Investigating Sex-Specific Effects of Cocaine Exposure on NAc Glutamate Transmission

by

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Dedication

To my three favorite boys: Jameson, Jaiden and Jackson.

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Abstract

Substance use disorder (SUD) is a health problem, with relatively few effective treatments particularly in the case of psychostimulant drugs like cocaine. Preclinical intravenous drug self-administration models have been useful for understanding the neural and behavioral pathophysiology of SUD. However, very little is known about sex differences in addiction. Additionally, current self-administration models need to be improved, especially in regard to modeling human patterns of drug use.

Within the reward system, the nucleus accumbens (NAc) mediates reward and motivational processes, and changes in NAc function contribute to addiction. Specifically, enhancements in glutamate transmission in the NAc have been established to mediate some addiction-like behaviors in rodents. Although, in humans there are sex differences in the development and persistence of addiction there have been very few preclinical studies investigating sex differences in glutamate plasticity following different regiments of cocaine exposure.

Chapter 2 examines the effects of sex on behavioral sensitization and glutamatergic transmission. We found that females showed a stronger acute locomotor response to cocaine than males. Furthermore, locomotor sensitization was seen in both male and female rats that had repeated cocaine exposure. However the magnitude of sensitization (i.e., the change in cocaine-induced locomotor activity on day 1 vs day 8) displayed no significant sex differences. Interestingly, despite robust sensitization in both sexes, only males showed enhancements in glutamate transmission, specifically an enhancement in spontaneous excitatory post synaptic current frequency, but no changes in amplitude or pair pulse ratio. Finally, regardless of sex, calcium permeable AMPAR (CP-AMPAR) mediated transmission was similar to drug naïve animals.

In Chapter 3, we directly compare the effects of long access (LgA) vs intermittent access (IntA) self-administration on the incubation of cocaine craving and NAc core CP-AMPAR transmission in male and female rats. We found that both LgA and IntA

experience result in clear escalation of cocaine intake. However, LgA males increased cocaine infusions to a greater magnitude than LgA females, whereas there were no sex differences in escalation within IntA. Furthermore, both male and female animals show similar magnitude of incubation of craving in both LgA and IntA groups. Lastly, we see an upregulation of CP-AMPAR transmission following LgA and IntA compared to drug naïve rats. Potential sex differences and cell-type specificity are discussed further in this chapter.

Given that there is overlap in neural and behavioral alterations induced by food and cocaine, Chapter 4 examines the effects of junk-food and junk-food deprivation on MSN intrinsic excitability and glutamate transmission in females. Rats were given free access to junk-food and followed by a short junk-food deprivation period (1-2 days) and then NAc function was assessed. We found increases in NAc glutamate transmission, and reductions in neuronal excitability. This effect required a junk-food deprivation period. However, these effects were transient as they did not persist after 14-16 days of junk-food deprivation.

Together these studies examine how the factors of sex and pattern of drug exposure affect plasticity in the NAc. They also begin to address potential cell-type specificity and how effects of essential reinforcers like food may or may not differ from drugs of abuse like cocaine.

Chapter 1: Introduction

1.1 Addiction in the United States

The U.S. Department of Health and Human Services estimates 19.7 million Americans 12 years and older struggle with substance use disorder (SUD) and of those that battle with SUD, 40-60% of them relapse. This highlights that substance use is an immense and persistent phenomenon. It has been well established that drug-taking in it of itself is not addiction, because not all individuals who use drugs in their lifetime go on to uncontrolled drug use. Instead, SUD is when drug-taking and seeking behaviors are compulsive and persistent even in the presence of adverse consequences (Koob and Le Moal, 2005; Kranzler and Li, 2008). The complexity of the disease state often can make it difficult to diagnose. Currently, diagnosis is made using the DSM-V-TR for SUD. Decades of research has helped create and define the criteria for the DSM-V-TR which now contains 11 characteristics of behaviors consistent with SUD. The severity of the diagnosis depends on how many criteria an individual possesses ranging from mild SUD, meeting 2-3 criteria, to severe SUD, meeting 6 or more of the criteria. The DSM-V-TR criteria can be found in Table 1.1. These criteria and how they relate to modeling addiction-like behaviors in rodents will be discussed in greater detail below.

Researchers have long been trying to understand the mechanisms that lead to the transition and persistence of addiction. In the field there are two main theories of addiction that interestingly hypothesize the development of addiction from opposite directions. The first theory, incentive sensitization, posits that repeated drug use sensitizes the neural system that mediates motivation for drug (Robinson and Berridge, 1993; Berridge and Robinson, 2016). The neural system associated with motivation for drug or wanting the drug is distinct from that of liking the drug. This idea of the distinct separation of wanting and liking arose from an experiment in the late 1980's. Berridge et al. (1989) depleted brain dopamine by neurochemically lesioning dopamine-producing neurons in the VTA to assess how dopamine depletion affected taste reactivity in rats.

They found that the facial reactivity that is caused by sweetness in rats, which are classified as liking reactions, remained intact. However, the motivation to seek out food reward was completely abolished (Berridge et al., 1989). This suggests that the animals still derived pleasure from the taste of food, but now had no desire or motivation to seek it out. This study along with many others helped define the development of incentive sensitization and determine that the dopamine system facilitates this process (Robinson and Berridge, 1993; Berridge and Robinson, 1998; Robinson and Berridge, 2001; Berridge and Robinson, 2016). It has long been postulated that the brain's dopamine system plays a key role in reward and motivation (Wise, 1985; Koob and Le Moal, 1997). Therefore, incentive sensitization theorizes that addiction occurs because the 'wanting' system becomes sensitized (due to a hyper reactive dopaminergic state in response to drug cues and drug itself). This drives continued pursuit of drug (i.e., enhanced drug wanting) that is not dependent on liking (Robinson and Berridge, 1993; Berridge and Robinson, 2016).

The other theory of addiction proposes that negative reinforcement resulting from a shift in hedonic allostasis is the driving factor for the development of addiction (Wise and Koob, 2014). Hedonic allostasis is a change in drug reward set point caused by repeated drug exposure, such that more drug is necessary to produce the same desired rewarding effects and overcome anhedonia (Koob and Le Moal, 2001). Hedonic allostasis is driven by two processes; a-process, where the drug effect is primarily positive, and b-process where the primary drug effect is negative. Continued drug-taking shifts the balance from the a-process to the b-process. This is accomplished through the development of drug tolerance and decreased dopamine transmission in the reward system, while simultaneous there is an amplification of the stress system and anhedonia (Weiss et al., 1992; Koob and Le Moal, 2001; Koob and Le Moal, 2008; Lüscher and Malenka, 2011; Koob, 2015). Therefore, drug-taking is maintained because an individual wants to decrease and minimize the negative withdrawal or affective state (b-process).

1.2 Cocaine use and pharmacology

There are many illicit drugs used by people that could be discussed in great detail, however, for this dissertation the primary focus will be on cocaine as it is one of the more commonly abused drugs in the United States. It is estimated that 1 million Americans are dependent on cocaine (Administration, 2017). Although cocaine use is highly prevalent, it does not affect men and women equally. For example, cocaine use is more common in men than women (Administration, 2017). This sex difference holds true in SUDs as well, where men have higher rates of SUD than women (Compton et al., 2007; Rehm and Shield, 2019). However, although women are initially less likely to use drugs, after their first exposure they tend to increase their consumption more rapidly and end up in rehabilitation centers in less time from initial drug exposure than men (Brady and Randall, 1999; McCance-Katz et al., 1999; Greenfield et al., 2010; Bobzean et al., 2014). This is known as a telescoping effect. Furthermore, women are more likely to experience relapse than men (Becker et al., 2017). This may be due in part to women exhibiting greater withdrawal symptoms than males (Sinha et al., 2006; Hudson and Stamp, 2011).

Currently, cocaine is a Schedule II drug. This means the abuse liability is high, however, there are known medical uses. Although it is not common in medical practice, it is approved for adult nasal mucosal surgery as an anesthetic (Reigstad and Reigstad, 2021). Cocaine is most often in powered form as cocaine hydrochloride. In this form it can be snorted, applied topically in the mouth, or dissolved and injected intravenously (IV; (Warner, 1993). In addition, the powered form can be easily converted into a free base to create what is known as crack cocaine, which is smoked (Smart, 1991).

The primary mechanism of action of cocaine is blocking the dopamine transporter, which inhibits dopamine reuptake from the synapse. Additionally, it also binds and blocks norepinephrine and serotonin transporters, which leads to an increase in their respective neurotransmitters in the synapse (Zimmerman, 2012). It is thought that increases in endorphin or endocannabinoid neurotransmitters cause the resulting euphoric effects of cocaine. Cocaine's neurobiological and behavioral effects and duration of action depend on the dose and route of administration, but generally its effects last between 15 minutes to one hour in both humans and rodents (Jatlow, 1987;

Donroe and Tetrault, 2017). The half-life of cocaine in humans is about 1 hour after IV infusion or 0.9-1.5 hours after nasal or oral administration (Inaba, 1989; Warner, 1993). Hydrolysis of cocaine happens rapidly in the liver by carboxylesterases and butyrylcholinesterase (Brzezinski et al., 1994; Kamendulis et al., 1996).

Due to cocaine's effects on many neurotransmitter systems, it has diverse effects on the body. Blockade of norepinephrine reuptake leads to activation of the sympathetic system resulting in vasocontraction, tachycardia, mydriasis and hyperthermia (Lathers et al., 2010). Effects of cocaine on the central nervous system results in increased alertness, loss of appetite, and increased energy, which are largely mediated by dopamine and serotonin (Gawin and Ellinwood Jr, 1988).

The most severe adverse reaction to cocaine use is death by overdose. Recent data from the CDC estimate that death after cocaine use has increased by 54% from 2019 to 2021 (Hedegaard H, 2021). Overdose risk increases with chronic cocaine use because users develop tolerance to the euphoric effects of cocaine as well as incentive motivation, which results in compulsive urge to take more drug after a single dose (Warner, 1993; Koob and Le Moal, 2001; Robinson et al., 2013). The problem arises because simultaneously other systems are sensitizing. For example, repeated cocaine exposure leads to sensitization of cocaine induced seizures (Karler et al., 1989; Marley et al., 1991b, a). This is problematic because as a user increases their frequency of use or dose they become more sensitive to the convulsant effects of the drug and are more likely to experience a seizure which can lead to death (Post and Weiss, 1988). Even though cocaine poses a significant health risk, there are currently no approved treatments for cocaine substance use disorder.

1.3 Animal models used to study addiction

1.3.1 Non-contingent drug administration

Non-contingent (i.e., experimenter-administered) drug administration has provided a deep understanding of drug-induced behavioral adaptations that may foster the transition to addiction. Of particular relevance here is the development of psychomotor sensitization, which is enhanced psychomotor activity after repeated

intermittent exposure of a stimulus. For example, repeated non-contingent cocaine exposure results in increased locomotor behavior to the same dose of the drug (Post and Rose, 1976; Post et al., 1981; Crombag et al., 1999). This is of particular importance because the brain regions that mediate psychomotor sensitization, to some extent, overlap with the regions that are important for the development and persistence of addiction (Wise and Bozarth, 1987; Kalivas and Stewart, 1991; Ferrario et al., 2010).

The development and characteristics of locomotor sensitization are influenced by factors such as, but not limited to, dose and sex. For example, repeated exposure to low doses of cocaine (10-20 mg/kg, i.p.) increases locomotor behavior in rodents. This is in contrast to higher doses of cocaine (~40 mg/kg, i.p.), which results in decreased locomotor behavior, but increased stereotyped behaviors (Kuczenski et al., 1991; O'Dell et al., 1996). Stereotyped behaviors included repetitive head, limb, and oral movements and play a competing role with locomotion (Robinson and Becker, 1986; Flagel and Robinson, 2007). Importantly, the development of stereotyped behaviors indicates a greater drug effect. Psychomotor sensitization is often characterized by a transition from behavior dominated by locomotion to behavior dominated by stereotyped patterns of activity in response to the same dose of cocaine (a greater drug effect). In addition to dose, sex plays a key role the behavioral response to cocaine exposure; this will be discussed in greater detail below (Section *1.4.6 Sex differences in addiction-like behaviors in rodents*).

Psychomotor sensitization has been associated with neuroplasticity that is thought to contribute to the transition to addiction. For example, animals that receive prior exposure to addictive drugs that result in sensitization later facilitates their acquisition of self-administration (Horger et al., 1990; Piazza et al., 1990), show stronger cocaine conditioned place preference (Lett, 1989; Shippenberg and Heidbreder, 1995), and escalate their cocaine intake more quickly than saline pre-treated controls (Ferrario and Robinson, 2007). Furthermore, behavioral expression of sensitization is long lasting, such that enhancements in locomotor activity can be seen as long as a year after cessation of drug exposure (Paulson et al., 1991). This highlights that the development of psychomotor sensitization causes long-lasting changes

associated with drug-induced neuroplasticity that mediate incentive motivation processes (Robinson and Berridge, 1993; Vanderschuren and Pierce, 2010).

1.3.2 Contingent drug administration

Contingent (i.e., self-administered) IV drug administration is the current standard to study behavioral changes associated with addiction. As previously mentioned, drug-taking in it of itself is not addiction, therefore, there has been much effort to develop animal models that are able to distinguish between mere drug-taking vs what might be considered compulsive or uncontrolled drug use. Self-administration models began by utilizing what is now known as short access (ShA) self-administration procedures. ShA procedures consist of 1-2 hours of unrestricted access to drug, however, under these conditions it takes months for addiction-like behaviors to emerge (Deroche-Gamonet et al., 2004). Ahmed and colleagues (1998) simply increased the amount of time per session that animals had drug access, and they noted a drastic increase in the development of addiction-like behaviors (Ahmed and Koob, 1998; Vanderschuren and Everitt, 2004). Thus, widespread use of extended or long access self-administration (LgA) began in the late 90's.

Under LgA conditions, animals have unrestricted drug access for 6+ hours per session. LgA self-administration has since been the gold standard for investigating neurobiological adaptations associated with the development of addiction-like behaviors. However, after nearly a decade, it was proposed that perhaps LgA does not mirror drug-taking patterns in humans. Humans do not maintain a high level of prolonged use. Rather users tend to cycle frequently between use and abstinence known as binges (Cohen and Sas, 1994; Simon et al., 2001). This realization led to the development of intermittent access (IntA) procedures (Ward et al., 1997; Zimmer et al., 2012). Cocaine IntA self-administration typically alternates between a 5-minute long drug available period followed by a 25-minute long no drug available period; this pattern is repeated across 3-6 hours. This pattern of drug use limits the total amount of drug intake similar to ShA (Zimmer et al., 2012; Kawa et al., 2016) but produces spiking drug concentrations in the brain compared to consistent levels seen in LgA or ShA (Zimmer et al., 2012). The spiking drug concentrations in the brain is thought to be clinically

relevant (Beveridge et al., 2012). Interestingly, animal models indicate that IntA self-administration produces similar or in some cases more robust addiction-like behaviors compared to LgA self-administration. This will be discussed in further detail below.

1.4 Addiction-like behaviors in rodents

To study addiction, addiction-like behaviors in rodents must first be clearly defined. In humans, the DSM-V-TR criteria for SUD (Table 1.1) are used to determine if an individual has transitioned to problematic drug use (discussed below). Using the same criteria in rodents, it is possible to see parallel behaviors. This is of particular importance because the development and persistence of addiction cannot be studied or understood without valid animal models. Researchers have primarily focused their efforts on escalation of drug intake, motivation to obtain drug, continued drug-seeking under adverse consequences, and measuring drug-craving and drug-seeking behavior as proxies to measure addiction-like behaviors in rodents. These criteria will be defined below and discussed in the context of the aforementioned cocaine self-administration models: ShA, LgA, and IntA. However, it is worthwhile to note that to some extent these behaviors can all be considered measures of motivation. So to break them into these discrete categories, as is done here, is simply for ease of discussion. For example, enhanced drug-seeking behavior would indicate enhanced motivation to obtain drug, as would continued drug-seeking under adverse consequences. However, these behavioral measurements capture different psychological aspects of motivated behavior and to some extent different aspects of neural function. Lastly, it's important to mention that some of these addiction-like behaviors have influence on others. For example, rats that exhibit escalation are more motivated to obtain drug as determined by break-point compared to animals that do not escalate intake (Paterson and Markou, 2003; Wee et al., 2008). Thus, IV self-administration in animals can lead to multiple addiction-like behaviors mirroring the complexities in human SUD.

1.4.1 Escalation of intake

In humans, one behavioral feature that defines problematic drug use is taking the substance in larger amounts or for longer than was intended. Similarly, rodent models of self-administration can lead to engagement of drug-taking that increases over time, also known as escalation (Deroche-Gamonet et al., 2004; Belin et al., 2008; Edwards and Koob, 2013). Specifically, under LgA and IntA cocaine self-administration conditions rats increase their cocaine intake across sessions, whereas ShA animals maintain steady intake (Wise and Bozarth, 1987; Ahmed and Koob, 1998).

1.4.2 Motivation to obtain drug

Individuals with SUD are motivated to continue to find and take drugs, therefore it is vital to understand how drug consumption can cause shifts in motivation. Motivation in rodents is commonly measured using "progressive ratio" schedules of reinforcement (Hodos, 1961). Initially, animals are trained to self-administer an IV infusion of cocaine by engaging with an active nose-poke or lever. Next, they transition to progressive ratio conditions, where the number of times the animals needs to actively respond to obtain a single infusion increases exponentially until the animal reaches a "break-point". The break-point is the last ratio completed that results in the final delivery of drug. This value is used to quantify how hard an animal is willing to work, or how motivated they are, to obtain drug. Therefore, it can be said that animals who are less motivated will have lower break-points, and vice versa.

Another procedure to measure motivation in animals utilizes concepts from behavioral-economics (Bickel et al., 1995). This model uses economic theory to analytically assess how the demand for cocaine changes as a function of price, i.e., how the amount of cocaine consumed varies a function of price, operationalized as the number of active responses (Hursh et al., 1988; Bentzley et al., 2013). Plotting consumption as a function of price generates a demand curve where three important variables can be obtained: Pmax, Q0, and α. Pmax is the price at which maximum responding occurs, Q0 describes the consumption when cost is very low, and α is the rate of consumption decline as price increases (Bentzley et al., 2013). Interestingly, a recent study conducted by James et al. (2019) directly compared the effects of ShA, LgA and IntA self-administration on demand for cocaine. While both IntA and LgA

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experience results in enhanced motivation compared to ShA as measured by Pmax and α, IntA experience resulted in even greater demand for cocaine than in the LgA group (James et al., 2019). Consistently it has been shown that there is greater motivation following IntA or LgA conditions compared to ShA conditions using demand or progressive ratio (Hao et al., 2010; Algallal et al., 2020). Some studies also report a greater increase in motivation using demand and progressive ratio following IntA conditions compared to LgA conditions (Kawa et al., 2016; Allain et al., 2018; Algallal et al., 2020; Beasley et al., 2023).

1.4.3 Continued drug-seeking under adverse consequences

A major problem many individuals face with continued drug use is harmful consequences such as adverse health consequences or negative outcomes on social, family, or work life. To best model this in rodents, researchers often use physical pain as an adverse consequence. Animals are trained to self-administer drug followed by a period where active responding is intermittently paired with a foot shock. Interestingly, animals that have experienced LgA conditions will continue to drug-seek even when drug-seeking results in the delivery of an electrical shock. However, animals under ShA conditions will not as readily continue to drug-seek in the presence of the foot shock (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Sorge and Stewart, 2005). Furthermore, animals following IntA cocaine self-administration conditions are willing to accept a higher maximum intensity of foot-shock than rats following LgA or ShA conditions (James et al., 2019), consistent with enhanced motivation after IntA self-administration experience.

1.4.4 Drug-seeking

Humans abstaining from drug use can be trigger to relapse by factors such as the drug itself (Jaffe et al., 1989), drug-associated cues (O'Brien et al., 1992), stress (Sinha, 2001), or experiencing withdrawal symptoms (Wikler, 1973). To model relapse in rodents' researchers utilize drug-seeking behavioral paradigms. One common method is extinction-reinstatement (de Wit and Stewart, 1981). In this model, following self-administration animals first undergo extinction. Under extinction conditions, animals are

tested in the absence of drug, which allows for extinction of active-operant responding. Following extinction, animals undergo a reinstatement test. They are re-exposed to the drug, a cue previously associated with drug delivery, or are exposed to acute stress to assess how much drug-seeking is "reinstated", i.e., number of active responses after these stimuli compared to the end of extinction (Shaham et al., 2003). One benefit of this method is that humans and rodents alike will reinstate drug-seeking behavior under similar conditions. However, one major limitation is that humans never undergo operant extinction (Marlatt, 1996; Epstein and Preston, 2003). Additionally, extinction-reinstatement may not be the best model to understand the neuroplasticity associated with relapse because extinction training itself causes neuroadaptations in the same systems that drugs alter to produce addiction, the mesocorticolimbic systems (Shalev et al., 2002; Self et al., 2004; Torregrossa et al., 2008).

Another method that assesses drug-seeking behavior is assessing drug-seeking behavior across different time points during forced abstinence. Animals are first trained to self-administer a drug that is paired with a discrete cue. They then undergo forced abstinence where they are subject to drug-seeking tests at varying time points. During testing animals are brought back to the operant testing chambers and instead of the active response leading to the cue and IV drug infusion, only the cue is presented. The amount of drug-seeking is indicated by the number of active responses, with low levels of responding indicating low drug-seeking and high responding indicating high drug-seeking behavior (Venniro et al., 2016; Wolf, 2016). Interestingly, when rats are tested early in withdrawal (e.g., withdrawal day 1-3) they exhibit low drug-seeking behavior. This is in contrast to when they are tested later in withdrawal (e.g., withdrawal day 14 or 30) where drug-seeking behavior is greater compared to early withdrawal. This has been termed "incubation" of craving (Neisewander et al., 2000; Grimm et al., 2001; Lu et al., 2004). Incubation of craving is elevated as long as 6 months after cessation of drug-taking (Lu et al., 2004). This phenomenon is also displayed in humans, where there is a progressive enhancement of drug craving across abstinence (Hunt et al., 1971; Vanderschuren and Everitt, 2004), which importantly suggests clinical relevance. In rodents, incubation of craving is not limited to cocaine, but has also been observed in animals that self-administer methamphetamine (Shepard et al., 2004), alcohol

(Bienkowski et al., 2004), nicotine (Abdolahi et al., 2010), and heroine (Pickens et al., 2011). Both male and female rats that experience LgA or IntA cocaine self-administration show incubation of craving (Nicolas et al., 2019; Nicolas et al., 2022), whereas animals that have experienced ShA conditions do not (Alonso et al., 2022).

1.4.5 Incubation in non-drug reinforcers

Drugs are not the only reinforces that result in incubation of craving. Interestingly, humans struggling with disordered eating have intense food cravings, which result in relapse to disordered eating (Boswell and Kober, 2016). Similar to humans, this has been shown in rodent models using sucrose (Grimm et al., 2002; Grimm et al., 2005) and standard or high fat chow (Krasnova et al., 2014; Darling et al., 2016). Although, in many cases incubation of food craving seems similar to drug, it does not appear quite as long lasting as incubation of craving for drugs (Grimm, 2020). Historically, when investigating drug-induced alterations in reward circuitry food has been used as a control. The thought was any food-induced change that is also seen after drugs exposure must not be relevant to addiction. Therefore, drug and diet induced neuronal alterations will be discussed in further detail below as well as data Chapter 2, 3 and 4.

1.4.6 Sex differences in addiction-like behaviors in rodents

As previously mentioned, there are clear sex differences in both the development and persistence of SUD in humans, and there are many parallels in rodents. For example, females exhibit greater acute behavioral effects of cocaine as well as greater behavioral sensitization than male rats (Glick and Hinds, 1984; Van Haaren and Meyer, 1991). Furthermore females sensitize at lower doses than male rats (Post et al., 1981). Sex differences are also well described following contingent administration. Specifically female rats more readily acquire drug self-administration (Lynch and Carroll, 1999; Campbell et al., 2002), escalate their drug intake more rapidly (Roth and Carroll, 2004; Anker and Carroll, 2011), and show a higher propensity to initiate cocaine-seeking behavior following a withdrawal period (Anker and Carroll, 2010; Becker and Koob, 2016; Nicolas et al., 2019).

Perhaps unsurprisingly, these sex differences in the response to cocaine are in part mediated by gonadal hormones. Studies have utilized both intact animals as well as ovariectomy in females and castration in males to investigate the role of gonadal hormones on the acute locomotor activation effects of cocaine as well as the effects on sensitization. Ovariectomy attenuates acute cocaine induced locomotor behavior however, when ovariectomized females are treated with estradiol it leads to an enhancement in the acute effects of cocaine on locomotor behavior (Sell et al., 2000; Van Swearingen et al., 2013). Interestingly, intact females in estrus display greater acute effects of cocaine on locomotion than compared to females in other phases of the cycle (Sell et al., 2000). Furthermore, the development of behavioral sensitization is influenced by gonadal hormones. Ovariectomized females treated with estrogen display an enhanced locomotor sensitization compared to ovariectomized females (with no hormone treatment), castrated males and intact males (Peris et al., 1991; Hu and Becker, 2003). However, nonhormone treated ovariectomized females displayed greater locomotor behavior compared to castrated and intact males (Hu and Becker, 2003). This indicates that although hormones influence the development of sensitization, there are distinct differences in sensitization behavior between sex even when hormones are not present. The role of sex in the development of behavioral sensitization and subsequent neuroplasticity is further discussed in Chapter 2.

Gonadal hormones also play a role in self-administration behaviors. Specifically estradiol facilitates acquisition of cocaine self-administration in ovariectomized females (Lynch et al., 2001; Jackson et al., 2006; Becker and Hu, 2008; Anker and Carroll, 2011). However, even when ovariectomized females do not receive hormone replacement they still acquire cocaine self-administration more readily than both intact and castrated males (Hu and Becker, 2003). Additionally, ovariectomized females treated with estradiol show greater magnitude of escalation than ovariectomized females not treated with replacement hormone and intact females (Larson et al., 2007). Lastly, intact females display higher breakpoints (Roberts et al., 1989) as well as higher cue-induced drug-seeking behavior (Kerstetter et al., 2008; Nicolas et al., 2019) while in estrus compared to other cycle phases and males. Further investigation of sex specific alterations in behavioral and brain could provide insight into how to best treat individuals

with SUD, and, if necessary, for sex-specific treatments. Although, there are numerous behavioral studies done in both males and females, there are very few female neurobiological studies in the context of plasticity induced by self-administered drugs. These sex difference will be discussed in further detail below.

1.5 Reward circuitry: the nucleus accumbens structure and circuitry

Mesocorticolimbic circuits are comprised of many brain regions that mediate reward and motivation. The reward system includes the ventral tegmental area (VTA), medial prefrontal cortex (mPFC), the basolateral amygdala (Gong et al.), and the paraventricular thalamus (PVT) which all project to the nucleus accumbens (NAc). The NAc is often referred to as the limbic-motor interface, which allows the NAc to translate motivation into action (Mogenson et al., 1980; Sesack and Grace, 2010). The NAc receives information from the limbic system, which is important for initiating goal directed behavior (Mogenson and Huang, 1973). This is accomplished by sending the information to the motor systems, which includes the globus pallidus, a major target of NAc projections (Swanson and Cwan, 1975). It has widely been accepted that the NAc is a key region for reward and motivation and that changes in this region are believed to underlie addiction (Kalivas and Volkow, 2005; Lüscher and Malenka, 2011).

The NAc, or the ventral striatum, can be anatomically divided into two regions, the core and the shell, each with its own distinct structure, circuitry, and function (Záborszky et al., 1985; Wright and Groenewegen, 1996; Meredith et al., 2008). The NAc shell is the outer region of the NAc and surrounds the core. The core mediates cocaine-seeking elicited by cues whereas the shell mediates unconditioned drug responses (Parkinson et al., 1999; Cardinal et al., 2003; Milton and Everitt, 2012). The function of the NAc is highly dependent upon the signals that it receives, which involves many neurotransmitters including glutamate, dopamine, GABA, norepinephrine, and serotonin. However only dopamine and glutamate will be discussed in this dissertation.

Interestingly, one commonality between most drugs of abuse is they acutely increase dopamine in the NAc in both humans and rodents (Di Chiara and Imperato, 1988; Volkow et al., 2002). Dopamine cell bodies project from the VTA directly to the

NAc onto the main output neurons, GABAergic medium spiny neurons (MSNs). MSNs comprise 90% of all neurons in the NAc and primarily form two distinct populations based on the type of dopamine receptor they express, dopamine receptor 1 (D1) or dopamine receptor 2 (D2). Dopamine receptors are G protein-coupled receptors that positively and negatively couple to adenylyl cyclase, where D1 couples to G_s and D2 couples to $G_{i/o}$ (Felder et al., 1991; Corvol et al., 2001). Importantly in the dorsal striatum D1- and D2-MSNs have distinct projections that form what is known as the direct and indirect pathways, respectively. The direct pathway projects directly to the globus pallidus and substantia nigra pars reticulum (Montaron et al., 1996), whereas the indirect pathway projects to the dorsal lateral ventral pallidum and then to the globus pallidus (Gerfen et al., 1990; Le Moine and Bloch, 1995). However, in the ventral striatum the direct and indirect pathway are not as clearly segregated. D1-MSNs in the NAc project via the direct pathway and indirect pathway whereas, D2-MSNs seem to project exclusively through the indirect pathway (Wolf and Tseng, 2012; Smith et al., 2013; Heinsbroek et al., 2017).

1.6 Roles of NAc in behavior

Interestingly, D1- and D2-MSNs seem to have opposite effects in psychostimulant-induced behaviors (Allichon et al., 2021). For example, in the dorsal striatum chemogenetic inhibition of D2-MSNs enhances drug-seeking behavior (Bock et al., 2013) whereas chemogenetic inhibition of D1-MSNs reduces drug-seeking behavior (Yager et al., 2019). Similarly, in the NAc core inhibition of D2-MSNs increased drug-seeking behavior, whereas activation of D1-MSNs enhance drug-seeking behavior (Heinsbroek et al., 2017). Given that these data suggest the functional role of D1- and D2-MSNs in drug-seeking behavior are opposite, and that these two populations largely project via different circuits this may have important implications for their roles in relapse and drug-seeking behavior. Therefore, studies in Chapter 3 investigate how cocaine influences alterations within D1- and D2-MSN populations.

Although dopamine has been shown to be an important modulator of MSNs, it alone is insufficient to drive firing of MSNs (Lüscher and Malenka, 2011). For example,

a study conducted by Cornish and Kalivas (2000) assessed reinstatement behavior in rats trained to self-administer cocaine. Prior to the reinstatement session, animals either received an NAc intracranial injection of fluphenazine, a dopamine receptor antagonist, or CNQX, an AMPA receptor antagonists, to allow for assessment of the role of these receptors in the expression of reinstatement behavior. Interestingly, fluphenazine injection did not reduce reinstatement, however, CNQX attenuated reinstatement behavior (Cornish and Kalivas, 2000). Furthermore, cocaine reinstatement is associated with an increase in extracellular glutamate in the NAc core (McFarland et al., 2003). Together this highlights that glutamate plays an important role in mediating addiction-like behaviors. Therefore, the studies done in this dissertation have investigated the role of glutamatergic plasticity in the NAc core after both cocaine exposure (Chapters 2 and 3) and diet manipulation (Chapter 4).

Glutamatergic projections from the medial prefrontal cortex (mPFC), basolateral amygdala (BLA), paraventricular thalamus (PVT), and the VTA all converge onto MSNs in the NAc (Sesack and Grace, 2010). Each input seems to be linked to varying behavioral outputs. For example, the BLA projection is responsible for mediating cue-induced reward seeking behavior (Ambroggi et al., 2008). This is in contrast to the PVT projection to the NAc, which is vital for the acquisition of cocaine self-administration, such that blocking the PVT to NAc projection reduces drug intake during acquisition. However, this same manipulation did not prevent learning of the active/inactive discrimination (Neumann et al., 2016). These data highlight the complexity of glutamatergic input specificity on addiction-like behaviors (see also section *1.6.3 drug-induced glutamate plasticity* below for additional information about how cocaine influences glutamate transmission).

1.6.1 Sex differences in MSN anatomy and function

There are sex difference in MSN anatomy and function within the NAc. Intact females have greater spine density and spine head size in the NAc core than males (Forlano and Woolley, 2010). This may be due in part to circulating gonadal hormones, since it has been demonstrated that ovarian hormones influence spine density and maturity. Specifically, estradiol has been shown to decrease spine density and spine

maturity in females (Staffend et al., 2011; Peterson et al., 2015). Basal NAc excitatory transmission is enhanced in females compared to males and MSN intrinsic excitability varies with the cycle in females, resulting in complex sex differences in NAc activity (Proaño et al., 2018; Alonso-Caraballo and Ferrario, 2019). For example, NAc miniature excitatory post synaptic current (mEPSC) frequency is similar in males and females when recordings are made from females in the diestrus phase of the cycle, but is enhanced in females vs males when recordings are made from females in proestrus or estrus (Proaño et al., 2018). Historically, there has been a lack of females included in preclinical research, which has led to a gap in knowledge about female behavior and physiology. Recent efforts have been implemented to encourage researchers to include females as part of their research. Understanding basal differences in males and females as well as drug-induced changes in brain and behavior are vital to understanding how sex may influence processes that mediate development of addiction and relapse.

1.6.2 Glutamate and AMPARs

Glutamate is the primary excitatory neurotransmitter in the brain and binds to glutamate receptors; specifically, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) will be discussed here. AMPARs are ionotropic receptors that are composed of four subunits, GluA1-4, each encoded by different yet highly homologous genes. These subunits combine in different stoichiometries to form homo- or hetero-tetramers to generate receptors each with distinct properties. Expression of these subunits is developmentally regulated and highly brain region specific (Hansen et al., 2021). In the NAc, AMPARs are primarily composed of GluA1/2 or GluA2/3 subunits, however there are low levels (~10%) of receptors that lack the GluA2 subunit which are composed of GluA1 homomers or GluA1/3 subunits (Geiger et al., 1995; Reimers et al., 2011). Importantly it is the GluA2 subunit that provides the cation specificity (Hollmann et al., 1991; Burnashev et al., 1992). This specificity comes from a single amino acid residue known as the Q/R site. This site is subject to RNA editing which leads to replacement of the glutamine with an arginine and when arginine is present it renders the channel impermeable to calcium (Hume et al., 1991). Importantly, >99% of GluA2 in the adult brain is edited to arginine (Sommer et al., 1991). Therefore, when the GluA2

subunit is present AMPARs are permeable to sodium and potassium and are calcium impermeable (CI-AMPARs), which again makes up the majority of AMPARs in the NAc. AMPARs lacking the GluA2 subunit are permeable to calcium, and are therefore called calcium permeable AMPARs (CP-AMPARs) (Sprengel and Seeburg, 1993).

Trafficking of AMPARs in and out of the synapse is a dynamic and rapid process that is important for receptor function and synaptic transmission (Huganir and Nicoll, 2013). Recruitment of AMPARs to the synapse requires insertion of AMPARs via exocytosis into extrasynaptic sites, which is followed by lateral diffusion into the synapse where receptors can then either be retained or migrate back to extrasynaptic sites (Borgdorff and Choquet, 2002; Opazo and Choquet, 2011). It has been demonstrated that AMPARs undergo exocytosis and endocytosis from extrasynaptic sites rather than directly from the synapse (Ashby et al., 2004).

