Examination of Addiction Relevant Psychomotor Activation and Incentive Motivation Following Intermittent Access to Cocaine Self-Administration

By

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Dedication

To my mom: because of you I am

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Abstract

Many people have tried an addictive drug at some point in their lifetime, but only a small percentage of these individuals develop compulsive patterns of drug use that define a substance abuse disorder. For ethical reasons, amongst many others, it is difficult to study the transition from casual to compulsive use in humans. As a result, a number of preclinical self-administration models have been developed to investigate the neurobiological and psychological mechanisms underlying addiction susceptibility. For over a decade, long access (LgA; 6-h sessions of continuous drug availability) self-administration was the most widely used preclinical model of addiction, the results of which suggest that high and escalated drug intake is necessary for the development of addiction-like behavior. Human cocaine users, however, rarely engage in sustained high-level use and instead opt for a more sporadic pattern of administration. Recently, intermittent access (IntA; 5-min Drug-Available periods separated by 25-min No Drug-Available periods) was developed to better model human cocaine use patterns and the results from this model show more robust addiction-like behavior despite less drug consumption. Intermittent patterns of use have also been shown to result in sensitization to the psychomotor activating effects of drugs. This behavioral plasticity is thought to reflect, in part, changes in dopamine (DA) neurotransmission, and therefore, may provide insights into the development of addiction. Currently, the growing literature on IntA suggests that IntA better models the transition to addiction but very little is known about the ability of IntA to produce psychomotor sensitization. Early studies of intermittent, experimenter-administered cocaine revealed several characteristics

of psychomotor sensitization, inclusive of, but not limited to: (1) greater expression after extended vs acute withdrawal, (2) greater expression in females compared to males, and (3) cross-sensitization to other drugs of abuse.

In Chapter II, we found that self-administered cocaine under IntA conditions produced psychomotor sensitization with similar characteristics. Specifically, our first experiment revealed a left shift in the dose-effect function (indicative of psychomotor sensitization) in animals with IntA but not LgA experience when tested one day after discontinuation of self-administration (withdrawal day 1). Following extended withdrawal (30 days) from IntA, an even greater drug effect was observed, as indicated by focused stereotyped head movements. Robust psychomotor sensitization was also observed following extended withdrawal from LgA, with neither stereotypy ratings nor frequencies differing from IntA animals. In our second experiment, we utilized IntA-Limited to ensure similar consumption in females and males and assessed psychomotor sensitization within and between subjects. We found that females with IntA-Limited experience increased locomotor activity to a greater extent across sessions than males with IntA-Limited experience. Even though both females and males expressed psychomotor sensitization in the self-administration context, only females with IntA-Limited experience expressed psychomotor sensitization outside of the self-administration context compared to sex-matched, drug-exposed controls. In a separate experiment, we also found a greater locomotor response to d-Amphetamine challenge doses in animals with IntA cocaine self-administration experience compared to acquisition-only controls. Lastly, in Chapter III, we found comparable incentive sensitization, as assessed by a behavioral economic approach, in females and males that was positively correlated with the extent of psychomotor sensitization expressed following IntA-Limited.

These results add to the literature showing that the IntA model is especially effective at producing the neurobehavioral sensitization thought to underlie the transition to addiction, and thus support an incentive-sensitization view of addiction.

Chapter I

General Introduction

Transitioning from Drug Use to Abuse

Over half of American adults have used an addictive, illicit drug at some point in their lifetime (SAMHSA, 2019). Following the first drug experience, some individuals may never use the drug again, some may continue to use the drug in a recreational manner, and some individuals will develop a substance abuse disorder (SUD). According to the diagnostic and statistical manual of mental disorders (DSM), a SUD is diagnosed when drug use interferes with daily functioning to the detriment of self and society, amongst other criteria. In 2018, only 3% of the population (aged 12 or older) met the criteria for an illicit drug use disorder (SAMHSA, 2019). Even though the percentage of individuals that transition from casual to compulsive drug use is small, illicit drug use costs nearly $200 billion in lost work productivity, health care, and crime each year (NDTA, 2011). Further, since 2011, deaths by drug poisoning have outnumbered deaths by suicide, homicide, firearms, and motor vehicle crashes, making drug poisoning the leading cause of injury death in the United States (NDTA, 2018). Drug poisoning deaths reached the highest recorded level in 2017 (this is the latest available data), with approximately 192 people dying each day (NDTA, 2019). These devastating consequences of drug use and addiction have motivated extensive research investigation on susceptibility, i.e. understanding the psychological and neurobiological mechanisms underlying the transition from drug use to abuse.

