, a separate set of male OP and OR rats were given access to chow or high-fat diet for 8 weeks (OP-Chow N = 8, OP-8wkHF N = 8, OR-Chow N = 6, OR-8wkHF N = 4). Rats were then tested in the elevated plus maze and body composition was determined.

Experiment 3: Effects of a junk-food diet on anxiety-like behaviors.

Because different dietary sources of calories can produce distinct outcomes in neural function (Murphy et al., 2014), we also determined the effect of 4 weeks of free access to a sugary, fatty, junk-food diet on anxiety-like behavior in male obesity-prone and obesity-

resistant rats (OP-Chow N = 4, OP-JF N = 6, OR-Chow N = 4, OR-JF N = 6). In addition, body composition was assessed at baseline and after the 4-week diet manipulation. Behavioral measures in both the elevated plus maze and open field were made only after diet manipulation. In addition, in order to determine whether differences in anxiety-like behavior may be due to reduced overall exploratory behavior in obesity-prone vs. obesity-resistant rats, locomotor activity in a novel environment was also assessed in these same rats.

Experiment 4: Anxiety-like behaviors in female OP vs. OR rats.

Here we assessed anxiety-like behaviors prior to, and after, the spontaneous divergence of weight gain in obesity-prone and obesity-resistant female rats. All animals remained on chow diet throughout the experiment. One set of females was tested in the elevated plus maze at a time when there were no differences in total body weight (OP N = 10, OR N = 10). In addition, two separate groups of rats were tested after spontaneous weight gain in obesity-prone rats in the open field (OR N=6, OP N=6) and elevated plus maze (OR N = 9, OP N = 9). Plasma leptin levels were used as an indirect measure of adiposity in these groups. Given that anxiety-like behavior is known to vary across the cycle, estrous cycle phase was monitored daily for a minimum of 10 days in a subset of females tested in the elevated plus maze. This was achieved by observing vaginal epithelial cell cytology and precopulatory and copulatory behaviors (Marcondes et al., 2002). In order to avoid additional potential stress on the test day, vaginal lavages were conducted after elevated plus maze in order to confirm estrous phase on the day of testing (OR metestrus and diestrus (M+D) N = 6; OR proestrus and estrus (P+E) N = 3; OP M+D N = 5, OP P+E N = 4).

Experiment 5: Effects of a junk-food or high-fat diet on anxiety-like behaviors in outbred male rats.

Outbred Sprague Dawley male rats were given either a chow (N = 10), junk-food (N = 10), or 60% high-fat diet (N = 10; 60% fat) for 48 days before determining body weight and evaluating anxiety-like behaviors in the elevated plus maze as described above.

Results

Figure A.1 shows a Gantt chart outlining experimental groups, treatment duration, primary measures, and the number of subjects in each group for each experiment. In addition, average daily food intake for each group within a given experiment is shown in Table A.1. Because animals were pair or group housed, food intake data are shown as daily averages based on food intake per cage divided by the number of animals in the cage and indicate general differences between groups and diet conditions. Data are presented as caloric intake, rather than mass, to facilitate comparisons across diets with different caloric density (e.g., high fat and chow). Food intake was comparable across studies including male OP and OR animals, with OP animals consuming more than their OR counterparts (Table A.1). Interestingly, food intake was highest in outbred animals (Experiment 5). The reason for this difference is unclear, as outbred animals were age matched to OP/OR rats and housed under similar conditions, albeit at a different institution.

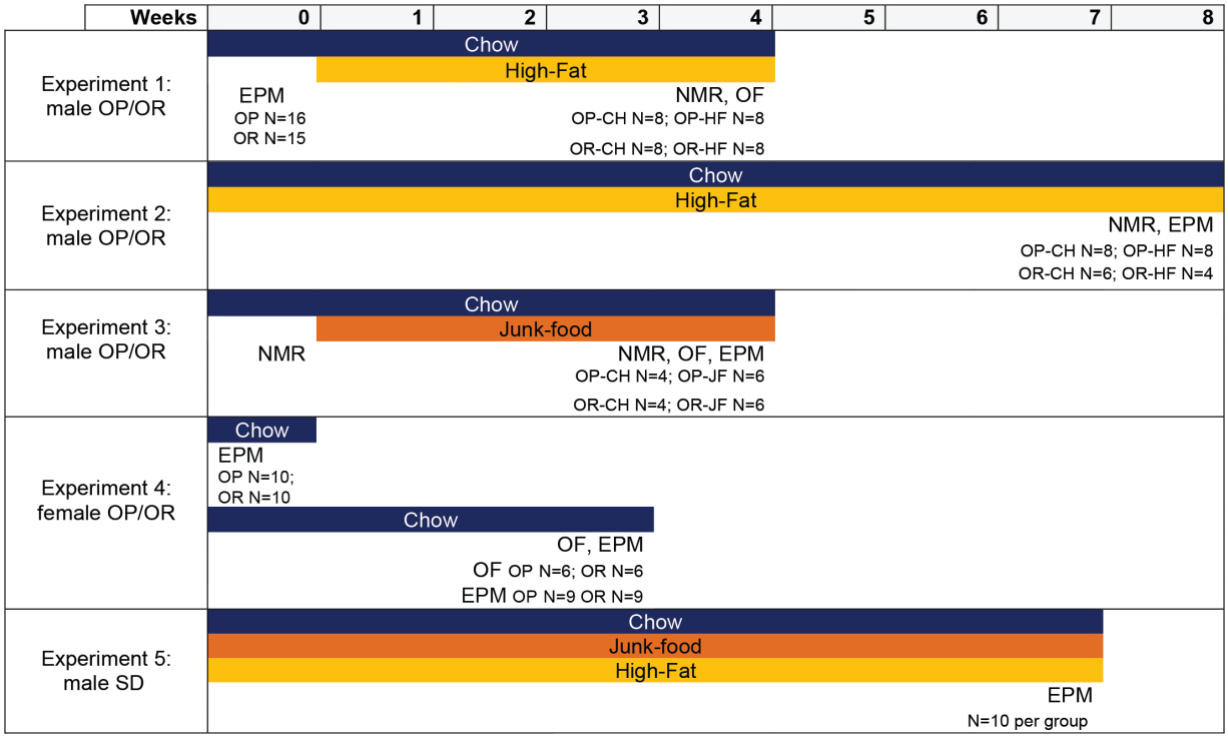

Fig. A.1. Experiment timelines. Timelines show number of animals used for each experiment and for each measure within an experiment. In addition, the timelines demonstrate which measures were performed for each experiment, and when each measure was performed EPM = Elevated plus maze; OF = Open Field; CH = Chow; HF = High Fat; JF = Junk-food

		Chow		High Fat		Junk-food	
		OR	OP	OR	OP	OR	OP
Exp. 1	average	121	130.9	129	152.3	-	-
	SEM	3.129	3.354	3.532	9.074	-	-
Exp. 2	average	115.4	127.3	99.34	119.4	-	-
	SEM	2.697	1.207	0.7095	3.293	-	-
Exp. 3	average	117.7	126.6.2	-	-	159.5	179.3
	SEM	2.269	8.346	-	-	6.488	3.041
		outbred males		outbred males		outbred males	
Exp. 5	average	164.4		159.3		203.9	
	SEM	2.783		4.141		4.041	

Table A.1. Average daily food intake as kilocalories per day.

Anxiety-like behaviors develop with weight gain in obesity-prone male rats

Figure A.2 shows weight and anxiety-like behavior in the same set of male obesity-prone and obesity-resistant rats before and after 4 weeks of chow or high-fat diet consumption. During baseline testing, weight (Fig. A.2A) and anxiety-like behavior in the elevated plus maze (Fig. A.2B-F) were similar in obesity-prone and obesity-resistant groups. These same rats were then tested in the open field after 4 weeks of free access to chow or high-fat diet (HF). As expected, obesity-prone rats given chow or high-fat diet were significantly heavier than obesity-resistant rats (Fig. A.2G: Two-way ANOVA main effect of group: $F_{(1, 12)} = 15.33$, $p < 0.01$), had more fat mass (Fig. A.2H: Two-way ANOVA main effect of group: $F_{(1, 12)} = 36.52$, $p < 0.001$), and less lean mass (Fig. A.2I: Two-way ANOVA main effect of group: $F_{(1,12)} = 22.54$, $p < 0.001$) than obesity-resistant rats. In addition, high fat diet did not produce any increases in weight or fat mass in obesity-resistant rats. Although there were visual trends towards increased fat mass in OP-HF vs OP-Chow groups, these were not statistically significant (Fig. A.2H; post-hoc OP-CH vs OP-HF p=0.2). This is not entirely unexpected given the short duration of diet exposure and pair housing conditions, which slow down weight gain (unpublished observation, CRF). When tested after weight gain, obesity-prone rats showed significantly stronger anxiety-like behavior compared to obesity-resistant groups, regardless of diet manipulation. Specifically, obesity-prone rats spent less time in center (Fig. A.2J: Two-way ANOVA main effect of group: $F_{(1, 28)} = 17.49$, $p < 0.001$), entered the center fewer times (Fig. A.2K: Two-way ANOVA main effect of

group: $F_{(1, 28)} = 17.79$, $p < 0.001$), and made fewer traverses (Fig. A.2L: Two-way RM ANOVA main effect of group: $F_{(1, 28)} = 16.42$, $p < 0.001$) compared to obesity-resistant rats in chow or high-fat groups. No significant interaction between 4 week high-fat diet and measure of anxiety-like behavior were found. Thus, data show that although anxiety-like behavior is initially comparable between obesity-prone and obesity-resistant rats, differences between these strains emerge with weight gain only in obesity-prone rats (see also discussion).

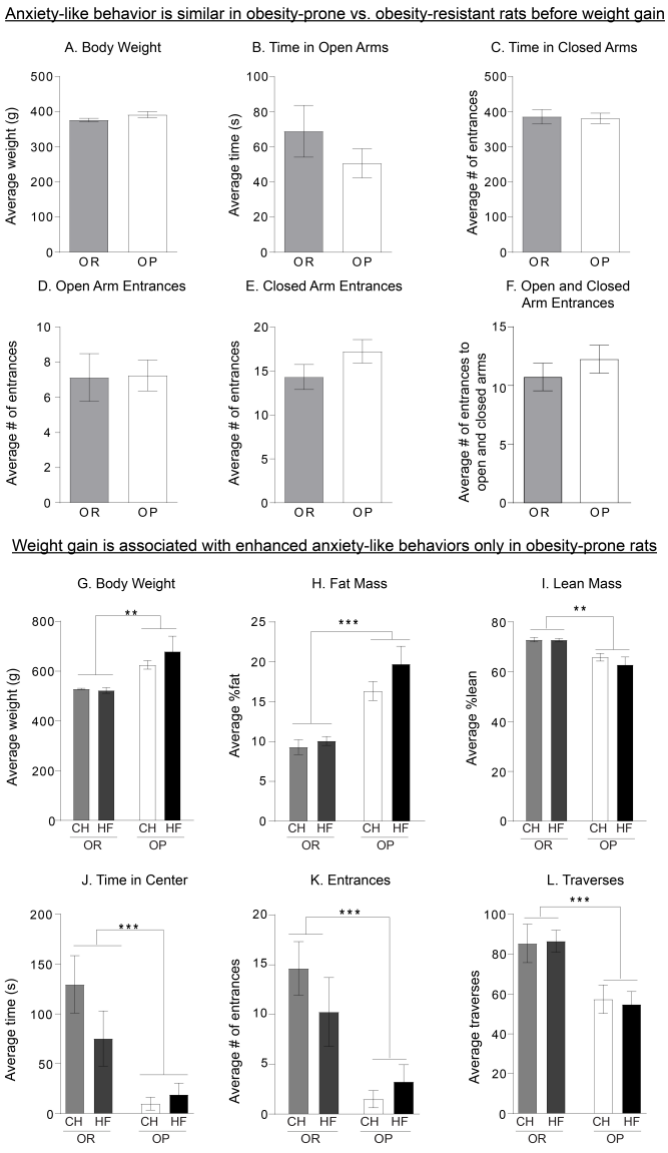

Fig. A.2. Anxiety-like behaviors emerge with spontaneous weight gain in male obesity-prone rats. A) Average weight at the time of elevated plus maze testing is similar between obesity-prone (OP) and obesity-resistant groups (OR). B–F) Measurements of anxiety-like behavior in the elevated plus maze were similar in obesity-prone and obesity-resistant groups. G–I) Weight and adiposity in the same obesity-prone and obesity-resistant rats after 4weeks of high-fat (HF) or regular chow (CH)diet. G–I) Obesity-prone rats were heavier, had more fat mass, and less lean mass than the obesity-resistant rats in both chow and high-fat diet fed groups. J–L) Measurements of anxiety-like behaviors in the open field after 4weeks of high-fat diet. Anxiety-like behavior was enhanced in obesity-prone vs. obesity-resistant rats following spontaneous weight gain chow or high-fat diet. Data are shown as mean ± SEM. * = $p < 0.05$, *** =$p < 0.001$.