Importantly, it is the subunit composition of the receptor that determines the specific trafficking pattern. For example, evidence from hippocampal neurons indicates that GluA1/2 receptors are inserted in an activity dependent manner, whereas GluA2/3 receptors are recruited constitutively (Hayashi et al., 2000; Passafaro et al., 2001; Shi et al., 2001; Kakegawa et al., 2004; Lee et al., 2004). These different trafficking patterns are mediated through distinct phosphorylation of residues on the C-terminal tail of AMPAR subunits (Shi et al., 2001; Anggono and Huganir, 2012).

AMPAR subunits have divergent C-terminal tails, long (GluA1) verse short (GluA2/3) tail length, which allows for tightly regulated modulation. Interestingly it has been shown that activity dependent insertion of GluA1/2 is highly dependent upon GluA1. Studies using GluA1 phosphorylation deficient mice indicate that GluA1 and more specifically, phosphorylation of GluA1 at S845 and S831 are necessary for synaptic plasticity (Lee et al., 2003). It was later determined that phosphorylation of GluA1 happens in a stepwise fashion. Specifically, GluA1/2 receptors are inserted into extrasynaptic membranes which is dependent on C-terminal phosphorylation of GluA1 subunit at S845 by protein kinase A (Roche et al., 1996; Esteban et al., 2003; Derkach et al., 2007). Once in the extraynaptic membrane, the migration of GluA1/2 into the synapse is dependent on activation of NMDA receptors and subsequent calcium influx. This results in phosphorylation of GluA1 at S831 by protein kinase A and

calcium/calmodulin dependent protein kinase II (Barria et al., 1997) and synaptic insertion of the AMPAR.

Further confirming that GluA1 is necessary for activity dependent insertion, studies indicate that the absence of the GluA1 subunit results in constitutive activity of the receptor (i.e., GluA2 homomers or GluA2/3 receptors) and that knockout of GluA2 and GluA3 results in impairments in basal synaptic transmission (Shi et al., 2001; Meng et al., 2003). Together this highlights that phosphorylation allows for precise dynamic trafficking of specific AMPARs (GluA1/2 or GluA2/3) to and from the synapse.

Subunit composition also affects the function of the receptor. AMPARs exhibit fast activation and deactivation, therefore signaling happens within milliseconds (Edmonds et al., 1995; Silver et al., 1996; Salazar et al., 2020). Regardless of composition, AMPARs at negative potentials pass current inward, whereas at positive potentials current flows outwards this is because the reversal potential is at 0 mV. CI-AMPARs show a linear I/V relationship, passing current equally at positive and negative potentials (Jonas et al., 1994; Geiger et al., 1995). This is in contrast to CP-AMPARs, which are susceptible to blockage by the endogenous intracellular polyamines spermine and spermidine particularly at positive potentials (Bowie and Mayer, 1995; Donevan and Rogawski, 1995). This renders the receptors inwardly rectifying, therefore passing more current at negative than positive potentials.

There are antagonists that selectively block CP-AMPARs. One of particular relevance to studies conducted here is 1-naphthylacetyl spermine (Naspm). Naspm is a synthetic analog of the Joro spider toxin (ASAMI et al., 1989). It is a highly selective, but reversible blocker of CP-AMPARs with an IC_{50} of 0.33 µM (Koike et al., 1997). The blockade, similar to that of endogenous polyamines, is voltage dependent (Koike et al., 1997; Twomey et al., 2018) which indicates that Naspm is able to block CP-AMPARs at negative potentials, but at positive potentials blockage is much less. Additionally, Naspm is use dependent, therefore the receptors need to be activated and opened for Naspm to exert its effects. The ability of Naspm to reduce AMPA-mediated transmission is the primary way researchers have examined the contribution of CP-AMPARs to synaptic transmission. Therefore, in Chapters 2-4 Naspm is used in my studies where I ask how different stimuli (cocaine and junk-food) alters NAc CP-AMPAR transmission.

1.6.3 Drug-induced glutamate plasticity

AMPAR transmission in the NAc core has been linked to behavioral sensitization as well as cue-induced drug-seeking, i.e., 'incubation' of craving (Kalivas and Volkow, 2005; Wolf and Ferrario, 2010; Wolf, 2016). Repeated exposure to cocaine alters AMPAR expression and synaptic transmission. However, the specific AMPAR upregulation depends on the type of drug administration. For example, non-contingent cocaine administration that results in behavioral sensitization followed by withdrawal increases the surface expression GluA1 and GluA2 subunits (Boudreau and Wolf, 2005; Boudreau et al., 2007). Furthermore, behavioral sensitization and withdrawal results in enhanced AMPAR transmission compared to saline treated animals (Kourrich et al., 2007; McCutcheon et al., 2011). Together this suggests CI-AMPARs are upregulated following non-contingent cocaine administration, yet there had yet to be a direct assessment of CP-AMPAR mediated transmission following non-contingent cocaine administration. Furthermore, very few studies were conducted in females. Therefore, Chapter 2 investigates the effect of sex on behavioral sensitization and AMPAR transmission in the NAc core.

The pattern of NAc AMPAR upregulation associated with behavioral sensitization is currently understood to be distinct from changes in NAc AMPARs found after self-administration. Specifically, LgA self-administration and protracted withdrawal results in increased GluA1, but not GluA2 surface expression as well as an upregulation of CP-AMPAR mediated transmission in the NAc core (Conrad et al., 2008; Kawa et al., 2022). Importantly, CP-AMPARs have been shown to mediate incubation of craving, such that blocking or pharmacological removal of CP-AMPARs in the NAc results in attenuation of the incubation of craving (Conrad et al., 2008; Loweth et al., 2014; Kawa et al., 2022). However, there were no studies investigating the effects of IntA self-administration and withdrawal on glutamate transmission in the NAc, even though it is well established that animals under IntA conditions experience incubation of craving (Nicolas et al., 2019; Nicolas et al., 2022). Furthermore, although MSNs can be divided into two types (D1 vs D2 discussed above in section *1.6 Roles of NAc in behavior*), little is known about NAc cell-type specific changes following self-administration. Therefore, Chapter 3

investigates MSN cell-type specific changes in the NAc core following LgA and IntA in male and female rats.

1.6.4 Junk-food induced glutamate plasticity

Interestingly there is overlap in the neural systems that mediate motivation for drugs of abuse and nondrug reinforcers such as food. Specifically, the same mesocorticolimbic circuitry that mediates drug-seeking and addiction also mediates food-seeking behavior (Cardinal et al., 2002; Bäckström and Hyytiä, 2007; Berridge, 2018; Derman and Ferrario, 2018; Ferrario, 2020). Specifically, NAc activity is required for cue-triggered food-seeking and incubation of craving (Kelley, 2004; Conrad et al., 2008; Crombag et al., 2008; Dingess et al., 2017; Derman and Ferrario, 2018). Consistent with this, in obesity-prone rats 10 days of junk-food diet followed by junk-food deprivation resulted in increased CP-AMPAR mediated transmission in the NAc core in males (Oginsky et al., 2016; Alonso-Caraballo et al., 2021). Interestingly no changes in CP-AMPAR mediated transmission were seen in female rats under these conditions (Alonso-Caraballo et al., 2021). Although both cocaine and junk-food enhance CP-AMPARs in the NAc core there are a few differences between these two stimuli. For example, the amount of time it takes for the upregulation of CP-AMPARs. Cocaine conditions require ~14 days of withdrawal, whereas junk-food conditions only required a 24 hour junk-food deprivation time period for the upregulation to occur (Oginsky et al., 2016). In addition, there are sex differences between junk-food and cocaine exposure. Specifically, cocaine induced CP-AMPAR mediated transmission happens readily in both males and females following LgA self-administration conditions (Kawa et al., 2022), however, following a junk-food diet and 14 days of deprivation females do not show enhancements in CP-AMPAR mediated transmission. No studies had yet investigated if CP-AMPAR mediated transmission was upregulated after 24 hours of deprivation in females, therefore studies in Chapter 4 investigate effects of junk-food deprivation in females.

In addition to post-synaptic changes, a junk-food diet alters MSN intrinsic properties. For example, eating a junk-food diet reduces MSN excitability in males

(Oginsky and Ferrario, 2019), however, junk-food diet induced changes in MSN function in females had yet to be investigated and were examined in Chapter 4.

1.7 Experimental Objectives

Sex differences in humans with SUD as well as parallel sex differences in rodent models of addiction have been well established. Importantly, drug-induced neuroplasticity in the NAc is thought to contribute to the transition to addiction. Specifically, both psychomotor sensitization and enhancements in cue-induced drug-seeking have been tied to glutamate plasticity in the NAc core. It appears that the route of drug administration may influence glutamate plasticity in males, however, this is poorly understood. Additionally, it is unclear how the factor of sex may impact cocaine-induced glutamate plasticity. Therefore, the overall goal of the work described in this thesis is to explore how different regiments of cocaine exposure (i.e., those with different pharmacokinetics: non-contingent i.p., LgA or IntA self-administration) affect NAc glutamatergic plasticity in both males and females.

To begin, Chapter 2 examines the effects of sex on behavioral sensitization and glutamate plasticity in the NAc core following experimenter administered cocaine. Chapter 3 expands on understanding how the pattern of intake, utilizing LgA and IntA self-administration, effects incubation of craving and concurrent changes in NAc glutamate transmission. Studies here include the examination of sex differences and NAc MSN cell-type specificity with an emphasis on CP-AMPAR plasticity. Lastly, as CP-AMPARs increases are specifically thought to mediate incubation of drug-seeking, effects of junk-food NAc plasticity in females were also examined in Chapter 4.

	DSM-5-TR Substance Use Disorder
1	Taking the substance in larger amounts or for longer than you're meant to
2	Wanting to cut down or stop using the substance but not managing to
3	Spending a lot of time getting, using, or recovering from use of the substance
4	Cravings and urges to use the substance
5	Not managing to do what you should at work, home, or school because of substance use
6	Continuing to use, even when it causes problems in relationships
7	Giving up important social, occupational, or recreational activities because of substance use
8	Using substances again and again, even when it puts you in danger
9	Continuing to use, even when you know you have a physical or psychological problem that could have been caused or made worse by the substance
10	Needing more of the substance to get the effect you want (tolerance)
11	Development of withdrawal symptoms, which can be relieved by taking more of the substance

Table 1.1 The DSM-V-TR criteria used for assessment of SUD.

References

Abdolahi A, Acosta G, Breslin FJ, Hemby SE, Lynch WJ (2010) Incubation of nicotine seeking is associated with enhanced protein kinase A-regulated signaling of dopamine-and cAMP-regulated phosphoprotein of 32 kDa in the insular cortex. European Journal of Neuroscience 31:733-741.

Administration SAaMHS (2017) Results from the 2016 National Survey on Drug Use and Health. In: Center for Behavioral Health Statistics and Quality. Rockville, MD.

Ahmed SH, Koob GF (1998) Transition from moderate to excessive drug intake: change in hedonic set point. Science 282:298-300.

Algallal H, Allain F, Ndiaye NA, Samaha AN (2020) Sex differences in cocaine self-administration behaviour under long access versus intermittent access conditions. Addict Biol 25:e12809.

Allain F, Bouayad-Gervais K, Samaha AN (2018) High and escalating levels of cocaine intake are dissociable from subsequent incentive motivation for the drug in rats. Psychopharmacology (Berl) 235:317-328.

Allichon MC, Ortiz V, Pousinha P, Andrianarivelo A, Petitbon A, Heck N, Trifilieff P, Barik J, Vanhoutte P (2021) Cell-Type-Specific Adaptions in Striatal Medium-Sized Spiny Neurons and Their Roles in Behavioral Responses to Drugs of Abuse. Front Synaptic Neurosci 13:799274.

Alonso IP, O'Connor BM, Bryant KG, Mandalaywala RK, España RA (2022) Incubation of cocaine craving coincides with changes in dopamine terminal neurotransmission. Addict Neurosci 3.

Alonso-Caraballo Y, Ferrario CR (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. Horm Behav 116:104583.

Alonso-Caraballo Y, Fetterly TL, Jorgensen ET, Nieto AM, Brown TE, Ferrario CR (2021) Sex specific effects of "junk-food" diet on calcium permeable AMPA receptors and silent synapses in the nucleus accumbens core. Neuropsychopharmacology 46:569-578.

Ambroggi F, Ishikawa A, Fields HL, Nicola SM (2008) Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. Neuron 59:648-661.

Anggono V, Huganir RL (2012) Regulation of AMPA receptor trafficking and synaptic plasticity. Curr Opin Neurobiol 22:461-469.

Anker JJ, Carroll ME (2010) Reinstatement of cocaine seeking induced by drugs, cues, and stress in adolescent and adult rats. Psychopharmacology (Berl) 208:211-222.

Anker JJ, Carroll ME (2011) Females are more vulnerable to drug abuse than males: evidence from preclinical studies and the role of ovarian hormones. Curr Top Behav Neurosci 8:73-96.

ASAMI T, KAGECHIKA H, HASHIMOTO Y, SHUDO K, MIWA A, KAWAI N, NAKAJIMA T (1989) Acylpolyamines mimic the action of joro spider toxin (JSTX) on crustacean muscle glutamate receptors. Biomedical research 10:185-189.

Ashby MC, De La Rue SA, Ralph GS, Uney J, Collingridge GL, Henley JM (2004) Removal of AMPA receptors (AMPARs) from synapses is preceded by transient endocytosis of extrasynaptic AMPARs. J Neurosci 24:5172-5176.

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 (Berl) 192:571-580.

Barria A, Derkach V, Soderling T (1997) Identification of the Ca2+/calmodulin-dependent protein kinase II regulatory phosphorylation site in the α-amino-3-hydroxyl-5-methyl4-isoxazole-propionate-type glutamate receptor. Journal of Biological Chemistry 272:32727-32730.

Beasley MM, Tunstall BJ, Kearns DN (2023) Intermittent access cocaine self-administration produces context-specific escalation and increased motivation. Drug Alcohol Depend 245:109797.

Becker JB, Hu M (2008) Sex differences in drug abuse. Front Neuroendocrinol 29:36-47.

Becker JB, Koob GF (2016) Sex Differences in Animal Models: Focus on Addiction. Pharmacol Rev 68:242-263.

Becker JB, McClellan ML, Reed BG (2017) Sex differences, gender and addiction. J Neurosci Res 95:136-147.

Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ (2008) High impulsivity predicts the switch to compulsive cocaine-taking. Science 320:1352-1355.

Bentzley BS, Fender KM, Aston-Jones G (2013) The behavioral economics of drug self-administration: a review and new analytical approach for within-session procedures. Psychopharmacology (Berl) 226:113-125.

Berridge KC (2018) Evolving Concepts of Emotion and Motivation. Front Psychol 9:1647.

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 28:309-369.

Berridge KC, Robinson TE (2016) Liking, wanting, and the incentive-sensitization theory of addiction. Am Psychol 71:670-679.

Berridge KC, Venier IL, Robinson TE (1989) Taste reactivity analysis of 6-hydroxydopamine-induced aphagia: implications for arousal and anhedonia hypotheses of dopamine function. Behav Neurosci 103:36-45.

Beveridge T, Wray P, Brewer A, Shapiro B, Mahoney J, Newton T, Haile C, De La Garza I (2012) Analyzing human cocaine use patterns to inform animal addiction model development. In: Published abstract for the College on problems of drug dependence annual meeting, Palm Springs, CA.

Bickel WK, DeGrandpre RJ, Higgins ST (1995) The behavioral economics of concurrent drug reinforcers: a review and reanalysis of drug self-administration research. Psychopharmacology 118:250-259.

Bienkowski P, Rogowski A, Korkosz A, Mierzejewski P, Radwanska K, Kaczmarek L, Bogucka-Bonikowska A, Kostowski W (2004) Time-dependent changes in alcohol-seeking behaviour during abstinence. European Neuropsychopharmacology 14:355-360.

Bobzean SA, DeNobrega AK, Perrotti LI (2014) Sex differences in the neurobiology of drug addiction. Exp Neurol 259:64-74.

Bock R, Shin JH, Kaplan AR, Dobi A, Markey E, Kramer PF, Gremel CM, Christensen CH, Adrover MF, Alvarez VA (2013) Strengthening the accumbal indirect pathway promotes resilience to compulsive cocaine use. Nat Neurosci 16:632-638.

Borgdorff AJ, Choquet D (2002) Regulation of AMPA receptor lateral movements. Nature 417:649-653.

Boswell RG, Kober H (2016) Food cue reactivity and craving predict eating and weight gain: a meta-analytic review. Obesity reviews 17:159-177.

Boudreau AC, Wolf ME (2005) Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. J Neurosci 25:9144-9151.

Boudreau AC, Reimers JM, Milovanovic M, Wolf ME (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. J Neurosci 27:10621-10635.

Bowie D, Mayer ML (1995) Inward rectification of both AMPA and kainate subtype glutamate receptors generated by polyamine-mediated ion channel block. Neuron 15:453-462.

Brady KT, Randall CL (1999) Gender differences in substance use disorders. Psychiatr Clin North Am 22:241-252.

Brzezinski MR, Abraham TL, Stone CL, Dean RA, Bosron WF (1994) Purification and characterization of a human liver cocaine carboxylesterase that catalyzes the production of benzoylecgonine and the formation of cocaethylene from alcohol and cocaine. Biochemical pharmacology 48:1747-1755.

Burnashev N, Monyer H, Seeburg PH, Sakmann B (1992) Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. Neuron 8:189-198.

Campbell UC, Morgan AD, Carroll ME (2002) Sex differences in the effects of baclofen on the acquisition of intravenous cocaine self-administration in rats. Drug Alcohol Depend 66:61-69.

Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2002) Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev 26:321-352.

Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2003) The contribution of the amygdala, nucleus accumbens, and prefrontal cortex to emotion and motivated behaviour. In: International Congress Series, pp 347-370: Elsevier.

Cohen P, Sas A (1994) Cocaine use in Amsterdam in non deviant subcultures. Addiction Research 2:71-94.

Compton WM, Thomas YF, Stinson FS, Grant BF (2007) Prevalence, Correlates, Disability, and Comorbidity of DSM-IV Drug Abuse and Dependence in the United States: Results From the National Epidemiologic Survey on Alcohol and Related Conditions. Archives of General Psychiatry 64:566-576.

Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, Marinelli M, Wolf ME (2008) Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature 454:118-121.

Cornish JL, Kalivas PW (2000) Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. J Neurosci 20:RC89.

Corvol J, Studler J, Schonn J, Girault J, Herve D (2001) Gαolf is necessary for coupling D1 and A2a receptors to adenylyl cyclase in the striatum. Journal of neurochemistry 76:1585-1588.

Crombag H, Mueller H, Browman K, Badiani A, Robinson T (1999) A comparison of two behavioral measures of psychomotor activation following intravenous amphetamine or cocaine: dose-and sensitization-dependent changes. Behavioural pharmacology 10:205-213.

Crombag HS, Sutton JM, Takamiya K, Holland PC, Gallagher M, Huganir RL (2008) A role for alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid GluR1 phosphorylation in the modulatory effects of appetitive reward cues on goal-directed behavior. Eur J Neurosci 27:3284-3291.

Darling RA, Dingess PM, Schlidt KC, Smith EM, Brown TE (2016) Incubation of food craving is independent of macronutrient composition. Sci Rep 6:30900.

de Wit H, Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. Psychopharmacology (Berl) 75:134-143.

Derkach VA, Oh MC, Guire ES, Soderling TR (2007) Regulatory mechanisms of AMPA receptors in synaptic plasticity. Nat Rev Neurosci 8:101-113.

Derman RC, Ferrario CR (2018) Enhanced incentive motivation in obesity-prone rats is mediated by NAc core CP-AMPARs. Neuropharmacology 131:326-336.

Deroche-Gamonet V, Belin D, Piazza PV (2004) Evidence for addiction-like behavior in the rat. Science 305:1014-1017.

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85:5274-5278.

Dingess PM, Darling RA, Derman RC, Wulff SS, Hunter ML, Ferrario CR, Brown TE (2017) Structural and Functional Plasticity within the Nucleus Accumbens and Prefrontal Cortex Associated with Time-Dependent Increases in Food Cue-Seeking Behavior. Neuropsychopharmacology 42:2354-2364.

Donevan SD, Rogawski MA (1995) Intracellular polyamines mediate inward rectification of Ca(2+)-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. Proc Natl Acad Sci U S A 92:9298-9302.

Donroe JH, Tetrault JM (2017) Substance Use, Intoxication, and Withdrawal in the Critical Care Setting. Crit Care Clin 33:543-558.

Edmonds B, Gibb AJ, Colquhoun D (1995) Mechanisms of activation of glutamate receptors and the time course of excitatory synaptic currents. Annu Rev Physiol 57:495-519.

Edwards S, Koob GF (2013) Escalation of drug self-administration as a hallmark of persistent addiction liability. Behav Pharmacol 24:356-362.

Epstein DH, Preston KL (2003) The reinstatement model and relapse prevention: a clinical perspective. Psychopharmacology (Berl) 168:31-41.

Esteban JA, Shi SH, Wilson C, Nuriya M, Huganir RL, Malinow R (2003) PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. Nat Neurosci 6:136-143.

Felder CC, Williams HL, Axelrod J (1991) A transduction pathway associated with receptors coupled to the inhibitory guanine nucleotide binding protein Gi that amplifies ATP-mediated arachidonic acid release. Proceedings of the National Academy of Sciences 88:6477-6480.

Ferrario CR (2020) Why did I eat that? Contributions of individual differences in incentive motivation and nucleus accumbens plasticity to obesity. Physiol Behav 227:113114.

Ferrario CR, Robinson TE (2007) Amphetamine pretreatment accelerates the subsequent escalation of cocaine self-administration behavior. Eur Neuropsychopharmacol 17:352-357.

Ferrario CR, Li X, Wang X, Reimers JM, Uejima JL, Wolf ME (2010) The role of glutamate receptor redistribution in locomotor sensitization to cocaine. Neuropsychopharmacology 35:818-833.

Flagel SB, Robinson TE (2007) Quantifying the psychomotor activating effects of cocaine in the rat. Behavioural pharmacology 18:297-302.

Forlano PM, Woolley CS (2010) Quantitative analysis of pre- and postsynaptic sex differences in the nucleus accumbens. J Comp Neurol 518:1330-1348.

Gawin FH, Ellinwood Jr EH (1988) Cocaine and other stimulants. New England Journal of Medicine 318:1173-1182.

Geiger JR, Melcher T, Koh D-S, Sakmann B, Seeburg PH, Jonas P, Monyer H (1995) Relative abundance of subunit mRNAs determines gating and Ca2+ permeability of AMPA receptors in principal neurons and interneurons in rat CNS. Neuron 15:193-204.

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma Jr FJ, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429-1432.

Glick SD, Hinds PA (1984) Sex differences in sensitization to cocaine-induced rotation. Eur J Pharmacol 99:119-121.

Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME, Heintz N (2003) A gene expression atlas of the

central nervous system based on bacterial artificial chromosomes. Nature 425:917-925.

Greenfield SF, Back SE, Lawson K, Brady KT (2010) Substance abuse in women. Psychiatr Clin North Am 33:339-355.

Grimm J, Shaham Y, Hope B (2002) Effect of cocaine and sucrose withdrawal period on extinction behavior, cue-induced reinstatement, and protein levels of the dopamine transporter and tyrosine hydroxylase in limbic and cortical areas in rats. Behavioural pharmacology 13:379.

Grimm JW (2020) Incubation of food craving in rats: A review. J Exp Anal Behav 113:37-47.

Grimm JW, Fyall AM, Osincup DP (2005) Incubation of sucrose craving: effects of reduced training and sucrose pre-loading. Physiol Behav 84:73-79.

Grimm JW, Hope BT, Wise RA, Shaham Y (2001) Neuroadaptation. Incubation of cocaine craving after withdrawal. Nature 412:141-142.

Hansen KB, Wollmuth LP, Bowie D, Furukawa H, Menniti FS, Sobolevsky AI, Swanson GT, Swanger SA, Greger IH, Nakagawa T, McBain CJ, Jayaraman V, Low CM, Dell'Acqua ML, Diamond JS, Camp CR, Perszyk RE, Yuan H, Traynelis SF (2021) Structure, Function, and Pharmacology of Glutamate Receptor Ion Channels. Pharmacol Rev 73:298-487.

Hao Y, Martin-Fardon R, Weiss F (2010) Behavioral and functional evidence of metabotropic glutamate receptor 2/3 and metabotropic glutamate receptor 5 dysregulation in cocaine-escalated rats: factor in the transition to dependence. Biol Psychiatry 68:240-248.

Hayashi Y, Shi S-H, Esteban JA, Piccini A, Poncer J-C, Malinow R (2000) Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science 287:2262-2267.

Hedegaard H MA, Spencer MR, Warner M (2021) Drug overdose deaths in the United States, 1999–2020. . In. Hyattsville, MD: NCHS Data Brief.

Heinsbroek JA, Neuhofer DN, Griffin WC, Siegel GS, Bobadilla A-C, Kupchik YM, Kalivas PW (2017) Loss of plasticity in the D2-accumbens pallidal pathway promotes cocaine seeking. Journal of Neuroscience 37:757-767.

Hodos W (1961) Progressive ratio as a measure of reward strength. Science 134:943-944.

Hollmann M, Hartley M, Heinemann S (1991) Ca2+ permeability of KA-AMPA—gated glutamate receptor channels depends on subunit composition. Science 252:851-853.

Horger BA, Shelton K, Schenk S (1990) Preexposure sensitizes rats to the rewarding effects of cocaine. Pharmacol Biochem Behav 37:707-711.

Hu M, Becker JB (2003) Effects of sex and estrogen on behavioral sensitization to cocaine in rats. J Neurosci 23:693-699.

Hudson A, Stamp JA (2011) Ovarian hormones and propensity to drug relapse: a review. Neuroscience & Biobehavioral Reviews 35:427-436.

Huganir RL, Nicoll RA (2013) AMPARs and synaptic plasticity: the last 25 years. Neuron 80:704-717.

Hume RI, Dingledine R, Heinemann SF (1991) Identification of a site in glutamate receptor subunits that controls calcium permeability. Science 253:1028-1031.

Hunt WA, Barnett LW, Branch LG (1971) Relapse rates in addiction programs. Journal of clinical psychology.

Hursh SR, Raslear TG, Shurtleff D, Bauman R, Simmons L (1988) A cost-benefit analysis of demand for food. Journal of the experimental analysis of behavior 50:419-440.

Inaba T (1989) Cocaine: pharmacokinetics and biotransformation in man. Canadian journal of physiology and pharmacology 67:1154-1157.

Jackson LR, Robinson TE, Becker JB (2006) Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. Neuropsychopharmacology 31:129-138.

Jaffe JH, Cascella NG, Kumor KM, Sherer MA (1989) Cocaine-induced cocaine craving. Psychopharmacology 97:59-64.

James MH, Stopper CM, Zimmer BA, Koll NE, Bowrey HE, Aston-Jones G (2019) Increased Number and Activity of a Lateral Subpopulation of Hypothalamic Orexin/Hypocretin Neurons Underlies the Expression of an Addicted State in Rats. Biol Psychiatry 85:925-935.

Jatlow PI (1987) Drug of abuse profile: cocaine. Clinical chemistry 33:66B-71B.

Jonas P, Racca C, Sakmann B, Seeburg PH, Monyer H (1994) Differences in Ca2+ permeability of AMPA-type glutamate receptor channels in neocortical neurons caused by differential GluR-B subunit expression. Neuron 12:1281-1289.

Kakegawa W, Tsuzuki K, Yoshida Y, Kameyama K, Ozawa S (2004) Input-and subunit-specific AMPA receptor trafficking underlying long-term potentiation at hippocampal CA3 synapses. European Journal of Neuroscience 20:101-110.

Kalivas PW, Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Brain Res Rev 16:223-244.

Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162:1403-1413.

Kamendulis LM, Brzezinski MR, Pindel EV, Bosron WF, Dean RA (1996) Metabolism of cocaine and heroin is catalyzed by the same human liver carboxylesterases. Journal of pharmacology and experimental therapeutics 279:713-717.

Karler R, Petty C, Calder L, Turkanis SA (1989) Proconvulsant and anticonvulsant effects in mice of acute and chronic treatment with cocaine. Neuropharmacology 28:709-714.

Kawa AB, Bentzley BS, Robinson TE (2016) Less is more: prolonged intermittent access cocaine self-administration produces incentive-sensitization and addiction-like behavior. Psychopharmacology (Berl) 233:3587-3602.

Kawa AB, Hwang E-K, Funke JR, Zhou H, Costa-Mattioli M, Wolf ME (2022) Positive allosteric modulation of mGlu1 reverses cocaine-induced behavioral and synaptic plasticity through the integrated stress response and oligophrenin-1. Biological Psychiatry 92:871-879.

Kelley AE (2004) Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. Neurosci Biobehav Rev 27:765-776.

Kerstetter KA, Aguilar VR, Parrish AB, Kippin TE (2008) Protracted time-dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. Psychopharmacology (Berl) 198:63-75.

Koike M, Iino M, Ozawa S (1997) Blocking effect of 1-naphthyl acetyl spermine on Ca(2+)-permeable AMPA receptors in cultured rat hippocampal neurons. Neurosci Res 29:27-36.

Koob GF (2015) The dark side of emotion: the addiction perspective. Eur J Pharmacol 753:73-87.

Koob GF, Le Moal M (1997) Drug abuse: hedonic homeostatic dysregulation. Science 278:52-58.

Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24:97-129.

Koob GF, Le Moal M (2005) Plasticity of reward neurocircuitry and the'dark side'of drug addiction. Nature neuroscience 8:1442-1444.

Koob GF, Le Moal M (2008) Addiction and the brain antireward system. Annu Rev Psychol 59:29-53.

Kourrich S, Rothwell PE, Klug JR, Thomas MJ (2007) Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. J Neurosci 27:7921-7928.

Kranzler HR, Li TK (2008) What is addiction? Alcohol Res Health 31:93-95.

Krasnova IN, Marchant NJ, Ladenheim B, McCoy MT, Panlilio LV, Bossert JM, Shaham Y, Cadet JL (2014) Incubation of methamphetamine and palatable food craving after punishment-induced abstinence. Neuropsychopharmacology 39:2008-2016.

Kuczenski R, Segal DS, Aizenstein ML (1991) Amphetamine, cocaine, and fencamfamine: relationship between locomotor and stereotypy response profiles and caudate and accumbens dopamine dynamics. Journal of Neuroscience 11:2703-2712.

Larson EB, Anker JJ, Gliddon LA, Fons KS, Carroll ME (2007) Effects of estrogen and progesterone on the escalation of cocaine self-administration in female rats during extended access. Experimental and clinical psychopharmacology 15:461.

Lathers CM, Tyau L, Spino M, Agarwal I (2010) Cocaine-induced seizures, arrhythmias and sudden death. Sudden Death in Epilepsy: Forensic and Clinical Issues:169.

Le Moine C, Bloch B (1995) D1 and D2 dopamine receptor gene expression in the rat striatum: sensitive cRNA probes demonstrate prominent segregation of D1 and D2 mRNAs in distinct neuronal populations of the dorsal and ventral striatum. J Comp Neurol 355:418-426.

Lee HK, Takamiya K, Han JS, Man H, Kim CH, Rumbaugh G, Yu S, Ding L, He C, Petralia RS, Wenthold RJ, Gallagher M, Huganir RL (2003) Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. Cell 112:631-643.

Lee SH, Simonetta A, Sheng M (2004) Subunit rules governing the sorting of internalized AMPA receptors in hippocampal neurons. Neuron 43:221-236.

Lett BT (1989) Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. Psychopharmacology 98:357-362.

Loweth JA, Scheyer AF, Milovanovic M, LaCrosse AL, Flores-Barrera E, Werner CT, Li X, Ford KA, Le T, Olive MF, Szumlinski KK, Tseng KY, Wolf ME (2014) Synaptic depression via mGluR1 positive allosteric modulation suppresses cue-induced cocaine craving. Nat Neurosci 17:73-80.

Lu L, Grimm JW, Hope BT, Shaham Y (2004) Incubation of cocaine craving after withdrawal: a review of preclinical data. Neuropharmacology 47 Suppl 1:214-226.

Lüscher C, Malenka RC (2011) Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. Neuron 69:650-663.

Lynch WJ, Carroll ME (1999) Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology (Berl) 144:77-82.

Lynch WJ, Roth ME, Mickelberg JL, Carroll ME (2001) Role of estrogen in the acquisition of intravenously self-administered cocaine in female rats. Pharmacol Biochem Behav 68:641-646.

Marlatt GA (1996) Models of relapse and relapse prevention: A commentary.

Marley RJ, Witkin JM, Goldberg SR (1991a) A pharmacogenetic evaluation of the role of local anesthetic actions in the cocaine kindling process. Brain Res 562:251-257.

Marley RJ, Witkin JM, Goldberg SR (1991b) Genetic factors influence changes in sensitivity to the convulsant properties of cocaine following chronic treatment. Brain Res 542:1-7.

McCance-Katz EF, Carroll KM, Rounsaville BJ (1999) Gender differences in treatment-seeking cocaine abusers--implications for treatment and prognosis. Am J Addict 8:300-311.

McCutcheon JE, Wang X, Tseng KY, Wolf ME, 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. J Neurosci 31:5737-5743.

McFarland K, Lapish CC, Kalivas PW (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 23:3531-3537.

Meng Y, Zhang Y, Jia Z (2003) Synaptic transmission and plasticity in the absence of AMPA glutamate receptor GluR2 and GluR3. Neuron 39:163-176.

Meredith GE, Baldo BA, Andrezjewski ME, Kelley AE (2008) The structural basis for mapping behavior onto the ventral striatum and its subdivisions. Brain Struct Funct 213:17-27.

Milton AL, Everitt BJ (2012) The persistence of maladaptive memory: addiction, drug memories and anti-relapse treatments. Neurosci Biobehav Rev 36:1119-1139.

Mogenson GJ, Huang YH (1973) The neurobiology of motivated behavior. Prog Neurobiol 1:55-83.

Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 14:69-97.

Montaron M, Deniau J, Menetrey A, Glowinski J, Thierry A (1996) Prefrontal cortex inputs of the nucleus accumbens-nigro-thalamic circuit. Neuroscience 71:371-382.

Neisewander JL, Baker DA, Fuchs RA, Tran-Nguyen LT, Palmer A, Marshall JF (2000) Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. Journal of Neuroscience 20:798-805.

Neumann PA, Wang Y, Yan Y, Wang Y, Ishikawa M, Cui R, Huang YH, Sesack SR, Schlüter OM, Dong Y (2016) Cocaine-Induced Synaptic Alterations in Thalamus to Nucleus Accumbens Projection. Neuropsychopharmacology 41:2399-2410.

Nicolas C, Russell TI, Shaham Y, Ikemoto S (2022) Dissociation Between Incubation of Cocaine Craving and Anxiety-Related Behaviors After Continuous and Intermittent Access Self-Administration. Frontiers in neuroscience 15:824741.

Nicolas C, Russell TI, Pierce AF, Maldera S, Holley A, You ZB, McCarthy MM, Shaham Y, Ikemoto S (2019) Incubation of Cocaine Craving After Intermittent-Access Self-administration: Sex Differences and Estrous Cycle. Biol Psychiatry 85:915-924.

O'Brien CP, Childress AR, McLellan AT, Ehrman R (1992) Classical conditioning in drug-dependent humans. Ann N Y Acad Sci 654:400-415.