Nearly all drugs of abuse share the ability to transiently increase dopamine neurotransmission within reward pathways, contributing to the reinforcing, incentive motivational properties of the drug (Wise and Bozarth 1987; Di Chiara and Imperato 1988). Most drugs of abuse can also induce varying degrees of psychomotor activation, dependent upon factors such as dose, route of administration, sex, and environment (Robinson and Becker 1986; Badiani and Robinson 2004; Samaha and Robinson 2005). Importantly, there are differences between drug effects following a single exposure compared with repeated administration. There are, of course, many illicit drugs, but here I will specifically focus on cocaine, which is currently considered a resurgent drug threat in the US (NDTA, 2019).

Following chronic administration of cocaine, dopamine neurotransmission and psychomotor activation has been shown to increase (sensitization) or decrease (tolerance), largely depending on whether cocaine is administered intermittently or continuously (for reviews, Post 1980; Kawa et al. 2019a) Sensitization and tolerance both represent forms of plasticity, and these types of changes in the brain and behavior are thought to be critical to our understanding of the progression of addiction. However, whether tolerance- or sensitization-related neurobehavioral plasticity is associated with the transition to addiction remains a point of discussion. Until recently, the long access animal model of self-administration was considered the best preclinical model of addiction due to its ability to produce tolerance-related changes observed in humans. In contrast, the newly introduced intermittent access model produces more robust addiction-like behavior, such as motivation for cocaine and drug cue-induced reinstatement, but instead results in sensitization-related neurobehavioral plasticity that has yet to be fully characterized.

The studies presented in this dissertation explore the expression of behavioral

sensitization produced by various preclinical models of addiction. Much of what we know about how drugs influence the brain and behavior was discovered by the passive administration of drugs to animals, and will thus be described first given its continued utility in both experiments presented here and elsewhere. This will be followed by an extensive discussion of the evolution of drug self-administration procedures, focusing on the translational value of the observed outcomes.

Non-Contingent Drug Administration in Rats

A Brief History

One of the earliest reports assessing experimenter-administered drug in rats was conducted in Germany. Joël and Ettinger (1926) reported a biphasic behavioral pattern characterized by an initial decrease in motility, followed by an increase in locomotor activity and gnawing after exposure to a high dose of an opioid, as well as withdrawal phenomena during drug abstinence. Soon after, additional descriptive animal morphine "addiction" experiments were published using dogs (although, reports of drug exposure experiments in dogs appear as early as 1864), cats, rabbits, and monkeys (Plant and Pierce 1928; Tatum et al. 1929; Kolb and DuMez 1931). At this time, addiction was defined as "a condition developed through the effects of repeated actions of a drug such that its use becomes a need and cessation of its action causes mental or physical disturbances" (Tatum and Seevers 1931), earning it the abstinence syndrome moniker.

Given that hyperirritability was a prominent symptom during drug withdrawal, Sollmann suggested assessing changes in irritability (i.e, struggle responsiveness, as first described by Barlow in 1932) during the drug exposure period and after withdrawal to Himmelsbach et al. (1935). In the experiment, they induced irritability in rats, both pre- and post-injection and

throughout withdrawal, by restraining the animal in a supine position that still allowed for movement. Each struggle was then recorded, resulting in the first quantitative method to assess physical drug dependence. Animals were given daily subcutaneous (SC) injections of one of three opium derivatives (morphine, codeine, or heroin) for five to six weeks, with increased hyperirritability prior to the injection being interpreted as the presence of addiction. The same observation post-injection was interpreted as tolerance to the sedative and cataleptic effects of the drug. Following this exposure period, the drug was replaced by tap water to induce withdrawal that continued nine to thirteen days, ending when hyperirritability levels returned to a normal level. They report the presence of addiction following repeated administration of all three opium derivatives, demonstrated by a progressive increase in the median number of struggles per minute pre-injection (with the exception of 1-2 animals per drug).