Diet-induced obesity is not sufficient to enhance anxiety-like behavior in obesity-resistant male rats.

Figure A.3 shows measures of obesity (A-C) and anxiety-like behavior in the elevated plus maze following chow or 8 weeks of high-fat diet (OR-Chow, OR-HF, OP-Chow, OP-HF; D-F). As expected, obesity-prone rats were heavier (Fig. A.3A: Two-way ANOVA main effect of group: $F_{(1, 22)} = 88.71$, $p < 0.0001$), had more fat mass (Fig. A.3B: main effect of group: $F_{(1, 22)} = 44.42$, $p < 0.0001$), and less lean mass (Fig. A.3C: main effect of group: $F_{(1, 22)} = 58.88$, $p < 0.0001$) compared to obesity-resistant rats. In addition, while high-fat diet produced significant elevations in all measures of obesity in both obesity-prone and obesity-resistant groups (Two-way ANOVA; Fig. A.3A: main effect of diet: $F_{(1, 22)} = 107.9$, $p < 0.0001$; Fig. 3B: main effect of diet: $F_{(1, 22)} = 84.15$, $p < 0.0001$; Fig. 3C: main effect of diet: $F_{(1, 22)} = 73.28$, $p < 0.0001$), direct comparisons between groups given high-fat diet, show that obesity-prone rats gained significantly more fat mass than obesity-resistant rats (Fig. A.3B: Sidak's multiple comparison test, $p <0.05$). Furthermore, prolonged exposure to high-fat diet also produced significant increases in fat mass in obesity-resistant groups compared to their chow fed counterparts (Fig A.3B: Sidak's multiple comparison test, $p <0.01$). Although the magnitude of increased adiposity was less in obesity-resistant vs. obesity-prone rats given high-fat, fat mass, weight, and lean mass were comparable between obesity-resistant rats given high-fat diet and obesity-prone rats maintained on chow. Thus, 8 weeks of high-fat diet was sufficient to induce moderate obesity in obesity-resistant rats that was comparable to spontaneously occurring obesity in the obesity-prone group. Despite this, diet-induced obesity did not alter anxiety-like behavior in the obesity-resistant group (Fig. A.3D-F), although greater anxiety-like behavior in obesity-prone vs. resistant groups was still present (Two-way ANOVA; Fig. A.3D Time in open arms: main effect of group: $F_{(1, 22)} = 39.48$, $p < 0.0001$; Fig. A.3E Time in closed arms: main effect of group: $F_{(1, 22)} = 32.2$, $p < 0.0001$; Fig. 3F Open arm entrances: main effect of group: $F_{(1, 22)} = 45.6$, $p < 0.0001$).

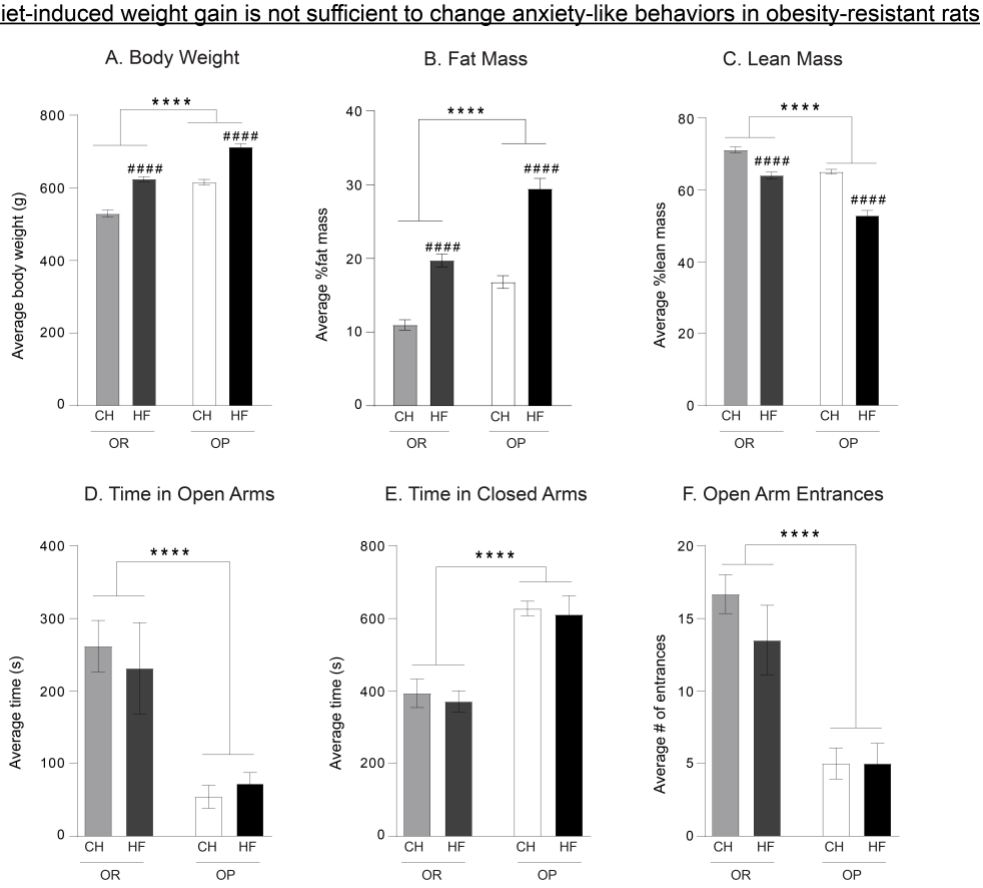

Fig. A.3. Diet-induced weight gain is not sufficient to enhance anxiety-like behavior in obesity-resistant rats. A–C) Weight, fat mass, and lean mass of obesity-prone and obesity-resistant rats given chow (CH) or high-fat diet for 8 weeks (HF). Eight weeks of high-fat diet was sufficient to induce increases in weight and adiposity, and reductions in lean mass in obesity-resistant rats that are comparable to spontaneous weight gain in obesity-prone rats given chow. In addition, high-fat diet produced additional weight gain, adiposity, and reductions in lean mass in obesity-prone rats compared to their chow-fed counterparts. D–F) Measurements of anxiety-like behavior in the elevated plus maze. Anxiety-like behavior in obesity-resistant rats was unaffected by increases in weight and fat mass, whereas anxiety-like behavior was greater in obesity-prone rats regardless of diet manipulation. Data are shown as mean ± SEM. #### = $p < 0.0001$ (chow vs. high-fat within group); **** =$p < 0.0001$ (OR vs. OP).

Junk-food diet does not alter anxiety-like behavior in obesity-prone or obesity-resistant rats.

Figure A.4 shows measures of adiposity, anxiety-like behavior, and locomotor activity after 4 weeks of free access to chow or junk-food diet. The organization of the graphs here differs from above such that chow and junk-food groups are arranged together on the X axis in order to better highlight differences between obesity-prone and obesity-resistant rats within diet condition. Obesity-prone and obesity-resistant rats given junk-food were significantly heavier than chow groups (Fig. A.4A: Two-way ANOVA: main effect of diet $F_{(1,16)} = 8.771$, $p < 0.01$), and obesity-prone rats were significantly heavier

than their obesity-resistant counterparts (Fig. A.4A: Two-way ANOVA: main effect of group, $F_{(1, 16)} = 11.68$, $p < 0.01$). Furthermore, obesity-prone rats given junk-food were significantly heavier than all other groups (Fig. A.4A: Tukey post hoc $p < 0.05$). Fasted plasma insulin levels were greater in obesity-prone vs. obesity-resistant groups (Fig. A.4B: Two-way ANOVA: main effect of group, $F_{(1,16)} = 5.868$, $p = 0.03$). Body composition was determined within subjects before and after diet manipulation (Fig. A.4C). Fat mass was similar between all groups prior to diet manipulation (Fig. 4C "Pre-JF"). However, 4 weeks of junk-food diet was sufficient to significantly increase fat mass in both obesity-prone and obesity-resistant groups (Fig. A.4C: T wo-way ANOVA: main effect of diet $F_{(1,16)} = 29.52$; $p < 0.0001$), with trends towards greater increases in obesity-prone groups. Consistent with effects observed in the previous two experiments (Fig. A.2 and Fig. A.3), obesity-prone rats again displayed significantly stronger anxiety-like behavior than obesity-resistant rats in the elevated plus maze and open field, with no effect of diet in either group (Fig. A.4D open field: Two-way ANOVA: main effect of group $F_{(1,16)} = 17.51$, $p < 0.001$; Fig. A.4E elevated plus maze: Two-way ANOVA: main effect of group, $F_{(1,16)} = 40.09$, $p < 0.0001$; data not shown include: EPM time in open arms, Two-way ANOVA main effect of group: $F_{(1,16)}= 8.04$, $p<0.05$; OF Center Entrances, Two-way ANOVA main effect of group: $F_{(1,16)}= 15.6$, $p<0.01$; and OF Traverses, Two-way ANOVA main effect of group: $F_{(1,16)}= 16.28$, $p<0.01$). In order to determine whether differences in anxiety-like behavior may be due to reductions in overall exploratory behavior in obesity-prone vs. obesity-resistant rats, we assessed locomotor activity in a novel testing environment in this same group of rats (Fig. A.4F). No significant differences in locomotor activity were found between any groups, and all rats showed normal habituation (Fig. A.4F: Two-way RM ANOVA: no main effect of group $p = 0.053$, or group x time interaction $p = 0.6410$; significant main effect of time $F_{(8,120)} = 36.81$ $p < 0.0001$). Thus, differences in anxiety-like behavior described above are not likely due to differences in exploratory behavior.