O'Dell LE, Khroyan TV, Neisewander JL (1996) Dose-dependent characterization of the rewarding and stimulant properties of cocaine following intraperitoneal and intravenous administration in rats. Psychopharmacology 123:144-153.

Oginsky MF, Ferrario CR (2019) Eating "junk food" has opposite effects on intrinsic excitability of nucleus accumbens core neurons in obesity-susceptible versus -resistant rats. Journal of Neurophysiology 122:1264-1273.

Oginsky MF, Goforth PB, Nobile CW, Lopez-Santiago LF, Ferrario CR (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 41:2977-2986.

Opazo P, Choquet D (2011) A three-step model for the synaptic recruitment of AMPA receptors. Mol Cell Neurosci 46:1-8.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity byd-amphetamine. Journal of Neuroscience 19:2401-2411.

Passafaro M, Piëch V, Sheng M (2001) Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. Nat Neurosci 4:917-926.

Paterson NE, Markou A (2003) Increased motivation for self-administered cocaine after escalated cocaine intake. Neuroreport 14:2229-2232.

Paulson PE, Camp DM, Robinson TE (1991) Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. Psychopharmacology 103:480-492.

Peris J, Decambre N, Coleman-Hardee ML, Simpkins JW (1991) Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal [3H]dopamine release. Brain Res 566:255-264.

Peterson BM, Mermelstein PG, Meisel RL (2015) Estradiol mediates dendritic spine plasticity in the nucleus accumbens core through activation of mGluR5. Brain Struct Funct 220:2415-2422.

Piazza PV, Deminiere JM, le Moal M, Simon H (1990) Stress-and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain research 514:22-26.

Pickens CL, Airavaara M, Theberge F, Fanous S, Hope BT, Shaham Y (2011) Neurobiology of the incubation of drug craving. Trends Neurosci 34:411-420.

Post RM, Rose H (1976) Increasing effects of repetitive cocaine administration in the rat. Nature 260:731-732.

Post RM, Weiss SR (1988) Psychomotor stimulant vs. local anesthetic effects of cocaine: role of behavioral sensitization and kindling. NIDA Res Monogr 88:217-238.

Post RM, Lockfeld A, Squillace KM, Contel NR (1981) Drug-environment interaction: context dependency of cocaine-induced behavioral sensitization. Life sciences 28:755-760.

Proaño SB, Morris HJ, Kunz LM, Dorris DM, 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 120:1356-1373.

Rehm J, Shield KD (2019) Global Burden of Disease and the Impact of Mental and Addictive Disorders. Current Psychiatry Reports 21:10.

Reigstad A, Reigstad O (2021) [The history of local anaesthesia – from coca leaves to effective analgesia]. Tidsskr Nor Laegeforen 141.

Reimers JM, Milovanovic M, Wolf ME (2011) Quantitative analysis of AMPA receptor subunit composition in addiction-related brain regions. Brain Res 1367:223-233.

Roberts D, Bennett S, Vickers G (1989) The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. Psychopharmacology 98:408-411.

Robinson M, Robinson T, Berridge K (2013) Chapter 39. Incentive Salience and the Transition to Addiction. In, pp 391-399.

Robinson TE, Becker JB (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain research reviews 11:157-198.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247-291.

Robinson TE, Berridge KC (2001) Incentive-sensitization and addiction. Addiction 96:103-114.

Roche KW, O'Brien RJ, Mammen AL, Bernhardt J, Huganir RL (1996) Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. Neuron 16:1179-1188.

Roth ME, Carroll ME (2004) Sex differences in the escalation of intravenous cocaine intake following long- or short-access to cocaine self-administration. Pharmacol Biochem Behav 78:199-207.

Salazar H, Mischke S, Plested AJ (2020) Measurements of the timescale and conformational space of AMPA receptor desensitization. Biophysical Journal 119:206-218.

Self DW, Choi KH, Simmons D, Walker JR, Smagula CS (2004) Extinction training regulates neuroadaptive responses to withdrawal from chronic cocaine self-administration. Learn Mem 11:648-657.

Sell SL, Scalzitti JM, Thomas ML, Cunningham KA (2000) Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. J Pharmacol Exp Ther 293:879-886.

Sesack SR, Grace AA (2010) Cortico-basal ganglia reward network: microcircuitry. Neuropsychopharmacology 35:27-47.

Shaham Y, Shalev U, Lu L, de Wit H, Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology (Berl) 168:3-20.

Shalev U, Grimm JW, Shaham Y (2002) Neurobiology of relapse to heroin and cocaine seeking: a review. Pharmacol Rev 54:1-42.

Shepard JD, Bossert JM, Liu SY, Shaham Y (2004) The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. Biological psychiatry 55:1082-1089.

Shi S, Hayashi Y, Esteban JA, Malinow R (2001) Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. Cell 105:331-343.

Shippenberg TS, Heidbreder C (1995) Sensitization to the conditioned rewarding effects of cocaine: pharmacological and temporal characteristics. J Pharmacol Exp Ther 273:808-815.

Silver RA, Colquhoun D, Cull-Candy SG, Edmonds B (1996) Deactivation and desensitization of non-NMDA receptors in patches and the time course of EPSCs in rat cerebellar granule cells. J Physiol 493 (Pt 1):167-173.

Simon SL, Richardson K, Dacey J, Glynn S, Domier CP, Rawson RA, Ling W (2001) A comparison of patterns of methamphetamine and cocaine use. Journal of addictive diseases 21:35-44.

Sinha R (2001) How does stress increase risk of drug abuse and relapse? Psychopharmacology 158:343-359.

Sinha R, Garcia M, Paliwal P, Kreek MJ, Rounsaville BJ (2006) Stress-induced cocaine craving and hypothalamic-pituitary-adrenal responses are predictive of cocaine relapse outcomes. Arch Gen Psychiatry 63:324-331.

Smart RG (1991) Crack cocaine use: a review of prevalence and adverse effects. The American journal of drug and alcohol abuse 17:13-26.

Smith RJ, Lobo MK, Spencer S, Kalivas PW (2013) Cocaine-induced adaptations in D1 and D2 accumbens projection neurons (a dichotomy not necessarily synonymous with direct and indirect pathways). Curr Opin Neurobiol 23:546-552.

Sommer B, Kohler M, Sprengel R, Seeburg PH (1991) RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. Cell 67:11-19.

Sorge RE, Stewart J (2005) The contribution of drug history and time since termination of drug taking to footshock stress-induced cocaine seeking in rats. Psychopharmacology 183:210-217.

Sprengel R, Seeburg PH (1993) The unique properties of glutamate receptor channels. FEBS Lett 325:90-94.

Staffend NA, Loftus CM, Meisel RL (2011) Estradiol reduces dendritic spine density in the ventral striatum of female Syrian hamsters. Brain Struct Funct 215:187-194.

Swanson LW, Cwan WM (1975) A note on the connections and development of the nucleus accumbens. Brain Res 92:324-330.

Torregrossa MM, Tang XC, Kalivas PW (2008) The glutamatergic projection from the prefrontal cortex to the nucleus accumbens core is required for cocaine-induced decreases in ventral pallidal GABA. Neurosci Lett 438:142-145.

Twomey EC, Yelshanskaya MV, Vassilevski AA, Sobolevsky AI (2018) Mechanisms of Channel Block in Calcium-Permeable AMPA Receptors. Neuron 99:956-968.e954.

Van Haaren F, Meyer ME (1991) Sex differences in locomotor activity after acute and chronic cocaine administration. Pharmacology Biochemistry and Behavior 39:923-927.

Van Swearingen AE, Sanchez CL, Frisbee SM, Williams A, Walker QD, Korach KS, Kuhn CM (2013) Estradiol replacement enhances cocaine-stimulated locomotion in female C57BL/6 mice through estrogen receptor alpha. Neuropharmacology 72:236-249.

Vanderschuren LJ, Everitt BJ (2004) Drug seeking becomes compulsive after prolonged cocaine self-administration. Science 305:1017-1019.

Vanderschuren LJ, Pierce RC (2010) Sensitization processes in drug addiction. Behavioral neuroscience of drug addiction:179-195.

Venniro M, Caprioli D, Shaham Y (2016) Animal models of drug relapse and craving: From drug priming-induced reinstatement to incubation of craving after voluntary abstinence. Prog Brain Res 224:25-52.

Volkow ND, Fowler JS, Wang GJ (2002) Role of dopamine in drug reinforcement and addiction in humans: results from imaging studies. Behav Pharmacol 13:355-366.

Ward AS, Haney M, Fischman MW, Foltin RW (1997) Binge cocaine self-administration in humans: intravenous cocaine. Psychopharmacology 132:375-381.

Warner EA (1993) Cocaine abuse. Ann Intern Med 119:226-235.

Wee S, Mandyam CD, Lekic DM, Koob GF (2008) α1-Noradrenergic system role in increased motivation for cocaine intake in rats with prolonged access. European Neuropsychopharmacology 18:303-311.

Weiss F, Paulus MP, Lorang MT, Koob GF (1992) Increases in extracellular dopamine in the nucleus accumbens by cocaine are inversely related to basal levels: effects of acute and repeated administration. Journal of Neuroscience 12:4372-4380.

Wikler A (1973) Dynamics of drug dependence: Implications of a conditioning theory for research and treatment. Archives of general psychiatry 28:611-616.

Wise RA (1985) The anhedonia hypothesis: Mark III. Behavioral and Brain Sciences 8:178-186.

Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94:469-492.

Wise RA, Koob GF (2014) The development and maintenance of drug addiction. Neuropsychopharmacology 39:254-262.

Wolf ME (2016) Synaptic mechanisms underlying persistent cocaine craving. Nat Rev Neurosci 17:351-365.

Wolf ME, Ferrario CR (2010) AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine. Neurosci Biobehav Rev 35:185-211.

Wolf ME, Tseng KY (2012) Calcium-permeable AMPA receptors in the VTA and nucleus accumbens after cocaine exposure: when, how, and why? Front Mol Neurosci 5:72.

Wright CI, Groenewegen HJ (1996) Patterns of overlap and segregation between insular cortical, intermediodorsal thalamic and basal amygdaloid afferents in the nucleus accumbens of the rat. Neuroscience 73:359-373.

Yager LM, Garcia AF, Donckels EA, Ferguson SM (2019) Chemogenetic inhibition of direct pathway striatal neurons normalizes pathological, cue-induced reinstatement of drug-seeking in rats. Addiction biology 24:251-264.

Záborszky L, Alheid GF, Beinfeld MC, Eiden LE, Heimer L, Palkovits M (1985) Cholecystokinin innervation of the ventral striatum: a morphological and radioimmunological study. Neuroscience 14:427-453.

Zimmer BA, Oleson EB, Roberts DC (2012) The motivation to self-administer is increased after a history of spiking brain levels of cocaine. Neuropsychopharmacology 37:1901-1910.

Zimmerman JL (2012) Cocaine intoxication. Crit Care Clin 28:517-526.

Chapter 2: Cocaine Induced Sensitization and Glutamate Plasticity in the Nucleus Accumbens Core: Effect of Sex

Abstract

The development and persistence of addiction is mediated in part by drug-induced alterations in nucleus accumbens (NAc) function. AMPA-type glutamate receptors (AMPARs) provide the main source of excitatory drive to the NAc and enhancements in transmission of calcium-permeable AMPARs (CP-AMPARs) mediate increased cue-triggered drug-seeking following prolonged withdrawal. Cocaine treatment regimens that result in psychomotor sensitization enhance subsequent drug-seeking and drug-taking behaviors. Furthermore, cocaine-induced locomotor sensitization followed by 14 days of withdrawal results in an increase in glutamatergic synaptic transmission. However, very few studies have examined cocaine-induced alterations in synaptic transmission of females or potential effects of experimenter administered cocaine on NAc CP-AMPAR mediated transmission in either sex. Male and female rats were given repeated systemic cocaine injections to induce psychomotor sensitization (15mg/kg, i.p. 1 injection/day, 8 days). Controls received repeated saline (1 mL/kg, i.p). After 14-16 days of withdrawal brain slices were prepared and whole-cell patch clamp approaches in the NAc core were used to measure spontaneous excitatory postsynaptic currents (sEPSC), paired pulse ratio, and CP-AMPAR transmission. Additional female rats from this same cohort were also given a challenge injection of cocaine at withdrawal day 14 to assess the expression of sensitization. Repeated cocaine produced psychomotor sensitization in both sexes. In males this was accompanied by an increase in sEPSC frequency, but not amplitude, and there was no effect on the paired pulse ratio. Males treated with cocaine and saline had similar sensitivity to Naspm. In contrast, in females

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there were no significant differences between cocaine and saline groups on any measure, despite females showing robust psychomotor sensitization both during the induction and expression phase. Overall, these data reveal striking sex differences in cocaine-induced NAc glutamate plasticity that accompany the induction of psychomotor sensitization. This suggests that the neural adaptations that contribute to sensitization vary by sex.

Introduction

Sex differences are reported both in the pattern of drug-taking behavior and the development of substance use disorders (Becker and Hu, 2008). For example, in humans, females transition to addiction more rapidly (Brady and Randall, 1999; Hernandez-Avila et al., 2004) and report stronger withdrawal effects than males (Robbins et al., 1999; Back et al., 2005; Hogle and Curtin, 2006). Parallel sex differences have been established in rodent models where female rats acquire cocaine self-administration at a faster rate (Lynch and Carroll, 1999; Hu and Becker, 2008), show a greater magnitude of escalation of cocaine intake (Algallal et al., 2019), and display a higher motivation to obtain cocaine after withdrawal than males (Kawa and Robinson, 2019a; Nicolas et al., 2019). These behavioral studies support the idea that the induction and expression of drug-induced alterations in brain function that underlie addiction vary with gonadal sex (Becker and Koob, 2016; Cornish and Prasad, 2021). Specifically, estradiol enhances both the acute locomotor effects of cocaine (Sell et al., 2000; Van Swearingen et al., 2013) and psychomotor sensitization in females (Sell et al., 2002; Hu and Becker, 2003). In contrast, male castration enhances locomotor activity after a single injection, but locomotor sensitization requires testosterone (Menendez-Delmestre and Segarra, 2011).

Drug-induced neuroplasticity thought to contribute to the transition to addiction is associated with the development of behavioral sensitization (Robinson and Berridge, 1993; Vezina, 2004). One manifestation of these alterations is the persistent enhancement in drug-induced psychomotor activity (i.e., psychomotor sensitization) following repeated exposure to addictive substances like cocaine (Carr et al., 2020).

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Also, animals sensitized to psychostimulant drugs more readily acquire psychostimulant self-administration (Piazza et al., 1990; Horger et al., 1992; Zhao and Becker, 2010), show stronger cocaine conditioned place preference (Lett, 1989), and escalate their cocaine intake more quickly than saline pre-treated controls (Ferrario and Robinson, 2007). Thus, psychomotor sensitization is also associated with incentive sensitization. Of particular relevance here, there are sex differences in both the induction of psychomotor (Robinson, 1984; Van Haaren and Meyer, 1991; Carr et al., 2020) and incentive sensitization (Kawa and Robinson, 2019b).

Both psychomotor sensitization and enhancements in cocaine-seeking behaviors have been linked to alterations in nucleus accumbens (NAc) glutamate neurotransmission (Vanderschuren and Kalivas, 2000; Ferrario et al., 2010; Dong et al., 2017). For example, experimenter-administered cocaine treatment regimens that result in psychomotor sensitization enhance excitatory transmission within the NAc shell and core of mice (Thomas et al., 2001; Jedynak et al., 2016) and increase the surface expression of GluA1 and GluA2 AMPAR subunits in the NAc of rats (Boudreau and Wolf, 2005; Ferrario et al., 2010). However, these studies were done exclusively in males. In addition, prolonged withdrawal from long-access cocaine-self-administration increases synaptic transmission mediated by calcium-permeable AMPARs (CP-AMPARs) in the NAc core, resulting in enhanced cue-associated drug-seeking (Conrad et al., 2008; Loweth et al., 2014). Yet, whether experimenter-administered cocaine treatments that produce psychomotor sensitization result in similar synaptic enhancements in either sex is unknown.

There are sex differences in medium spiny neuron (MSN) anatomy and function within the NAc. Intact females have greater spine density and spine head size in the NAc core then males (Forlano and Woolley, 2010). This may be due in part to circulating gonadal hormones, as estradiol specifically decreases spine density and spine maturity in females (Staffend et al., 2011; Peterson et al., 2015). Interestingly, these sex differences persist when animals are exposed to drugs of abuse. Specifically, repeated systemic cocaine exposure increases NAc spine density in both males and females, but the magnitude of this increase is greater in females than males (Wissman et al., 2011). In addition, there are sex differences in NAc MSN function. Basal

excitatory transmission is enhanced in females compared to males and MSN intrinsic excitability varies with the cycle in females, resulting in complex sex differences in MSN firing (Proaño et al., 2018; Alonso-Caraballo and Ferrario, 2019; Proaño et al., 2020). For example, NAc mEPSC frequency is similar in males and females when recordings are made from females in the diestrus phase of the cycle, but is enhanced in females vs males when recordings are made from females in proestrus or estrus. Together these data suggest that cocaine-induced NAc glutamatergic plasticity may differ in females vs males.

Therefore, in the current study we determined the effects of a sensitizing regimen of cocaine on NAc core glutamatergic synaptic transmission in male and female rats. All measures were made after 14-16 days of withdrawal from cocaine.

Materials and Methods

Subjects

Male and female outbred Sprague Dawley were purchased from Envigo (Indianapolis, IN) and were 55 days old upon arrival. Rats were allowed to acclimate to the colony room for one week, after which they were handled (once per day, 5 days) prior to start of the first habituation session (details below). Rats were pair housed by sex on a reverse 12-h light/dark cycle (lights off at 0800) and had free access to water and food. The estrous cycle was not monitored. Procedures were approved by The University of Michigan Committee on the Use and Care of Animals in accordance with AAALAC and AVMA guidelines.

Drugs and Reagents

Cocaine HCL was provided by the NIDA drug supply program. All other drugs and reagents were obtained from Sigma (St. Louis MO, USA) or Tocris (Minneapolis MN, USA).

Cocaine exposure

Rats were assigned to saline (sal) or cocaine (coc) treatment groups, counterbalanced by weight (M sal: N=19; M coc: N=19; F sal: N=24; F coc: N=24). All injections took place in locomotor activity chambers (22.86 cm × 44.45 cm × 28 cm) equipped with infrared beams around the perimeter. Rats were first habituated to the chambers and injection procedures beginning when they were ~67 days old. Briefly, 40 min after being placed in the chamber each animal received an injection of saline (0.9%, 1ml/kg, i.p.) and remained in the chamber for 60 minutes. This procedure was repeated on two consecutive days. Rats then received 8 consecutive days of either saline or cocaine (15 mg/kg, i.p.) injections, as previously described (Ferrario et al., 2010). Briefly, rats were placed in the locomotor chambers for 40 minutes, they were then given an injection of saline or cocaine and returned to their home cage 1.5 hours later. After the 8th injection rats were left in their home cages undisturbed for 14-16 days. After this, some rats were used for whole-cell patch-clamp recordings while a subset of females were used to examine the expression of psychomotor sensitization in response to a cocaine challenge. Psychomotor activity was assessed by quantifying the total number of beam breaks per 5 min during each session, as an index of locomotion. Time to peak locomotor activity was assessed as the time (per 5 mins) it took to reach the largest number of beam breaks.

Cocaine challenge

Due to the absence of an effect on sEPSC's in females, despite the development of psychomotor sensitization during the induction phase, we wanted to rule out a possible abatement of sensitization after 14-16 days of withdrawal. Therefore, a subset of female rats pre-treated with cocaine or saline were given a cocaine challenge on WD14-16 (F sal pre-treated N=6; F coc pre-treated N=6). Procedures were based on Oginsky et al. 2016 (Oginsky et al., 2016). 14-16 days after the last pre-treatment session, females from cocaine and saline groups were placed back into the locomotor chambers and given increasing doses of cocaine, starting with saline, followed by 7.5mg/kg and 15 mg/kg cocaine. These injections were given at 40 minutes, 80

minutes, and 140 minutes after animals were placed in the chamber, respectively. Locomotor activity was assessed as the total number of beam breaks per 5 min. Females remained in the locomotor boxes for 1.5 hours after the final injection.

Whole-cell patch clamp recordings

Established whole-cell patch clamping approaches were used (Nieto et al., 2023). Briefly, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), brains were removed and placed in ice-cold oxygenated (95% O_2–5% CO_2) 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 7.45, 300-305 mOsm. Coronal slices (300 µm) containing the NAc were made on a vibratome (Leica Biosystems VT 1200, Buffalo Grove, IL, USA). Slices were allowed to recover in oxygenated aCSF (30 min, 37 °C), and then maintained at room temperature (30 min) prior to recording. For the recording aCSF, $CaCl_2$ was 2.5 mM and $MgCl_2$ was 1 mM. All recordings were made from the NAc core and conducted in the presence of the $GABA_A$ receptor antagonist, picrotoxin (50 µM). The NAc core was identified using the anterior commissure as a primary landmark (see Fig 2.2E cartoon). Medium spiny neurons (MSNs) were identified by cell body size (~15um in diameter) and by their capacitance (30-60pF) and membrane resistance (30-120mOhms) after break in. Due to required recording conditions it prevented other measures of membrane properties distinct to MSNs. Spontaneous excitatory post-synaptic currents (sEPSCs) were recorded at a holding potential of -70mV (5 min). For all recordings, pipettes were filled with (in mM): 140 CsCl, 10 HEPES, 2 $MgCl_2$, 5 Na^+-ATP, 0.6 Na^+-GTP, 2 QX-314, pH 7.3, 285 mOsm. Evoked EPSCs (eEPSCs) were elicited by local stimulation (0.02 to 0.30 mA square pulses, 0.1 ms, delivered every 20s) using a bipolar electrode placed about 300 µm lateral to recorded neurons. The minimum amount of current needed to elicit a synaptic response with less than 20% variability in amplitude was used. If more than 0.30 mA was required, the neuron was not used for analysis. eEPSCs were recorded at -70 mV before and after application of the CP-AMPAR selective antagonist Naspm (200 µM). Paired pulse ratio recordings were recorded at -70 mV. The inter-stim-interval was 20 ms and the ratio was calculated

as the amplitude of the second peak divided by the first. For all data analysis, only cells with an access resistance of less than 30 MΩ were used. Cell parameters (capacitance and membrane resistance) were recorded at the start and end of data collection and only cells with less than 20% change across time were included. Recordings were made at 14-16 days after the last cocaine or saline injection and alternated between slices from males or females and from rats in the saline or cocaine group each day (note that no more than 3 cells were collected from the same rat for a given measure; the number of cells per group are given in the results below).

Analysis and Statistics

Evoked and paired pulse responses were analyzed using Clampfit 10.7 (Molecular Devices). sEPSCs were analyzed using MiniAnalysis (Synaptosoft V.6.0.7; amplitude threshold of 5 pA; decay threshold of <10 msec) and verified by hand. The minimum detected sEPSC amplitude was set to 5pA. Comparisons were made between data collected within the same cohort of animals (i.e., that received saline or cocaine side by side). T-test, two-way, and three-way ANOVAs using standard general linear models (GLM) or mixed model residual maximum likelihood (REML) followed by Sidak's and Tukey's post-hoc comparisons were used (Prism 9, GraphPad, San Diego, CA). Interpretation of p-values is based on guidelines set forth by the American Statistical Association (Wasserstein and Lazar, 2016). Experimenters were not blind to grouping during data acquisition but were during analysis. Ns for electrophysiological measures are reported in the results and based on expected effect size and variance of our primary measures.

Results

Repeated systemic cocaine results in locomotor sensitization in male and female rats

The experimental timeline is shown in Figure 2.1A. Females are known to be more sensitive to the acute locomotor-activating effects of cocaine than males (Van Haaren and Meyer, 1991; Hu and Becker, 2008). Therefore, we examined total beam

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breaks in response to the first cocaine injection in females vs males (Fig 2.1B). Consistent with previous reports, females showed a stronger acute locomotor response to cocaine than males with significantly greater cocaine-induced locomotion in females than males on day 1 of cocaine exposure (Two-way ANOVA, main effect of sex $F_{(1,82)}=57.96$, $p<0.01$; main effect of drug $F_{(1,82)}=44.84$, $p<0.01$; drug x sex interaction $F_{(1,82)}=24.83$, $p<0.01$: Sidak's post-test coc treated males vs coc treated females, $p<0.01$).

Figure 2.1C,D show beam breaks per 5 min interval after an i.p. injection of saline or cocaine, as an index of locomotion, following the first (day 1) and the last (day 8) injection in males and females, respectively (M sal: N=19; M coc: N=19; F sal: N=24; F coc: N=24). As expected, in both sexes cocaine produced a significant increase in locomotor activity compared to animals receiving saline injection (Fig 2.1C: three-way REML ANOVA, main effect of treatment $F_{(1,72)}=53.62$, $p<0.01$; Fig 2.1D: three-way REML ANOVA, main effect of treatment $F_{(91,46)}=128.8$, $p<0.01$). The development of locomotor sensitization was assessed by comparing cocaine-induced locomotion in response to the first vs last injection of cocaine within sex. In males, locomotor activity was greater on day 8 than day 1 in rats given repeated cocaine injections, indicative of locomotor sensitization (Fig 2.1C: three-way REML ANOVA, main effect of time $F_{(27,1901)}=25.58$, $p<0.01$; no effect of day $F_{(1,72)}=53.62$, $p=0.06$; time x day x treatment interaction $F_{(27,1901)}=10.69$, $p<0.01$; Tukey post-test coc day 1 vs coc day 8 $p<0.01$ min 40-70). Sensitization was also seen in females, with a greater locomotor response to cocaine on day 8 vs day 1 (Fig 2.1D: three-way REML ANOVA, main effect of time $F_{(27,1242)}=46.00$, $p<0.01$; no effect of day $F_{(1,46)}=0.2497$, $p=0.62$; time x day x treatment interaction $F_{(27,1148)}=4.85$, $p<0.01$; Tukey post-test coc day 1 vs coc day 8 $p \leq 0.02$ min 40-50. However, when comparisons in the magnitude of sensitization were made across sex (i.e., the change in cocaine-induced locomotor activity on day 1 vs day 8), no significant sex differences were found (Fig 2.1C,D total beam breaks post cocaine day 1 vs day 8 [95 min]: Two-way REML ANOVA, main effect of day $F_{(1, 80)}=4.45$, $p<0.05$; main effect of sex $F_{(1,80)}=63.97$, $p<0.0001$; no sex x day interaction $F_{(1, 80)}=0.83$, $p=0.36$). This was due in part to the large locomotor response to the first cocaine injection in

females, and possibly to the emergence of stereotypy in females by the 8th injection (AMC; see also discussion).

Early studies established that psychomotor sensitization is not only characterized by changes in the magnitude of locomotor activity, but also in the rapidity of onset of psychomotor activity including locomotion (Post and Rose, 1976). Thus, the time to peak locomotor activity on day 1 vs day 8 was used as a second measure of sensitization in cocaine-treated rats, as this measure may be influenced less by the emergence of stereotyped behaviors (Fig 2.1E). Consistent with locomotor results the time to peak was faster on day 8 than day 1 (Fig 2.1E: two-way REML ANOVA, main effect of day $F_{(1,39)}$=11.85, p<0.01). In addition, time to peak was slower in females than males, regardless of day (Fig 2.1E: main effect of sex $F_{(1,41)}$=9.97, p<0.01; no significant sex x day interaction p=0.55). Thus overall, repeated cocaine treatment produced psychomotor sensitization in both sexes, and there was a sex difference in the acute locomotor response on day 1 of cocaine treatment, but no difference in the magnitude of sensitization across sex, at least based on these measures.
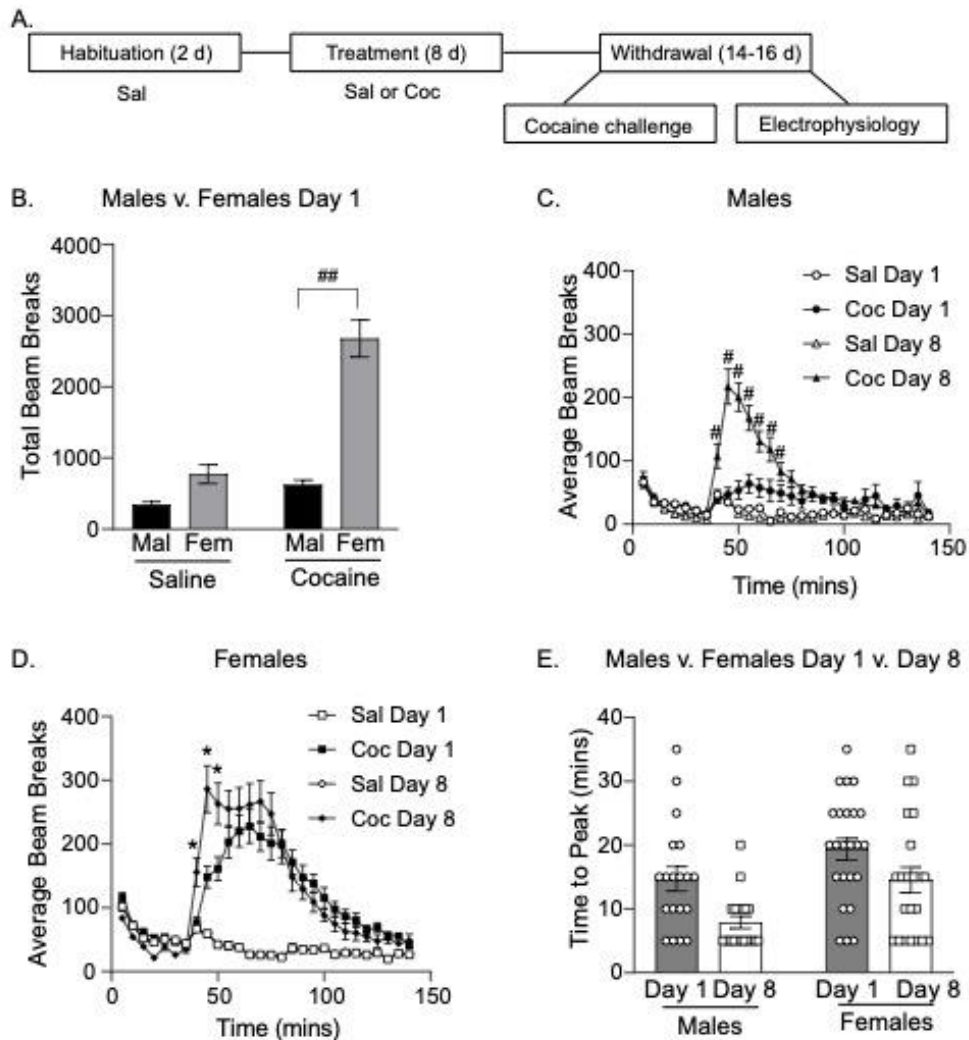
Figure 2.1 Effects of repeated systemic cocaine on psychomotor behavior in males and females. A) Schematic displaying the experimental timeline. B) Comparison of locomotor activity on day 1 between males (M) and females (F). Females showed a stronger response to the acute locomotor-activating effects of cocaine compared to males. C) Male and D) female locomotor behavior on day 1 vs day 8. Animals in cocaine groups (coc) increased locomotor behavior from day 1 to day 8 compared to saline (sal) treated controls. E) Time to peak locomotor activity on day 1 (D1) and day 8 (D8) was faster following repeated cocaine treatment. Tukey's post-test *p≤0.02; ## = Sidak's post-test ##p<0.01. All data shown as mean ± SEM.

Cocaine exposure and withdrawal did not enhance CP-AMPAR transmission

Figure 2.2 shows NAc core CP-AMPAR transmission measured 14-16 days after the discontinuation of cocaine or saline treatments (M sal: N=4 rats, 10 cells; M coc: N=5 rats, 8 cells; F sal: N=5 rats, 11 cells; F coc: N=5 rats, 7 cells). Figure 2.2A (males) and 2.2B (females) show the time course of eEPSC amplitude before (baseline; 10 min) and after bath application of the CP-AMPAR antagonist, Naspm (10 min), in saline and

cocaine treated groups. Naspm produced similar decreases in eEPSC amplitude in males and females, regardless of whether they were treated with saline or cocaine (Fig 2.2C: two-way ANOVA, no effect of treatment $F_{(1,32)}=0.68$, p=0.42; no effect of sex $F_{(1,32)}=0.86$, p=0.36). Example traces before (black) and after (red) Naspm are shown in panel D. Overall, 8 days of cocaine exposure followed by a withdrawal period did not result in changes in CP-AMPAR mediated-transmission in either sex (location of cell recordings is depicted in panel E).
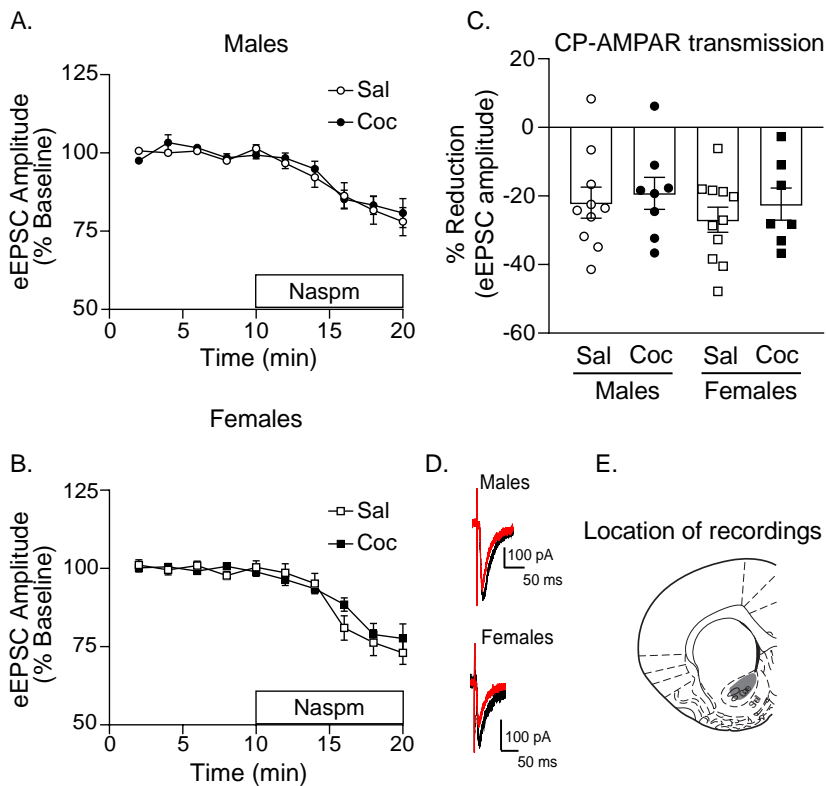

Figure 2.2 Effects of repeated systemic cocaine and subsequent withdrawal on calcium permeable-AMPAR (CP-AMPAR) mediated transmission. Time course showing effects of bath application of CP-AMPAR antagonist Naspm on eEPSC amplitude recordings in A) males and B) females. C) Percent reduction in eEPSC amplitude by Naspm. Naspm resulted in no changes in eEPSC amplitude in males or females regardless of treatment (average of the last two minutes of drug wash on) Two-way ANOVA no effect of treatment $F_{(1,32)}=0.68$, p=0.42; no effect of sex $F_{(1,32)}=0.86$, p=0.36. D) Example traces in males and females. E) Cartoon depiction of where recordings were made within the slice (shaded region).