Within the first week of repeated administration, tolerance was detected as post-injection struggles increased in the morphine and codeine groups, but not heroin due to the induction of a pronounced cataleptic state. After two to three weeks, however, the morphine and codeine groups peaked and leveled-off. This was thought to reflect gradual tolerance to the sedative effects but very little tolerance to the cataleptic effects that possibly prevented further increases in the struggle response. Although not quantified, other forms of behavioral tolerance were also observed following repeated administration of the drugs, inclusive of a decreased latency to reposition from supine placement (interpreted to mean the duration of action of the drug was shorter), increased sensitivity to auditory and painful stimuli and forced gnawing to the point of self-mutilation. During the extended withdrawal period, a slight increase in pre-injection irritability was observed soon after discontinuation of drug administration in two of the six groups, but otherwise gradually declined. A similar trend of increased irritability followed by a

gradual decrease was observed post-injection for all but one of the groups.

The authors concluded that the method employed produced results comparable to clinical observations, e.g. animals showed increased irritability during acute withdrawal and behavioral tolerance that is characteristic of addiction. They also noted that, "It may be emphasized that appearance of abstinence phenomena after withdrawal of the drug is the sine qua non of addiction" (Himmelsbach et al. 1935, p. 186). This emphasis, however, was problematic due to observations of repeated relapse after physical withdrawal symptoms had subsided. Furthermore, a number of other potentially addictive controlled substances were also shown not to produce physical dependence, namely marihuana, amphetamine, and cocaine (Vogel 1952).

Evolution of Cocaine Addiction. Surprisingly, "pure addiction" to cocaine was considered rare in the 1950s and it was determined that "Tolerance to cocaine does not develop; rather, increased sensitivity to the effects of the drug occurs. There are no true withdrawal symptoms" (Isbell and White 1953, p. 564). As such, psychostimulants like amphetamine and cocaine were reported to only produce "habituation", which refers to psychological dependence driven by the desire to relieve emotional discomfort (see next section for discussion of amphetamines). Then, psychological addictions were considered relatively minor clinical conditions amenable to treatments with psychotherapy. When cocaine use began to increase in 1970s, the National Institute of Drug Abuse (NIDA) increased their support for cocaine research, labeling it high priority because so little was known about the substance (Cocaine: 1977, NIDA Research Monograph series). Based on evidence accumulated over a 4 year period, the NIDA monograph series concluded that the American public was not suffering greatly from cocaine use, despite previous claims.

In the early 1980s, however, patients began to increasingly cite cocaine as their direct

cause of compulsive drug taking and reason for seeking treatment. In 1986, a model of cocaine withdrawal was proposed and reported three distinct phases: crash, withdrawal, and extinction (Gawin and Kleber 1986). The crash phase was typically experienced within hours to days of last use and was characterized by symptoms such as dysthymia, restlessness, hypersomnia, and even irritability, but no cravings to use. Strong cravings, however, emerged during the withdrawal phase that lasted 1-10 weeks. During this time, symptomology slightly worsened and expanded to include other disturbances such as poor concentration and erratic sleep. These symptoms started to decrease during the extinction phase, with normative functioning returning within 28 weeks. This increasing awareness of "pure addiction" to cocaine of course led to a surge in preclinical research examining the effects of cocaine, mostly in rats. Those critical to the experiments presented here are described in further detail below.

Psychomotor Activating Effects of Acute Cocaine

As with opioids and many other drugs of abuse, cocaine administration results in changes in locomotor activity and stereotyped behavior, both of which are forms of psychomotor activity (Wise and Bozarth 1987). Observation of this behavior in the rat appears to have been first reported by Downs and Eddy (1932). Following the administration of a high dose of cocaine (75 mg/kg) administered intraperitoneal (IP), stereotyped head movements (then referred to as "weaving movements of the head") were observed. The term "stereotype" was first used by Hauschild (1938) to described the abnormal behavior observed following amphetamine administration, but was later used to describe behavior with little variation (Randrup and Munkvad 1967). In the early 1960s, a number of studies were published detailing the behavioral characteristics of amphetamine in rats (for review, see Robinson and Becker 1986) As a result, much of what is discussed below regarding cocaine administration was first reported following

studies on amphetamine. Once cocaine became a priority, researchers sought to determine if it would produce similar behavioral characteristics.