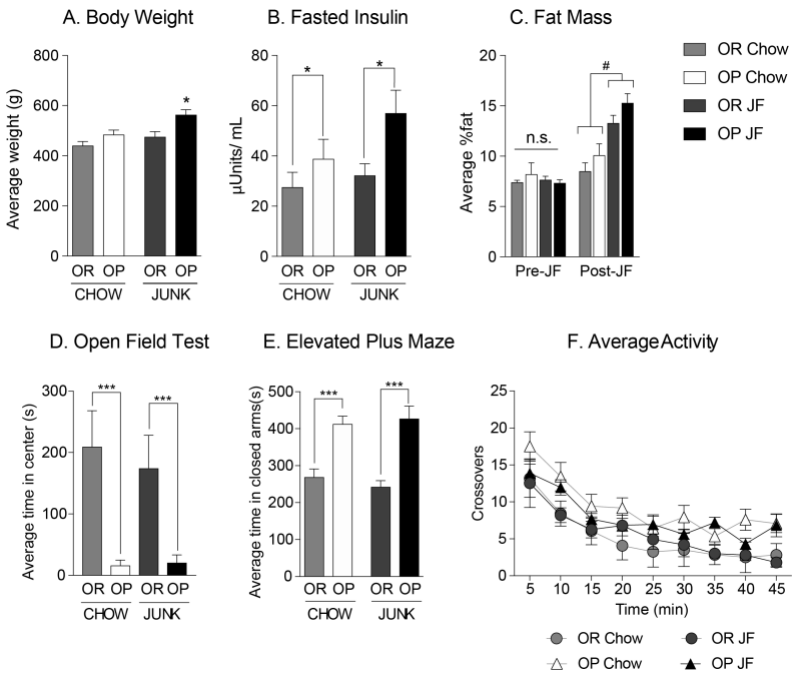

Fig. A.4. Junk-food diet does not alter anxiety-like behavior in obesity-prone or obesity-resistant rats. A–C) Weight, fasted insulin and fat mass following 4 weeks of free access to chow or junk-food (JF). Junk-food diet resulted in significant increases in weight only in obesity-prone rats, however fat mass was significantly increased by junk-food in obesity-prone and obesity-resistant groups compared to chow. Furthermore, fasted plasma insulin levels were elevated in obesity-prone vs. obesity-resistant groups, regardless of diet. D) Average time spent in the center during open field testing following 4 weeks of chow or junk-food diet. Junk-food did not alter anxiety-like behaviors in either group, but the obesity-prone groups spent significantly less time in the center than obesity-resistant groups. E) Average time spent in closed arms during elevated plus maze testing. Junk-food did not alter anxiety-like behaviors in either group, but the obesity-prone groups spent significantly more time in the closed arm than obesity-resistant groups. F) Average locomotor activity in a novel environment. Locomotor activity was similar between obesity-prone and obesity-resistant groups, regardless of diet or strain. Data are shown as mean ± SEM. * = $p < 0.05$, *** = $p < 0.001$ (OP vs. OR), # = $p < 0.05$ (JF vs. Chow).

Anxiety-like behavior in female obesity-prone rats is greater compared to obesity-resistant females.

Figure A.5 shows measures of adiposity and anxiety-like behavior in female obesity-prone and obesity-resistant rats maintained on chow. Consistent with data in males, no differences in anxiety-like behavior were found when body weight was similar between groups (Fig. A.5A-E). However, greater anxiety-like behavior was found in obesity-prone vs. obesity-resistant females that differed in weight (Fig. A.5F: Two-tailed unpaired t-test, $t_{(28)} = 2.868$, $p < 0.01$). Specifically, in the open field test, obesity-prone females tended to spend less time in the center (Fig. A.5H: Two-tailed unpaired t-test, $t_{(8)} = 2.181$, $p = 0.06$), and made significantly fewer entries to the center (Fig. A.5I: Two-tailed unpaired t-test, $t_{(8)}=2.666$, $p < 0.05$) than obesity-resistant females. No differences in traverses were found between groups (Fig. A.5J), thus group differences are not likely attributable to

differences in exploratory behavior. Similarly, in the elevated plus maze obesity-prone females spent significantly less time in the open arms (Fig. A.5K: Two-tailed unpaired t-test, $t_{(16)} = 2.496$, $p < 0.05$), and more time in the closed arms (Fig. A.5L: Two-tailed unpaired t-test, $t_{(16)} = 2.22$, $p < 0.05$) than obesity-resistant females. Although there were apparent trends, there were no statistical differences in the number of open arm entrances (Fig. A.5M: Two-tailed unpaired t-test, $t_{(16)} = 1.56$, $p = 0.14$). Despite differences in weight, no differences in plasma leptin concentrations (an indirect measure of adiposity) were observed between obesity-prone and obesity-resistant female rats (Fig. A.5G).

Potential effects of the cycle on behavior in the elevated plus maze were also assessed. Consistent with data above, obesity-prone females spent less time in open arms (Fig. A.5N: Two-way ANOVA: main effect of group, $F_{(1,14)} = 4.47$, $p = 0.05$, no group x cycle interaction $F_{(1,14)} = 0.644$, $p = 0.43$), more time in the closed arms (Fig. A.5O: Two-way ANOVA: main effect of group, $F_{(1,14)} = 4.61$, $p = 0.05$; no group x cycle interaction $F_{(1,14)} = 0.133$, $p = 0.72$), and showed no differences in open arm entrances (Fig. A.5M) compared to obesity-resistant females regardless of estrous cycle phase. Furthermore, there were some apparent trends for anxiety-like behavior to be greater during proestrus/estrus than metestrus/dietestrus in both groups (Two-way ANOVA: time in closed arms, main effect of cycle ($F_{(1,14)} = 3.93$, $p = 0.067$). In sum, similar to effects seen in males, differences in anxiety-like behavior were present only following weight gain in female obesity-prone vs. obesity-resistant rats, this group difference did not appear to vary across the cycle, although cycle phase may modulate anxiety-like behavior in both groups.

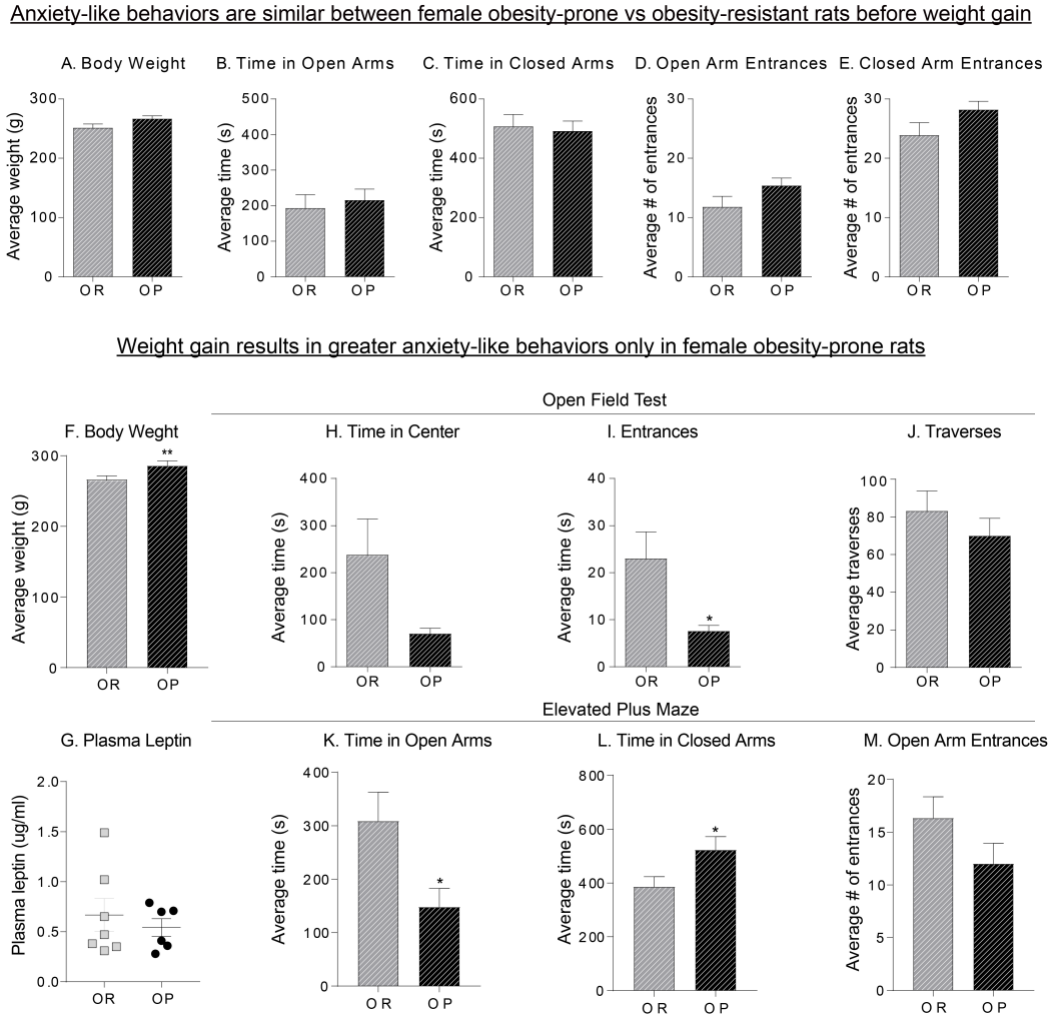

Fig. A.5. Similar to males, anxiety-like behaviors emerge with spontaneous weight gain in obesity-prone females and anxiety-like behavior varied across the cycle in both groups. A) Average body weight is initially similar between obesity-prone and obesity-resistant female rats. B–E) In the absence of weight differences, anxiety-like behavior in the elevated plus maze does not differ between obesity-prone and obesity-resistant females. F) Similar to males, obesity-prone females become heavier than obesity-resistant females as they age. G) Average plasma leptin concentrations did not differ between groups. H–J) Measures of anxiety-like behavior in open field test. Obesity-prone females tend to spend less time in the center, and made significantly fewer entries to the center than obesity-resistant females. K–M) Measures of anxiety-like behavior in elevated plus maze indicate that obesity-prone females display increased anxiety-like behaviors. N–O) Measures of anxiety-like behaviors in elevated plus maze by estrous cycle phase; metestrus and diestrus (M + D), proestrus and estrus (P + E). Obesity-prone females spent less time in the open arms and more time in the closed arms compared to obesity-resistant rats, regardless of estrous cycle phase. Data are shown as mean ± SEM. * = $p < 0.05$, **= $p < 0.01$

There is a positive correlation between adiposity and anxiety-like behavior in obesity-prone male, but not female rats. Figure A.6 shows pooled data from studies above using the elevated plus maze (male OP N = 32, female OP N = 10, male OR N = 24, female OR N = 13), and compares the relationship between time spent in the closed arm vs. body weight (Fig. A.6A,C) or plasma leptin levels (Fig. A.6B,D). These measures were chosen because we obtained leptin levels from both females and males, the largest number of rats were tested in the elevated plus maze, and because circulating leptin levels are proportional to fat mass. Strong positive correlations were found in male obesity-prone rats between time spent in the closed arms and weight (Fig. A.6A: r_2 = 0.5744; $p < 0.0001$) as well as time spent in the closed arms and plasma leptin levels (Fig. A.6B: r_2 = 0.2093; p = 0.0097). No relationship was observed between these factors in obesity-resistant males (Fig. A.6A: r_2 = 0.00087, p=0.89; Fig. 6B: r_2 = 0.058, p=0.27). In contrast, there was no relationship between weight or plasma leptin levels and time spent in the closed arms in female rats (Fig. A.6C: r_2 = 0.032, p=0.59; Fig. 5D: r_2 = 0.0001, p=0.92).