Cocaine exposure and withdrawal results in sex-specific alterations in sEPSCs

Figure 2.3 shows sEPSC frequency (A,B) and amplitude (C,D) following withdrawal from repeated cocaine or saline treatment in both sexes (M sal: N=5 rats, 15

cells; M coc: N=4 rats, 13 cells; F sal: N=6 rats, 11 cells; F coc: N=5 rats, 12 cells; representative traces shown in panel G). sEPSC frequency was greater in males than in females regardless of treatment (Fig 2.3A: two-way ANOVA, main effect of sex $F_{(1,47)}=8.56$, $p<0.01$; no significant drug x sex interaction, $p=0.15$). In addition, there was a significant main effect of drug (Fig 2.3A: $F_{(1,47)}=9.66$, $p<0.01$) that was driven by an increase in sEPSC frequency in males treated with cocaine, with no difference between cocaine and saline-treated females (Sidak's post-test: males sal vs coc, $p<0.01$; females sal vs coc, $p=0.54$). In addition, males treated with cocaine show a clear shift in the sEPSC frequency distribution with more events occurring with shorter inter-event intervals (Fig 2.3B upper panel: two-way RM ANOVA REML, main effect of drug $F_{(1,25)}=6.68$, $p<0.01$), but no such shift in sEPSC frequency distribution was found in females (Fig 2.3B lower panel: two-way RM ANOVA REML, no main effect of drug $F_{(1,12)}=0.74$, $p=0.41$). Thus, cocaine treatment resulted in an increase in sEPSC frequency in males, but not females.

When average sEPSC amplitudes were examined, no differences were found across treatment groups or sexes (Fig 2.3C: no effect of treatment $F_{(1,47)}=1.76$, $p=0.19$; no effect of sex $F_{(1,47)}=0.68$, $p=0.41$; no interaction $F_{(1,47)}=0.53$, $p=0.47$). Similarly, the amplitude distributions remained unchanged in both sexes following cocaine (Fig 2.3D: males no effect of treatment $F_{(1,242)}=0.01$, $p=0.92$; females no effect of treatment $F_{(1,126)}=0.13$, $p=0.72$). Finally, given that there was an increase in sEPSC frequency in males, we next measured the paired pulse ratio to determine if the increase in frequency was associated with an increased probability of pre-synaptic glutamate release (Fig 2.3E: M sal: N=3 rats, 10 cells; M coc: N=3 rats, 6 cells; representative traces shown in panel F). However, the paired pulse ratio was similar in males treated with cocaine or saline (unpaired two-tailed t-test, $t_{(14)}=0.56$, $p=0.58$).

Although many studies have established that psychomotor sensitization persists for many weeks even after a single injection (Robinson, 1984; Pierce and Kalivas, 1997), it's possible that the absence of an effect of cocaine treatment on glutamate transmission in females could be due to an abatement of sensitization after withdrawal. To address this possibility, a subset of cocaine and saline treated females were given a cocaine challenge on withdrawal days 14-16 (F sal pre-treated N=6; F coc pre-treated

N=6). Cocaine pre-treated females showed stronger cocaine-induced locomotion across both doses tested compare to saline pre-treated females receiving cocaine for the first time (Fig 2.3H: two-way RM ANOVA, main effect of pre-treatment, $F_{(1,10)}=6.42$, $p=0.03$; main effect of time $F_{(45,450)}=15.77$, $p<0.01$; no interaction $F_{(45,450)}=1.28$, $p=0.11$). This highlights that females pre-treated with cocaine show lasting behavioral sensitization at WD 14-16. Therefore, the absence of effects on NAc excitatory transmission in females is not likely due to an abatement of sensitization.
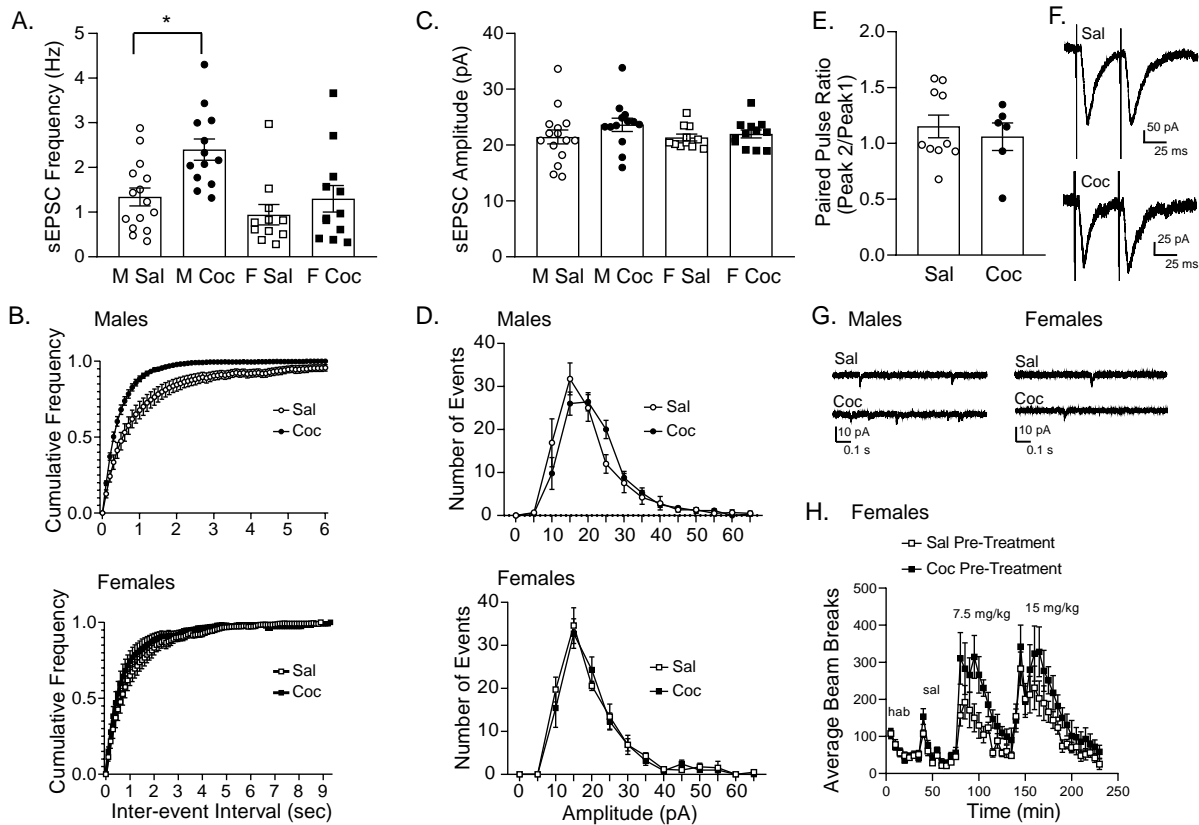

Figure 2.3 Effects of repeated systemic cocaine and subsequent withdrawal on spontaneous excitatory post synaptic currents (sEPSCs) and paired pulse ratio. A) Average sEPSC frequency in cocaine (coc) and saline (sal) pre-treated groups. In males, cocaine treatment increased sEPSC frequency compared to saline treatment. No effects were found in females. B) Cumulative sEPSC frequency distribution in males (top) and females (bottom). Males treated with cocaine show a clear shift in the sEPSC frequency distribution with more events occurring with shorter inter-event intervals, but no such shift in sEPSC frequency distribution was found in females. C) Average sEPSC in cocaine and saline pre-treated groups. sEPSC amplitude was unaffected by cocaine treatment in both groups. E) sEPSC Amplitude distribution in males (top) and females (bottom). F) Paired pulse ratio in males. Cocaine treatment in males did alter the paired pulse ratio. G. Representative paired pulse ratio trace in saline and cocaine treated males. H) Representative sEPSC traces in males (top) and females (bottom) from saline- and cocaine-treated groups. I) Locomotor activity in response to cocaine on withdrawal day 14-16 in saline- or cocaine-pre-treated females. Cocaine pre-treated females showed stronger cocaine-induced locomotion at both doses tested compared to saline pre-treated females receiving cocaine for the first time. * = Sidak's post-test p<0.01; #=main effect of pre-treatment p=0.03

Discussion

We examined the effects of a sensitizing regimen of cocaine treatments on NAc core glutamatergic transmission after 14-16 days of withdrawal in male and female rats. There were no effects of cocaine treatment on CP-AMPAR mediated transmission in either sex. However, males, but not females, treated with cocaine showed an increase in sEPSC frequency. Together these data show that despite the development of robust psychomotor sensitization in both sexes, NAc excitatory transmission was enhanced only in males.

Prior studies established that females show a greater enhancement in locomotor activity both acutely and following repeated cocaine injections compared to male rats (Robinson and Becker, 1982; Van Haaren and Meyer, 1991; Hu and Becker, 2008). These sex differences in the response to cocaine rely on gonadal hormones. Specifically, estradiol enhances the acute locomotor effects of cocaine (Sell et al., 2000; Van Swearingen et al., 2013) and facilitates psychomotor sensitization in females (Sell et al., 2002; Hu and Becker, 2003). Furthermore, ovariectomizing females attenuates both the acute (Van Swearingen et al., 2013) and sensitizing effects of cocaine (Sell et al., 2000). Here, females were more sensitive to the acute psychomotor-activating effects of cocaine than males (Fig 2.1B), consistent with prior studies. Unexpectedly, we did not find evidence for greater psychomotor sensitization in females compared to males. It's unlikely that this is due to procedural differences as the dose and regimen used here is similar to that used in studies where sex differences in the magnitude of sensitization were observed (Van Haaren and Meyer, 1991; Hu and Becker, 2003). However, on the 8th day of cocaine injection some females showed brief bouts of in place stereotyped head movements (unpublished observation, AMC). This can interfere with the ability of locomotor-based measures to capture sensitization (Flagel and Robinson, 2007). Thus, it's possible that the automated beam break measure used here may be an under-estimate of the overall magnitude of psychomotor activity in females (Flagel et al., 2007; Carr et al., 2020).

Previous studies in male rats found increases in the surface expression of GluA1 and GluA2 AMPAR subunits in the NAc following cocaine withdrawal (Boudreau and

Wolf, 2005; Ferrario et al., 2010). Although these changes in protein expression suggest enhancements in NAc AMPARs, direct measures of AMPAR synaptic transmission were not made. Here we found no effects of cocaine treatment on CP-AMPAR-mediated transmission (Fig 2.2) or on sEPSC amplitude in males (Fig 2.3C-D). The absence of a change in NAc CP-AMPAR transmission is consistent with results from recordings in the NAc shell of male mice after withdrawal from a sensitizing regimen of cocaine (Kourrich et al., 2007). These data are also consistent with the idea that prolonged withdrawal from long-access cocaine self-administration is required for the synaptic recruitment of these receptors (Conrad et al., 2008; McCutcheon et al., 2011), rather than cocaine exposure per se. The absence of any effect on sEPSC amplitude here is somewhat surprising in light of enhancements in NAc core mEPSC amplitude in male mice (Jedynak et al., 2016) and increases in surface protein expression of AMPAR subunits in rat NAc (Boudreau and Wolf, 2005; Ferrario et al., 2010). The former could be due to species differences (mice vs rats here) or other methodological differences (e.g., sagittal vs coronal sections, internal recording solution, and miniature vs spontaneous EPSCs). However, increases in protein expression and null effects on sEPSC amplitude are not mutually exclusive; it's possible for increases in NAc AMPAR subunit protein expression to result in the accumulation of AMPARs at extra-synaptic sites without resulting in increases in synaptic AMPAR transmission (Gao and Wolf, 2007; Wolf and Tseng, 2012; Alonso-Caraballo et al., 2021).

The primary effect of cocaine we found in males was an increase in sEPSC frequency (Fig 2.3A-B) without a concurrent change in the PPR (Fig 2.3E). This pattern is consistent with increases in dendritic spine density, which is expected to result in more synaptic contacts and thus an increase in sEPSC frequency, but does not require a change in release probability at individual synapses (Glasgow et al., 2019). Indeed, it's well-established that passive and self-administered cocaine increases dendritic spine density and excitatory synapse number within the NAc of males and females (Robinson and Kolb, 1999; Ferrario et al., 2005; Alcantara et al., 2011). Furthermore, Wissman et al. (2011) also found concurrent increases in NAc dendritic spine density and mEPSC frequency with no changes in PPR following cocaine exposure and withdrawal in males (Wissman et al., 2011). Thus, data here are consistent with prior

results examining effects of experimenter administered cocaine and subsequent withdrawal on NAc core excitatory synaptic transmission in males.

Surprisingly, despite showing robust sensitization, no effects of cocaine on NAc core glutamatergic transmission were found in females (Fig 2.2, 2.3). One possible explanation for the absence of effects is that sensitization may have abated by the time recordings were made on withdrawal day 14-16. We examined this possibility by re-exposing females pretreated with cocaine or saline to cocaine on withdrawal day 14-16 and evaluated the expression of locomotor sensitization. Females pre-treated with cocaine showed a stronger locomotor response to cocaine than saline-pretreated controls, confirming that behavioral sensitization persisted through the withdrawal period (Fig 2.3G). Therefore, given that the behavioral response is a manifestation of alterations in mesolimbic function (Berridge and Robinson, 1998), the absence of effects on synaptic transmission are not likely due to a loss of sensitization.

To our knowledge, only one previous study similar to the present report has been conducted in females (Wissman et al., 2011). They reported a greater increase in NAc core mEPSC frequency in female rats compared to male rats treated with cocaine. The dose of cocaine they used was the same as that used here (15 mg/kg), but their treatment regimen was much longer (1 injection/day, 5 days per week for 5 weeks) and the period of withdrawal (17-21 days) was a little longer than here (1 injection/day, 8 days, 14-16 days of withdrawal). This could suggest that females need more exposure to cocaine to drive changes in glutamatergic transmission in the NAc compared to males. Additionally, Wissman et al. (2011) reported disruptions in the estrous cycle during the cocaine sensitization regimen, which corresponded to reductions in cocaine induced locomotor activity. We did not monitor the cycle in our study, but we did not observe any reductions in locomotor activity after repeated injections in females. In addition, 2 injections per day of 15 mg/kg for 5 days is reported to not be sufficient to disrupt the female cycle in rats (Sell et al., 2002). Thus, it seems unlikely that the more modest dosing regimen used here (1 injection/day 15mg/kg for 8 days) produced disruptions in the cycle. It is possible that some degree of cycle disruption (and presumably of ovarian hormone fluctuations) may be required to observe alterations in mEPSC frequency in the NAc. This idea is consistent with the ability of estradiol to

rapidly decrease mEPSC frequency in the NAc core of females but not males (Krentzel et al., 2019). Perhaps naturally occurring fluctuations in estradiol are sufficient to dampen the ability of cocaine to induce increases in sEPSC frequency in females. Conversely, cocaine treatment regimens that disrupt the cycle and concurrent fluctuations in estradiol would be expected to remove this protection. This possibility should be directly tested in future studies. Finally, it should be noted that mEPSCs recorded in Wissman et al., (2011) and sEPSCs (recorded here) may be capturing different aspects of synaptic transmission and/or different populations of synaptic inputs. This is mitigated somewhat by the use of coronal sections in our study (which contain glutamate terminals, but lack axon initial segments and cell bodies from glutamate inputs, limiting the likelihood of action potential driven synchronous release), but nonetheless could contribute to the differences observed between these studies.

References

Alcantara AA, Lim HY, Floyd CE, Garces J, Mendenhall JM, Lyons CL, Berlanga ML (2011) Cocaine- and morphine-induced synaptic plasticity in the nucleus accumbens. Synapse 65:309-320.

Algallal H, Allain F, Ndiaye N, Samaha A-N (2019) Sex differences in cocaine self-administration behaviour under long access versus intermittent access conditions. Addiction Biology 25.

Alonso-Caraballo Y, Ferrario CR (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. Horm Behav 116:104583.

Alonso-Caraballo Y, Fetterly TL, Jorgensen ET, Nieto AM, Brown TE, Ferrario CR (2021) Sex specific effects of "junk-food" diet on calcium permeable AMPA receptors and silent synapses in the nucleus accumbens core. Neuropsychopharmacology 46:569-578.

Back SE, Brady KT, Jackson JL, Salstrom S, Zinzow H (2005) Gender differences in stress reactivity among cocaine-dependent individuals. Psychopharmacology (Berl) 180:169-176.

Becker JB, Hu M (2008) Sex differences in drug abuse. Front Neuroendocrinol 29:36-47.

Becker JB, Koob GF (2016) Sex Differences in Animal Models: Focus on Addiction. Pharmacol Rev 68:242-263.

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 28:309-369.

Boudreau AC, Wolf ME (2005) Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. J Neurosci 25:9144-9151.

Brady KT, Randall CL (1999) Gender differences in substance use disorders. Psychiatr Clin North Am 22:241-252.

Carr CC, Ferrario CR, Robinson TE (2020) Intermittent access cocaine self-administration produces psychomotor sensitization: effects of withdrawal, sex and cross-sensitization. Psychopharmacology (Berl) 237:1795-1812.

Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, Marinelli M, Wolf ME (2008) Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature 454:118-121.

Cornish JL, Prasad AA (2021) Sex Differences in Substance Use Disorders: A Neurobiological Perspective. Front Glob Womens Health 2:778514.

Dong Y, Taylor JR, Wolf ME, Shaham Y (2017) Circuit and Synaptic Plasticity Mechanisms of Drug Relapse. J Neurosci 37:10867-10876.

Ferrario CR, Robinson TE (2007) Amphetamine pretreatment accelerates the subsequent escalation of cocaine self-administration behavior. Eur Neuropsychopharmacol 17:352-357.

Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE (2005) Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. Biol Psychiatry 58:751-759.

Ferrario CR, Li X, Wang X, Reimers JM, Uejima JL, Wolf ME (2010) The role of glutamate receptor redistribution in locomotor sensitization to cocaine. Neuropsychopharmacology 35:818-833.

Flagel SB, Robinson TE (2007) Quantifying the psychomotor activating effects of cocaine in the rat. Behav Pharmacol 18:297-302.

Flagel SB, Watson SJ, Robinson TE, Akil H (2007) Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. Psychopharmacology 191:599-607.

Forlano PM, Woolley CS (2010) Quantitative analysis of pre- and postsynaptic sex differences in the nucleus accumbens. J Comp Neurol 518:1330-1348.

Gao C, Wolf ME (2007) Dopamine Alters AMPA Receptor Synaptic Expression and Subunit Composition in Dopamine Neurons of the Ventral Tegmental Area Cultured with Prefrontal Cortex Neurons. The Journal of Neuroscience 27:14275.

Glasgow SD, McPhedrain R, Madranges JF, Kennedy TE, Ruthazer ES (2019) Approaches and Limitations in the Investigation of Synaptic Transmission and Plasticity. Frontiers in Synaptic Neuroscience 11.

Hernandez-Avila CA, Rounsaville BJ, Kranzler HR (2004) Opioid-, cannabis- and alcohol-dependent women show more rapid progression to substance abuse treatment. Drug Alcohol Depend 74:265-272.

Hogle JM, Curtin JJ (2006) Sex differences in negative affective response during nicotine withdrawal. Psychophysiology 43:344-356.

Horger BA, Giles MK, Schenk S (1992) Preexposure to amphetamine and nicotine predisposes rats to self-administer a low dose of cocaine. Psychopharmacology 107:271-276.

Hu M, Becker JB (2003) Effects of sex and estrogen on behavioral sensitization to cocaine in rats. J Neurosci 23:693-699.

Hu M, Becker JB (2008) Acquisition of cocaine self-administration in ovariectomized female rats: Effect of estradiol dose or chronic estradiol administration. Drug and Alcohol Dependence 94:56-62.

Jedynak J, Hearing M, Ingebretson A, Ebner SR, Kelly M, Fischer RA, Kourrich S, Thomas MJ (2016) Cocaine and Amphetamine Induce Overlapping but Distinct Patterns of AMPAR Plasticity in Nucleus Accumbens Medium Spiny Neurons. Neuropsychopharmacology 41:464-476.

Kawa AB, Robinson TE (2019a) Sex differences in incentive-sensitization produced by intermittent access cocaine self-administration. Psychopharmacology 236:625-639.

Kawa AB, Robinson TE (2019b) Sex differences in incentive-sensitization produced by intermittent access cocaine self-administration. Psychopharmacology (Berl) 236:625-639.

Kourrich S, Rothwell PE, Klug JR, Thomas MJ (2007) Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. J Neurosci 27:7921-7928.

Krentzel AA, Barrett LR, Meitzen J (2019) Estradiol rapidly modulates excitatory synapse properties in a sex- and region-specific manner in rat nucleus accumbens core and caudate-putamen. J Neurophysiol 122:1213-1225.

Lett BT (1989) Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. Psychopharmacology 98:357-362.

Loweth JA, Scheyer AF, Milovanovic M, LaCrosse AL, Flores-Barrera E, Werner CT, Li X, Ford KA, Le T, Olive MF, Szumlinski KK, Tseng KY, Wolf ME (2014) Synaptic depression via mGluR1 positive allosteric modulation suppresses cue-induced cocaine craving. Nat Neurosci 17:73-80.

Lynch WJ, Carroll ME (1999) Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology (Berl) 144:77-82.

McCutcheon JE, Wang X, Tseng KY, Wolf ME, 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. J Neurosci 31:5737-5743.

Menendez-Delmestre R, Segarra AC (2011) Testosterone is essential for cocaine sensitization in male rats. Physiol Behav 102:96-104.

Nicolas C, Russell TI, Pierce AF, Maldera S, Holley A, You ZB, McCarthy MM, Shaham Y, Ikemoto S (2019) Incubation of Cocaine Craving After Intermittent-Access Self-administration: Sex Differences and Estrous Cycle. Biol Psychiatry 85:915-924.

Nieto AM, Catalfio AM, Papacostas Quintanilla H, Alonso-Caraballo Y, Ferrario CR (2023) Transient effects of junk food on NAc core MSN excitability and glutamatergic transmission in obesity-prone female rats. Obesity 31:434-445.

Oginsky MF, Maust JD, Corthell JT, Ferrario CR (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 (Berl) 233:773-784.

Peterson BM, Mermelstein PG, Meisel RL (2015) Estradiol mediates dendritic spine plasticity in the nucleus accumbens core through activation of mGluR5. Brain Struct Funct 220:2415-2422.

Piazza PV, Deminiere JM, le Moal M, Simon H (1990) Stress-and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain research 514:22-26.

Pierce RC, Kalivas PW (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Res Brain Res Rev 25:192-216.

Post RM, Rose H (1976) Increasing effects of repetitive cocaine administration in the rat. Nature 260:731-732.

Proaño SB, Krentzel AA, Meitzen J (2020) Differential and synergistic roles of 17β-estradiol and progesterone in modulating adult female rat nucleus accumbens core medium spiny neuron electrophysiology. J Neurophysiol 123:2390-2405.

Proaño SB, Morris HJ, Kunz LM, Dorris DM, 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 120:1356-1373.

Robbins SJ, Ehrman RN, Childress AR, O'Brien CP (1999) Comparing levels of cocaine cue reactivity in male and female outpatients. Drug Alcohol Depend 53:223-230.

Robinson TE (1984) Behavioral sensitization: Characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats. Psychopharmacology 84:466-475.

Robinson TE, Becker JB (1982) Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247-291.

Robinson TE, Kolb B (1999) Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. Eur J Neurosci 11:1598-1604.

Sell SL, Thomas ML, Cunningham KA (2002) Influence of estrous cycle and estradiol on behavioral sensitization to cocaine in female rats. Drug Alcohol Depend 67:281-290.

Sell SL, Scalzitti JM, Thomas ML, Cunningham KA (2000) Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. J Pharmacol Exp Ther 293:879-886.

Staffend NA, Loftus CM, Meisel RL (2011) Estradiol reduces dendritic spine density in the ventral striatum of female Syrian hamsters. Brain Struct Funct 215:187-194.

Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001) Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. Nature Neuroscience 4:1217-1223.

Van Haaren F, Meyer ME (1991) Sex differences in locomotor activity after acute and chronic cocaine administration. Pharmacology Biochemistry and Behavior 39:923-927.

Van Swearingen AED, Sanchez CL, Frisbee SM, Williams A, Walker QD, Korach KS, Kuhn CM (2013) Estradiol replacement enhances cocaine-stimulated locomotion in female C57BL/6 mice through estrogen receptor alpha. Neuropharmacology 72:236-249.

Vanderschuren LJ, Kalivas PW (2000) Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl) 151:99-120.

Vezina P (2004) Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. Neurosci Biobehav Rev 27:827-839.

Wasserstein RL, Lazar NA (2016) The ASA statement on p-values: context, process, and purpose. In, pp 129-133: Taylor & Francis.

Wissman AM, McCollum AF, Huang GZ, Nikrodhanond AA, Woolley CS (2011) Sex differences and effects of cocaine on excitatory synapses in the nucleus accumbens. Neuropharmacology 61:217-227.

Wolf ME, Tseng KY (2012) Calcium-permeable AMPA receptors in the VTA and nucleus accumbens after cocaine exposure: when, how, and why? Front Mol Neurosci 5:72.

Zhao W, Becker JB (2010) Sensitization enhances acquisition of cocaine self-administration in female rats: Estradiol further enhances cocaine intake after acquisition. Hormones and Behavior 58:8-12.

Chapter 3: Effects of Intermittent vs Long Access Cocaine Self-Administration on NAc CP-AMPAR Transmission

Abstract

The development and persistence of addiction is mediated partly by drug-induced alterations in the function of the nucleus accumbens (NAc). AMPA-type glutamate receptors provide the main source of excitatory drive to the NAc, and enhancements in transmission of calcium-permeable AMPAR (CP-AMPARs) within the NAc following long access (LgA) cocaine self-administration mediates the incubation of craving, a progressive increase in drug-seeking across withdrawal. Recent evidence indicates intermittent access (IntA) self-administration produces more robust addiction-like behaviors compared to LgA, but effects on NAc glutamatergic transmission are unknown. Furthermore, medium spiny neurons (MSNs) are divided into two types (D1 vs D2). However, little is known about NAc cell-type specific changes following self-administration. Therefore, we examined the effects of IntA vs LgA cocaine self-administration on incubation and NAc glutamatergic transmission in D1- and D2-MSNs in male and female rats. Rats underwent 5 days of training (0.40mg/kg/infusion) followed by 10 days of LgA or IntA cocaine self-administration. Drug naïve rats handled similarly to experimental groups served as controls. Some rats were used for within subject incubation testing at withdrawal day 1, 30 and 45. A separate group of rats were used to measure the effects of LgA and IntA experience on CP-AMPAR mediated transmission at withdrawal day 30-35. IntA and LgA self-administration followed by withdrawal resulted in similar patterns of incubation across both sex. Additionally, Naspm sensitivity was enhanced in LgA and IntA groups compared to drug naïve animals. Initial results suggest effects of Naspm were similar in D1 and D2 cells and

between sex. Overall, these data reveal that despite less drug consumption the IntA group display a similar magnitude of incubation of craving and NAc glutamate plasticity to LgA.

Introduction

Intravenous drug self-administration in rodents is the most accepted preclinical approach to studying drug-induced changes in psychological and neurobiological functions that may result in addiction. However, it is known that not all self-administration experiences produce behavioral features consistent with addiction (Ahmed and Kenny, 2011). For example, under short access (ShA) self-administration conditions (1-2 hours of continuous access) rats do not escalate their drug intake or show progressive enhancements in drug-seeking behaviors (Ahmed and Koob, 1998; Ferrario et al., 2005; Hao et al., 2010). Therefore, there has been considerable effort to develop self-administration procedures (mainly in rodents) that lead to the development of these addiction-like behaviors (Deroche-Gamonet et al., 2004; Kawa et al., 2019; Samaha et al., 2021).

The current gold standard for studying addiction-like behaviors in rodents is long access (LgA) self-administration. LgA conditions allow animals to freely administer drug, such as cocaine, for 6+ hours on a fixed ratio 1 schedule. Animals who've experienced LgA self-administration reliably develop several addiction-like behaviors (Ahmed and Koob, 1998; Paterson and Markou, 2003; Deroche-Gamonet et al., 2004). For example, LgA cocaine self-administration followed by withdrawal results in drug-seeking behavior that increases as a function of drug withdrawal. This is in contrast to animals that experience ShA conditions; these animals maintain consistent responding across withdrawal (Ferrario et al., 2005; Ahmed and Kenny, 2011; Purgianto et al., 2017). This progressive enhancement in drug-seeking behavior across withdrawal has been termed "incubation" and is thought to mirror enhanced drug craving across prolonged abstinence in humans (Hunt et al., 1971; Vanderschuren and Everitt, 2004). Additionally, animals that experience LgA cocaine self-administration escalate their cocaine intake. However, animals under ShA conditions keep cocaine responding stable (Ahmed and

Koob, 1998; Roth and Carroll, 2004). Furthermore, animals that experience LgA self-administration continue to pursue cocaine when faced with adverse consequences, such as pairing infusions with a foot-shock (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Sorge and Stewart, 2005).

More recently, intermittent access (IntA) self-administration has been proposed to better model patterns of drug-taking in humans because humans do not maintain a high level of prolonged use but rather recreational drug users tended to cycle frequently between use and abstinence (Cohen and Sas, 1994; Ward et al., 1997; Simon et al., 2001; Zimmer et al., 2012). Therefore, to mimic this pattern of drug-taking in rodents the IntA model of self-administration alternates between 5-minutes of a drug available period followed by 25-minutes of a no drug available period for 6 hours (Zimmer et al., 2011; Zimmer et al., 2012). This pattern of drug availability limits the total amount of drug intake, similar to that of ShA but also produces spiking drug concentrations in the brain compared to consistent levels seen in LgA or ShA (Zimmer et al., 2012; Kawa et al., 2016). Despite much less overall drug consumption, the IntA experience produces addiction-like behaviors comparable to or greater in magnitude than LgA (Kawa et al., 2016; Nicolas et al., 2019; Carr et al., 2020). For example, IntA cocaine self-administration followed by withdrawal results in incubation of craving (Nicolas et al., 2019). The magnitude of the incubation of cocaine craving following IntA self-administration is greater than that seen following LgA self-administration when male and female rats are tested at withdrawal day 2 vs withdrawal day 29 (Nicolas et al., 2019; Nicolas et al., 2022). However, longer withdrawal times had not been examined.

Incubation of craving is associated with specific neurobiological adaptations. Specifically, LgA self-administration that results in incubation of craving results in an enhancement of calcium permeable AMPARs (CP-AMPARs) in the NAc core (Conrad et al., 2008; McCutcheon et al., 2011; Loweth et al., 2014; Kawa et al., 2022). Furthermore, blockade or pharmacological removal of CP-AMPARs through activation of metabotropic glutamate receptors prevents the expression of incubation in male and female rats (Conrad et al., 2008; Loweth et al., 2014; Kawa et al., 2022). Importantly, enhancements in CP-AMPAR mediated transmission are not seen following ShA cocaine self-administration (Purgianto et al., 2013), which indicates that CP-AMPARs

are specific to the emergence of addiction-like behaviors and not simply drug exposure. Withdrawal dependent increases in NAc core CP-AMPARs following LgA cocaine self-administration have been extensively characterized in males, but only one study has assessed CP-AMPAR mediated transmission following LgA cocaine self-administration in females (Kawa et al., 2022). Furthermore, to date there are no studies investigating the effects of IntA cocaine self-administration on CP-AMPAR mediated transmission in either sex.

Recent evidence suggests that CP-AMPAR mediated transmission enhancements following cocaine self-administration may be cell-type specific. For example, in the NAc shell of mice, dopamine 1 receptor (D1) containing medium spiny neurons (MSNs) have an increase in CP-AMPAR following 30 days of withdrawal from either ShA or LgA cocaine self-administration. This is in contrast to dopamine 2 receptor (D2) containing MSNs that show no upregulation in CP-AMPARs following either ShA or LgA (Pascoli et al., 2014; Terrier et al., 2016). Currently, there are no studies in rodents investigating the effects of LgA or IntA cocaine self-administration on D1- or D2-containing MSNs in the NAc core.

Here we directly compared the effects of LgA vs IntA self-administration on the incubation of cocaine craving across prolonged withdrawal (1, 30 and 45 days) in both sexes, and determined the degree to which withdrawal from these self-administration experiences enhanced NAc core CP-AMPARs in D1- and D2-MSNs of male and female rats.

Materials and methods

Animals

Adult male and female LE-Drd1[em1(iCre)Berke] (RRRC #00856; Drd1-Cre) and LE-A2a[em1(iCre)Berke] (RRRC #00857; A2a-Cre) transgenic rats were bred in house to wild type Long Evans rats (Envigo). Targeting of the A2a promotor was selected over the D2 promotor because A2a receptors are selectively expressed on post-synaptic MSNs, however, D2 receptors are found both pre and post-synaptically as well as other striatal cells (Alcantara et al., 2003; Pettibone et al., 2019). Offspring were genotyped by a

commercial vendor (TransnetYX) using knock-in insertion-spanning primers. Both Drd1-Cre and A2a-Cre rats were then crossed with homozygous LE-ROSA26[em1(CAG-LSL-TdTomato)Rrrc] (#00938; Td-Tomato), resulting in offspring expressing Td-tomato within Cre+ (Drd1 or A2a cells). All rats used in these studies were offspring from the Td-tomato X Cre crosses. Rats were pair housed unless described otherwise and were maintained on a reverse 12-h light/dark cycle (lights off at 0800). All rats were obtained from the Rat Resource and Research Center (Columbia, MO). Procedures were approved by The University of Michigan Committee on the Use and Care of Animals in accordance with AAALAC and AVMA guidelines.

Intravenous catheter surgery

Animals ~55 days old were surgically implanted with an intravenous (IV) catheter as previously described (Ferrario et al., 2005; Carr et al., 2020). Briefly, rats were anesthetized with isoflurane (inhalation, 2-4%), and an indwelling catheter was secured into the right jugular vein. The catheter exited via a port just above the shoulder blades. Carprofen was given at the start of the surgery and once daily for two days following surgery (5 mg/kg, SC). For 10 days post-surgery catheters were flushed daily with 0.2 ml of sterile saline containing 5 mg/ml gentamicin sulfate (Vedco, MO). Then for the duration of the experiment catheters were flushed daily with 0.2 ml sterile saline. During the withdrawal period catheters were no longer flushed. Following surgery all animals were singly housed. Experimental timeline is shown in Figure 3.1A.

Cocaine self-administration

The day prior to the experiment, rats (N=76, 43 males and 33 females) were weighed and food restricted to 95-105% of that body weight during self-administration, but returned to *ad lib* feeding during withdrawal. They had free access to water throughout. Rats began cocaine self-administration training 7-10 days after surgical recovery. Training was conducted in standard operate chambers equipped with two nose-poke ports (active and inactive), a red house light, and a cue light above the active port (Med Associates, St Albans, VT). Responses in the active port produced an IV cocaine infusion (0.4 mg/kg/infusion, in 50μl of sterile saline delivered over 2.6 seconds)

on a fixed-ratio 1 (FR1) schedule. Each infusion was accompanied by a 5 second discrete cue light presentation and a 20 second timeout period. Responses in the inactive nose-port had no programmed consequence, but were recorded. Each animal received 5 consecutive training sessions that ended when rats obtained 20 infusions, or 1 hour had passed. After training, animals were divided into LgA or IntA groups counter-balanced by the average number of active nose-pokes made during the last three days of training, such that LgA and IntA groups were matched for active responding and number of infusions taken during initial training.