Indeed, Scheel-Krüger (1972) found comparable psychomotor activity following cocaine and amphetamine administration. He reported that within 3-4-min after the administration of an IP dose of 25 mg/kg cocaine, there was a large increase in the intensity and frequency of locomotion and rearing (standing on hind legs with front paws elevated). Another administration of the same dose of cocaine 60-min after the first dose produced the same behavioral response in the majority of the rats, but 3 of the 12 rats also engaged in amphetamine-like stereotyped sniffing. Here, sniffing was only considered to be stereotyped if the rat was in a crouched posture and performed the behavior on a lower portion of the wall or while moving the head from side to side in a seated position on the floor. Sniffing behavior can also be accompanied by licking and biting (of the cage or self) following high doses, but that was not observed in any of the animals administered cocaine. Although not acknowledged by the author, this report provided an early indication that repeated administration of the same dose of cocaine could also result in a larger observed drug effect (i.e., the transition from a hyperactive ambulatory response to stereotypy).

Effects of Repeated Cocaine on Psychomotor Activation

A few years later, Post and Rose (1976) systematically assessed how repeated administration of cocaine affected psychomotor activation. Rats were given 10 mg/kg IP cocaine once daily for 5 days/week. Compared to saline-controls, only cocaine animals significantly increased locomotion (as measured by horizontal photocell counts) and rearing (as measured by vertical photocell counts) over the course of 14 injections. More specifically, the time course of individual injections revealed an increase in both peak effect and duration. For example, 10-min

following injection 5, horizontal counts peaked at ~900, remained elevated for an additional 10-min, and then decreased to ~600 in the following 10-min. In comparison, 10-min following injection 10, horizontal counts reached ~1400, slightly increased in the next 10-min (~1500), returned to ~1400 in the following 10-min and then gradually declined. Activity levels returned to baseline within 90-min following both injections, but activity was higher overall following the later injection.

Following injection 14, however, stereotypy ratings increased and locomotion trends were essentially the complete opposite. Rather than a rapid increase and peak during the first 10-20-min, horizontal counts increased slowly and peaked around 70-min post injection 15. This trend is explained by the earlier initiation of repetitive movements (such as rearing, movement from corner to corner, head bobbing and circling) that were greater in intensity and longer in duration (up to 15-min) following repeated cocaine administration. When a similar progressive increase in hyperactivity and stereotypy was observed following repetitive amphetamine administration, it was thought to be a conditioning effect (Tilson and Rech 1973). To test this theory, all rats received saline for injection 25 and those with chronic cocaine administration did not exhibit greater psychomotor activation than saline controls. Differences in psychomotor activity did, however, remerge when cocaine was again administered to those with a history of exposure. The authors concluded:

> Our data thus suggest that repetitive administration of cocaine is capable of producing increasing hyperactivity and stereotypy in some conditions of administration. Whether tolerance can be manifested may depend not only on the specific parameters studied, but also on the intermittent rather than continuous drug administration.
> (Post and Rose 1976, pp. 731-732)

The progressive increase in psychomotor activation observed following repeated drug administration has in-fact been referred to as reverse-tolerance, but it is now more commonly

referred to as sensitization.

Characteristics of Psychomotor Sensitization

Behaviorally, a notable observation regarding psychomotor sensitization was that mice expressing sensitization to morphine or d-amphetamine later showed an enhanced response to cocaine (Shuster et al. 1975, 1977). The term "cross-sensitization" was used to describe this phenomenon. The purpose of the latter aforementioned experiment, however, was to assess several characteristics of cocaine sensitization, inclusive of, but not limited to, persistence, the effect of dose, and specificity (Shuster et al. 1977). As such, the authors measured running activity following repeated injections of cocaine (pre-treatment) and later exposed the cocaine-pretreated mice to d-amphetamine. Mice given 20 mg/kg IP cocaine daily for 5 days displayed a four-fold increase in running activity 60-min following an additional injection of cocaine administered 18 days after the first injection. They also reported that mice pretreated with 20 mg/kg IP cocaine (one injection a day for 4 days) expressed sensitization to the same dose as far out as 102 days after the first injection. Additionally, groups of mice were pretreated with 10, 20, 30, or 40 mg/kg IP cocaine (one injection a day for 3 days) and then exposed to a test dose of 20 mg/kg IP cocaine. They found that mice with a higher pretreatment dose also had a greater running response to the test dose.