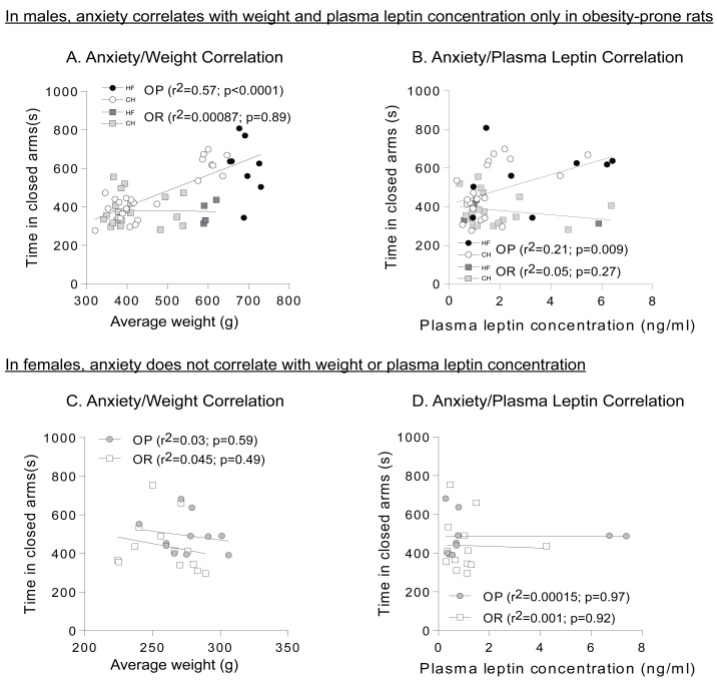

Fig. A.6. Weight and adiposity are positively correlated with anxiety-like behavior only in male obesity-prone rats. A) Relationship between weight and time spent in closed arms in males. A significant correlation is present in obesity-prone, but not obesity-resistant rats. B) Relationship between plasma leptin concentration and time spent in closed arms in males. A significant correlation is present in obesity-prone, but not obesity-resistant rats. C) Relationship between weight and time spent in closed arms in females. D) Relationship between plasma leptin concentration and time spent in closed arms in females. No significant correlations were found in females.

Diet-induced obesity does not alter anxiety-like behavior in outbred male rats.

Figure A.7 shows body weight and measures of anxiety-like behavior in the elevated plus maze in separate groups of outbred male rats given prolonged access to chow, junk-food, or high-fat diet. As expected, high-fat fed rats were significantly heavier than chow or junk-food fed groups (Fig. A.7A: One-way ANOVA: $F_{(2,27)} = 13.52$, $p < 0.0001$; Tukey's post-test: Chow vs. HF: $p < 0.01$; JF vs. HF: $p < 0.0001$). However, no differences were observed between any group for time spent in open arms (Fig. A.7B: One-way ANOVA: $F_{(2, 20)} = 1.70$, $p = 0.21$), time spent in closed arms (Fig. A.7C: One-way ANOVA: $F_{(2, 20)} = 0.47$, $p = 0.63$), or number of open arm entrances (Fig. A.7D: One-way ANOVA: $F_{(2, 20)} = 0.50$, $p = 0.62$).

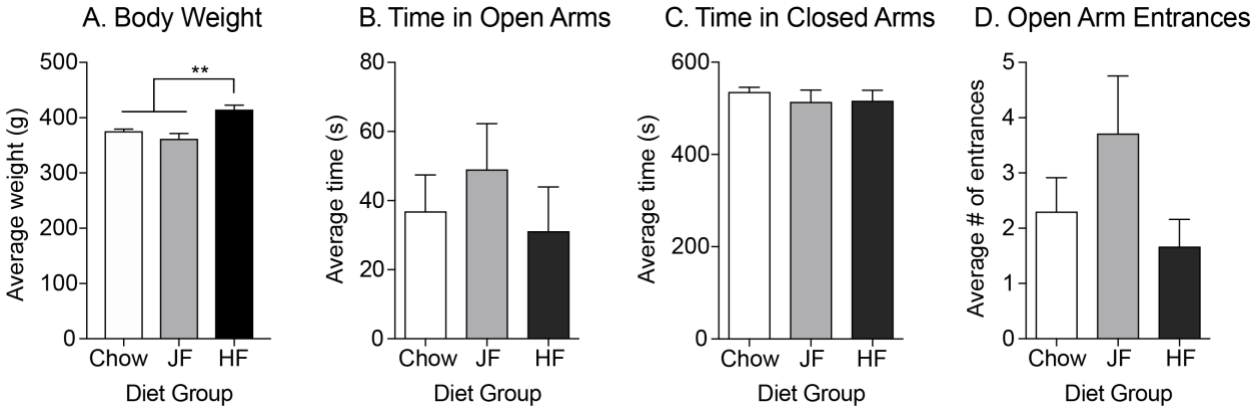

Fig. A. 7. Prolonged junk-food or high-fat diet (48 days) does not alter anxiety-like behavior in outbred male rats. A) Average body weight following 48 days of free access to chow, junk-food (JF) or high-fat (HF) diet. Animals given a high-fat diet were significantly heavier than those fed either chow or junk-food. B–D) Measures of anxiety-like behavior in the elevated plus maze. In outbred males, junk-food or high-fat diet did not alter anxiety-like behavior compared to chow-fed animals. Data are shown as mean ± SEM. ** = $p < 0.01$.

Discussion

Previous studies have demonstrated that anxiety and obesity are often comorbid in both humans (Daumit et al., 2003; Dickerson et al., 2006; Gariepy et al., 2010) and in rodent models (Vogel et al., 2017 Sharma et al., 2013; Sivanathan et al., 2015). However, it remains difficult to determine whether obesity leads to the development of anxiety, whether basal differences in anxiety may promote obesity, or a combination of the two. Here, we utilized both selectively bred and outbred rats to examine the relationship between pre-existing vs. obesity-induced increases in anxiety-like behavior. Weight gain and fat mass vary from 'overweight' to 'obese' to 'obesity accompanied by metabolic dysregulation'. Each of these designations is not one explicit state, but rather is a representation of key stages across a continuum. Based on weight gain, fasted insulin levels, and fat mass, young adult obesity-prone and obesity-resistant rats begin within the normal range of the outbred rodent population (Vollbrecht et al., 2015; Lillie et al., 1996). As they age, obesity-prone rats diverge from their obesity-resistant counterparts, accumulating more fat and showing mild elevations in fasted plasma insulin levels that do not yet constitute metabolic dysregulation (i.e., overweight; Vollbrecht et al., 2015; Levin, 2007). However, when given a high-fat diet, obesity-prone rats rapidly increase their fat mass and become metabolically compromised, though this effect varies by diet composition and duration of diet consumption (see Giles et al., 2016 for review). While many studies utilizing high fat diets and/or obesity-prone rats provide access for 6-8 weeks, we observe trends towards differences in adiposity and weight as early as 4 weeks, with some (but not all) cohorts reaching statistical significance even at this early timepoint. Many previous studies have utilized single housing, which can increase food intake and decrease activity levels, leading to faster rises in weight. Coincidentally, single housing can also have significant effects of anxiety-like behaviors which led us to maintain our animals in pair or group housing. Obesity-resistant rats also gain fat mass on a high-fat diet, reaching levels of fat mass that are comparable to that of 'overweight' obesity-prone rats given standard lab chow, but that have not yet transitioned to a metabolically compromised state (see below). Thus, this model is useful for examining behavioral differences in obesity-prone vs. -resistant populations prior to and after the development of obesity. We found that in both males and females, increases in anxiety-like behavior

emerged with the development of obesity in obesity-prone but not obesity-resistant rats. These data suggest that interactions between genetic predisposition and alterations accompanying weight gain promote anxiety in some susceptible individuals.

Relationships between predisposition, weight gain, and anxiety-like behaviors in males:

We found that measures of anxiety-like behavior in the elevated plus maze are similar in obesity-prone and obesity-resistant male rats prior to weight gain (Fig. A.2A-F). However, when these same rats were tested again after 4 weeks of high-fat diet or spontaneous weight gain, anxiety-like behavior in the open field was enhanced in obesity-prone vs. obesity-resistant groups (Fig. A.2G-L). No statistically significant effect of diet was observed within the obesity-prone rats; however, this appears to be a floor effect with animals on both a chow and high fat diet entering the center very infrequently. It is possible that a different paradigm might be used in the future to tease apart subtler effects of diet manipulation within this strain. Similar differences in anxiety-like behaviors were found in a separate cohort of rats tested in the elevated plus maze following 8 weeks of high-fat diet consumption, corroborating our initial observation (see also below). Furthermore, in males the degree of anxiety-like behavior correlated with fat mass and fasted plasma leptin levels (Fig. A.6A-B). These data appear to be in contrast to a recent study by Vogel et al. (2017), which found mild elevations in anxiety-like behaviors in obesity-prone rats prior to any diet manipulation, but no correlation between weight and behavior. This could be due to differences in the lines of rats used, as rats in the current study were offspring of breeders originally obtained from Taconic, whereas rats in the study by Vogel et al. (2017) were obtained from Charles River, or to the use of weight gain previously vs. fat mass, plasma insulin, and plasma leptin levels used here. For example, we have found that elevations in peripheral insulin levels precede observable weight differences between obesity-prone and obesity-resistant rats (Vollbrecht et al., 2015, 2016). Furthermore, differences in anxiety-like behavior in the Vogel paper were less robust than those observed here. This supports the idea of a continuum in the relationship between anxiety-like behaviors and alterations accompanying weight gain. Consistent with this, Vogel et al. (2017) also found greater anxiety-like behaviors in

Zucker rats compared to obesity-prone animals, possibly due to greater obesity and associated peripheral dysregulation.

Following our initial 4-week diet manipulation studies, we determined whether moderate obesity was sufficient to enhance anxiety-like behaviors in *obesity-resistant* rats. To do this, a second set of obesity-resistant and obesity-prone rats were fed a high-fat diet for 8 weeks, leading to significant weight gain in obesity-resistant rats compared to their chow fed counterparts, and increases in fat mass that were comparable to that of obesity-prone rats fed a chow diet (Fig. A.3A-C). As was seen previously, compared to obesity-resistant rats given chow, anxiety-like behavior of obesity-prone rats was enhanced (Fig. A.3D-F). However, despite having similar fat mass, weight, and fasted leptin levels to chow-fed obesity-prone rats who showed high levels of anxiety-like behavior, anxiety-like behavior remained unaffected in obesity-resistant rats given high-fat diet (Fig. A.3D-F). This suggests that weight gain and accompanying alterations, independent of susceptibility, are not sufficient to enhance these measures of anxiety.

We next examined effects of a sugary, fatty junk-food diet on anxiety-like behaviors. Junk-food diet led to significant increases in weight in both strains when compared to chow fed counterparts, and stronger anxiety-like behavior was again observed in obesity-prone groups, regardless of diet (Fig. A.4). However, as with the high-fat diet, obesity-resistant rats failed to display any changes in anxiety-like behaviors. This further suggests that diet and weight gain are not sufficient to induce anxiety in obesity-resistant rats, even in the presence of increased adiposity. Given the heterogeneity of effects of different foods on anxiety, we cannot rule out the possibility that a different diet composition may be required to induce anxiety-like behaviors in obesity-resistant rats. For example, diets made from animal fats and trans fats may be more anxiogenic than saturated fats (Mizunoya et al., 2013; for review see Murphy and Mercer, 2013). However, both diets used here are composed largely of animal and soy-based fat, which have been linked to anxiogenic effects (Ulrich-Lai et al., 2015 for review). None-the-less, our data do show that increases in fat mass or consumption of fatty, sugary foods in obesity-resistant rats are not in-and-of-themselves sufficient to enhance anxiety.