Immediately following training, animals underwent 10 consecutive days of LgA (N=34; M=18, F=16) or IntA (N=42; M=25, F=17) as previously described (Ferrario et al., 2005; Kawa et al., 2016). For both self-administration paradigms active and inactive nose-pokes were recorded throughout. LgA sessions consisted of 6 hours of continuous access to cocaine and conditions were identical to training sessions. IntA sessions consisted of alternating between 5-minute drug available periods followed by a 25-minute no drug available periods for a total of 6 hours (12 cycles of 5-min drug available and 25-min no drug available periods). The no drug available periods were signaled by the illumination of the house light. Drug available periods were signaled by the house light turning off. As during training, active responses resulted in an infusion of cocaine and illumination of the active nose-poke port for 5 seconds. There was no explicit timeout period, but another infusion could not be triggered until the cue-light turned off. After 10 sessions of IntA or LgA rats were assigned to either undergo within subject incubation testing or slice electrophysiology studies (see timeline Fig 3.1A).

Drug naïve control animals (N=11; 8 males, 13 females) did not undergo surgery. However, prior to the start of the experiment, animals were weighed and food restricted to 95-105% of that body weight. Controls were handled similar and experienced the same amount of time outside the rodent housing colony as experimental groups.

Monitoring the estrous cycle

The estrous cycle was monitored daily throughout self-administration as well as immediately prior to incubation testing and slice preparation for electrophysiological recordings. Estrous cycle phase was determined by observations of vaginal epithelial

cell cytology as previously described, (Alonso-Caraballo and Ferrario, 2019; Nieto et al., 2023). Briefly, epithelial cells were collected by vaginal lavage (during the dark phase) and visualized using an inverted light microscope (Olympus CKX53) under bright-field conditions. 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.

Incubation Testing

Rats assigned to incubation testing (i.e., drug-seeking in the absence of cocaine) were tested at both withdrawal day 1 and 30 (LgA N=22, 13 males, 9 females; IntA N=27, 17 males, 10 females) with a subset of these rats tested a third time at withdrawal day 45 (LgA N=19, 11 males, 8 females; IntA N=23, 13 males, 10 females). Given that rats underwent repeated testing, no electrophysiological measures were made from these rats. During this 1-hour test session, responding in the previously active port led to a 5 second presentation of the cue previously paired with cocaine infusions, but not cocaine. Responding in the previously active nose-port was the operational measure of cocaine-seeking. Responses in the inactive port were recorded but had no consequences. All rats used in incubation studies were offspring from the Td-tomato X Cre crosses including both Cre+ and Cre- animals.

Whole-cell patch-clamp recordings

Rats that were destined for electrophysiology studies remained in their home cage until withdrawal day 30-35 (Drug naïve N=11, LgA N=11, IntA N=15). Brain slices containing the NAc were prepared as previously described (Oginsky et al., 2016; Fetterly et al., 2021). Briefly, coronal sections (300 µm) were prepared using a vibratome (Lecia Biosystems). Slices recovered in oxygenated artificial cerebrospinal fluid for 30 mins at 37 °C, (aCSF; in mM: 122.5 NaCl, 25 $NaHCO_3$, 12.5 Glucose, 1 NaH_2PO_4, 1 L-ascorbic acid, 2.5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$; 295-305 mOsm, pH 7.45). Slices were then maintained at room temperature (30 minutes) prior to recording.

All electrophysiology studies used established whole-cell patch clamp approaches (Ferrario et al., 2011; Fetterly et al., 2021; Catalfio et al., 2023). For the recording aCSF, $CaCl_2$ was 2.5 mM and $MgCl_2$ was 1 mM. For all recordings, pipettes were filled with (in mM): 140 CsCl, 10 HEPES, 2 $MgCl_2$, 5 Na^+-ATP, 0.6 Na^+-GTP, 2 QX-314, pH 7.3, 285 mOsm. All recordings were made from the NAc core and conducted in the presence of the $GABA_A$ receptor antagonist, picrotoxin (50 µM). The NAc core was identified using the anterior commissure as a primary landmark (see Fig 3.3C). MSNs were identified by cell body size (~15 µm in diameter), capacitance (30–60pF), and membrane resistance (30–120 mOhms) after break in. Drd1-Cre+ and A2a-Cre+ neurons were identified by Td-tomato expression that was visualized using epifluorescence (BioLED Light Source BLS-Series Mightex) and passed through a mCherry filter cube for identification (see Fig 3.3C). Cells with the NAc core in Drd1-Cre+ slices expressing Td-tomato were considered D1-MSNs, whereas cells that were negative for Td-tomato were considered D2-MSNs, and vice versa in slices from A2a-Cre+ rats.

Evoked EPSCs (eEPSCs) were elicited by local stimulation (0.02 to 0.30 mA square pulses, 0.1 ms, delivered every 20 s) using a bipolar electrode placed about 300 µm lateral to recorded neurons. The minimum amount of current needed to elicit a synaptic response with less than 20% variability in amplitude was used. If more than 0.30 mA was required, the recording was terminated. eEPSCs were recorded at −70 mV before and after application of the CP-AMPAR selective antagonist Naspm (200 µM) (ASAMI et al., 1989; Koike et al., 1997). Cell parameters (capacitance and membrane resistance), as well as access resistance were recorded throughout data collection and only cells with less than 20% change across time were included in analyses. Recordings alternated between slices from males or females and from rats in the drug naïve, LgA, or IntA groups each day (note that no more than 4 cells, two from each cell-type, were collected from the same rat).

Drugs and reagents

Cocaine HCl was provided by the NIDA drug supply program. All other drugs and reagents were obtained from Sigma (St. Louis MO, USA) or Tocris (Minneapolis MN,

USA).

Analysis and statistics

All recordings were made using Clampex 10.7-11.1 and analyzed using Clampfit 10.7-11.1 (Molecular Devices). Two-way, and three-way ANOVAs using standard general linear models (GLM) or mixed model residual maximum likelihood (REML) followed by Sidak's, Tukey's, Dunnett's multiple comparisons, or Fisher's LSD post hoc comparisons were used (Prism 9, GraphPad, San Diego, CA). Interpretation of *p*-values is based on guidelines set forth by the American Statistical Association (Wasserstein and Lazar, 2016). Experimenters were not blind to grouping during data acquisition but were during analysis. Ns for electrophysiological measures are reported in the results and were based on expected effect size and variance of our primary measures.

Results

Effects of LgA and IntA self-administration on drug consumption and escalation

Figure 3.1 shows instrumental responding and drug intake during initial acquisition (B) and subsequent LgA or IntA (C-E). During initial acquisition, all rats readily acquired cocaine self-administration, preferentially responding in the active vs inactive nose-poke port (Fig 3.1B: Two-way RM ANOVA, main effect of port $F_{(1,150)}$=72.11, p<0.01). Potential sex differences during acquisition were also evaluated (Fig 3.1B), with slightly greater active responding in males compared to females (Fig 3.1B: Three-way RM ANOVA, main effect of sex $F_{(1,74)}$=3.02, p=0.09). This trend was opposite to what was expected, but active and inactive responding between males and females did not differ by the last day of training.

Next, rats were assigned to LgA and IntA groups, counter-balanced for drug intake during initial acquisition. Figure 3.1C shows infusions in males and females taken during LgA and IntA sessions. As expected, the LgA group took more infusions than the IntA group (Fig 3.1C: Three-way RM ANOVA, main effect of SA model $F_{(1,713)}$=6.36, p=0.01). We next evaluated escalation by assessing the number of infusions taken during the first vs last session of LgA and IntA, both groups display clear escalation (Fig

3.1E: Two-way REML ANOVA, main effect of session $F_{(1,69)}=49.63$, $p<0.01$). Given that the LgA group took more infusions than the IntA group we assesses the effects of sex on escalation within group (Fig 3.1C). Surprisingly, males in the LgA group show a larger magnitude of escalation compared to females (sex x time interaction $F_{(9,283)}=1.96$, $p=0.04$), however, there were no sex differences in the IntA group (main effect of sex $F_{(1,40)}=0.33$, $p=0.57$; sex x time interaction, $F_{(9,358)}=0.26$, $p=0.98$). Additionally, there were no sex differences in the total number of infusions taken by either self-administration group (Fig 3.1D: Two-way ANOVA, main effect of sex $F_{(1,72)}=0.66$, $p=0.42$). Importantly, genotype had no effect on initial acquisition or LgA and IntA self-administration behavior (Supplemental Fig 3.5A-C).
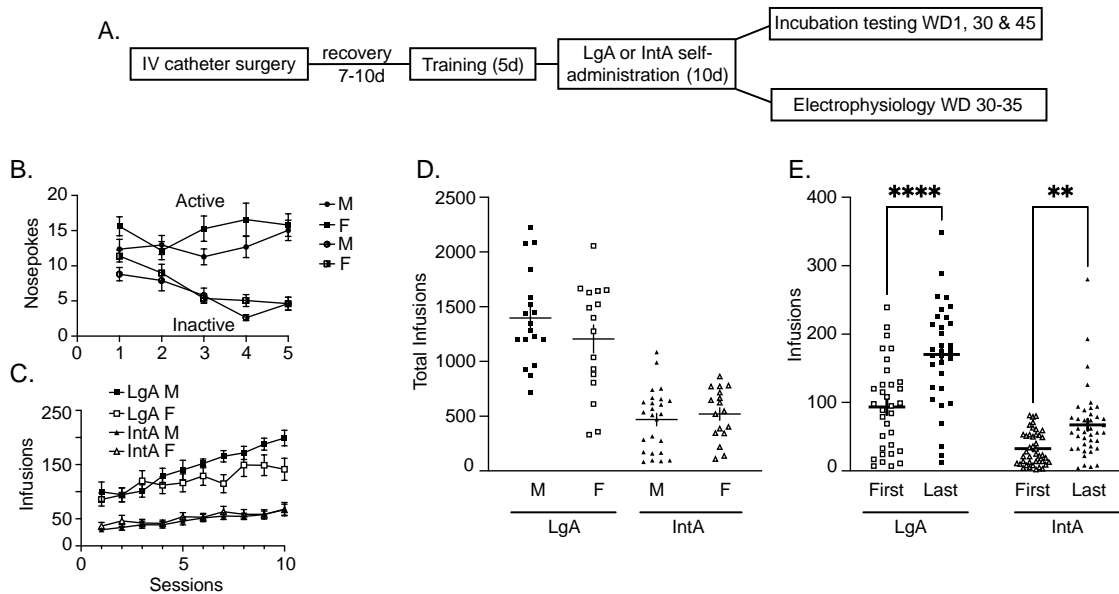

Figure 3.1 LgA and IntA cocaine self-administration behavior. A) Schematic of experimental timeline. B) The number of active and inactive nose-pokes across session in males and females. Animals distinguished between active and inactive nose-poke ports. Males and females did not differ in the number of active and inactive nose-pokes. C) The number of infusions taken across sessions in LgA and IntA separated by males and females within each group. Both LgA and IntA groups escalate cocaine intake. LgA males increase infusions more than LgA females. There was no difference between sex in escalation in the IntA group. D) The total number of infusions during self-administration. There is no sex difference in total infusions taken within SA model. E) LgA and IntA increased the number of infusions from the first to last session. **p=0.0031, ****p<0.0001

Effects of long and intermittent access cocaine self-administration on incubation

Rats were tested for incubation of drug-seeking on withdrawal day 1, 30 and 45 (Fig 3.2). Drug-seeking increased from withdrawal day 1 to withdrawal day 30 and this enhancement was maintained at withdrawal day 45 (Fig 3.2A: Three-way REML

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ANOVA, main effect of time $F_{(2,94)}=12.74$, p<0.01; time x port interaction $F_{(2,80)}=15.58$, p<0.01; Dunnett's multiple comparisons LgA withdrawal day 1 vs 30 p<0.01, withdrawal day 1 vs 45 p <0.01; IntA withdrawal day 1 vs 30 p=0.01, withdrawal day 1 vs 45 p<0.01). This time-dependent increase in drug-seeking was similar in LgA and IntA groups (Main effect of group $F_{(1,80)}=0.58$, p=0.04; group x time interaction $F_{(2,80)}=0.02$, p=0.79). In addition, responses on the inactive lever were low and stable across testing (Two-way REML ANOVA, main effect of time $F_{(1.514,65.86)}=1.06$, p=0.34; SA model $F_{(1,47)}=0.13$, p=0.72; SA model x time interaction $F_{(2,87)}=0.12$, p=0.89). Given that the incubation of drug-seeking is defined by time dependent increases in active responding, we used this measure to evaluate potential sex differences in this effect (Fig 3.2B). Both sexes showed similar increases in active responding across withdrawal (Fig 3.2B: Three-way REML ANOVA, main effect of time $F_{(2,88)}=15.83$, p<0.01; main effect of sex $F_{(1,45)}=0.06$, p=0.82, time x sex interaction $F_{(2,88)}=0.13$ p=0.88). Thus, IntA self-administration produced similar enhancements in drug-seeking behavior compared to LgA self-administration in both sexes. There was no effect of genotype on incubation of craving (Supplemental Fig 3.5D).
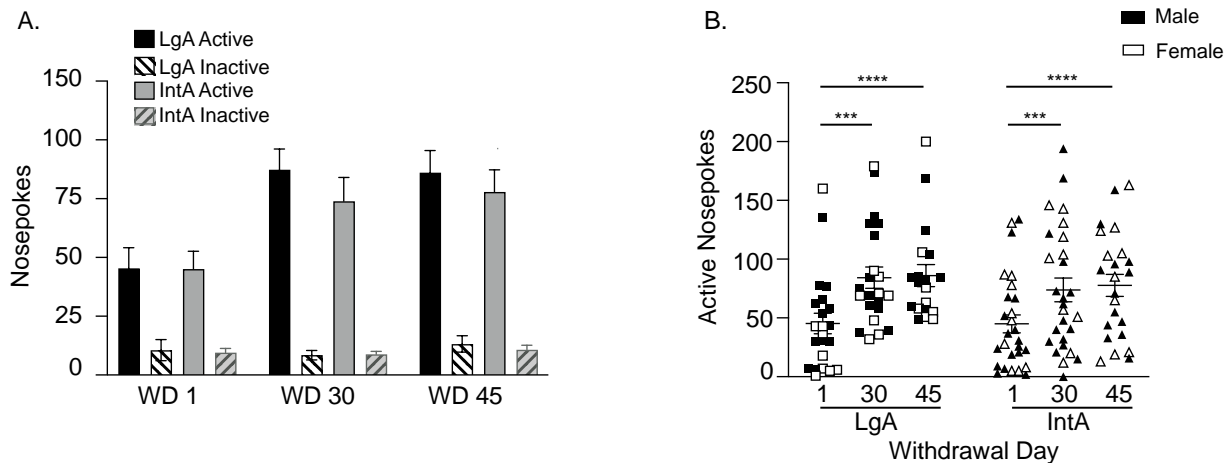

Figure 3.2 IntA cocaine self-administration results in incubation of craving to a similar magnitude to LgA in both males and females. A) The number of active and inactive nose-poke port across withdrawal. LgA and InA increase the number of active nose-pokes from withdrawal 1 to 30 and 45. Inactive nose-pokes remain low in both groups. B) The number of active nose-pokes separated by self-administration model and sex. LgA males (closed squares) and LgA females (open squares) increase the number of active nose-pokes across withdrawal day to a similar extent. IntA males (closed triangle) and IntA females (open triangle) increase responding on the active nose-port across withdrawal day similarly. ***p=0.0004, ****p<0.0001

Effects of long and intermittent access cocaine self-administration on NAc CP-AMPAR mediated transmission

Images of Td-tomato positive and negative cells and the recording area are shown in Figure 3.3C. Importantly, using measuring of intrinsic firing properties we confirmed that Td-tomato positive and negative cells are MSNs (Fig 3.3A; see also discussion).

Figure 3.3A and B show NAc core CP-AMPAR-mediated transmission measured 30-35 days after the last IntA or LgA self-administration session (Drug naïve N=12 rats (M=4, F=8), 21 cells (13=D1, 8=D2); LgA N=10 rats (M=4, F=6), 25 cells (D1=15, D2=10); IntA N=15 rats (M=8, F=7), 27 cells (D1=13, D2=14). As stated above, we considered Drd1-Cre Td-tomato positive cells D1-MSNs, while Td-tomato negative cells were considered D2-MSNs (and vice versa for slices from A2A-Cre x Td-tomato crosses). Thus, D1 and D2 cells could both be obtained from the same rat.

We first examined the effect of prolonged withdrawal from IntA vs LgA self-administration, including the factors of sex and cell-type. Both IntA and LgA self-administration increased CP-AMPAR-mediated transmission compared to drug naïve controls (Fig 3.3A: Three-way ANOVA, main effect of group $F_{(2,60)}=3.45$, p=0.04; Fisher's LSD drug naïve vs LgA: $t_{(60)}=2.47$, p=0.02; drug naïve vs IntA: $t_{(60)}=2.18$, p=0.03), with similar effects in both sexes and cell-types (main effect of sex $F_{(1,60)}=0.81$, p=0.37; main effect of cell-type $F_{(1,60)}=0.79$, p=0.38; group x sex interaction $F_{(2,60)}=0.52$, p=0.60; group x cell-type interaction $F_{(2,60)}=1.13$, p=0.33; group x sex x cell-type interaction $F_{(2,60)}=0.49$, p=0.61). Figure 3.3B shows average eEPSC amplitude, as a percentage of baseline, before (baseline; 10 min) and after bath application of the Naspm (10 min) collapsed across sex and cell-type, with example traces before (black) and after (red) Naspm for each group shown in the inset. Overall, these data indicate that IntA self-administration followed by 30-35 days of withdrawal results in enhanced CP-AMPAR mediated transmission similar to LgA self-administration.
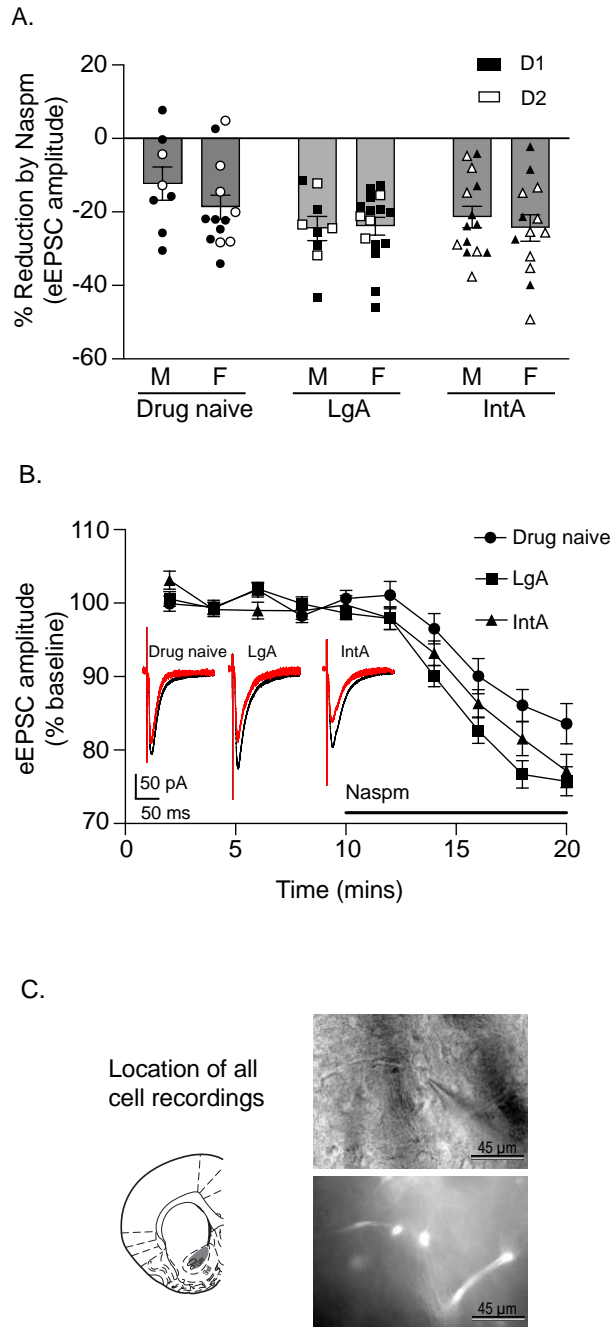
Figure 3.3 LgA or IntA followed by 30-35 days of withdrawal increase CP-AMPAR mediated transmission. A) The eEPSC amplitude displayed as a percent reduction by Naspm. Naspm decreased eEPSC amplitude similarly in D1- and D2-MSNs within group. Naspm decreased eEPSC amplitude similarly in males and females within group. B) Time course showing effects of bath application of CP-AMPAR antagonist Naspm in drug naïve, LgA, and IntA animals. Example traces before (black) and after (red) Naspm. C) Cartoon depiction of where recordings were made within the slice (shaded region) and example image of a Td-tomato positive cell under both fluorescent and brightfield conditions

Effects of the cycle on incubation and CP-AMPAR mediated transmission

Female cycle was monitored as described in the methods during self-administration, prior to incubation testing, and prior to slice preparation for electrophysiology studies. Cycle monitoring was not conducted during the withdrawal period. Due to unforeseen circumstances not all females tested for incubation have cycle data (withdrawal day: 1 N=11, 30 N=10, 45 N=11), therefore, we are unable to definitively determine effects of cycle on incubation. Trends in the data will be reported here. On withdrawal day 1, regardless of cycle, responding was low. There is an enhancement in responding at withdrawal day 30, which seems to be driven by females in proestrus (P) and estrus (E). However, the opposite is true at withdrawal day 45 where the enhancement in active nose-pokes is within the females in metestrus (M) and diestrus (D). At withdrawal day 45 females in P/E have similar responding to withdrawal day 1 (Fig 3.4A).

Cycle data collected from the females prior to electrophysiology studies is underpowered as well (naïve N=8 rats, 11 cells; LgA N=7 rats, 15 cells; IntA N=7 rats, 11cells), therefore, trends in the data will be reported. Consistent with the prior data including both males and females (Fig 3.3A) females show slight enhancements in Naspm sensitivity in LgA and IntA groups compared to the drug naïve group (Fig 3.4B). To our knowledge this is the first assessment of CP-AMPAR mediated transmission across the cycle. Drug naïve females seem to have similar Naspm sensitivity across cycle. LgA females in P/E may have a slight increase in Naspm sensitivity compared to females in M/D. Interestingly IntA seems to have the opposite trend, a slight reduction in CP-AMPAR mediated transmission in P/E compared to M/D (Fig 3.4B).
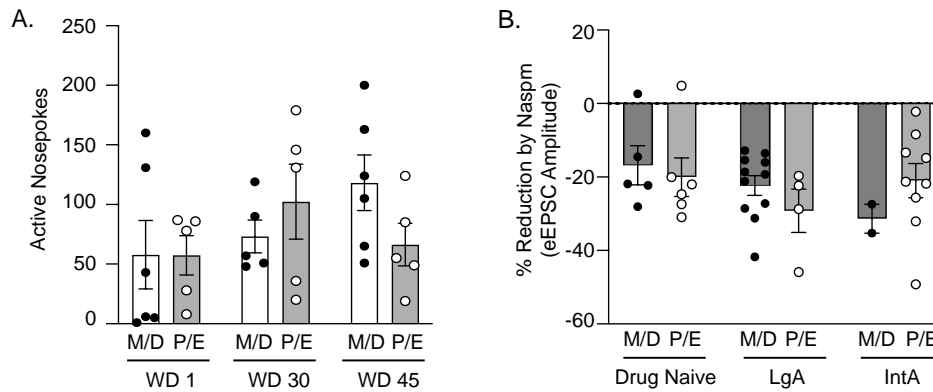
Figure 3.4 Effects of cycle on incubation and Naspm sensitivity. A) Active nose-pokes in females separated by cycle within testing day (1, 30 and 45). Incubation of craving is seen in females at withdrawal day 30 and 45 compared to day 1. Enhanced responding is seen within P/E compared to M/D at withdrawal day 30. This is reversed at withdrawal day 45 where enhanced responding is seen in M/D compared to P/E. B) The eEPSC amplitude displayed as a percent reduction by Naspm in females. CP-AMPAR mediated transmission is enhanced in LgA and IntA groups compared to drug naïve. Naspm sensitivity is slightly enhanced in P/E compared to M/D in the LgA group and P/E is reduced compared to M/D in the IntA group.

Discussion

We examined the effects of LgA and IntA cocaine self-administration on incubation and CP-AMPAR mediated transmission in the NAc core after withdrawal in male and female Drd1-Cre and A2a-Cre crossed with Td-tomato transgenic rats. Both LgA and IntA groups displayed escalation of drug intake (Fig 3.1E). Within group, IntA males and females escalated to a similar magnitude, whereas LgA males exhibited greater escalation than LgA females (Fig 3.1C). Furthermore, there were no sex differences in total drug intake within self-administration groups (Fig 3.1D). Some animals were used to assess incubation at withdrawal day 1, 30, and 45. Both LgA and IntA self-administration resulted in increased drug-seeking at withdrawal day 30 and 45 compared to day 1 (Fig 3.2A) with similar effects in males and females (Fig 3.2B). Lastly, we assessed the effects of LgA and IntA self-administration on CP-AMPAR mediated transmission. LgA and IntA groups show increased Naspm sensitivity compared to drug naïve animals (Fig 3.3A-B) with initial data suggesting similar effects in Drd1-Cre and A2a-Cre males and females (Fig 3.3A). Together, these data highlight that addiction-like behaviors such as incubation and associated increases in NAc CP-AMPAR mediated transmission can be achieved with much less drug consumption and a vastly different pattern of drug intake, which may have implications for how to best

model addiction to get a more comprehensive understanding of neurobiological changes associated with addiction.

Animals were trained to self-administer cocaine for 5 days. Under these conditions, animals learned to distinguish between the active and inactive nose-poke (Fig 3.1B). Following training, rats underwent LgA and IntA cocaine self-administration. Consistent with previous reports (Algallal et al., 2020), the LgA group consumed more cocaine than the IntA group (Fig 3.1C-D). This is not surprising given that LgA animals receive 6 hours of continuous cocaine access and IntA animals only have a total of 1 hour of drug availability spaced out across 6 hours. Perhaps what is more striking given the total drug consumption differences is that both LgA and IntA groups demonstrated clear escalation of drug intake (Fig 3.1E). Our finding here is in line with previous reports of escalation of intake following both LgA and IntA self-administration experience (Ahmed and Koob, 1998; Ahmed and Koob, 1999; Ferrario et al., 2005; Allain and Samaha, 2019). Prior studies established that females escalate their drug intake to a greater extent than males under LgA and IntA self-administration conditions (Roth and Carroll, 2004; Kawa et al., 2019). Unexpectantly, we did not see any sex differences in escalation under IntA conditions and found the opposite in LgA conditions, where males increased cocaine consumption to a greater extent than females. Although this was surprising this was not completely unprecedented. For example, Algallal et al. (2019) report similar magnitude of escalation in males and females following IntA. Additionally, previous studies indicate that LgA conditions resulted in similar escalation between males and females (Nicolas et al., 2019). The reason for the differences remains unclear, however, this may be due to procedural differences such as dose, number of training days, or duration of sessions. Additionally, this may due to cycle effects in females (discussed further below).

Successful completion of LgA or IntA self-administration allowed for us to assess within subject incubation of craving at withdrawal day 1, 30, and 45. These time points were chosen because previous studies have shown that following LgA cocaine self-administration, drug-seeking is low at withdrawal day 1 and is elevated at 30 and 45 (Lu et al., 2004). However, to date assessment of drug-seeking following IntA conditions had only been done at withdrawal day 1 and 28-30 (Nicolas et al., 2019; Alonso et al.,

2022), so it was unknown if this effect is as long lasting as LgA. As expected, both groups show enhanced drug-seeking behavior from withdrawal day 1 to 30 (Fig 3.2). Surprisingly, unlike previous studies directly comparing the effects LgA and IntA cocaine self-administration on incubation of craving, we did not see an enhancement in incubation within the IntA group compared to LgA group (Nicolas et al., 2019; Nicolas et al., 2022). This may be due to procedural differences such as dose used. Nicolas et al (2019, 2022) used a higher dose (0.75mg/kg) than what was used in the current study (0.40mg/kg). Additionally, training sessions were 2 hours for 7 days (Nicolas et al. 2019; 2022) as compared to 1 hour for 5 days (studies done here). Together these procedural differences may account for the difference in magnitude of incubation.

To our knowledge, for the first time these studies directly compare LgA and IntA self-administration on incubation of craving at withdrawal day 45, where we see similar enhancement in drug-seeking in LgA and IntA animals (Fig 3.2). Together, this indicates that both LgA and IntA result in long-lasting incubation of craving. Importantly, under LgA conditions incubation of craving has consistently been shown to coincide with an upregulation of both GluA1 subunit expression and CP-AMPAR mediated transmission (Conrad et al., 2008; Ferrario et al., 2011; McCutcheon et al., 2011; Loweth et al., 2014). Furthermore, studies indicate that CP-AMPARs in the NAc core mediate incubation of craving behavior (Conrad et al., 2008; Loweth et al., 2014; Kawa et al., 2022). This would suggest that under IntA conditions we would see similar enhancements in this aspect of glutamate transmission.

With that in mind we went on to assess CP-AMPAR mediated transmission after LgA and IntA self-administration followed by 30-35 days of withdrawal. Our studies here indicate that LgA self-administration followed by withdrawal increases CP-AMPAR mediated transmission compared to drug naïve animals (Fig 3.3A-B), consistent with previous findings (Conrad et al., 2008; Purgianto et al., 2013; Loweth et al., 2014; Kawa et al., 2022). To our knowledge, results here are the first to demonstrate that IntA self-administration followed by withdrawal increased Naspm sensitivity compared to drug naïve animals and that the magnitude of Naspm reduction is comparable to the LgA group (Fig 3.3A-B). Currently the consensus in the field is that continuous LgA cocaine self-administration followed by prolonged withdrawal is necessary for the upregulation of

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CP-AMPARs in the NAc given that ShA cocaine self-administration and experimenter administered cocaine do not result in similar enhancements (McCutcheon et al., 2011; Purgianto et al., 2013; Catalfio et al., 2023). However, we demonstrate here that IntA conditions upregulate CP-AMPARs, therefore enhancements in CP-AMPARs is not limited to continuous cocaine self-administration or total cocaine intake. Lastly, this would suggest that blocking CP-AMPARs in the NAc after IntA self-administration will attenuate drug-seeking behavior during prolonged withdrawal, however, future studies will be needed to directly assess this.

In the NAc, MSNs primarily form two distinct populations based off the type of dopamine receptor present, D1- or D2-containing MSNs. D1 and D2 receptors have been demonstrated to have opposing roles in drug-seeking behavior (Lobo and Nestler, 2011; Kravitz et al., 2012; Pascoli et al., 2014; Allichon et al., 2021). For example, global knockout of D1 receptors reduces the willingness of an animal to self-administer cocaine (Caine et al., 2007), whereas global knockout of D2 receptors increases self-administration of cocaine (Caine et al., 2002). Interestingly, these distinct cell populations also exhibit opposing cocaine-induced effects. For example, experimenter administered cocaine and withdrawal results in reduced membrane excitability in D1-MSNs, increased frequency of miniature excitatory post synaptic currents (mEPSC), and decreased frequency of inhibitory post synaptic currents (mIPSC), while D2-MSNs exhibit decreased frequency of mEPSC and no changes in mIPSCs (Kim et al., 2011). Cell-type differences are also seen following self-administration. For example, studies in the NAc shell indicate ShA and LgA cocaine self-administration followed by 30 days of withdrawal results in increased CP-AMPAR mediated transmission specifically in D1-MSNs in male mice (Pascoli et al., 2014; Terrier et al., 2016).

Importantly, studies investigating the neural mechanisms of incubation have determined CP-AMPAR upregulated in the NAc core mediates incubation of craving in male and female rats (Conrad et al., 2008; Loweth et al., 2014; Kawa et al., 2022), but currently no cell-type specific changes in CP-AMPAR mediated transmission in the NAc core have been investigated. Therefore, we wanted to assess potential differences in CP-AMPAR mediated transmission in D1- and D2-MSNs in the NAc core after LgA and IntA. As previously mentioned, targeting of the A2a promotor was selected over the D2

promotor because A2a receptors are selectively expressed in MSNs, however, D2 receptors are found both pre and post-synaptically as well as on other striatal cells. Interestingly, preliminary data indicate a similar effect of LgA and IntA self-administration on CP-AMPAR mediated transmission in both cell-types (Fig 3.3A).

To date, one study has investigated glutamate transmission after LgA cocaine self-administration followed by 30 days of withdrawal in females. Consistent with that study (Kawa et al., 2022), our data indicate that CP-AMPAR mediated transmission following withdrawal from LgA self-administration is similar in males and females. We next sought to assess potential sex differences in IntA. Interestingly, similar to LgA, we found no effect of sex on Naspm sensitivity in IntA animals (Fig 3.3A). Since, we saw no effect of sex on self-administration or drug-seeking behavior it may be unsurprising there were no differences in CP-AMPAR mediated transmission within group. However, it is important to note we may be missing some effects of cell-type because of the number of cells per group. This is particularly relevant if there are sex by cell-type interactions, which we are underpowered to fully evaluate.

Female cycle has been shown to alter NAc MSN function and glutamate transmission in drug naïve animals (Proaño et al., 2018; Alonso-Caraballo and Ferrario, 2019; Proaño and Meitzen, 2020). For example, NAc mEPSC frequency is similar in males and females when recordings are made from females in the diestrus phase of the cycle, but is enhanced in females compared to males when recordings are made from females in proestrus or estrus. However, currently no studies have investigated the effects of cycle on CP-AMPARs. Studies done here indicate that there may be an interaction with cocaine self-administration and cycle (Fig 3.4B) such that there are enhancements in CP-AMPAR mediated transmission in P/E following LgA conditions and withdrawal and the opposite effects following IntA conditions. Although this data is currently underpowered, this would suggest that the self-administration pattern (continuous access vs intermittent access) may influence patterns of trafficking CP-AMPARs in and out of the synapse. Future studies should be done to confirm or refute these preliminary findings.

As stated in the introduction, many previous studies have shown clear sex differences in cocaine self-administration behaviors. For example females more readily

acquire cocaine self-administration (Lynch and Carroll, 1999; Hu et al., 2004; Algallal et al., 2020), tend to self-administer more cocaine (Roberts et al., 1989; Roth and Carroll, 2004), show greater escalation (Kawa and Robinson, 2019; Algallal et al., 2020), and drug-seeking behavior (Kerstetter et al., 2008; Nicolas et al., 2019) than males. Therefore, we assessed whether rats in our studies show distinct sex-differences across self-administration and incubation. Interestingly, we see no effect of sex on cocaine acquisition, total drug consumption, or incubation of craving. Surprisingly in the LgA group we see greater escalation in males than females, however, we see no effect of sex on escalation in the IntA group. Although, this was surprising given the extensive literature that indicates females in particular show greater enhancements in drug-taking and seeking behavior, there are studies that also show no sex differences in drug-taking behavior. For example, there are previous reports of no sex differences in training (Kawa et al., 2019; Nicolas et al., 2019; Carr et al., 2020; Kawa et al., 2022), cocaine self-administration (Nicolas et al., 2019; Kawa et al., 2022), and incubation of cocaine craving (Kawa et al., 2022). This suggests that sex differences may be nuanced and experimental design may play a role in the emergence of these differences between sex.

The studies mentioned above use a wide variety of ways to train the animals to self-administer cocaine including, but not limited to, food training prior to cocaine self-administration and set infusion criteria before moving onto LgA or IntA. It is also worth noting that these studies use different strains of rats (Wistar, Sprague Dawley) than what we used here (Long Evans). Therefore, it is possible that there are slight differences among the varying strains. Lastly, previous studies that investigated sex differences in acquisition, drug intake, and drug-seeking behavior indicate that females, specifically in estrus, drive the sex differences seen. For example, incubation of craving is similar in females in metestrus, diestrus and proestrus to males, but females in estrus display a greater magnitude of incubation of craving at withdrawal day 15 and 48 (Kerstetter et al., 2008; Nicolas et al., 2019; Corbett et al., 2021). Our studies here did not have enough females in estrus during incubation testing for definitive results (Fig 3.4A), but this may be why currently we see no effects of sex on incubation.

Conclusions

These studies set out to compare and contrast incubation of drug-seeking and NAc core CP-AMPAR mediated transmission following withdrawal from LgA and IntA cocaine self-administration. Here we show that LgA and IntA groups display similar incubation of craving at withdrawal day 30 and 45 even though total drug consumption was vastly different. Furthermore, CP-AMPAR mediated transmission is enhanced similarly in LgA and IntA groups compared to the drug naïve group. Our data here indicate that prolonged cocaine access is not a necessary feature for the development of incubation of craving or coinciding upregulation of CP-AMPAR mediated transmission. This suggests that the pharmacokinetics and the pattern of drug consumption, not just total intake, can drive the recruitment of CP-AMPARs. This has important implications for how we model addiction and for the potential mechanism of the development and persistence of addiction-like behaviors.

Supplemental Figure

A.

B.

LgA

C.

IntA

D.
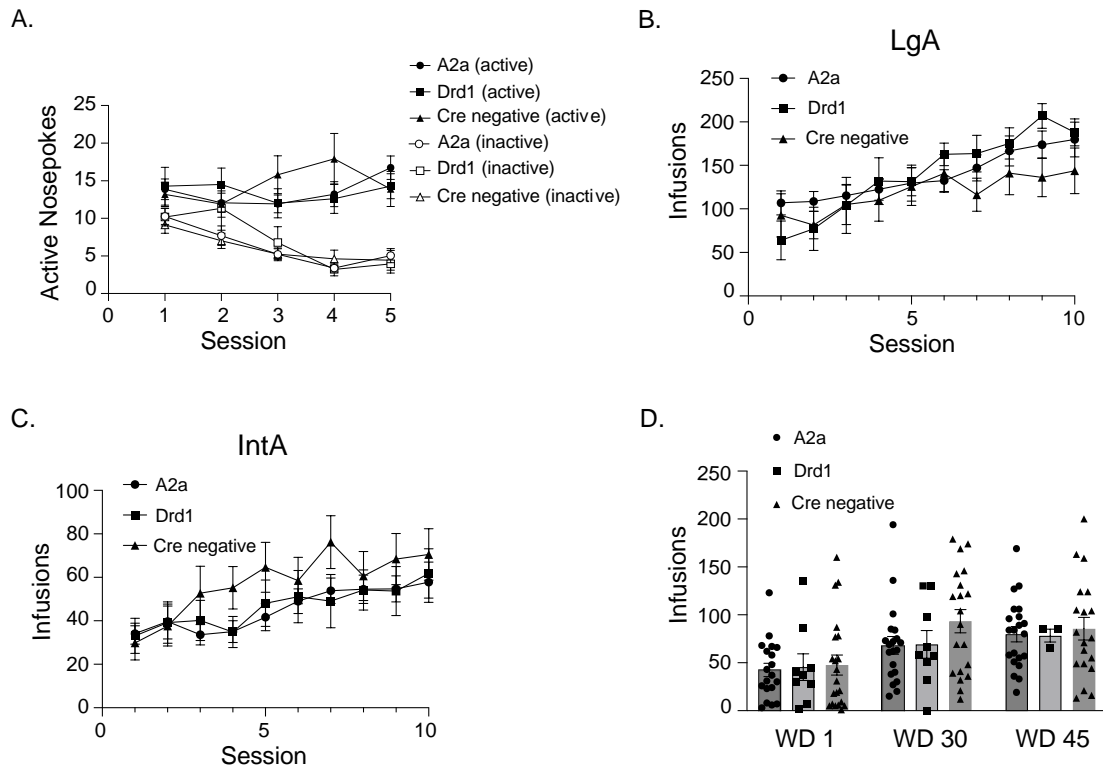
Figure 3.5 Genotype did not have any effect on self-administration behavior or incubation. Animals distinguished between active and inactive nose-pokes. The number of active and inactive nose-pokes did not differ between genotype. B-C) The number of infusions across sessions for LgA (B) and IntA (C). Animals increased infusions across sessions and there was no effect of genotype. D) There are no effect of genotype on drug-seeking behavior after protracted withdrawal. Animals increase the number of active nose-pokes from WD1 to WD30 and WD45.

References

Ahmed SH, Koob GF (1998) Transition from moderate to excessive drug intake: change in hedonic set point. Science 282:298-300.

Ahmed SH, Koob GF (1999) Long-lasting increase in the set point for cocaine self-administration after escalation in rats. Psychopharmacology 146:303-312.

Ahmed SH, Kenny PJ (2011) Cracking the molecular code of cocaine addiction. Ilar j 52:309-320.

Alcantara AA, Chen V, Herring BE, Mendenhall JM, Berlanga ML (2003) Localization of dopamine D2 receptors on cholinergic interneurons of the dorsal striatum and nucleus accumbens of the rat. Brain research 986:22-29.

Algallal H, Allain F, Ndiaye NA, Samaha AN (2020) Sex differences in cocaine self-administration behaviour under long access versus intermittent access conditions. Addict Biol 25:e12809.

Allain F, Samaha AN (2019) Revisiting long-access versus short-access cocaine self-administration in rats: intermittent intake promotes addiction symptoms independent of session length. Addict Biol 24:641-651.

Allichon MC, Ortiz V, Pousinha P, Andrianarivelo A, Petitbon A, Heck N, Trifilieff P, Barik J, Vanhoutte P (2021) Cell-Type-Specific Adaptions in Striatal Medium-Sized Spiny Neurons and Their Roles in Behavioral Responses to Drugs of Abuse. Front Synaptic Neurosci 13:799274.

Alonso IP, O'Connor BM, Bryant KG, Mandalaywala RK, España RA (2022) Incubation of cocaine craving coincides with changes in dopamine terminal neurotransmission. Addict Neurosci 3.

Alonso-Caraballo Y, Ferrario CR (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. Horm Behav 116:104583.

ASAMI T, KAGECHIKA H, HASHIMOTO Y, SHUDO K, MIWA A, KAWAI N, NAKAJIMA T (1989) Acylpolyamines mimic the action of joro spider toxin (JSTX) on crustacean muscle glutamate receptors. Biomedical research 10:185-189.

Caine SB, Negus SS, Mello NK, Patel S, Bristow L, Kulagowski J, Vallone D, Saiardi A, Borrelli E (2002) Role of dopamine D2-like receptors in cocaine self-administration: studies with D2 receptor mutant mice and novel D2 receptor antagonists. J Neurosci 22:2977-2988.

Caine SB, Thomsen M, Gabriel KI, Berkowitz JS, Gold LH, Koob GF, Tonegawa S, Zhang J, Xu M (2007) Lack of self-administration of cocaine in dopamine D1 receptor knock-out mice. J Neurosci 27:13140-13150.

Carr CC, Ferrario CR, Robinson TE (2020) Intermittent access cocaine self-administration produces psychomotor sensitization: effects of withdrawal, sex and cross-sensitization. Psychopharmacology (Berl) 237:1795-1812.

Catalfio AM, Fetterly TL, Nieto AM, Robinson TE, Ferrario CR (2023) Cocaine-induced sensitization and glutamate plasticity in the nucleus accumbens core: effects of sex. Biology of Sex Differences 14:41.

Cohen P, Sas A (1994) Cocaine use in Amsterdam in non deviant subcultures. Addiction Research 2:71-94.

Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, Marinelli M, Wolf ME (2008) Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature 454:118-121.

Corbett CM, Dunn E, Loweth JA (2021) Effects of sex and estrous cycle on the time course of incubation of cue-induced craving following extended-access cocaine self-administration. ENeuro 8.

Deroche-Gamonet V, Belin D, Piazza PV (2004) Evidence for addiction-like behavior in the rat. Science 305:1014-1017.

Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE (2005) Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. Biol Psychiatry 58:751-759.

Ferrario CR, Loweth JA, Milovanovic M, Ford KA, Galiñanes GL, Heng LJ, Tseng KY, Wolf ME (2011) Alterations in AMPA receptor subunits and TARPs in the rat nucleus accumbens related to the formation of Ca^{2+}-permeable AMPA receptors during the incubation of cocaine craving. Neuropharmacology 61:1141-1151.

Fetterly TL, Oginsky MF, Nieto AM, Alonso-Caraballo Y, Santana-Rodriguez Z, Ferrario CR (2021) Insulin Bidirectionally Alters NAc Glutamatergic Transmission: Interactions between Insulin Receptor Activation, Endogenous Opioids, and Glutamate Release. J Neurosci 41:2360-2372.

Hao Y, Martin-Fardon R, Weiss F (2010) Behavioral and functional evidence of metabotropic glutamate receptor 2/3 and metabotropic glutamate receptor 5 dysregulation in cocaine-escalated rats: factor in the transition to dependence. Biol Psychiatry 68:240-248.

Hu M, Crombag HS, Robinson TE, Becker JB (2004) Biological basis of sex differences in the propensity to self-administer cocaine. Neuropsychopharmacology 29:81-85.

Hunt WA, Barnett LW, Branch LG (1971) Relapse rates in addiction programs. Journal of clinical psychology.

Kawa AB, Robinson TE (2019) Sex differences in incentive-sensitization produced by intermittent access cocaine self-administration. Psychopharmacology (Berl) 236:625-639.

Kawa AB, Bentzley BS, Robinson TE (2016) Less is more: prolonged intermittent access cocaine self-administration produces incentive-sensitization and addiction-like behavior. Psychopharmacology (Berl) 233:3587-3602.

Kawa AB, Allain F, Robinson TE, Samaha AN (2019) The transition to cocaine addiction: the importance of pharmacokinetics for preclinical models. Psychopharmacology (Berl) 236:1145-1157.

Kawa AB, Hwang E-K, Funke JR, Zhou H, Costa-Mattioli M, Wolf ME (2022) Positive allosteric modulation of mGlu1 reverses cocaine-induced behavioral and synaptic plasticity through the integrated stress response and oligophrenin-1. Biological Psychiatry 92:871-879.

Kerstetter KA, Aguilar VR, Parrish AB, Kippin TE (2008) Protracted time-dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. Psychopharmacology (Berl) 198:63-75.

Kim J, Park BH, Lee JH, Park SK, Kim JH (2011) Cell type-specific alterations in the nucleus accumbens by repeated exposures to cocaine. Biol Psychiatry 69:1026-1034.

Koike M, Iino M, Ozawa S (1997) Blocking effect of 1-naphthyl acetyl spermine on Ca(2+)-permeable AMPA receptors in cultured rat hippocampal neurons. Neurosci Res 29:27-36.

Kravitz AV, Tye LD, Kreitzer AC (2012) Distinct roles for direct and indirect pathway striatal neurons in reinforcement. Nat Neurosci 15:816-818.

Lobo MK, Nestler EJ (2011) The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons. Front Neuroanat 5:41.

Loweth JA, Scheyer AF, Milovanovic M, LaCrosse AL, Flores-Barrera E, Werner CT, Li X, Ford KA, Le T, Olive MF, Szumlinski KK, Tseng KY, Wolf ME (2014) Synaptic depression via mGluR1 positive allosteric modulation suppresses cue-induced cocaine craving. Nat Neurosci 17:73-80.

Lu L, Grimm JW, Hope BT, Shaham Y (2004) Incubation of cocaine craving after withdrawal: a review of preclinical data. Neuropharmacology 47 Suppl 1:214-226.

Lynch WJ, Carroll ME (1999) Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology (Berl) 144:77-82.

McCutcheon JE, Wang X, Tseng KY, Wolf ME, 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. J Neurosci 31:5737-5743.

Nicolas C, Russell TI, Shaham Y, Ikemoto S (2022) Dissociation Between Incubation of Cocaine Craving and Anxiety-Related Behaviors After Continuous and Intermittent Access Self-Administration. Frontiers in neuroscience 15:824741.

Nicolas C, Russell TI, Pierce AF, Maldera S, Holley A, You ZB, McCarthy MM, Shaham Y, Ikemoto S (2019) Incubation of Cocaine Craving After Intermittent-Access Self-administration: Sex Differences and Estrous Cycle. Biol Psychiatry 85:915-924.

Nieto AM, Catalfio AM, Papacostas Quintanilla H, Alonso-Caraballo Y, Ferrario CR (2023) Transient effects of junk food on NAc core MSN excitability and glutamatergic transmission in obesity-prone female rats. Obesity 31:434-445.

Oginsky MF, Goforth PB, Nobile CW, Lopez-Santiago LF, Ferrario CR (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 41:2977-2986.

Pascoli V, Terrier J, Espallergues J, Valjent E, O'Connor EC, Lüscher C (2014) Contrasting forms of cocaine-evoked plasticity control components of relapse. Nature 509:459-464.

Paterson NE, Markou A (2003) Increased motivation for self-administered cocaine after escalated cocaine intake. Neuroreport 14:2229-2232.

Pettibone JR, Yu JY, Derman RC, Faust TW, Hughes ED, Filipiak WE, Saunders TL, Ferrario CR, Berke JD (2019) Knock-In Rat Lines with Cre Recombinase at the Dopamine D1 and Adenosine 2a Receptor Loci. eNeuro 6.

Proaño SB, Meitzen J (2020) Estradiol decreases medium spiny neuron excitability in female rat nucleus accumbens core. Journal of neurophysiology 123:2465-2475.

Proaño SB, Morris HJ, Kunz LM, Dorris DM, 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 120:1356-1373.

Purgianto A, Weinfeld ME, Wolf ME (2017) Prolonged withdrawal from cocaine self-administration affects prefrontal cortex- and basolateral amygdala-nucleus accumbens core circuits but not accumbens GABAergic local interneurons. Addict Biol 22:1682-1694.

Purgianto A, Scheyer AF, Loweth JA, Ford KA, Tseng KY, Wolf ME (2013) Different adaptations in AMPA receptor transmission in the nucleus accumbens after short vs long access cocaine self-administration regimens. Neuropsychopharmacology 38:1789-1797.

Roberts DC, Loh EA, Vickers G (1989) Self-administration of cocaine on a progressive ratio schedule in rats: dose-response relationship and effect of haloperidol pretreatment. Psychopharmacology 97:535-538.

Roth ME, Carroll ME (2004) Sex differences in the escalation of intravenous cocaine intake following long- or short-access to cocaine self-administration. Pharmacol Biochem Behav 78:199-207.

Samaha AN, Khoo SY, Ferrario CR, Robinson TE (2021) Dopamine 'ups and downs' in addiction revisited. Trends Neurosci 44:516-526.

Simon SL, Richardson K, Dacey J, Glynn S, Domier CP, Rawson RA, Ling W (2001) A comparison of patterns of methamphetamine and cocaine use. Journal of addictive diseases 21:35-44.

Sorge RE, Stewart J (2005) The contribution of drug history and time since termination of drug taking to footshock stress-induced cocaine seeking in rats. Psychopharmacology 183:210-217.

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 41:1779-1789.

Vanderschuren LJ, Everitt BJ (2004) Drug seeking becomes compulsive after prolonged cocaine self-administration. Science 305:1017-1019.

Ward AS, Haney M, Fischman MW, Foltin RW (1997) Binge cocaine self-administration in humans: intravenous cocaine. Psychopharmacology 132:375-381.

Wasserstein RL, Lazar NA (2016) The ASA statement on p-values: context, process, and purpose. In, pp 129-133: Taylor & Francis.

Zimmer BA, Dobrin CV, Roberts D (2011) Brain-cocaine concentrations determine the dose self-administered by rats on a novel behaviorally dependent dosing schedule. Neuropsychopharmacology 36:2741-2749.

Zimmer BA, Oleson EB, Roberts DC (2012) The motivation to self-administer is increased after a history of spiking brain levels of cocaine. Neuropsychopharmacology 37:1901-1910.

Chapter 4: Transient Effects of Junk-Food on NAc core MSN Excitability and Glutamatergic Transmission in Obesity Prone Female Rats

Abstract

The nucleus accumbens (NAc) plays critical roles in eating and food-seeking in rodents and humans. Diets high in fats and sugars ('junk-food') produce persistent increases in NAc function in male obesity-prone rats. Here we examine effects of junk-food and junk-food deprivation on NAc core medium spiny neuron (MSN) excitability and glutamate transmission in females. Obesity-prone female rats were given access to ad lib junk-food for 10 days and recordings were made from MSNs in the NAc core immediately or after a short (27-72 hour) or long (14-16 day) junk-food deprivation period in which rats were returned to ad lib standard chow. Controls remained on chow throughout. Whole-cell slice electrophysiology was used to examine MSN intrinsic membrane and firing properties, and glutamatergic transmission. We found that intrinsic excitability is reduced while glutamatergic transmission is enhanced after the short, but not long, junk-food deprivation period. A brief junk-food deprivation period was necessary for increases in NAc CP-AMPAR transmission and sEPSC frequency. This study reveals that females are protected from long-lasting effects of sugary, fatty foods on MSN neuronal function and provides evidence for sex specific effects on plasticity in brain centers that influence food-seeking and feeding behavior.

Introduction

The nucleus accumbens (NAc) plays critical roles in eating and food-seeking. For example, NAc activity is required for cue-triggered food-seeking in non-obese rats and involves both dopamine and glutamate transmission (Setlow et al., 2002; Kelley, 2004; Balleine, 2005). In men and women, the magnitude of NAc activation in response to

food cues corresponds to future weight gain (Demos et al., 2012), and this activation is stronger in individuals with obesity (Stoeckel et al., 2008; Jensen and Kirwan, 2015). Thus, recent preclinical studies examining the neurobiology of obesity and over-eating have focused on diet-induced alterations in NAc function within populations that are obesity-prone or obesity-resistant, to best model human obesity susceptibility (Alonso-Caraballo et al., 2018; Ferrario, 2020). However, the majority of these studies have used males, despite established roles of ovarian hormones in feeding and energy expenditure (Palmer and Clegg, 2015) and mounting evidence that neural mechanisms underlying seemingly similar behaviors differ by sex (Tronson, 2018).

The NAc is comprised predominantly of medium spiny projection neurons (MSNs). The activity of these cells is influenced by their intrinsic properties and by ongoing neurotransmission. Within MSNs, inwardly-rectifying potassium currents help maintain a hyperpolarized state, while fast transient potassium currents influence action potential firing following depolarization (Nisenbaum and Wilson, 1995). Dopamine receptor activation bi-directionally modulates these intrinsic properties (Perez et al., 2006; Zhao et al., 2016), and can indirectly influence glutamatergic transmission (Surmeier et al., 2007; Planert et al., 2013; Cao et al., 2018). AMPA type glutamate receptors (AMPARs) provide the main source of excitation to the NAc. Disruption of AMPAR synaptic trafficking blocks cue-triggered motivation for sucrose in non-obese mice (Crombag et al., 2008) as does pharmacological AMPAR or calcium-permeable AMPARs (CP-AMPAR) blockade within the NAc core (Di Ciano et al., 2001; Derman and Ferrario, 2018a). Thus, food-seeking behaviors are influenced by MSN intrinsic properties and excitatory drive to the NAc.

Eating diets high in fats and sugars (i.e., junk-food) alters NAc core function, and these effects are more pronounced in obesity-prone rats, which model at-risk human populations (Alonso-Caraballo et al., 2018; Ferrario, 2020). For example, eating a junk-food diet reduces MSN intrinsic excitability in obesity-prone, but not obesity-resistant males (Oginsky and Ferrario, 2019). In addition, junk-food increases NAc CP-AMPAR transmission in obesity-prone, but not obesity-resistant males (Oginsky et al., 2016; Alonso-Caraballo et al., 2021). This increase requires a junk-food free period (24 hours) and persists for at least 14 days after junk-food removal (Alonso-Caraballo et al., 2021).

In contrast, the same diet regimen and 2-week junk-food deprivation period does not alter NAc core CP-AMPAR transmission in obesity-prone females (Alonso-Caraballo et al., 2021). However, shorter time points following junk-food deprivation were not examined in females, and prior studies did not examine effects of junk-food alone vs junk-food followed by deprivation in females.

In the present study, females were given free access to junk-food or chow for 10 days. Recordings were then made with and without a period of junk-food deprivation (24-48 hrs or 14-16 days). This was done to determine persistence of junk-food effects, and to establish whether removal of junk-food is needed for NAc plasticity. We found reductions in intrinsic excitability and increases in NAc glutamatergic transmission following the short junk-food deprivation period (24-48 hrs), but these effects did not persist following the long deprivation period (14-16 days). In addition, junk-food deprivation was required for increased CP-AMPAR transmission, but not for enhancements in sEPSC amplitude. As a whole, the data suggest that while the general pattern of junk-food and junk-food deprivation effects are somewhat similar across sex, in females these effects are transient and return to levels comparable to chow after long-term junk-food removal.

Materials and Methods

Subjects

Adult female selectively-bred obesity-prone (OP) rats (Levin et al., 1997) bred in house were used for all studies. Rats were ~55 days old at the start of the experiment, housed on a reverse 12-h light/dark cycle (lights off at 0800), had free access to water and food, and were group housed unless otherwise noted. Procedures were approved by The University of Michigan Committee on the Use and Care of Animals in accordance with AAALAC and AVMA guidelines.

Diet Manipulation:

The junk-food diet consisted of a mash of Ruffles™ potato chips (40 g), Chips

Ahoy!™ chocolate chip cookies (130 g), Nesquik™ chocolate powder (130 g), Jiff™ peanut butter (130 g), powdered Lab Diet 5001 (200 g) and 180 ml of water (19.6% fat, 14% protein, and 58% carbohydrates; 4.5 kcal/g) and was made in house (Oginsky et al., 2016). Body weight and food intake were measured daily. Rats were maintained on this diet for 10 days, after which recordings were made immediately, or junk-food was removed and replaced with standard lab chow (i.e. junk-food deprivation; Lab Diet 5001: 4 kcal/g; 4.5% fat, 23% protein, 48.7% carbohydrates; % of caloric content) for either 14-16 days or 24-72 hours. Thus only one type of food was available during each phase. Controls remained on standard chow throughout. This timing was chosen to determine persistence of junk-food effects, and to establish whether removal of junk-food is needed for NAc plasticity. To maintain feasibility of whole-cell recordings, separate cohorts were used for each time point after junk-food exposure and groups were counterbalanced for starting weight.

Cycle Monitoring

Estrous cycle phase was determined by daily observations of vaginal epithelial cell cytology, precopulatory, and copulatory behaviors (Marcondes et al., 2001; Alonso-Caraballo and Ferrario, 2019). Epithelial cells were collected by vaginal lavage (1-2 hours after the start of the dark phase) and visualized using an inverted light microscope (Olympus CKX53) under bright-field. Recordings were made during the metestrus/diestrus phase of the cycle unless otherwise noted. These phases were chosen because this is when motivation for food, food intake, and cue-triggered food-seeking are highest in females (Asarian and Geary, 2006; Palmer and Clegg, 2015; Alonso-Caraballo and Ferrario, 2019).

Whole-cell patch clamp recordings

Established whole-cell patch clamping approaches were used (Alonso-Caraballo and Ferrario, 2019; Oginsky and Ferrario, 2019; Alonso-Caraballo et al., 2021). Briefly, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), brains were removed and

placed in ice-cold oxygenated (95% O_2–5% CO_2) aCSF containing (in mM): 125 NaCl, 25 NaHCO3, 12.5 glucose, 1.25 NaH2PO4, 3.5 KCl, 1 L-ascorbic acid, 0.5 CaCl2, 3 MgCl2, pH 7.45, 300-305 mOsm. Coronal slices (300 µm) containing the NAc were made on a vibratome (Leica Biosystems, Buffalo Grove, IL, USA). Slices were allowed to recover in oxygenated aCSF (30 min, 37 °C), and then maintained at room temperature (30 min) prior to recording. For the recording aCSF, CaCl2 was 2.5 mM and MgCl2 was 1 mM. All recordings were conducted in the presence of the GABA$_A$ receptor antagonist, picrotoxin (50 µM). For recordings of intrinsic properties and sEPSCs pipettes were filled with a solution containing (in mM): 130 K-gluconate, 10 KCl, 1 EGTA, 2 Mg2+-ATP, 0.6 Na+-GTP and 10 HEPES, pH 7.45, 285 mOsm. MSNs were identified based on their hyperpolarized resting membrane potential and distinct firing pattern in response to square pulse current injections (-200 to +400pA, 500ms). Current/voltage (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 required to elicit an action potential. Spontaneous excitatory post-synaptic currents (sEPSCs) were recorded at a holding potential of -70mV (5 min). For recordings of CP-AMPARs, pipettes were filled with (in mM): 140 CsCl, 10 HEPES, 2 MgCl2, 5 Na+-ATP, 0.6 Na+-GTP, 2 QX314, pH 7.3, 285 mOsm for evoked responses. Evoked EPSCs (eEPSCs) were elicited by local stimulation (0.02 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 <20% variability in amplitude was used. If >0.30 mA was required, the neuron was discarded. eEPSCs were recorded at -70 mV before and after application of the CP-AMPAR selective antagonist Naspm (200 µM) (Oginsky and Ferrario, 2019; Alonso-Caraballo et al., 2021). For all data analysis, only cells with an access resistance of less than 30 MΩ were used. Cell parameters (capacitance and membrane resistance) were recorded at the start and end of data collection and only cells with less than a 20% change across time were included in analyses.

Recordings alternated between slices from rats in the chow and junk-food group each day. Intrinsic membrane properties and action potentials, and sEPSC were measured on day 14-16 of junk-food deprivation. Intrinsic membrane properties and action potentials, sEPSCs and CP-AMPAR-mediated transmission were measured after 24-72 hours of junk-food deprivation, and sEPSCs and CP-AMPAR-mediated transmission were measured without junk-food deprivation.

Analysis and Statistics

Evoked responses and intrinsic excitability data were analyzed using Clampfit 10.7 (Molecular Devices). sEPSCs were analyzed using MiniAnalysis (Synaptosoft V.6.0.7) and verified by hand. Comparisons were made between data collected within the same cohort of animals (i.e., given chow or junk-food side by side). Two-tailed t-tests, Mann-Whitney U Tests, Mixed-effects models, and one- or two-way ANOVAs with Sidak's post-hoc comparisons were used (Prism 9, GraphPad, San Diego, CA). Interpretation of p-values is based on guidelines set forth by the American Statistical Association (Wasserstein and Lazar, 2016). Experimenters were not blind to grouping during data acquisition but were during analysis. All final Ns are reported in the results and based on expected effect size and variance of our primary measures.

Results

We first assessed persistent effects of junk-food and subsequent deprivation on MSN intrinsic excitability and glutamatergic transmission. Rats were given junk-food for 10 days followed by a 14-16-day return to standard lab chow (i.e., "deprivation"), or standard chow throughout prior to whole cell patch clamp recordings (see timeline, Fig 4.1A).

During junk-food exposure, obesity-prone females gained more weight than chow controls (Fig 4.1B: two-way RM ANOVA, time x diet interaction $F_{(10,100)}=5.13$, $p<0.0001$, Sidak's multiple comparison's test: days 6-10: $p<0.05$). However, by the recording day, the weights of chow- and junk-food groups were comparable (Mann-Whitney U Test:

U=8, p=0.35; data not shown). This occurred because although rats in the junk-food group ate more when junk-food was available, they ate significantly less chow during the deprivation period than controls (Fig 4.1C,D; C: two-way RM ANOVA group x period interaction: $F_{(19,190)}=4.88$, p<0.0001; Sidak's multiple comparison's test: day 11: p<0.01; D: two-way RM ANOVA group x period interaction: $F_{(1,10)}=71.37$, p<0.0001; Sidak's multiple comparison's test Chow vs JF: during diet exposure, p=0.07; after deprivation period: p=0.02).

When recordings were made on deprivation day 14-16 (Chow: 14 cells from 3 rats, JF: 15 cells from 4 rats), there were no differences in membrane response to current injections (Fig 4.1E: two-way RM ANOVA: $F_{(16,432)}=0.55$, p=0.92), input resistance (Fig 4.1F: two-tailed unpaired t-test: $t_{(27)}=0.97$, p=0.34), or rheobase (Fig 4.1G: two-tailed unpaired t-test: $t_{(27)}=0.08$, p=0.93) between cells from chow and junk-food rats. While there were not strong effects on MSN firing, there was a small but significant reduction in the number of action potentials fired in response to higher current injections in cells from rats given junk-food vs chow (Fig 4.1H: two-way RM ANOVA: $F_{(16,432)}=2.02$, p=0.01).
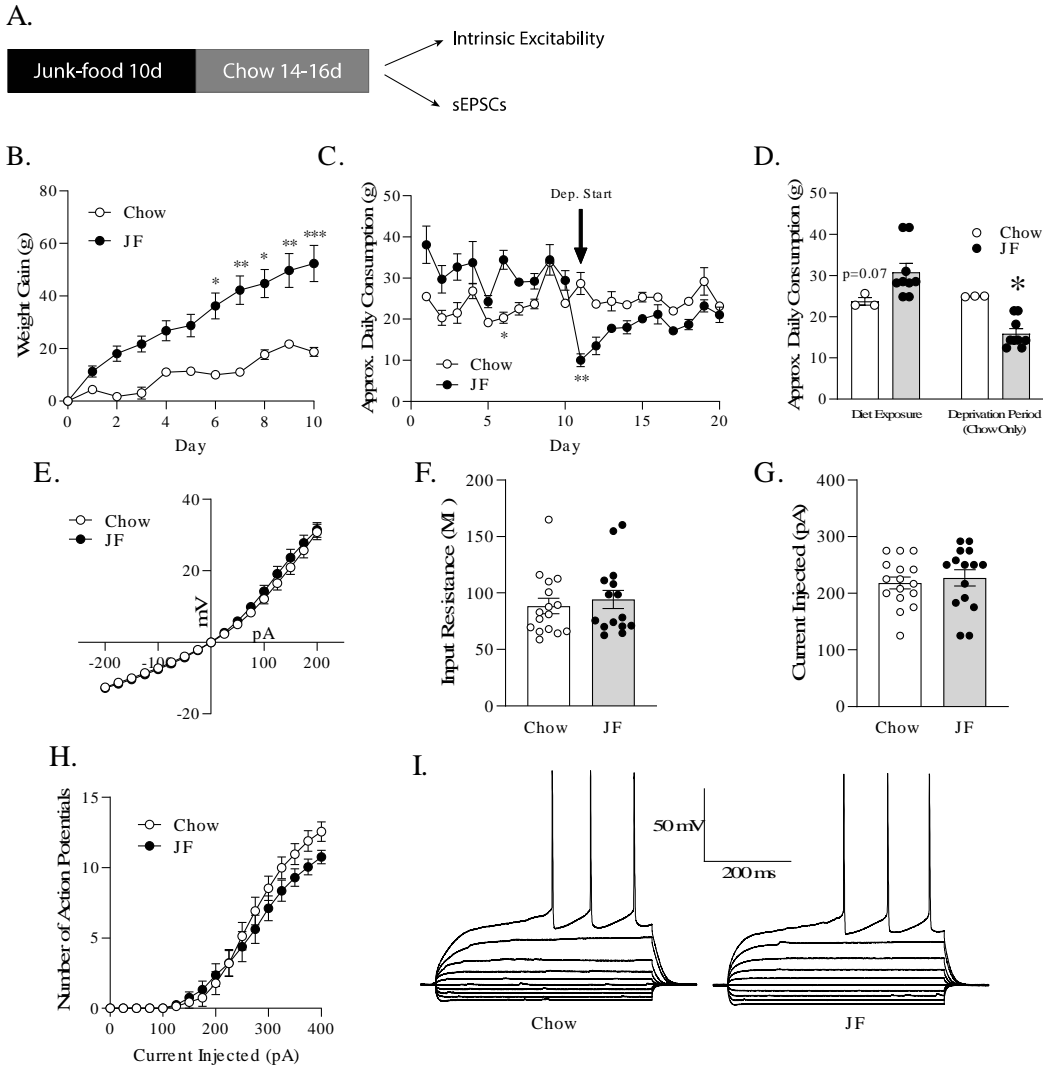
Figure 4.1 Effect of junk-food followed by long deprivation on MSN intrinsic excitability. A) Experimental timeline. B) Weight gain across time. Rats in the junk-food group (JF) gained more weight than chow controls. C, D) Average daily food intake. Compared to chow controls, obesity-prone female rats given junk-food ate more during the diet manipulation, but less chow during the deprivation period. E) Change in membrane potential across current injection. F) Average input resistance. G) Average rheobase. H) Number of action potentials elicited by each current injection. I) Example recordings from chow (left) and junk-food (right) cells. All data shown as average ±SEM, *=p<0.05, **=p<0.01, ***=p<0.001

When sEPSC were recorded (Chow: 9 cells from 3 rats, JF:17 cells from 9 rats), no differences were observed in average frequency (Fig 4.2A: Mann-Whitney U Test: U=100.5, p=0.96) or amplitude (Fig 4.2B: Mann-Whitney U Test: U=75, p=0.24) between groups. Similarly, the distributions for frequency and amplitude were unchanged. Thus overall, junk-food and deprivation did not produce long-lasting changes in MSN intrinsic excitability or glutamatergic transmission in females.
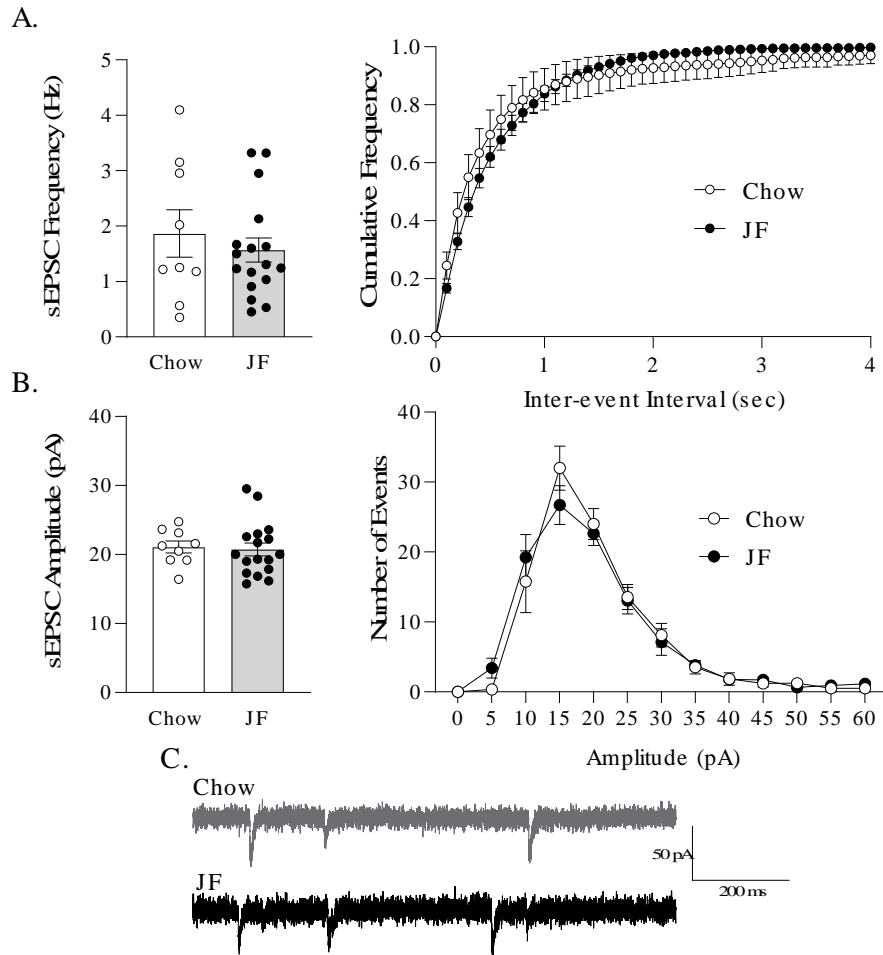
Figure 4.2 Effect of junk-food followed by long deprivation on sEPSC frequency and amplitude. A) Average frequency (left) and cumulative frequency distribution (right) of sEPSCs. B) Average amplitude (left) and amplitude distribution (right) of sEPSCs. C) Representative traces of sEPSCs from chow and junk-food (JF) groups.