The role of obesogenic diets and obesity in the development of anxiety-like behaviors is rather complex. It has been recently demonstrated that chronic consumption of high-fat diets increases anxiety- and depressive-like behaviors, heightens the HPA axis and it is responsible for biochemical changes in brain reward circuitry (Sharma & Fulton, 2013). However, highly-palatable diets have different effects on the development of anxiety-like behaviors, depending on the type of diet, length of administration, age that it was fed and whether the rats became obese. For example, some studies have shown that feeding highly-palatable diets to recently weaned rats and during the pre-pubertal periods can be anxiolytic (de Lima Marcolin et al., 2012). In addition, another study showed that limited sucrose intake has stress-relieving properties (Ulrich-Lai et al., 2011). In contrast, adult rats fed a high-fat diet and that developed an obesity phenotype showed increase anxiety-like behaviors (Souza et al., 2007). Hence, the anxiolytic or anxiogenic effects of highly-palatable foods will depend on the administration of the diet, age of the animal, additional stressors, individual susceptibility to obesity and quality of prior experiences to the behavioral test.

In our experiments, increases in anxiety-like behavior in obesity-prone rats were generally similar whether induced by spontaneous weight gain, high-fat, or junk-food diet. This may be due to floor effects, in which case the lack of an effect of diet may be due to the sensitivity of the measure. However, it is important to note that while no differences in these behaviors were seen on average, there was a positive correlation between anxiety-like behaviors and plasma leptin levels as well as fat mass in obesity-prone males (Fig. A.6A,B). This suggests that there is not simply an all-or-none relationship between obesity and anxiety, but rather that anxiety-like behavior may scale with physiological alterations that accompany obesity, at least in males (see below for discussion of results in female). Of course, we cannot rule out an age effect in the current study, something that should be addressed in future studies.

We also examined the relationship between obesity and anxiety-like behaviors in outbred male Sprague-Dawley rats given chow, junk-food or high-fat diet for 48 days. The inability of the JF diet to induce significant weight gain may be attributable to the similarities in caloric content between the JF diet and a standard chow diet (4.5 kcal/g and 4.07 kcal/g respectively), or a result of insufficient heterogeneity in susceptibility to weight gain in this

particular cohort. No significant effects on anxiety-like behavior were observed between groups, although high-fat fed animals displayed significant increases in weight (Fig. A.7). This could suggest that differences observed in obesity-prone animals may be a result of not only weight gain, but also selective-breeding which is expected to amplify phenotypic differences. It is also interesting to note that similarities exist in anxiety-like behaviors between obesity-prone and outbred rats. The expected response of animals in our behavioral tests, and that observed in both outbred and OP rats, is to limit time spent in open, unprotected spaces. Obesity-resistant rats do not appear to follow this trend. Thus, it is possible that obesity-resistant rats are not as good at perceiving external cues that might be potentially harmful. In addition, we have observed that the obesity-resistant rats are not as good at engaging and perceiving food-associated cues (Derman & Ferrario, 2017). Hence, obesity-resistant rats may be lacking internal mechanisms to detect and engage with external cues that might be useful for their survival. Alternatively, the absence of an effect in outbred rats may simply have been due to insufficient heterogeneity in weight gain due to a relatively small outbred sample size (N=10/group). Additionally, we cannot rule out the possibility that running outbred animals during their light phase may have dampened overall responses, masking subtler behaviors. Thus, larger scale studies of outbred populations are needed before a firm conclusion can be drawn regarding the generalizability of the effects observed in obesity-prone rats.

Relationships between predisposition, weight gain, and anxiety-like behaviors in females:

To our knowledge, interactions between obesity and anxiety-like behaviors have not previously been examined in female selectively bred obesity-prone vs. obesity-resistant rats, even though females are at a higher risk for developing obesity and anxiety disorders (see Lopresti & Drummond, 2013 for review). Similar to males, obesity-prone female rats also develop anxiety-like behaviors alongside weight gain compared to female obesity-resistant rats (Fig. A.5). Thus, the overall pattern of behavioral data in females was similar to that of males. However, we did not find any correlation between weight, or leptin levels and anxiety-like behavior in females, while there were correlations between these measures and anxiety-like behavior in males. The absence of these correlations in females could simply be due to a lack of sensitivity in these measures, however if this

were the case, this should have equally affected data from males. Alternatively, these data could indicate that the mechanisms underlying the interaction between weight gain and enhanced anxiety differ between males and females. For example, neuroinflammation increases with obesity, and contributes to anxiety (see Guillemot-Legris & Muccioli, 2017 for review). However, males and females differ in their central and peripheral responses to inflammation. For example, in males, accumulation of fat in abdominal and visceral depots correlates with a deleterious metabolic profile that results in an inflammatory response. Whereas accumulation of subcutaneous fat in the gluteofemoral areas in females is correlated with a beneficial metabolic profile and a decrease in inflammatory responses (see Leeners et al., 2017 for review). Thus, the absence of correlations between overall fat mass and anxiety-like behavior in females may be an artifact of the general NMR measure used, which does not distinguish between different fat depots.

Although only a few studies have been conducted, data to date suggest that ovarian hormones can influence the expression of anxiety (Donner and Lowry, 2013). Here, we observed trends for greater anxiety-like behavior during proestrus/estrus compared to metestrus/diestrus that were similar in obesity-prone and obesity-resistant groups (Fig. A.5N,O). This is consistent with previous reports showing that anxiety-like behaviors increase in the proestrus and estrus phases under high light conditions similar to those used in the current study (Mora et al 1996), although the opposite relationship has been found when females are tested under low-light conditions (Donner & Lowry, 2013; Marcondes et al., 2001; Mora et al., 1996; ter Horst et al., 2012). However, regardless of any effect of the cycle on anxiety-like behavior, the data here show that behavioral differences between obesity-prone and obesity-resistant females cannot be explained by differences in behavior across the cycle.

What may be driving increases in anxiety-like behaviors?

While it is challenging to determine which of the many changes co-occurring with weight gain may mediate the observed increases in anxiety, positive correlations between plasma leptin levels and anxiety-like behaviors in obesity-prone, but not obesity-resistant rats may provide clues to potential mechanisms, at least in males. Leptin is involved in

the regulation of corticotropin releasing factor (CRF). Some have reported that increases in leptin lead to increases in CRF (Costa et al., 1997; Yamagata et al., 2013). Furthermore, elevated levels of CRF can increase anxiety-like behaviors in rodent models (See Koob, 1999 for review) and alterations in CRF systems have been reported in some anxiety disorders (Bangasser & Kawasumi, 2015; Kalin et al., 2016; Weber et al., 2016). A better understanding of potential changes in CRF signaling and peripheral signals associated with obesity and adiposity in the OP/OR model could be important in understanding the link between genetic susceptibility, obesity-development, and anxiety-like behaviors. Of course, many different hormones and neuromodulators are altered by obesity, thus it's unlikely that changes in leptin alone may account for behavioral differences. For example, insulin resistance either via knockout of the insulin receptor (Kleinridders et al., 2015) or via obesity-induced insulin resistance and inflammation (Soto et al., 2018) can also increase anxiety-like behaviors. Thus, future studies should address the potential role of insulin resistance, metabolic dysregulation, and inflammation in the effects reported here.

Finally, as suggested previously, future studies are necessary to tease apart potential contributions of aging vs increased adiposity to anxiety-like phenotypes that have been observed.

Summary:

Our data demonstrate interactions between susceptibility to obesity and anxiety-like behaviors in males and females. In multiple cohorts, baseline measures of anxiety-like behavior did not differ between obesity-prone and obesity-resistant rats, but differences emerged even with mild increases in adiposity in obesity-prone groups. Increases in fat mass were not sufficient to induce anxiety-like behaviors in either obesity-resistant or outbred rats. While we have put forth several plausible mechanisms by which obesity development may lead to anxiety-like behaviors, it is important to note that additional studies are needed to determine causal relationships, and that effects found in obesity-prone rats may be due to shared neurobiological factors or to dissociable neurobiological factors that are both influenced by a third factor. It remains to be determined whether selective breeding for an obesity-prone phenotype has co-selected for anxiety-like traits, although a lack of anxiety prior to obesity development suggests that at the least, these traits are related either directly or by a common, as yet unidentified, factor. These data suggest that susceptibility to obesity in combination with physiological alterations accompanying weight gain lead to enhancements in anxiety-like behaviors.

References

Albuquerque, D., Stice, E., Rodríguez-López, R., Manco, L., & Nóbrega, C. (2015). Current review of genetics of human obesity: from molecular mechanisms to an evolutionary perspective. *Molecular Genetics and Genomics*. https://doi.org/10.1007/s00438-015-1015-9

Alonso-Caraballo, Y., Jorgensen, E. T., Brown, T. E., & Ferrario, C. R. (2018). Functional and structural plasticity contributing to obesity: roles for sex, diet, and individual susceptibility. *Current Opinion in Behavioral Sciences*, *23*, 160–170. https://doi.org/10.1016/j.cobeha.2018.06.014

Avena, N. M., Bocarsly, M. E., Rada, P., Kim, A., & Hoebel, B. G. (2008). After daily bingeing on a sucrose solution, food deprivation induces anxiety and accumbens dopamine/acetylcholine imbalance. *Physiology and Behavior*, *94*(3), 309–315. https://doi.org/10.1016/j.physbeh.2008.01.008

Bangasser, D. A., & Kawasumi, Y. (2015). Cognitive disruptions in stress-related psychiatric disorders: A role for corticotropin releasing factor (CRF). *Hormones and Behavior*, *76*, 125–135. https://doi.org/10.1016/j.yhbeh.2015.04.003

Bocarsly, M. E., Fasolino, M., Kane, G. A., LaMarca, E. A., Kirschen, G. W., Karatsoreos, I. N., … Gould, E. (2015). Obesity diminishes synaptic markers, alters microglial morphology, and impairs cognitive function. *Proceedings of the National Academy of Sciences*, *112*(51), 201511593. https://doi.org/10.1073/pnas.1511593112

Bravo, J. A., Forsythe, P., Chew, M. V, Escaravage, E., Savignac, H. M., Dinan, T. G., … Cryan, J. F. (2011). Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proceedings of the National Academy of Sciences*, *108*(38), 16050–16055. https://doi.org/10.1073/pnas.1102999108

Costa, A., Poma, A., Martignoni, E., Nappi, G., Ur, E., & Grossman, A. (1997). Stimulation of corticotrophin-releasing hormone release by the obese (ob) gene product, leptin, from hypothalamic explants. *NeuroReport*, *8*(5), 1131–1134. https://doi.org/10.1097/00001756-199703240-00014

Cryan, J. F., & Sweeney, F. F. (2011). The age of anxiety: role of animal models of anxiolytic action in drug discovery. *British Journal of Pharmacology*, *164*(4), 1129–1161. https://doi.org/10.1111/j.1476-5381.2011.01362.x

Daumit, G. L., Clark, J. M., Steinwachs, D. M., Graham, C. M., Lehman, A., & Ford, D. E. (2003). Prevalence and correlates of obesity in a community sample of individuals with severe and persistent mental illness. *Journal of Nervous and Mental Disease*, *191*(12), 799–805. https://doi.org/10.1097/01.nmd.0000100923.20188.2d

Derman, R. C., & Ferrario, C. R. (2017). Enhanced incentive motivation in obesity-prone rats is mediated by NAc core CP-AMPARs. *Neuropharmacology*, *131*, 326–336. https://doi.org/10.1016/j.neuropharm.2017.12.039