It is possible that junk-food produces rapid changes in MSN physiology that return to baseline during the deprivation period. Therefore, we next determined whether effects on NAc MSN function are present following a shorter period of junk-food deprivation (24-72 hours; see timeline, Fig 4.3A). As above, obesity-prone female rats given junk-food (N=22) gained more weight (Fig 4.3B: Mixed-effects analysis time x diet interaction: $F_{(10,359)}=76.88$, $p<0.0001$; Sidak's multiple comparison's test: days 5-10: $p<0.01$), ate more when junk-food was available, but ate significantly less chow during the first 24-hours of the deprivation period than their chow counterparts (N=18; Fig 4.3C: diet exposure: two-tailed unpaired t-test: $t_{(28)}=2.10$, $p=0.04$; deprivation period: two-tailed unpaired t-test: $t_{(17)}=5.30$, $p<0.0001$). Note that separate t-tests were

conducted for Fig 4.3C because food intake during the deprivation period was only measured in a subset of rats (Chow N=8; JF N=11).

To ensure that we were not missing any effects, the cycle was monitored, and recordings were made in all phases (Chow M/D: 9 cells from 5 rats; Chow P/E: 12 cells from 6 rats; JF M/D: 7 cells from 6 rats; JF P/E: 8 cells from 4 rats). No cycle effects were found, and thus data were collapsed across phase (Fig 4.3 D-G). We found that junk-food followed by this short deprivation shifted I/V relationships at positive current injections compared to cells from chow controls (Fig 4.3D: two-way RM ANOVA group x current injection interaction: $F_{(16,544)}$= 5.5, p<0.0001; Sidak's multiple comparison p<0.01). In addition, input resistance was decreased (Fig 4.3E: two-tailed unpaired t-test, $t_{(34)}$=2.4, p=0.02), while rheobase was increased (Fig 4.3F: two-tailed unpaired t-test, $t_{(34)}$=2.1, p=0.04) in cells from junk-food vs chow groups. Consistent with these effects, we also observed reduced action potential firing in cells from junk-food vs chow groups (Fig 4.3G: two-way RM ANOVA main effect of group, $F_{(16,544)}$=2.56, p=0.08). Furthermore, these effects held when data were separated out by estrous cycle phase (I/V: M/D, two-way RM ANOVA group x current interaction: $F_{(16, 224)}$=1.99, p<0.02; P/E, two-way RM ANOVA group x current interaction: $F_{(16, 288)}$=3.38, p<0.001); number of action potentials: M/D, two-way RM ANOVA main effect of group: $F_{(16, 224)}$=3.03, p=0.001; P/E, two-way RM ANOVA main effect of group: $F_{(16, 288)}$=1.89, p=0.02; data not shown). Thus, junk-food followed by a brief deprivation resulted in a reduction in MSN intrinsic excitability across the cycle.
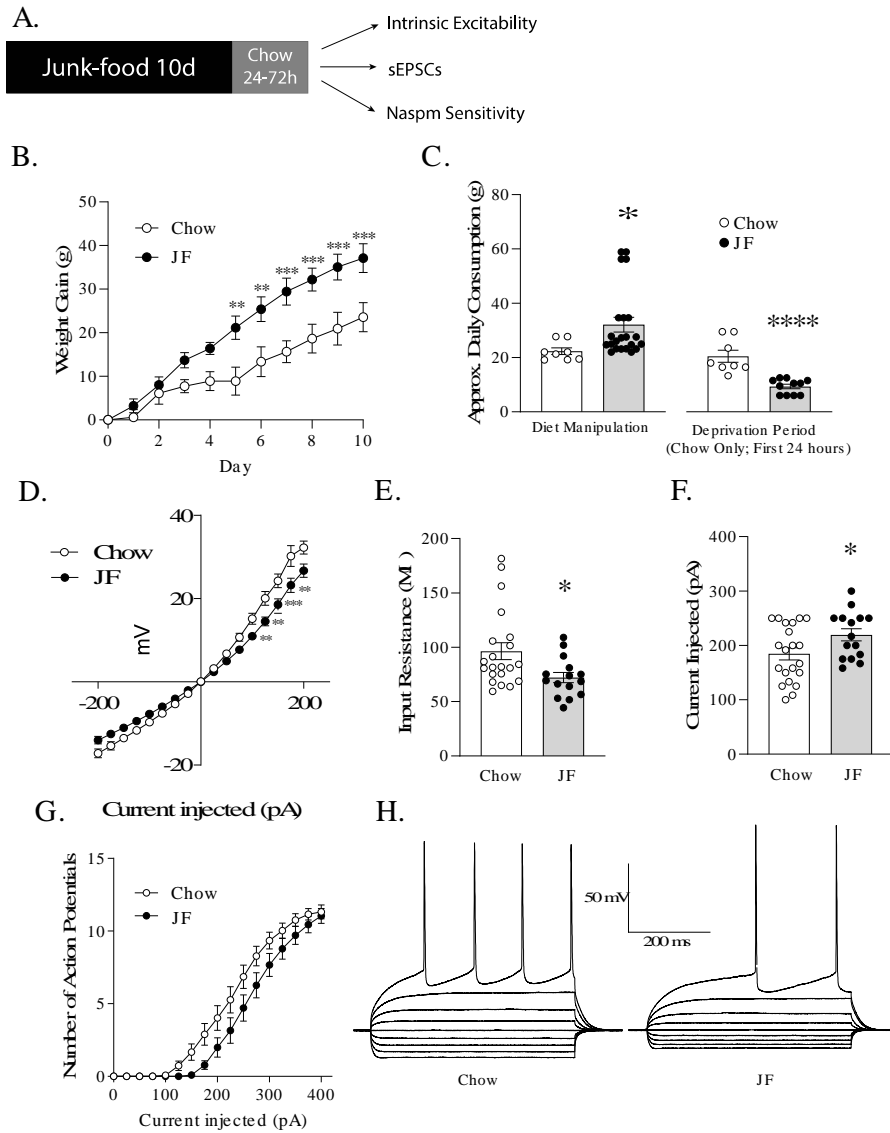
Figure 4.3 Effect of junk-food followed by short deprivation on MSN intrinsic excitability. A) Experimental timeline. B) Weight gain across time. Obesity-prone female rats given junk-food (JF) gained more weight across the diet manipulation than chow controls. C) Average daily food consumption in junk-food and chow groups. Compared to chow controls, obesity-prone female rats on junk-food ate more during the diet manipulation, but ate less chow during the deprivation period. D) Change in membrane potential across current injection. Changes in membrane potential in response to positive current injection were reduced in obesity-prone females given junk-food followed by a brief deprivation compared to controls. E) Average input resistance. Input resistance is decreased following junk-food consumption and a brief deprivation. F) Average rheobase. Rheobase is increased following junk-food consumption and a brief deprivation. G) Number of action potentials elicited by each current injection. Junk-food consumption followed by brief deprivation reduced in the number of action potentials fired at intermediate current injections compared to chow controls. H) Example traces from chow (left) and junk-food (right) cells. *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

We also examined effects of this same manipulation on NAc glutamatergic transmission. Bath application of the selective CP-AMPAR antagonist Naspm (Chow: 7

cells from 7 rats; JF: 10 cells from 9 rats) resulted in a larger reduction in eEPSC amplitude in cells from junk-food vs chow controls, indicative of a greater contribution from CP-AMPARs (Fig 4.4A: Mann-Whitney U Test: U=12, p=0.02). Furthermore, both sEPSC frequency (Fig 4.4C: Mann-Whitney U Test: U=48, p=0.04) and amplitude (Fig 4.4D: Mann-Whitney U Test: U=43, p=0.02) were significantly increased in cells from junk-food vs chow groups (Chow: 10 cells from 5 rats; JF: 18 cells from 11 rats). Thus, junk-food followed by a short deprivation period results in enhancements in NAc core excitatory transmission in females.
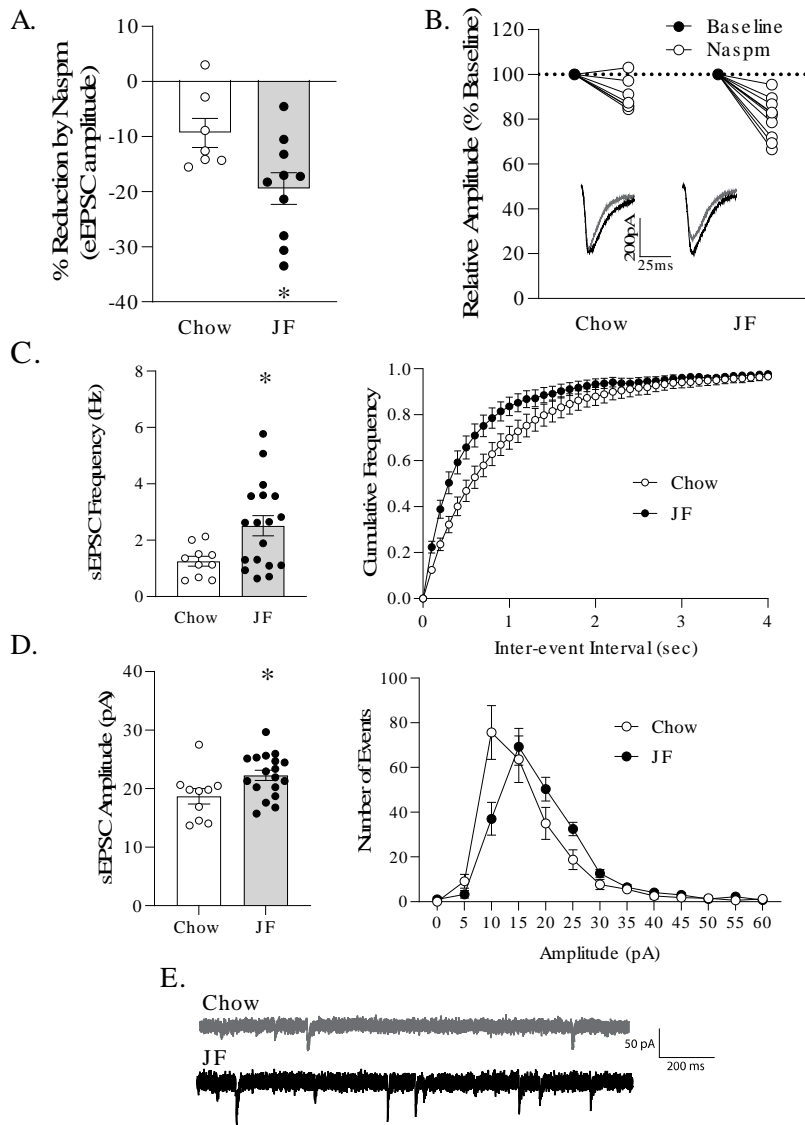
Figure 4.4 Effect of junk-food followed by short deprivation on glutamatergic transmission. A,B) Reduction in eEPSC amplitude following bath application of the CP-AMPAR antagonist Naspm. CP-AMPAR transmission was enhanced following junk-food consumption and a brief deprivation period compared to controls. Inset in B: black trace=before Naspm; gray trace=after Naspm. C) Average frequency (left) and cumulative frequency distribution (right) of sEPSCs.

sEPSC frequency is enhanced following junk-food consumption and a brief deprivation period in obesity-prone female rats. D Average amplitude (left) and amplitude distribution (right) of sEPSCs. sEPSC amplitude is increased following junk-food consumption and a brief deprivation period. E) Representative traces of sEPSCs from both groups. *=p<0.05.

Lastly, we evaluated CP-AMPAR mediated transmission and sEPSC amplitude and frequency following junk-food consumption without any deprivation period (see timeline, Fig 4.5A). As before, obesity-prone females given junk-food gained more weight (Fig 4.5B: Mixed Effect Model; main effect of diet, $F_{(1,9)}$=4.17, p=0.07; diet x time interaction $F_{(12,100)}$=4.02, p<0.001) and ate significantly more (5B Inset: Mann-Whitney U-Test: U=3, p=0.03) than chow controls. Interestingly, junk-food with no deprivation failed to alter CP-AMPAR transmission (Chow: 5 cells from 4 rats; JF: 4 cells from 3 rats; Fig 4.5C; Mann-Whitney U Test: U=10, p>0.99) or sEPSC frequency (Chow: 5 cells from 3 rats; JF: 9 cells from 5 rats; Fig 4.5D; Mann-Whitney U Test: U=14, p=0.30) compared to chow controls. However, sEPSC amplitude was increased in junk-food vs. chow groups (Fig 4.5E; Mann-Whitney U Test: U=5, p=0.02). Thus, a short deprivation period following junk-food consumption is necessary for enhancements in NAc CP-AMPAR transmission and sEPSC frequency, but not sEPSC amplitude.
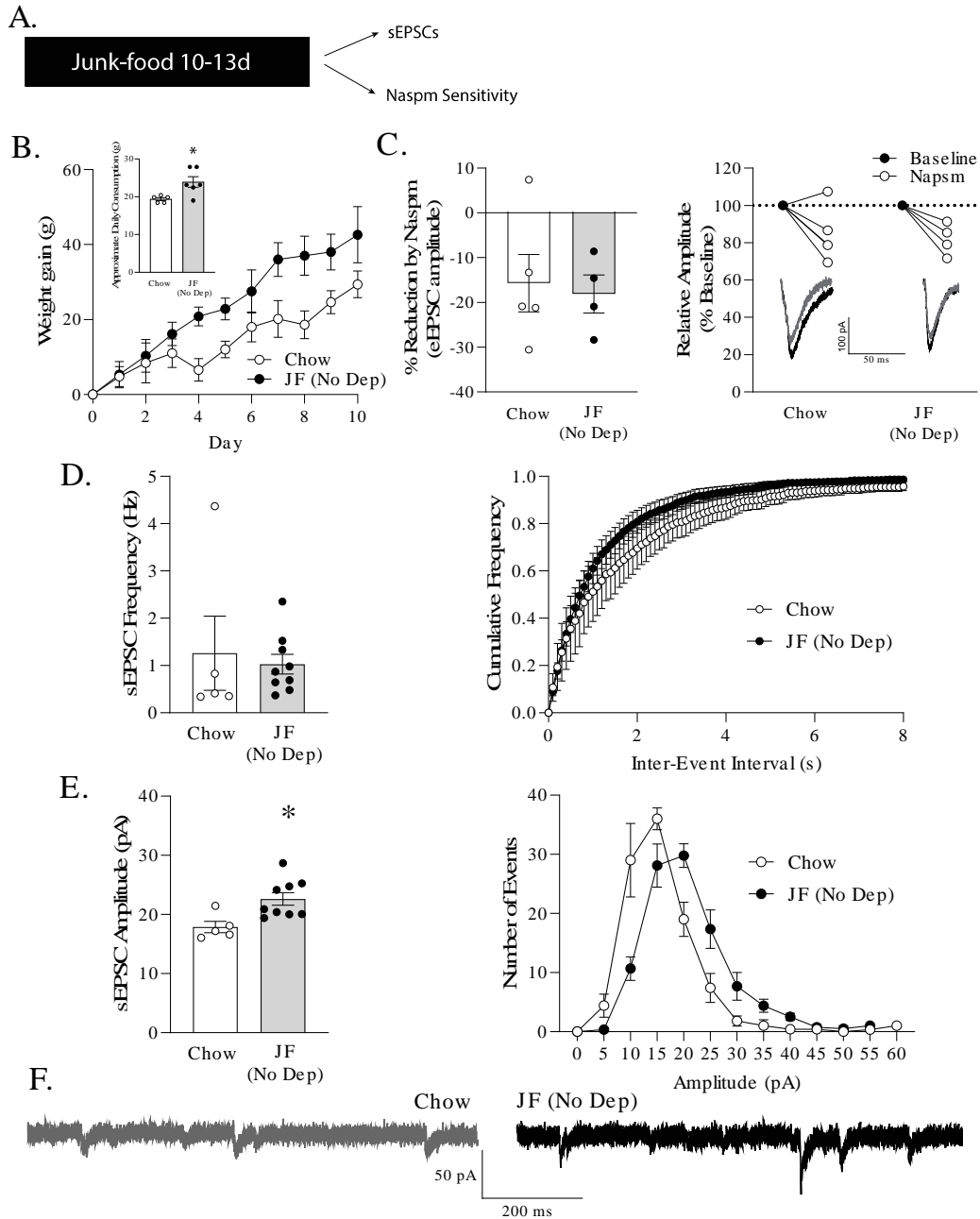
Figure 4.5 Effect of junk-food (no deprivation) on glutamatergic transmission. A) Experimental timeline. B) Weight gain across time. Obesity-prone female rats given junk-food (JF) gained more weight across time than chow controls. Inset shows average daily food consumption in junk-food and chow groups. Rats in the junk-food group ate more than rats in the chow group. C) Reduction in eEPSC amplitude following bath application of the CP-AMPAR antagonist Naspm (left); percent change from baseline (right). Junk-food without deprivation (No Dep) did not alter CP-AMPAR transmission compared to chow controls; black trace=before Naspm; gray trace=after Naspm. D) Average frequency (left) and cumulative frequency distribution (right) of sEPSCs. No group differences were found. E) Average amplitude (left) and amplitude distribution (right) of sEPSCs. sEPSC amplitude is increased after junk-food consumption compared to chow controls. F) Representative traces of sEPSCs in both groups. All data shown as average ±SEM. *=p<0.05.

Discussion

Effects of junk-food and subsequent deprivation on MSN intrinsic excitability in females

We began by evaluating the effects of a junk-food diet on MSN intrinsic excitability in obesity-prone females. We found that junk-food (10 days) followed by a 14-16 day deprivation period had no effect on MSN intrinsic excitability (Fig 4.1). However, after a brief period of deprivation (24-72 hrs), MSN excitability and firing were reduced compared to chow controls (Fig 4.3). Together, these data suggest that junk-food has transient effects on MSN intrinsic excitability in obesity-prone females that return to baseline in the absence of continued junk-food consumption.

In outbred Sprague Dawley female rats, and the selectively bred obesity-prone model used here, MSN excitability is greater in the metestrus/diestrus phase of the cycle compared to the proestrus/estrus phase (Proaño et al., 2018; Alonso-Caraballo and Ferrario, 2019). Given these small, but consistent shifts in intrinsic properties of MSNs with the cycle, we compared effects of junk-food on excitability when recordings were made in the proestrus/estrus vs metestrus/diestrus phases following short junk-food deprivation. A reduction in MSN excitability was present regardless of cycle phase (see results). Thus, effects following junk-food and deprivation were not strongly affected by cycle phase. Recordings after the longer deprivation were made only from animals in metestrus/diestrus. Although we cannot rule out potential cycle effects, we think it is unlikely that effects of the cycle impeded our ability to detect group differences at the longer deprivation time point, given that effects were detectable across the cycle following the short deprivation period.

Similar reductions in MSN excitability are found in obesity-prone males shortly after this same junk-food diet exposure (Oginsky and Ferrario, 2019). Thus, although there is evidence for basal sex differences in MSN excitability (Cao et al., 2018), reductions in excitability following junk-food and subsequent deprivation do not appear to be sex-specific. In the previous study using males, all food was removed from the home cage 14-16 hours prior to recording, whereas in the current study all females were given free access to chow for 24-72 hrs prior to recording. However, when junk-food is removed and rats are returned to *ad lib* chow, they voluntarily reduce their food

intake, largely refusing to eat standard lab chow for a period of 1-3 days before gradually resuming levels of chow consumption comparable to controls; this is seen in females here (Fig 4.1C, 4.3C) and in males from previous studies (Vollbrecht et al., 2015; Derman and Ferrario, 2018b). Thus, while complete fasting is not likely to be necessary for reductions in MSN excitability, we cannot rule out possible contributions of reduced food intake to effects of junk-food followed by deprivation. Further, it is possible that voluntary reductions in food intake during the deprivation period may produce a "stress" response that contributes to effects on excitability and glutamate transmission (discussed below). However, to our knowledge there are no data that examine effects of voluntary food restriction on HPA axis activation, or other measures of stress. Thus, this remains an outstanding question.

Effects of junk-food on glutamatergic transmission in females

The one previous study of NAc excitatory transmission conducted in females focused on relatively long-lasting effects of junk-food consumption. In that study, 10 days of eating junk-food followed by 14 days of junk-food deprivation did not alter NAc core CP-AMPAR transmission but produced slight increases in the AMPA/NMDA ratio (Alonso-Caraballo and Ferrario, 2019). Shorter deprivation periods were not examined. Consistent with previous results, we found no group differences in sEPSC amplitude or frequency following long deprivation (Fig 4.2). In addition, the recording conditions used here biased measures towards AMPAR-mediated EPSCs. Thus, the absence of effects on sEPSC amplitude suggest that previously reported shifts in the AMPA/NMDA ratio after this same regimen may be due to alterations in NMDAR-transmission (Alonso-Caraballo et al., 2021), although caution should be used when integrating results from spontaneous vs. evoked responses.

Following the short junk-food deprivation, we found increases in sEPSC frequency and amplitude and in CP-AMPAR transmission in junk-food vs. chow groups (Fig 4.4). This suggests that NAc excitatory transmission following junk-food consumption is indeed enhanced in females, but that this effect is transient and returns to baseline when rats are returned to *ad lib* chow for an extended period. In regard to

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effects on glutamate transmission, increases in sEPSC frequency are often indicative of increases in glutamate release, whereas increases in sEPSC amplitude are indicative of enhancements in postsynaptic receptor expression. The latter is consistent with CP-AMPAR up-regulation, and both are consistent with enhancements in excitatory transmission. However, changes in sEPSC frequency can also occur in the absence of changes in glutamate release. For example, Wissman et. al. (2011) found increases in mEPSC frequency with no differences in paired pulse ratio (a common measure of the probability of glutamate release) in MSNs of male and female rats following repeated experimenter-administered cocaine injections (Wissman et al., 2011). This suggested that increases in frequency were not due to increased glutamate release, but rather to increases in MSN spine density. Self-administration of high-fat food pellets increases the number of mushroom-type spines on MSNs in the NAc core of male rats (Dingess et al., 2017). Therefore, the increases in sEPSC frequency here could be due to increases in presynaptic glutamate release or increases in synaptic contacts. These possibilities can be examined in future studies.

Pertaining to post-synaptic transmission, we found increases in CP-AMPAR-mediated transmission in addition to increases in sEPSC amplitude following the short junk-food deprivation. Similar to reports in males (Conrad et al., 2008; Oginsky et al., 2016), CP-AMPARs mediated ~10% of the AMPA current in chow fed female controls, and a little over 20% of the current following junk-food consumption (Fig 4.4). Transient trafficking of CP-AMPARs is a normal part of synaptic plasticity thought to contribute to learning and memory, while persistent upregulation of CP-AMPAR expression and transmission is thought to induce nonconventional forms of synaptic remodeling that lead to pathological states including addiction (Ferrario, 2017; Cull-Candy and Farrant, 2021). For example, blockade of CP-AMPARs in the NAc core prevents the expression of cue-triggered food-seeking in obesity-prone male rats and blunts the incubation of cocaine craving in males (Conrad et al., 2008; Derman and Ferrario, 2018a). However, the transience in CP-AMPAR upregulation in females is in contrast to what we have previously seen in obesity-prone males, where increases in both CP-AMPAR surface expression and transmission are rapid and persistent (Oginsky et al., 2016; Alonso-Caraballo et al., 2021). Therefore, although junk-food increases CP-AMPARs in both

sexes, females appear to be protected from long-lasting diet-induced alterations in NAc function. This could suggest that potential behavioral effects of junk-food diet exposure, such as enhanced cue-triggered food-seeking found in males (Derman and Ferrario, 2018b), may also be transient in females.

Finally, we found that increases in CP-AMPAR transmission required a junk-food deprivation period in females. The same occurs in males, where removal of junk-food is also required for increases in NAc core CP-AMPAR transmission (Alonso-Caraballo et al., 2021). As mentioned above, whether voluntary reductions in food intake contribute to this effect is unknown. Indeed, what triggers the recruitment of CP-AMPARs, vs "standard" GluA1/2 containing AMPARs is also unknown see (Cull-Candy and Farrant, 2021). However, it's worth noting that increases in CP-AMPARs following cocaine consumption require a drug-free period, albeit longer (at least 30 days following cessation of cocaine self-administration) (Wolf and Tseng, 2012). Overall, data to date suggest that CP-AMPARs may be recruited in response to the absence of continued consumption of reinforcing stimuli, be they food or drug.

Although regulation of intrinsic excitability and glutamate transmission can be independent (Zhang and Linden, 2003; Turrigiano and Nelson, 2004), alterations in synaptic transmission often results in opposing changes in membrane excitability, and vice versa (Ishikawa et al., 2009; Vollbrecht et al., 2015). Therefore, it is possible that reductions in intrinsic excitability are a compensatory response to enhancements in glutamatergic drive onto MSNs. This hypothesis is supported by data showing that reducing excitatory input increases membrane excitability in MSNs of the NAc (Ishikawa et al., 2009). However, it is also possible that increased excitatory transmission is instead a compensatory response to initial experience-induced reductions in MSN excitability, for which there is also evidence (Burrone et al., 2002; Ibata et al., 2008). Nonetheless, the pattern of effects found here in females are consistent with overall enhancements in excitatory drive to the NAc.

What might be causing these transient effects in females?

When considering what might be driving sex-specific effects, one starting point is

the potential role of gonadal hormones. Naturally circulating ovarian hormones (estradiol and progesterone) influence food-seeking and feeding behaviors, and modulate neuroplasticity associated with alterations in motivation (Forlano and Woolley, 2010; Wissman et al., 2011; Proaño et al., 2018; Yoest et al., 2018; Alonso-Caraballo and Ferrario, 2019). Thus, the presence of ovarian hormones in the absence of continued junk-food consumption may help reverse the effects and return the system to baseline. This would be consistent with the ability of ovarian hormones to suppresses food-intake and reduce food-seeking behaviors (Asarian and Geary, 2006; Alonso-Caraballo and Ferrario, 2019). However, there are strikingly few studies of the effects of ovarian hormones on MSN synaptic transmission on which to build strong mechanistic hypotheses. Evidence suggests that circulating levels of progesterone and estradiol correlate with mEPSC frequency and amplitude measures (Proaño et al., 2020), and that NAc glutamatergic transmission increases during proestrus and estrus compared to other phases in naturally cycling females (Proaño et al., 2018). In addition, acute estradiol treatment of striatal slices from adult females produces rapid, but small reductions in mEPSC frequency and amplitude in the NAc core (Krentzel et al., 2019). Thus, it's possible that effects are transient in females, but not in males, due to ongoing fluctuations in ovarian hormones across the junk-food deprivation period. However, additional studies addressing fundamental physiological effects of ovarian hormones on NAc glutamatergic transmission and MSN excitability are needed.

In summary, junk-food consumption reduces MSN intrinsic excitability, increases NAc core glutamate transmission, and enhances CP-AMPAR-mediated transmission when followed by a brief period of deprivation. These effects are absent after a longer deprivation period. Thus, this study reveals that females are protected from long-lasting effects of sugary, fatty food consumption on NAc core function.

References

Alonso-Caraballo Y, Ferrario CR (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. Horm Behav 116:104583.

Alonso-Caraballo Y, Jorgensen ET, Brown T, Ferrario CR (2018) Functional and structural plasticity contributing to obesity: roles for sex, diet, and individual susceptibility. Curr Opin Behav Sci 23:160-170.

Alonso-Caraballo Y, Fetterly TL, Jorgensen ET, Nieto AM, Brown TE, Ferrario CR (2021) Sex specific effects of "junk-food" diet on calcium permeable AMPA receptors and silent synapses in the nucleus accumbens core. Neuropsychopharmacology 46:569-578.

Asarian L, Geary N (2006) Modulation of appetite by gonadal steroid hormones. Philos Trans R Soc Lond B Biol Sci 361:1251-1263.

Balleine BW (2005) Neural bases of food-seeking: affect, arousal and reward in corticostriatolimbic circuits. Physiol Behav 86:717-730.

Burrone J, O'Byrne M, Murthy VN (2002) Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. Nature 420:414-418.

Cao J, Willett JA, Dorris DM, 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. Front Endocrinol (Lausanne) 9:173.

Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, Marinelli M, Wolf ME (2008) Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature 454:118-121.

Crombag HS, Sutton JM, Takamiya K, Holland PC, Gallagher M, Huganir RL (2008) A role for alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid GluR1 phosphorylation in the modulatory effects of appetitive reward cues on goal-directed behavior. Eur J Neurosci 27:3284-3291.

Cull-Candy SG, Farrant M (2021) Ca(2+) -permeable AMPA receptors and their auxiliary subunits in synaptic plasticity and disease. J Physiol 599:2655-2671.

Demos KE, Heatherton TF, Kelley WM (2012) Individual differences in nucleus accumbens activity to food and sexual images predict weight gain and sexual behavior. J Neurosci 32:5549-5552.

Derman RC, Ferrario CR (2018a) Enhanced incentive motivation in obesity-prone rats is mediated by NAc core CP-AMPARs. Neuropharmacology 131:326-336.

Derman RC, Ferrario CR (2018b) 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. Physiol Behav 192:145-157.

Di Ciano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ (2001) Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. J Neurosci 21:9471-9477.

Dingess PM, Darling RA, Derman RC, Wulff SS, Hunter ML, Ferrario CR, Brown TE (2017) Structural and Functional Plasticity within the Nucleus Accumbens and Prefrontal Cortex Associated with Time-Dependent Increases in Food Cue-Seeking Behavior. Neuropsychopharmacology 42:2354-2364.

Ferrario CR (2017) Food Addiction and Obesity. Neuropsychopharmacology 42:361.

Ferrario CR (2020) Why did I eat that? Contributions of individual differences in incentive motivation and nucleus accumbens plasticity to obesity. Physiol Behav 227:113114.

Forlano PM, Woolley CS (2010) Quantitative analysis of pre- and postsynaptic sex differences in the nucleus accumbens. J Comp Neurol 518:1330-1348.

Ibata K, Sun Q, Turrigiano GG (2008) Rapid synaptic scaling induced by changes in postsynaptic firing. Neuron 57:819-826.

Ishikawa M, Mu P, Moyer JT, Wolf JA, Quock RM, Davies NM, Hu XT, Schlüter OM, Dong Y (2009) Homeostatic synapse-driven membrane plasticity in nucleus accumbens neurons. J Neurosci 29:5820-5831.

Jensen CD, Kirwan CB (2015) Functional brain response to food images in successful adolescent weight losers compared with normal-weight and overweight controls. Obesity (Silver Spring) 23:630-636.

Kelley AE (2004) Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. Neurosci Biobehav Rev 27:765-776.

Krentzel AA, Barrett LR, Meitzen J (2019) Estradiol rapidly modulates excitatory synapse properties in a sex- and region-specific manner in rat nucleus accumbens core and caudate-putamen. J Neurophysiol 122:1213-1225.

Levin BE, Dunn-Meynell AA, Balkan B, Keesey RE (1997) Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. Am J Physiol 273:R725-730.

Marcondes FK, Miguel KJ, Melo LL, Spadari-Bratfisch RC (2001) Estrous cycle influences the response of female rats in the elevated plus-maze test. Physiol Behav 74:435-440.

Nisenbaum ES, Wilson CJ (1995) Potassium currents responsible for inward and outward rectification in rat neostriatal spiny projection neurons. J Neurosci 15:4449-4463.

Oginsky MF, Ferrario CR (2019) Eating "junk food" has opposite effects on intrinsic excitability of nucleus accumbens core neurons in obesity-susceptible versus -resistant rats. Journal of Neurophysiology 122:1264-1273.

Oginsky MF, Goforth PB, Nobile CW, Lopez-Santiago LF, Ferrario CR (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 41:2977-2986.

Palmer BF, Clegg DJ (2015) The sexual dimorphism of obesity. Mol Cell Endocrinol 402:113-119.

Perez MF, White FJ, Hu XT (2006) Dopamine D(2) receptor modulation of K(+) channel activity regulates excitability of nucleus accumbens neurons at different membrane potentials. J Neurophysiol 96:2217-2228.

Planert H, Berger TK, Silberberg G (2013) Membrane properties of striatal direct and indirect pathway neurons in mouse and rat slices and their modulation by dopamine. PLoS One 8:e57054.

Proaño SB, Krentzel AA, Meitzen J (2020) Differential and synergistic roles of 17β-estradiol and progesterone in modulating adult female rat nucleus accumbens core medium spiny neuron electrophysiology. J Neurophysiol 123:2390-2405.

Proaño SB, Morris HJ, Kunz LM, Dorris DM, 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 120:1356-1373.

Setlow B, Holland PC, Gallagher M (2002) Disconnection of the basolateral amygdala complex and nucleus accumbens impairs appetitive pavlovian second-order conditioned responses. Behav Neurosci 116:267-275.

Stoeckel LE, Weller RE, Cook EW, 3rd, Twieg DB, Knowlton RC, Cox JE (2008) Widespread reward-system activation in obese women in response to pictures of high-calorie foods. Neuroimage 41:636-647.

Surmeier DJ, 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 Neurosci 30:228-235.

Tronson NC (2018) Focus on females: A less biased approach for studying strategies and mechanisms of memory. Curr Opin Behav Sci 23:92-97.

Turrigiano GG, Nelson SB (2004) Homeostatic plasticity in the developing nervous system. Nat Rev Neurosci 5:97-107.

Vollbrecht PJ, Nobile CW, Chadderdon AM, Jutkiewicz EM, Ferrario CR (2015) Pre-existing differences in motivation for food and sensitivity to cocaine-induced locomotion in obesity-prone rats. Physiol Behav 152:151-160.

Wasserstein RL, Lazar NA (2016) The ASA statement on p-values: context, process, and purpose. In, pp 129-133: Taylor & Francis.

Wissman AM, McCollum AF, Huang GZ, Nikrodhanond AA, Woolley CS (2011) Sex differences and effects of cocaine on excitatory synapses in the nucleus accumbens. Neuropharmacology 61:217-227.

Wolf ME, Tseng KY (2012) Calcium-permeable AMPA receptors in the VTA and nucleus accumbens after cocaine exposure: when, how, and why? Front Mol Neurosci 5:72.

Yoest KE, Quigley JA, Becker JB (2018) Rapid effects of ovarian hormones in dorsal striatum and nucleus accumbens. Horm Behav 104:119-129.

Zhang W, Linden DJ (2003) The other side of the engram: experience-driven changes in neuronal intrinsic excitability. Nat Rev Neurosci 4:885-900.

Zhao B, Zhu J, Dai D, Xing J, He J, Fu Z, Zhang L, Li Z, Wang W (2016) Differential dopaminergic regulation of inwardly rectifying potassium channel mediated subthreshold dynamics in striatal medium spiny neurons. Neuropharmacology 107:396-410.

Chapter 5: Discussion

Researchers have long been trying to understand the neuromechanisms that drive the development and persistence of addiction. Through this research key elements have been identified that are necessary for both the assessment of addiction-like behaviors in rodent models, as well as the drug-induced neurobiological adaptations associated with the addiction-like behaviors. One important consideration is the regiment of cocaine exposure, experimenter vs self-administration. Repeated non-contingent administration of cocaine leads to the development of psychomotor sensitization (Post and Rose, 1976; Crombag et al., 1999; Carr et al., 2020) and importantly, the neuroplasticity associated with psychomotor sensitization is thought to contribute to the transition to addiction (Robinson and Berridge, 1993; Vezina, 2004). However, it has been well established that drug exposure in it of itself is not addiction. Therefore, self-administration models have been the gold standard for investigating addiction-like behaviors in rodents. Through investigation of self-administration models it has been established that even when animals self-administer drug this does not always lead to addiction-like behaviors. For example, under short access self-administration (ShA) conditions rats do not escalate their drug intake or show progressive enhancements in drug-seeking behaviors (Ahmed and Koob, 1998; Ferrario et al., 2005; Hao et al., 2010). Therefore the use of long access self-administration (LgA) was developed, and importantly animals who've experienced LgA self-administration reliably develop several addiction-like behaviors (Ahmed and Koob, 1998; Paterson and Markou, 2003; Deroche-Gamonet et al., 2004). For example, LgA cocaine self-administration followed by withdrawal results in drug-seeking behavior that increases as a function of drug withdrawal.

Another important consideration is the sex of the subject. It is well established both in humans and in preclinical models of addiction that there are sex differences in both the development and persistence of addiction (Becker and Hu, 2008). For

example, women transition to addiction more rapidly (Brady and Randall, 1999; Hernandez-Avila et al., 2004) and are more likely to experience relapse than men (Becker et al., 2017). Similarly, female rats acquire cocaine self-administration at a faster rate (Lynch and Carroll, 1999; Hu and Becker, 2008) and display greater drug-seeking behavior following a withdrawal period than males (Kerstetter et al., 2008; Carroll and Anker, 2010; Nicolas et al., 2019). Perhaps unsurprisingly, these sex differences rely on gonadal hormones (Becker and Koob, 2016; Cornish and Prasad, 2021).

Importantly, both psychomotor sensitization and enhancements in cocaine-seeking behaviors have been linked to alterations in nucleus accumbens (NAc) glutamate transmission (Vanderschuren and Kalivas, 2000; Ferrario et al., 2010; Dong et al., 2017). For example, experimenter administered cocaine treatment regimens that result in psychomotor sensitization enhance excitatory transmission within the NAc shell and core of mice (Thomas et al., 2001; Jedynak et al., 2016) and increase the surface expression of GluA1 and GluA2 AMPAR subunits in the NAc of rats consistent with enhancements in CI-AMPARs (Boudreau and Wolf, 2005; Ferrario et al., 2010). In contrast, LgA self-administration results in an enhancement of GluA2-lacking CP-AMPARs in the NAc core (Conrad et al., 2008; McCutcheon et al., 2011; Loweth et al., 2014; Kawa et al., 2022). Together this highlights that the route of cocaine administration (experimenter administered vs. LgA IV self-administration) may alter glutamate plasticity in the NAc in distinct ways. However, no direct assessment on the effects of experimenter administered cocaine on CP-AMPAR had yet to be assessed. Furthermore, what effects, if any occur in females are poorly understood.

Recent evidence indicates that humans do not maintain a high level of prolonged drug intake within a single sitting. Instead, they tended to cycle frequently between use and abstinence known as binges (Cohen and Sas, 1994; Simon et al., 2001). Therefore, the IntA model was created to better mimic human use. It utilizes forced drug-free periods interspersed with drug-available periods (5-minutes of drug available followed by 25-minutes of no drug available typically for 6 hours) which forces the animal to cycle between use and abstinence more similarly to human patterns of drug-taking (Zimmer et al., 2011; Zimmer et al., 2012). Despite IntA self-administration producing addiction-

like behaviors comparable to or greater in magnitude than LgA self-administration (Kawa et al., 2016; Nicolas et al., 2019; Carr et al., 2020) the effects of IntA cocaine self-administration on glutamate plasticity were unknown. Therefore, studies conducted in this dissertation were done to assess how different regiments of cocaine exposure (i.e., those with different pharmacokinetics: experimenter administered, LgA and IntA) affect NAc glutamatergic plasticity in male and female rats.

Sex differences in NAc MSN function following experimenter administered cocaine

In Chapter 2 we investigated the effects of sex on behavioral sensitization and glutamatergic transmission. Unsurprisingly, females showed a stronger acute locomotor response to cocaine than males (Fig 2.1B). Furthermore, sensitization was seen in both male and female rats that had repeated cocaine exposure (Fig 2.1C-E). We were surprised however, that when comparisons in the magnitude of sensitization were made across sex (i.e., the change in cocaine-induced locomotor activity on day 1 vs day 8), no significant sex differences were found. We attributed this to the large locomotor response to the first cocaine injection in females, and possibly to the emergence of stereotypy in females by the 8th injection. Interestingly, despite robust sensitization in both sexes, only males showed enhanced NAc glutamate transmission, specifically enhancements in sEPSC frequency, but not amplitude or paired pulse ratio (Fig 2.3). Finally, regardless of sex CP-AMPAR mediated transmission was similar to drug naïve animals (Fig 2.2).

Chapter 2 revealed striking sex differences in glutamate plasticity even though both sexes show clear behavioral sensitization. This could be due to differences in gonadal hormones as there are known differences in MSN function across the cycle. For example, estradiol has been shown to rapidly decrease mEPSC frequency in the NAc core of females but not males (Krentzel et al. 2019). Furthermore, NAc mEPSC frequency is similar in males and females when recordings are made from females in the diestrus phase of the cycle, but is enhanced in females vs males when recordings are made from females in proestrus or estrus (Proaño et al., 2018). Together, this

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highlights that gonadal hormones modulate glutamate transmission; however, how cocaine may influence this process is not yet understood. It is possible we saw no effects in females sEPSC frequency or amplitude in our studies because effects of the cycle hindered our ability to detect group differences. Therefore, studies should investigate if there is an interaction between cocaine and ovarian hormones on glutamate transmissions. To examine this question ovariectomized (OVX) females, freely cycling females, and intact males should undergo the same cocaine regimen used in our studies in Chapter 2 (15mg/kg, i.p. 1 injection/day, 8 days) and controls would receive saline injections as described (1 mL/kg, i.p). Immediately followed by 14-16 days of withdrawal. Importantly, the cycle would be monitored in intact females throughout the experiment. Whole cell-patch clamp electrophysiology would be done to assess sEPSC frequency and amplitude. Data from intact females would be analyzed by cycle phase to determine if there were any cycle effects. One could imagine that OVX females that are treated with cocaine may display similar glutamatergic transmission alterations to cocaine treated males (increases in sEPSC frequency) compared to saline treated animals. Additionally, in freely cycling females opposite effects of cocaine on transmission could occur. For example, proestrus/estrus may lead to enhancements in sEPSC frequency whereas metestrus/diestrus could lead to a decrease in sEPSC frequency, which could account for the absence of an effect in females within Chapter 2 studies. Although this is speculative, these studies would help dissect if ovarian hormones influence cocaine's effects on glutamatergic transmission.

MSN neurons in the NAc receive and integrate glutamatergic and dopaminergic signals, ultimately leading to an output. However, in addition to those external signals, MSNs intrinsic properties help define if and how frequently the cell is going to initiate an action potential (Hille, 1992; Nicola et al., 2000). MSNs have a number of influences that govern their intrinsic properties. Inwardly-rectifying potassium currents (I_{KR}) help maintain their hyperpolarized state, whereas, fast transient potassium currents (I_A) influence action potential firing (Nisenbaum and Wilson, 1995). Furthermore, dopamine receptor activation indirectly modulates MSN excitability through direction action on I_{KR} and I_A (Perez et al., 2006; Zhao et al., 2016). Importantly, cocaine has been shown to not only result in synaptic changes, but also changes in MSN intrinsic excitability.

Specifically, 5 days of repeated systemic injections of cocaine in mice results in increased MSN firing capacity in the NAc core early in withdrawal (1-3 days), though by withdrawal day 14 firing capacity returns to baseline (Kourrich and Thomas, 2009). However, these studies were done exclusively in males. Alterations in synaptic transmission can results in opposing changes in membrane excitability, and vice versa (Ishikawa et al., 2009; Vollbrecht et al., 2015) but, regulation of intrinsic excitability and glutamate transmission can be independent (Zhang and Linden, 2003; Turrigiano and Nelson, 2004). Thus, the absence of an effect in synaptic transmission in cocaine treated females (Chapter 2) does not indicate there will be an absence of an effect in MSN excitability. Therefore, studies should be done to assess the effects of cocaine on MSN intrinsic excitability in females.

To examine this question male and female rats would undergo the same behavioral sensitization regimen (described above and in greater detail in Chapter 2). After a short (1-3 day) or long (14-16 day) period of withdrawal whole cell-patch clamp electrophysiology would be conducted to assess intrinsic excitability in both males and females.

Overall, the above described studies would provide information about how female gonadal hormones influence glutamate transmission following cocaine exposure as well as how cocaine exposure alters MSN excitability in females. Currently, knowledge about female physiology is underwhelming and there is even less information about how female neurocircuitry adapts in response to drugs of abuse. Given that in humans the development of addiction displays distinct sex differences, studies investigating drug effects in females are important for understanding the pathophysiology of addiction in females. This will shed light on whether the mechanisms driving addiction in males and females are distinct or overlapping, which will provide vital information for potential future therapies.

Effects of LgA vs IntA on NAc CP-AMPAR transmission

In Chapter 3, we directly compare the effects of LgA vs IntA self-administration on the incubation of cocaine craving across prolonged withdrawal (1, 30 and 45 days) in

males and females. Furthermore, we assessed NAc core CP-AMPAR mediated transmission in both sexes. Lastly, given that MSNs primarily form two populations based on the dopamine receptor present (D1- or D2-containing MSNs), which have been demonstrated to have opposing roles in drug-seeking behavior (Lobo and Nestler, 2011; Kravitz et al., 2012; Pascoli et al., 2014; Allichon et al., 2021), we assessed the effects of cell-type on CP-AMPAR transmission.

We found both LgA and IntA experience result in clear escalation (Fig 3.1E). However, surprisingly, LgA males increase infusions to a greater magnitude than LgA females, whereas there were no sex differences in escalation within IntA (Fig 3.1C). Furthermore, both male and female animals show similar magnitude of incubation behavior regardless of self-administration model at both withdrawal day 30 and 45 compared to withdrawal day 1 (Fig 3.2B). Lastly, we see an upregulation of CP-AMPAR mediated transmission following LgA and IntA compared to drug naïve rats (Fig 3.3A). Within group, data suggests CP-AMPAR mediated transmission was similar in males and females and similar between D1- and D2-MSNs. However, we are underpowered to assess if there is a sex by cell-type interaction.

Our data here indicate that prolonged cocaine access is not a necessary feature for the development of incubation of craving or coinciding upregulation of CP-AMPAR mediated transmission. We were surprised that the data suggests no effects of cell-type on CP-AMPAR mediated transmission. However, we may be missing some effects of cell-type because of the number of cells per group. This is particularly relevant because the data hints at a potential sex by cell-type interactions. Even though we are underpowered to draw a firm conclusion, I will discuss the trends in the data. Of note, males and females have been separated graphically (Fig 5.1A-B) for ease of discussion, however, male and female data were collected together therefore direct comparisons can be made. Interestingly, in the drug naïve group, male D1- and D2-MSNs have a similar reduction by Naspm, indicating that basal CP-AMPAR mediated transmission is similar between the two cell-types (Fig 5.1A). However, in drug naïve females, D1-MSNs display a greater reduction by Naspm than D2-MSNs, suggesting that the basal contribution of CP-AMPARs to transmission may be greater in D1- vs D2-MSN in females (Fig 5.1B). Interestingly, as discussed in Chapter 3, within the drug

naïve group, female cycle does not seem to affect CP-AMPAR mediated transmission (Fig 3.4B); that is, regardless of where females were in their cycle (metestrus/diestrus vs proestrus/estrus) CP-AMPAR mediated transmission was similar. Thus, it seems unlikely that the potential difference in CP-AMPAR mediated transmission in D1- vs D2-MSNs in drug naïve females is due to an effect of cycle. If indeed there are basal differences in CP-AMPAR transmission due to a sex by cell-type interaction, this would alter the interpretation of how LgA and IntA cocaine self-administration affect CP-AMPAR mediated transmission.

In males, the interpretation is straight forward. Within group, Naspm reduction seems to be similar, such that drug naïve animals display similar reductions by Naspm in D1- and D2-MSNs. Furthermore, LgA and IntA groups display a greater reduction by Naspm compared to the drug naïve group, and there seems to be no effect of cell-type. Together this indicates that in males, the LgA and IntA experience followed by protracted withdrawal results in similar upregulations in CP-AMPARs compared to drug naïve animals in both D1- and D2-MSNs (Fig 5.1A).

The interpretation in females is a little less uniform. Interestingly, regardless of group (drug naïve, LgA or IntA) CP-AMPAR mediated transmission is similar in D1-MSNs. However, D2-MSNs in the LgA and IntA group show enhancements in CP-AMPAR mediated transmission compared to the drug naïve group. This would suggest that LgA and IntA experience in females preferentially increases CP-AMPAR transmission in D2-MSNs, with no changes in D1-MSNs (Fig 5.1B).

Together data would suggest that LgA and IntA cocaine experience followed by withdrawal result in increased CP-AMPAR mediated transmission in both sexes. However, in males this occurs in both D1- and D2-MSN populations, whereas in females this upregulation may be selective to the D2-MSN population. Interestingly, this could mean that in females the mechanism driving incubation of craving is different than males (i.e., independent of CP-AMPAR mediated transmission) or that an enhancement in CP-AMPAR mediated transmission specifically in D2-MSN is sufficient in females to mediate incubation of craving. Future studies will determine the answers to these questions, however, this could provide valuable insight as to why females have a greater propensity to relapse than males.
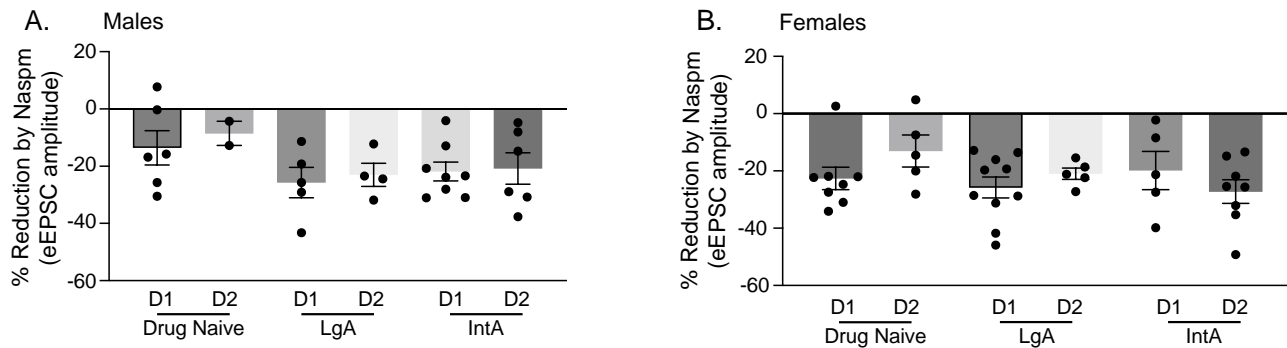
Figure 5.1 LgA or IntA followed by 30-35 days of withdrawal increases CP-AMPAR mediated transmission.The eEPSC amplitude displayed as a percent reduction by Naspm in A) males and B) females. Naspm decreased eEPSC amplitude similarly in D1- and D2-MSNs within group in males. In females regardless of group (drug naïve, LgA or IntA) D1-MSNs have no changes in CP-AMPAR mediated transmission. This is in contrast to D2-MSNs which show enhancements in CP-AMPAR mediated transmission in the LgA and IntA group compared to the drug naïve group.

One hypothesis about the mechanism by which CP-AMPARs are upregulated following LgA self-administration and withdrawal is through the process of generating silent synapses, which are synapses that have NMDARs but lack AMPARs (Kerchner and Nicoll, 2008). Interestingly, early in withdrawal animals generate *de novo* silent synapses, which leads to the recruitment of AMPARs to some of these newly made synapses, specifically CP-AMPARs (Huang et al., 2009; Koya et al., 2012; Lee et al., 2013). Furthermore, the blockade of CP-AMPARs restores high levels of silent synapses (Lee et al., 2013; Wright and Dong, 2020). This indicates that drug exposure and withdrawal are necessary for the generation of these silent synapses. But perhaps it is the intermittency, not the amount of drug per se that matters. For example, each new drug exposure could be its own salient event that drives synaptic changes. Under LgA conditions, there would only be one event per day because animals sustain high levels of infusions throughout the session, therefore cocaine levels in the brain are steady. However, with IntA conditions there are fluctuating cocaine levels in the brain due to the drug available and no drug available periods. Therefore, each spike (the peak and trough) of cocaine in the brain could be its own event driving synaptic changes. If this were the case, CP-AMPARs may be inserted into the nascent synapses earlier in IntA than LgA. One could imagine that this upregulation of CP-AMPARs could happen either earlier in withdrawal or possibly even with IntA sessions. This would suggest that the pattern of drug intake is driving the synaptic plasticity and that the

intermittency is a necessary feature for the enhancements in CP-AMPAR mediated transmission. Furthermore, given that it has been demonstrated that the upregulation of CP-AMPARs coincides with and mediates incubation of craving (Conrad et al., 2008; Loweth et al., 2014) it would be reasonable to predict that the IntA experience may result in more rapid incubation of craving than after a LgA experience. Therefore, studies should investigate the onset of incubation of craving and CP-AMPAR upregulation following IntA self-administration. To test this animals, would undergo training and IntA as described in Chapter 3 (5 days of training followed by 10 days of IntA, 0.4mg/kg/infusion). Since incubation of craving and assessment of CP-AMPAR mediated transmission has been well examined in LgA, LgA conditions would not need to be included in these studies. Immediately following IntA self-administration, assessment of incubation of craving at different time points in withdrawal would be done, withdrawal day 1,5, 7, 14, 21, 30, 45, 60 (incubation of craving assessment would be done as conducted in Chapter 3). Subsequent studies investigating CP-AMPAR transmission could be done based off of the findings of incubation data. These studies would identify how the pattern of drug-taking influences incubation of craving and insertion of CP-AMPARs. Understanding what drives both the behavioral and neuroadaptation is vital to understanding the mechanisms of addiction.

Overall, although the LgA procedure has provided the field with extensive knowledge about drug-induced behavioral adaptations and neuroplasticity, more recent studies indicate that the total drug consumption may not be as important as it originally was thought to be. In fact, it is possible that such large quantities of drug may overpower the more subtle differences in an individual's susceptibility to addiction or perhaps even nuances in mechanisms driving the transition to addiction.

Although, data suggests that LgA and IntA cocaine self-administration experience result in similar behavioral and neurobiological changes, this does not mean the mechanisms are the same. It is reasonable to postulate that how much drug is consumed or the pattern of drug consumption may alter the reward circuitry in different ways and still lead to similar behavioral changes. Given that LgA self-administration is not a good model for human drug consumption, further studies investigating the

neuroplasticity associated with IntA self-administration experience may be key for understanding the pathophysiology of addiction in humans.

Comparing effects of natural reinforcers to effects of cocaine

Historically, when investigating drug-induced alterations in reward circuitry food has been used as a control with the assumption that food-induced changes that are also seen after drugs exposure must not be relevant to addiction. Furthermore, evidence indicates there is overlap between food and drug-induced changes in both behavioral adaptations as well as neurobiological adaptations (Conrad et al., 2008; Oginsky et al., 2016; Derman and Ferrario, 2018). Specifically, it has been determined that junk-food increases NAc CP-AMPAR transmission in obesity-prone males following a 24 hours junk-food free period and persists for at least 14 days after junk-food removal (Oginsky et al., 2016; Alonso-Caraballo et al., 2021). However, the effects of junk-food in females following a short deprivation (24 hours) had yet to be investigated. Therefore in Chapter 4 we investigated the effects of junk-food and junk-food deprivation on MSN intrinsic excitability and glutamate transmission in females.

We found that females given 10 days of junk-food and 14-16 days of junk-food deprivation showed no changes in excitability or sEPSC frequency or amplitude compared to chow controls (Fig 4.1 and 4.2). However, at earlier deprivation time points (24-72 hours) junk-food resulted in reduced excitability and an enhancement CP-AMPAR mediated transmission in the NAc core compared to chow fed controls (Fig 4.3 and 4.4). Finally, we assessed whether the junk-food deprivation period was necessary to drive changes in glutamate transmission, which indeed data indicate it is necessary. When measures were made after junk-food but without deprivation we saw no effects of junk-food on sEPSC frequency or amplitude as well as no changes in CP-AMPAR mediated transmission (Fig 4.5). These data highlight that females experience a transient effect of junk-food on excitability and glutamate transmission, where the effect returns to baseline by 14-16 days of withdrawal.

Interestingly, this study highlights that the effects of junk-food are dependent upon sex. Males exposed to junk-food followed by a short deprivation period show

increases in CP-AMPAR mediated transmission, however, females seem to be protected from this long-lasting plasticity. Somewhat similarly, following experimenter administered cocaine females were also protected from glutamate plasticity in the NAc (discussed above and in Chapter 2). However, following LgA or IntA cocaine self-administration and withdrawal effects seem to be similar in both males and females, i.e., both experiences followed by withdrawal lead to upregulations in CP-AMPARs (discussed above and in Chapter 3). Together these data suggests that the threshold necessary to induce glutamate plasticity in females may be set higher than in males. Understanding the mechanism driving these sex specific differences may provide insight about why the development of addiction may be different between men and women.

Additionally, the male junk-food data is reminiscent of effects seen after cocaine self-administration (junk-food or cocaine exposure leading to upregulations in CP-AMPARs in the NAc). However the timeline of these effects are drastically different. Only a 24-hour junk-food deprivation period is necessary for the upregulation of CP-AMPARs in males (Oginsky et al., 2016; Alonso-Caraballo et al., 2021), whereas following cocaine exposure protracted withdrawal (~30 days) is required for upregulation of CP-AMPARs, at least following LgA self-administration (Conrad et al., 2008; McCutcheon et al., 2011; Purgianto et al., 2013). However, the onset of enhancements in CP-AMPARs following IntA self-administration remains unknown. As discussed above it is possible that CP-AMPARs are upregulated more rapidly and the timeline is more similar to that following junk-food exposure.

Comparing the drugs of abuse and food literature data so far suggests that drugs of abuse hijack at least some of the same circuitry that is necessary to ensure human survival, as it is essential to be motivated to eat and find food. Furthermore, under historical pretense it would suggest that since there are similarities (discussed above) between behavioral and brain alterations to food and drug that these alterations would not be specific to addiction. However, a recent study conducted in our lab concluded that behavioral differences in obesity susceptible populations seen in response to food and food-cues do not transfer to drug and drug-cues (Saraswat et al., 2023). This suggests that although there is overlap in some similar attributes of NAc glutamate

plasticity between food and drug, there seems to be a clear distinction between what is driving these behavioral responses.

Overall Conclusions

Together the data in this dissertation are examples of how the reward system adapts over time when the system is activated more than normal, i.e., activated by cocaine or junk-food. Given that both drugs of abuse and highly palatable foods alter the reward systems, it may be unsurprising that there are similarities found between them. Therefore, under this framework, I broadly think about addiction as a spectrum where perhaps highly palatable foods are addictive in similar ways that drugs may be. Future studies comparing and contrasting the nuanced differences between food and drug are needed to further develop insight in ways the reward system responds and adapts to better understand what is driving addiction. Perhaps revealing a common mechanism between these two distinct stimuli would move preclinical research forward at a faster rate and allow for better therapies across a wide range of "addictive" substances.

References

Ahmed SH, Koob GF (1998) Transition from moderate to excessive drug intake: change in hedonic set point. Science 282:298-300.

Allichon MC, Ortiz V, Pousinha P, Andrianarivelo A, Petitbon A, Heck N, Trifilieff P, Barik J, Vanhoutte P (2021) Cell-Type-Specific Adaptions in Striatal Medium-Sized Spiny Neurons and Their Roles in Behavioral Responses to Drugs of Abuse. Front Synaptic Neurosci 13:799274.

Alonso-Caraballo Y, Fetterly TL, Jorgensen ET, Nieto AM, Brown TE, Ferrario CR (2021) Sex specific effects of "junk-food" diet on calcium permeable AMPA receptors and silent synapses in the nucleus accumbens core. Neuropsychopharmacology 46:569-578.

Becker JB, Hu M (2008) Sex differences in drug abuse. Front Neuroendocrinol 29:36-47.

Becker JB, Koob GF (2016) Sex Differences in Animal Models: Focus on Addiction. Pharmacol Rev 68:242-263.

Becker JB, McClellan ML, Reed BG (2017) Sex differences, gender and addiction. J Neurosci Res 95:136-147.

Boudreau AC, Wolf ME (2005) Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. J Neurosci 25:9144-9151.

Brady KT, Randall CL (1999) Gender differences in substance use disorders. Psychiatr Clin North Am 22:241-252.

Carr CC, Ferrario CR, Robinson TE (2020) Intermittent access cocaine self-administration produces psychomotor sensitization: effects of withdrawal, sex and cross-sensitization. Psychopharmacology (Berl) 237:1795-1812.

Carroll ME, Anker JJ (2010) Sex differences and ovarian hormones in animal models of drug dependence. Hormones and behavior 58:44-56.

Cohen P, Sas A (1994) Cocaine use in Amsterdam in non deviant subcultures. Addiction Research 2:71-94.

Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, Marinelli M, Wolf ME (2008) Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature 454:118-121.

Cornish JL, Prasad AA (2021) Sex Differences in Substance Use Disorders: A Neurobiological Perspective. Front Glob Womens Health 2:778514.

Crombag H, Mueller H, Browman K, Badiani A, Robinson T (1999) A comparison of two behavioral measures of psychomotor activation following intravenous amphetamine or cocaine: dose-and sensitization-dependent changes. Behavioural pharmacology 10:205-213.

Derman RC, Ferrario CR (2018) Enhanced incentive motivation in obesity-prone rats is mediated by NAc core CP-AMPARs. Neuropharmacology 131:326-336.

Deroche-Gamonet V, Belin D, Piazza PV (2004) Evidence for addiction-like behavior in the rat. Science 305:1014-1017.

Dong Y, Taylor JR, Wolf ME, Shaham Y (2017) Circuit and Synaptic Plasticity Mechanisms of Drug Relapse. J Neurosci 37:10867-10876.

Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE (2005) Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. Biol Psychiatry 58:751-759.

Ferrario CR, Li X, Wang X, Reimers JM, Uejima JL, Wolf ME (2010) The role of glutamate receptor redistribution in locomotor sensitization to cocaine. Neuropsychopharmacology 35:818-833.

Hao Y, Martin-Fardon R, Weiss F (2010) Behavioral and functional evidence of metabotropic glutamate receptor 2/3 and metabotropic glutamate receptor 5 dysregulation in cocaine-escalated rats: factor in the transition to dependence. Biol Psychiatry 68:240-248.

Hernandez-Avila CA, Rounsaville BJ, Kranzler HR (2004) Opioid-, cannabis- and alcohol-dependent women show more rapid progression to substance abuse treatment. Drug Alcohol Depend 74:265-272.

Hille B (1992) Potassium channels and chloride channels. In Ionic Channels of Excitable Membrane. Edited by Hill B:130-133.

Hu M, Becker JB (2008) Acquisition of cocaine self-administration in ovariectomized female rats: Effect of estradiol dose or chronic estradiol administration. Drug and Alcohol Dependence 94:56-62.

Huang YH, Lin Y, Mu P, Lee BR, Brown TE, Wayman G, Marie H, Liu W, Yan Z, Sorg BA, Schlüter OM, Zukin RS, Dong Y (2009) In vivo cocaine experience generates silent synapses. Neuron 63:40-47.

Ishikawa M, Mu P, Moyer JT, Wolf JA, Quock RM, Davies NM, Hu XT, Schlüter OM, Dong Y (2009) Homeostatic synapse-driven membrane plasticity in nucleus accumbens neurons. J Neurosci 29:5820-5831.

Jedynak J, Hearing M, Ingebretson A, Ebner SR, Kelly M, Fischer RA, Kourrich S, Thomas MJ (2016) Cocaine and Amphetamine Induce Overlapping but Distinct

Patterns of AMPAR Plasticity in Nucleus Accumbens Medium Spiny Neurons. Neuropsychopharmacology 41:464-476.

Kawa AB, Bentzley BS, Robinson TE (2016) Less is more: prolonged intermittent access cocaine self-administration produces incentive-sensitization and addiction-like behavior. Psychopharmacology (Berl) 233:3587-3602.

Kawa AB, Hwang E-K, Funke JR, Zhou H, Costa-Mattioli M, Wolf ME (2022) Positive allosteric modulation of mGlu1 reverses cocaine-induced behavioral and synaptic plasticity through the integrated stress response and oligophrenin-1. Biological Psychiatry 92:871-879.

Kerchner GA, Nicoll RA (2008) Silent synapses and the emergence of a postsynaptic mechanism for LTP. Nat Rev Neurosci 9:813-825.

Kerstetter KA, Aguilar VR, Parrish AB, Kippin TE (2008) Protracted time-dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. Psychopharmacology (Berl) 198:63-75.

Kourrich S, Thomas MJ (2009) Similar neurons, opposite adaptations: psychostimulant experience differentially alters firing properties in accumbens core versus shell. J Neurosci 29:12275-12283.

Koya E, Cruz FC, Ator R, Golden SA, Hoffman AF, Lupica CR, Hope BT (2012) Silent synapses in selectively activated nucleus accumbens neurons following cocaine sensitization. Nat Neurosci 15:1556-1562.

Kravitz AV, Tye LD, Kreitzer AC (2012) Distinct roles for direct and indirect pathway striatal neurons in reinforcement. Nat Neurosci 15:816-818.

Lee BR, Ma Y-Y, Huang YH, Wang X, Otaka M, Ishikawa M, Neumann PA, Graziane NM, Brown TE, Suska A (2013) Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving. Nature neuroscience 16:1644-1651.

Lobo MK, Nestler EJ (2011) The striatal balancing act in drug addiction: distinct roles of direct and indirect pathway medium spiny neurons. Front Neuroanat 5:41.

Loweth JA, Scheyer AF, Milovanovic M, LaCrosse AL, Flores-Barrera E, Werner CT, Li X, Ford KA, Le T, Olive MF, Szumlinski KK, Tseng KY, Wolf ME (2014) Synaptic depression via mGluR1 positive allosteric modulation suppresses cue-induced cocaine craving. Nat Neurosci 17:73-80.

Lynch WJ, Carroll ME (1999) Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology (Berl) 144:77-82.

McCutcheon JE, Wang X, Tseng KY, Wolf ME, 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. J Neurosci 31:5737-5743.

Nicola SM, Surmeier J, Malenka RC (2000) Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. Annu Rev Neurosci 23:185-215.

Nicolas C, Russell TI, Pierce AF, Maldera S, Holley A, You ZB, McCarthy MM, Shaham Y, Ikemoto S (2019) Incubation of Cocaine Craving After Intermittent-Access Self-administration: Sex Differences and Estrous Cycle. Biol Psychiatry 85:915-924.

Nisenbaum ES, Wilson CJ (1995) Potassium currents responsible for inward and outward rectification in rat neostriatal spiny projection neurons. J Neurosci 15:4449-4463.

Oginsky MF, Goforth PB, Nobile CW, Lopez-Santiago LF, Ferrario CR (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 41:2977-2986.

Pascoli V, Terrier J, Espallergues J, Valjent E, O'Connor EC, Lüscher C (2014) Contrasting forms of cocaine-evoked plasticity control components of relapse. Nature 509:459-464.

Paterson NE, Markou A (2003) Increased motivation for self-administered cocaine after escalated cocaine intake. Neuroreport 14:2229-2232.

Perez MF, White FJ, Hu XT (2006) Dopamine D(2) receptor modulation of K(+) channel activity regulates excitability of nucleus accumbens neurons at different membrane potentials. J Neurophysiol 96:2217-2228.

Post RM, Rose H (1976) Increasing effects of repetitive cocaine administration in the rat. Nature 260:731-732.

Proaño SB, Morris HJ, Kunz LM, Dorris DM, 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 120:1356-1373.

Purgianto A, Scheyer AF, Loweth JA, Ford KA, Tseng KY, Wolf ME (2013) Different adaptations in AMPA receptor transmission in the nucleus accumbens after short vs long access cocaine self-administration regimens. Neuropsychopharmacology 38:1789-1797.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247-291.

Saraswat AA, Longyear LG, Kawa AB, Ferrario CR (2023) Cocaine-induced plasticity, motivation, and cue responsivity do not differ in obesity-prone vs obesity-resistant rats; implications for food addiction. Psychopharmacology (Berl) 240:853-870.

Simon SL, Richardson K, Dacey J, Glynn S, Domier CP, Rawson RA, Ling W (2001) A comparison of patterns of methamphetamine and cocaine use. Journal of addictive diseases 21:35-44.

Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001) Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. Nature Neuroscience 4:1217-1223.

Turrigiano GG, Nelson SB (2004) Homeostatic plasticity in the developing nervous system. Nat Rev Neurosci 5:97-107.

Vanderschuren LJ, Kalivas PW (2000) Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl) 151:99-120.

Vezina P (2004) Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. Neurosci Biobehav Rev 27:827-839.

Vollbrecht PJ, Nobile CW, Chadderdon AM, Jutkiewicz EM, Ferrario CR (2015) Pre-existing differences in motivation for food and sensitivity to cocaine-induced locomotion in obesity-prone rats. Physiol Behav 152:151-160.

Wright WJ, Dong Y (2020) Psychostimulant-Induced Adaptations in Nucleus Accumbens Glutamatergic Transmission. Cold Spring Harb Perspect Med 10.

Zhang W, Linden DJ (2003) The other side of the engram: experience-driven changes in neuronal intrinsic excitability. Nat Rev Neurosci 4:885-900.

Zhao B, Zhu J, Dai D, Xing J, He J, Fu Z, Zhang L, Li Z, Wang W (2016) Differential dopaminergic regulation of inwardly rectifying potassium channel mediated subthreshold dynamics in striatal medium spiny neurons. Neuropharmacology 107:396-410.

Zimmer BA, Dobrin CV, Roberts D (2011) Brain-cocaine concentrations determine the dose self-administered by rats on a novel behaviorally dependent dosing schedule. Neuropsychopharmacology 36:2741-2749.

Zimmer BA, Oleson EB, Roberts DC (2012) The motivation to self-administer is increased after a history of spiking brain levels of cocaine. Neuropsychopharmacology 37:1901-1910.