Dickerson, F. B., Brown, C. H., Kreyenbuhl, J. A., Fang, L., Goldberg, R. W., Wohlheiter, K., & Dixon, L. B. (2006). Obesity among individuals with serious mental illness. *Acta Psychiatrica Scandinavica*, *113*(4), 306–313. https://doi.org/10.1111/j.1600-0447.2005.00637.x

Donner, N. C., & Lowry, C. A. (2013). Sex differences in anxiety and emotional behavior. *Pflügers Archiv - European Journal of Physiology*, *465*(5), 601–626. https://doi.org/10.1007/s00424-013-1271-7

Gariepy, G., Nitka, D., & Schmitz, N. (2010). The association between obesity and anxiety disorders in the population: a systematic review and meta-analysis. *International Journal of Obesity (2005)*, *34*(3), 407–419. https://doi.org/10.1038/ijo.2009.252

Giles, E. D., Jackman, M. R., & MacLean, P. S. (2016). Modeling Diet-Induced Obesity with Obesity-Prone Rats: Implications for Studies in Females. *Frontiers in Nutrition*, *3*, 50. https://doi.org/10.3389/fnut.2016.00050

Gorski, J. N. (2006). Postnatal environment overrides genetic and prenatal factors influencing offspring obesity and insulin resistance. *AJP: Regulatory, Integrative and Comparative Physiology*, *291*(3), R768–R778. https://doi.org/10.1152/ajpregu.00138.2006

Guillemot-Legris, O., & Muccioli, G. G. (2017). Obesity-Induced Neuroinflammation: Beyond the Hypothalamus. *Trends in Neurosciences*. https://doi.org/10.1016/j.tins.2017.02.005

Hryhorczuk, C., Sharma, S., & Fulton, S. E. (2013). Metabolic disturbances connecting obesity and depression. *Frontiers in Neuroscience*, *7*, 177. https://doi.org/10.3389/fnins.2013.00177

Kalin, N. H., Fox, A. S., Kovner, R., Riedel, M. K., Fekete, E. M., Roseboom, P. H., … Oler, J. A. (2016). Overexpressing Corticotropin-Releasing Factor in the Primate Amygdala Increases Anxious Temperament and Alters Its Neural Circuit. *Biological Psychiatry*, *80*(5), 345–355. https://doi.org/10.1016/j.biopsych.2016.01.010

Kleinridders, A., Cai, W., Cappellucci, L., Ghazarian, A., Collins, W. R., Vienberg, S. G., … Kahn, C. R. (2015). Insulin resistance in brain alters dopamine turnover and causes behavioral disorders. *Proceedings of the National Academy of Sciences*, *112*(11), 3463–3468. https://doi.org/10.1073/pnas.1500877112

Koob, G. F. (1999). Corticotropin-releasing factor, norepinephrine, and stress. *Biological Psychiatry*, *46*(9), 1167–1180. https://doi.org/10.1016/j.bbagen.2009.07.018

Leeners, B., Geary, N., Tobler, P. N., & Asarian, L. (2017). Ovarian hormones and obesity. *Human Reproduction Update Advanced Access Publication On*, *23*(3), 300–321. https://doi.org/10.1093/humupd/dmw045

Levin, B. E. (2007). Why some of us get fat and what we can do about it. *The Journal of Physiology*, *583*(Pt 2), 425–30. https://doi.org/10.1113/jphysiol.2007.135434

Levin, B. E., Dunn-Meynell, A., Balkan, B., & Keesey, R. E. (1997). Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. *The American Journal of Physiology*, *273*, R725–R730. Retrieved from http://www.physiology.org/doi/pdf/10.1152/ajpregu.1997.273.2.R725

Lillie, L. E., Temple, N. J., & Florence, L. Z. (1996). Reference values for young normal Sprague-Dawley rats: Weight gain, hematology and clinical chemistry. *Human & Experimental Toxicology*, *15*(8), 612–616. https://doi.org/10.1177/096032719601500802

Lopresti, A. L., & Drummond, P. D. (2013). Obesity and psychiatric disorders: Commonalities in dysregulated biological pathways and their implications for treatment. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. https://doi.org/10.1016/j.pnpbp.2013.05.005

Luppino, F. S., De Wit, L. M., Bouvy, P. F., Stijnen, T., Cuijpers, P., Penninx, B. W. J. H., & Zitman, F. G. (2010, March 1). Overweight, obesity, and depression: A systematic review and meta-analysis of longitudinal studies. *Archives of General Psychiatry*. American Medical Association. https://doi.org/10.1001/archgenpsychiatry.2010.2

Mackos, A. R., Eubank, T. D., Parry, N. M. A., & Bailey, M. T. (2013). Probiotic lactobacillus reuteri attenuates the stressor-enhanced severity of citrobacter rodentium infection. *Infection and Immunity*, *81*(9), 3253–3263. https://doi.org/10.1128/IAI.00278-13

Madsen, A. N., Hansen, G., Paulsen, S. J., Lykkegaard, K., Tang-Christensen, M., Hansen, H. S., … Vrang, N. (2010). Long-term characterization of the diet-induced obese and diet-resistant rat model: A polygenetic rat model mimicking the human obesity syndrome. *Journal of Endocrinology*, *206*(3), 287–296. https://doi.org/10.1677/JOE-10-0004

Marcolin, M. de L., Benitz, A. de N. D., Arcego, D. M., Noschang, C., Krolow, R., & Dalmaz, C. (2012). Effects of early life interventions and palatable diet on anxiety and on oxidative stress in young rats. *Physiology & Behavior*, *106*(4), 491–8. https://doi.org/10.1016/j.physbeh.2012.03.025

Marcondes, F. K., Bianchi, F. J., & Tanno, A. P. (2002). Determination of the estrous cycle phases of rats: some helpful considerations. *Brazilian Journal of Biology*. https://doi.org/10.1590/S1519-69842002000400008

Marcondes, F. K., Miguel, K. J., Melo, L. L., & Spadari-Bratfisch, R. C. (2001). Estrous cycle influences the response of female rats in the elevated plus-maze test. *Physiology and Behavior*, *74*(4–5), 435–440. https://doi.org/10.1016/S0031-9384(01)00593-5

Mizunoya, W., Iwamoto, Y., Shirouchi, B., Sato, M., Komiya, Y., Razin, F. R., … Ikeuchi, Y. (2013). Dietary fat influences the expression of contractile and metabolic genes in rat skeletal muscle. *PLoS ONE*, *8*(11). https://doi.org/10.1371/journal.pone.0080152

Mora, S., Dussaubat, N., & Díaz-Véliz, G. (1996). Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. *Psychoneuroendocrinology*, *21*(7), 609–620. https://doi.org/10.1016/S0306-4530(96)00015-7

Morganstern, I., Ye, Z., Liang, S., Fagan, S., & Leibowitz, S. F. (2012). Involvement of cholinergic mechanisms in the behavioral effects of dietary fat consumption. *Brain Research*, *1470*, 24–34. https://doi.org/10.1016/j.brainres.2012.06.004

Murphy, M., & Mercer, J. G. (2013). Diet-regulated anxiety. *International Journal of Endocrinology*. Hindawi Publishing Corporation. https://doi.org/10.1155/2013/701967

Murphy, T., Dias, G. P., & Thuret, S. (2014). Effects of Diet on Brain Plasticity in Animal and Human Studies: Mind the Gap. https://doi.org/10.1155/2014/563160

Naneix, F., Tantot, F., Glangetas, C., Kaufling, J., Janthakhin, Y., Boitard, C., … Ferreira, G. (2017). Impact of Early Consumption of High-Fat Diet on the Mesolimbic Dopaminergic System. *Eneuro*, *4*(3), ENEURO.0120-17.2017. https://doi.org/10.1523/ENEURO.0120-17.2017

Oginsky, M. F., Maust, J. D., Corthell, J. T., & Ferrario, C. R. (2016). Enhanced cocaine-induced locomotor sensitization and intrinsic excitability of NAc medium spiny neurons in adult but not in adolescent rats susceptible to diet-induced obesity. *Psychopharmacology*, *233*(5), 773–784. https://doi.org/10.1007/s00213-015-4157-x

Ohland, C. L., Kish, L., Bell, H., Thiesen, A., Hotte, N., Pankiv, E., & Madsen, K. L. (2013). Effects of lactobacillus helveticus on murine behavior are dependent on diet and genotype and correlate with alterations in the gut microbiome. *Psychoneuroendocrinology*, *38*(9), 1738–1747. https://doi.org/10.1016/j.psyneuen.2013.02.008

Sharma, S., & Fulton, S. (2012). Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. https://doi.org/10.1038/ijo.2012.48

Sivanathan, S., Thavartnam, K., Arif, S., Elegino, T., & McGowan, P. O. (2015). Chronic high fat feeding increases anxiety-like behaviour and reduces transcript abundance of glucocorticoid signalling genes in the hippocampus of female rats. https://doi.org/10.1016/j.bbr.2015.02.036

Soto, M., Herzog, C., Pacheco, J. A., Fujisaka, S., Bullock, K., Clish, C. B., & Kahn, C. R. (2018). Gut microbiota modulate neurobehavior through changes in brain insulin sensitivity and metabolism. *Molecular Psychiatry*, 1. https://doi.org/10.1038/s41380-018-0086-5

Souza, C. G., Moreira, J. D., Siqueira, I. R., Pereira, A. G., Rieger, D. K., Souza, D. O., … Perry, M. L. S. (2007). Highly palatable diet consumption increases protein oxidation in rat frontal cortex and anxiety-like behavior. *Life Sciences*, *81*(3), 198–203. https://doi.org/10.1016/J.LFS.2007.05.001

ter Horst, J. P., de Kloet, E. R., Schächinger, H., & Oitzl, M. S. (2012). Relevance of Stress and Female Sex Hormones for Emotion and Cognition. *Cellular and Molecular Neurobiology*, *32*(5), 725–735. https://doi.org/10.1007/s10571-011-9774-2

Ulrich-lai, Y. M., Fulton, S., Wilson, M., Petrovich, G., & Rinaman, L. (2015). Stress exposure , food intake and emotional state. *Stress*, *00*(00), 1–19. https://doi.org/10.3109/10253890.2015.1062981

Ulrich-Lai, Y. M., Fulton, S., Wilson, M., Petrovich, G., & Rinaman, L. (2015). Stress exposure, food intake and emotional state. *Stress (Amsterdam, Netherlands)*, *18*(4), 381–99. https://doi.org/10.3109/10253890.2015.1062981

Vogel, H., Kraemer, M., Rabasa, C., Askevik, K., Adan, R. A. H., & Dickson, S. L. (2017). Genetic predisposition to obesity affects behavioral traits including food reward and anxiety-like behavior in rats. *Behavioural Brain Research*, *328*, 95–104. https://doi.org/10.1016/j.bbr.2017.02.037

Vollbrecht, P. J., Mabrouk, O. S., Nelson, A. D., Kennedy, R. T., & Ferrario, C. R. (2016). Pre-existing differences and diet-induced alterations in striatal dopamine systems of obesity-prone rats. *Obesity*, *24*(3), 670–677. https://doi.org/10.1002/oby.21411

Vollbrecht, P. J., Nobile, C. W., Chadderdon, A. M., Jutkiewicz, E. M., & Ferrario, C. R. (2015). Pre-existing differences in motivation for food and sensitivity to cocaine-induced locomotion in obesity-prone rats. *Physiology and Behavior*, *152*(Pt A), 151–160. https://doi.org/10.1016/j.physbeh.2015.09.022

Walsh, R. N., & Cummins, R. A. (1976). The Open-Field Test: a critical review. *Psychological Bulletin*, *83*(3), 482–504. https://doi.org/10.1037/0033-2909.83.3.482

Wardle, J., & Carnell, S. (2009). Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. *American Journal of Clinical Nutrition*, *87*(2), 398–404. https://doi.org/10.1007/s12160-009-9116-5

Weber, H., Richter, J., Straube, B., Lueken, U., Domschke, K., Schartner, C., … Reif, A. (2016). Allelic variation in CRHR1 predisposes to panic disorder: Evidence for biased fear processing. *Molecular Psychiatry*, *21*(6), 813–822. https://doi.org/10.1038/mp.2015.125

Yamagata, S., Kageyama, K., Akimoto, K., Watanuki, Y., Suda, T., & Daimon, M. (2013). Regulation of corticotropin-releasing factor and urocortin 2/3 mRNA by leptin in hypothalamic N39 cells. *Peptides*, *50*, 1–7. https://doi.org/10.1016/j.peptides.2013.09.010

Zender, R., & Olshansky, E. (2009). Women's Mental Health: Depression and Anxiety. *Nursing Clinics of North America*. https://doi.org/10.1016/j.cnur.2009.06.002

Zhao, G., Ford, E. S., Dhingra, S., Li, C., Strine, T. W., & Mokdad, a H. (2009). Depression and anxiety among US adults: associations with body mass index. *International Journal of Obesity (2005)*, *33*(2), 257–266. https://doi.org/10.1038/ijo.2008.268

APPENDIX B

Effects of Junk-Food Diet on NAc Core MSN Intrinsic Excitability in Obesity-Prone and Obesity-Resistant Female Rats.

In a separate study, we determined the effects of junk-food diet on NAc core MSN intrinsic excitability in female obesity-prone and obesity-resistant female rats. Our lab has previously reported that junk-food has opposite effects on intrinsic excitability of NAc core MSNs in obesity-prone compared to obesity-resistant male rats. For example, in obesity-prone male rats, junk-food decreased MSN excitability whereas in obesity-resistant rats, excitability was increased (Oginsky & Ferrario, 2019).

In this study, obesity-prone (n = 10 rats; 15 cells) and obesity-resistant (n = 11 rats; 17 cells) female rats were fed a junk-food diet for 10 days. Control groups of obesity-prone (n = 10 rats; 21 cells) and obesity-resistant (n = 10 rats; 16 cells) female rats were kept on a chow diet throughout the experiment. Following 24-72 hours of junk-food deprivation, whole cell patch clamp electrophysiology was conducted to measure intrinsic membrane properties of MSNs in the NAc core (see methods Chapter 1).

Consistent with our previous results in males (Oginsky et al., 2016), and in females (Alonso-Caraballo & Ferrario, 2019) the I/V curved showed a greater change in membrane potential in response to positive current injections in MSNs from obesity-prone vs. obesity-resistant groups fed a chow diet (Fig. B.1A: Two-way RM ANOVA group x current injection interaction: $F_{(16,560)} = 3.4$, $p < 0.0001$; Sidak's multiple comparison $p < 0.05$). Additionally, we also observed differences in the number of action potentials between obesity-prone and obesity-resistant rats (Fig. B.1B: Two-way RM ANOVA group x current injection interaction: $F_{(16,560)} = 3.6$, $p < 0.0001$; Sidak's multiple comparison $p < 0.05$). We did not find differences in threshold between obesity-prone and obesity-resistant rats fed a chow diet (Fig. B.1C). Furthermore, input resistance was greater in

obesity-prone rats compared to obesity-resistant (Fig. B.1D: Two-tailed unpaired t-test, $t_{(35)} = 2$, $p = 0.05$). However, no significant differences in rheobase were observed (Fig. B.1E: Two-tailed unpaired t-test, $p = 0.1$) between obesity-prone and obesity-resistant rats on a chow diet. No differences between obesity-prone and obesity-resistant rats were observed when both groups were fed a junk-food diet (Fig. B.1F-J).

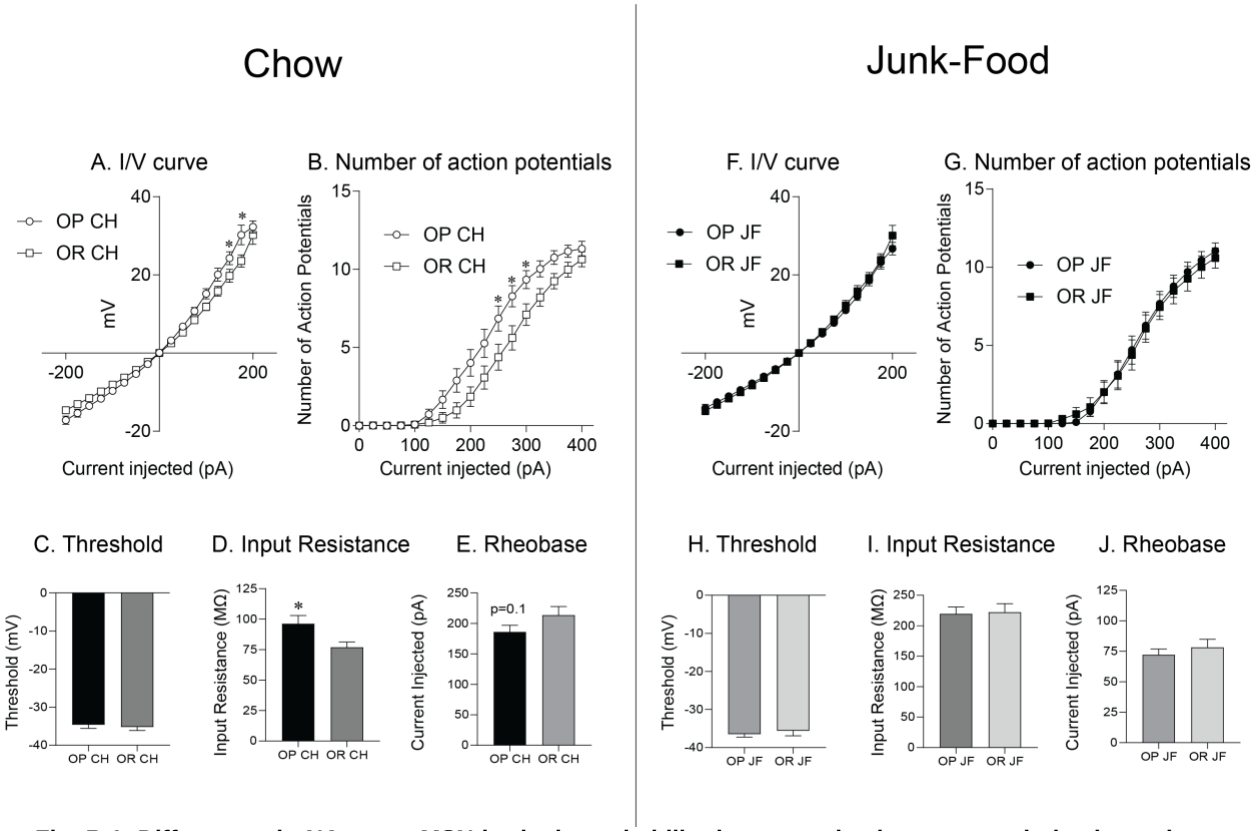

Fig. B.1. Differences in NAc core MSN intrinsic excitability between obesity-prone and obesity-resistant female rats in chow and junk-food diets. A) I/V curve of obesity-prone and obesity-resistant rats on a chow diet. Obesity-prone rats have greater shifts in membrane potential at positive currents compared to obesity-resistant rats. B) Number of action potentials between obesity-prone and obesity-resistant on a chow diet. Obesity-prone rats fired more action potentials compared to obesity-resistant at the same current injection. C. No differences in threshold between obesity-prone and obesity-resistant on chow. D) Input resistance was greater in obesity-prone fed a chow diet compared to obesity-resistant rats. E) No differences in rheobase between obesity-prone and obesity-resistant females fed a chow diet. F-J) No differences in MSN intrinsic excitability between obesity-prone and obesity-resistant rats fed a junk-food diet.

Similar to male obesity-prone rats, obesity-prone female rats had a reduction in excitability after junk-food diet followed by 24-72 hours of deprivation. Here we observed that there was a reduction in changes in membrane potential at positive currents in obesity-prone female rats fed a junk-food diet compared to their chow counterpart (Fig. B.2A: Two-way RM ANOVA group x current injection interaction: $F_{(16,544)} = 5.5$, $p < 0.0001$; Sidak's multiple comparison $p < 0.05$). Additional trends in the number of action potentials elicited between obesity-prone on chow vs. junk-food were also observed (Fig. B2B: Two-way RM ANOVA main effect of diet: chow vs. junk-food, $p = 0.08$). No differences in threshold were observed (Fig. B.2C: $p = 0.1$) Consistent with changes in I/V, obesity-prone fed a junk-food diet had a reduction in input resistance (Fig. B.2D: Two-tailed unpaired t-test, $t_{(34)} = 2.4$, $p = 0.02$) and an increase in rheobase (Fig. B2E: Two-tailed unpaired t-test, t $t_{(34)} = 2.1$, $p = 0.04$) compared to chow-fed obesity-prone female rats. We did not observe shifts in intrinsic excitability in obesity-resistant female rats fed a chow diet compared to junk-food (Fig. B2F-J).

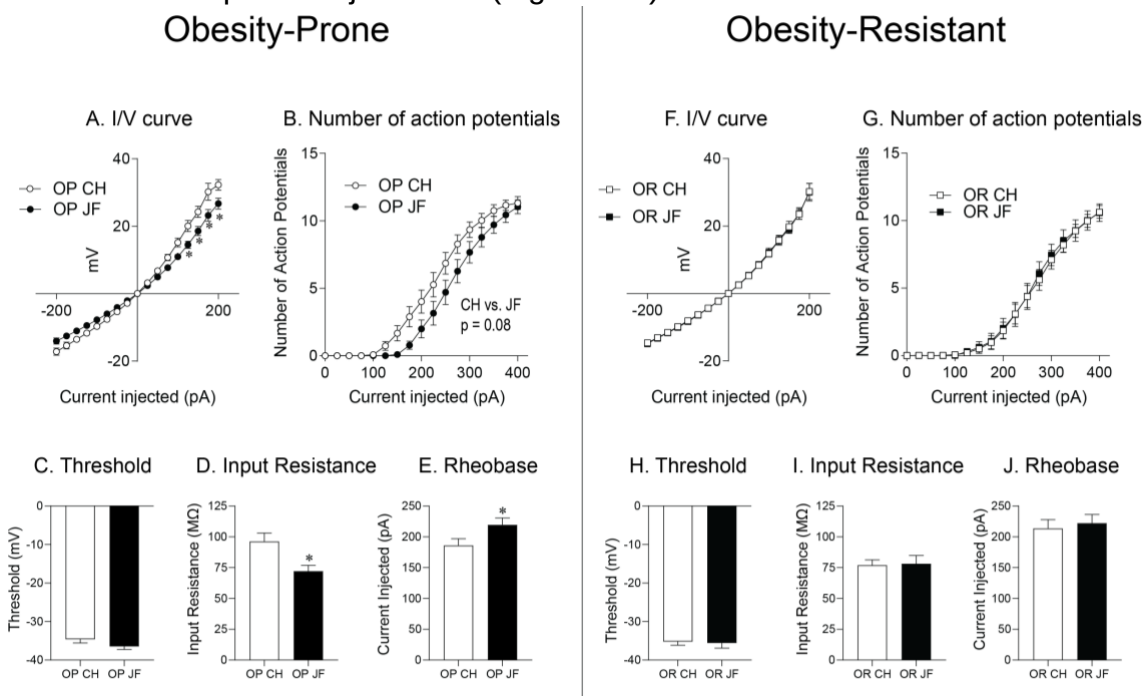

Fig. B.2. Effects of junk-food diet on NAc core MSN intrinsic excitability in obesity-prone and obesity-resistant female rats. A) I/V curve of obesity-prone fed a chow diet compared to junk-food. Obesity-prone rats on junk-food had a reduction in changes on membrane potential vs. chow-fed obesity-prone females. B) Differences in elicited number of action potentials between female obesity-prone on chow vs. junk-food diet. No significant differences were observed in female obesity-prone fed a chow vs. junk-food diet. C. No differences in threshold between female obesity-prone fed a chow diet vs. junk-food diet. D) Input resistance decreased in obesity-prone female rats fed a chow diet compared to junk-food. E) Rheobase increased after junk-food in obesity-prone females. F-J) No differences in MSN intrinsic excitability in obesity-resistant rats chow vs. junk-food.

In addition, we also determined the effects of the estrous cycle in modulating excitability in obesity-prone and –resistant rats fed a chow and a junk-food diet. Obesity-prone rats in metestrus/diestrus have greater excitability compared to obesity-resistant in the same phase of the estrous cycle. For example, a greater change in membrane potential was observed in response to positive current injections in MSNs from obesity-prone in metestrus/diestrus compared to obesity-resistant in metestrus/diestrus that were fed a chow diet (Fig. B.3A: Two-way RM ANOVA group x current injection interaction: $F_{(16,224)} = 3.6$, $p < 0.0001$; Sidak's multiple comparison $p < 0.05$). Additionally, we also observed differences in the number of action potentials between chow-fed obesity-prone and obesity-resistant in metestrus/diestrus (Fig. B.3B: Two-way RM ANOVA group x current injection interaction: $F_{(16,224)} = 3.0$, $p < 0.0001$; Sidak's multiple comparison $p < 0.05$). We did not find significant differences in input resistance between groups (Fig. B.3C: Two-tailed unpaired t-test, $t_{(14)} = 1.8$, $p = 0.09$). However, we saw a significant decrease in rheobase in obesity-prone compared to obesity-resistant rats in metestrus/diestrus (Fig. B.3D: Two-tailed unpaired t-test, $t_{(14)} = 2.3$, $p = 0.04$). No differences between groups were observed during proestrus/estrus phases of the estrous cycle (Fig. B.3E-H).

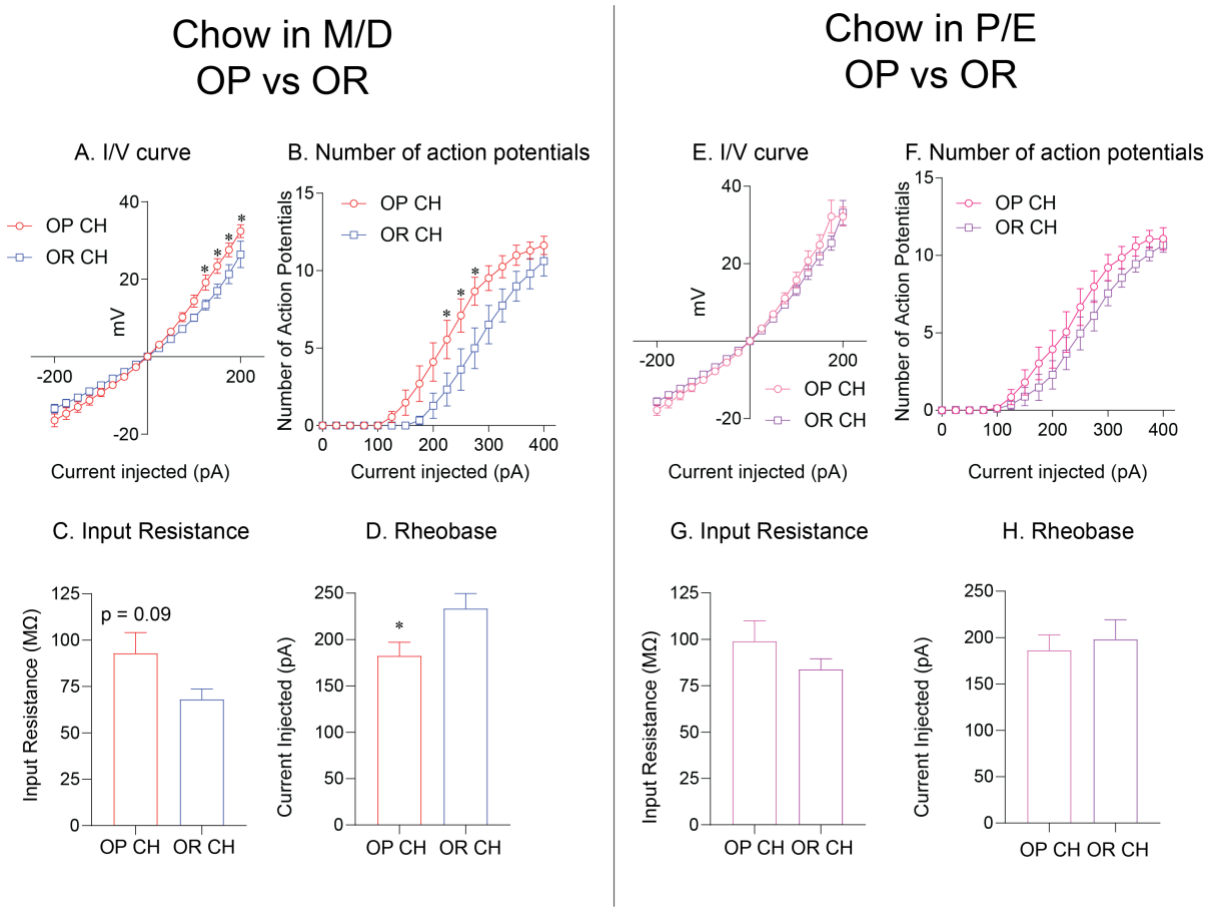

Fig. B.3. Estrous cycle phase effects on excitability in chow-fed obesity-prone and obesity-resistant rats. A) I/V curve of obesity-prone and obesity-resistant rats fed a chow diet and recorded during metestrus/diestrus and proestrus/estrus. Obesity-prone rats on metestrus/diestrus had greater changes on membrane potential vs. -resistant females. B) Differences in elicited number of action potentials between chow-fed obesity-prone and –resistant rats in metestrus/diestrus. Chow-fed obesity-prone female rats elicited more action potentials compared to obesity-resistant. C) No differences in input resistance. D) Rheobase was lower in chow-fed obesity-prone compared to obesity-resistant in metestrus/diestrus. E-H) No differences in intrinsic excitability were observed in chow-fed obesity-prone and obesity-resistant females during the proestrus/estrus phase of estrous cycle.

Furthermore, when making comparisons between junk-food-fed obesity-prone and obesity-resistant rats during metestrus/diestrus or proestrus/estrus, we did not find differences between groups (Fig B.4 A-H).

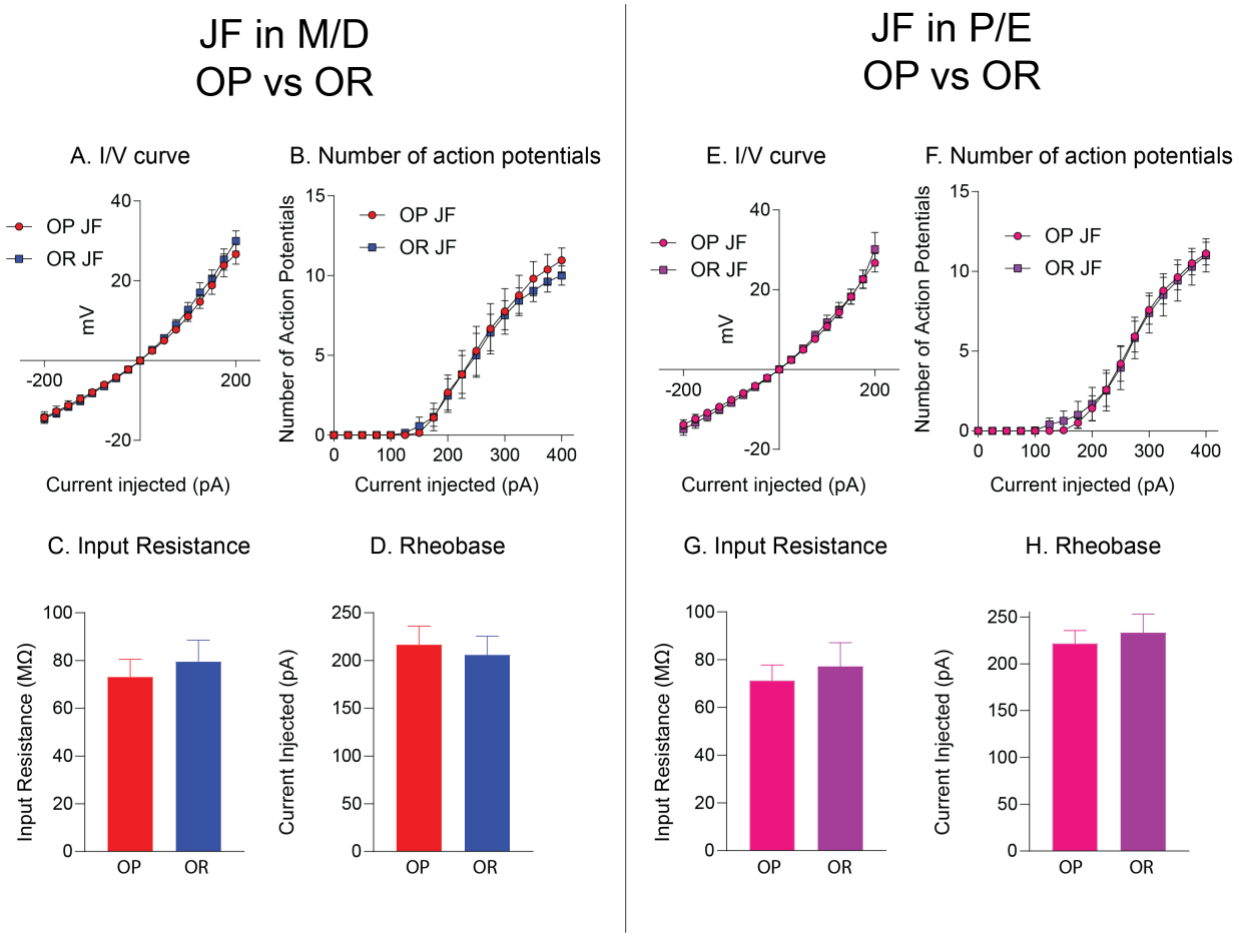

Fig. B.4. Estrous cycle phase effects on excitability in junk-food-fed obesity-prone and obesity-resistant rats. A-D) No differences in intrinsic excitability between junk-food-fed obesity-prone and obesity-resistant rats in metestrus/diestrus. E-H) No differences in intrinsic excitability between junk-food-fed obesity-prone and obesity-resistant rats in proestrus/estrus.