Interestingly, mice pretreated with 20 mg/kg IP cocaine (one injection a day for 3 days) did not show an increased running speed to 5 mg/kg IP d-amphetamine (this absence of cross-sensitization was also observed for morphine). In contrast, there are later reports that pretreatment with cocaine does produce cross-sensitization with methamphetamine in mice (Kaneto et al. 1988; Hirabayashi et al. 1991). These findings are corroborated by one of, if not, the first cocaine-pretreated rat cross-sensitization experiment (Akimoto et al. 1990). Rather than

focus on the behavior, these authors instead focused on the underlying neurobiological mechanisms. Given that a number of early reports implicated enhanced dopamine neurotransmission in behavioral sensitization (for review, see Robinson and Becker 1986), they assessed how pretreatment with cocaine affected methamphetamine-induced dopamine release during a later challenge. Compared to saline-pretreated rats, the methamphetamine challenge produced a greater increase in dopamine levels in cocaine-pretreated rats. The same effect was observed when rats were instead pretreated with methamphetamine and challenged with cocaine. While there is a more recent report also confirming that pretreatment with cocaine produces cross-sensitization to methamphetamine (Shanks et al. 2015), the literature regarding cross-sensitization between cocaine and other drugs is surprisingly limited and is thus investigated in Chapter II of this dissertation, along with examinations of persistence and sex differences.

Sex Differences in Psychomotor Sensitization

Unlike with cross-sensitization and persistence of sensitization, there is a very large literature on cocaine-induced psychomotor sensitization comparing female and male rats. First, cocaine was also shown to induce circling behavior (rotation), and females were more sensitive than males across several doses administered IP (Glick et al. 1983). To assess sex differences in sensitization, females and males were given 20 mg/kg IP cocaine on three separate occasions. Following the first injection, another injection of the same dose was administered after 1, 7, or 14 days. The last injection was administered after the same interval (1, 7, or 14 days after second injection). A sensitized rotation response was observed in females during the 1 and 7 day interval test for the second injection only. No other significant differences were found. This same experiment was also conducted with d-amphetamine and sensitization was not detected in either sex, which was inconsistent with a previous report (Robinson et al. 1982). The authors suggested

that the discrepancy might be due to procedural differences, but nonetheless concluded that sensitization more readily occurs in females than males following repeated cocaine administration. Later reports found that males express psychomotor sensitization following chronic cocaine administration, but the degree of sensitization was still far greater in females (for review, see Becker et al. 2006), supporting the notion of greater sensitization susceptibility in females.

Reinforcing Effects of Cocaine

There are very simple animal models to assess whether or not a drug is reinforcing. The most common non-contingent procedure, conditioned place preference (CPP), involves pairing one distinct context (for example, striped walls and grid floor) with drug administration. On the alternate day, another context (polka dot walls and mesh floor) is then paired with administration of a non-drug solution such as saline. This cycle is repeated several times, and then on the test day, animals are allowed to roam freely between the two compartments to determine which context is now preferred following the drug experience (thus relying on associative learning). If the animal spends more time in the drug-paired context, it is inferred that the drug had positive reinforcing effects.

In 1982, Spyraki et al. used CPP to assess the reinforcing properties of various doses of cocaine. They administered IP cocaine and saline on alternating days, with one injection being administered per day for eight total days (four injections each). The procedure was the same for each of the six doses of cocaine. The lowest dose of 0.625 mg/kg cocaine failed to produce a place preference. All other doses (1.25, 2.5, 5.0, 10.0, and 20.0 mg/kg) of cocaine, however, resulted in significantly greater time being spent in the cocaine-paired context. Interestingly, preference peaked at the 5.0 mg/kg dose of cocaine, and this was also the lowest dose that

produced significant increases in locomotor activity (2.5 mg/kg was the other low dose tested for locomotor activity). The higher dose of 10 mg/kg cocaine produced nearly twice as much hypermotility as the 5 mg/kg dose, but did not produce greater place preference. Nonetheless, these results indicated that cocaine was indeed reinforcing, even at lower doses.

Impact of Route of Administration on Reinforcing Effects of Cocaine

It was suggested that an IP cocaine-induced place preference was partly established by the peripheral local anesthetic effects of cocaine (not described here). To avoid the confound of such effect, Spyraki et al. (1987) assessed CPP using a different, and yet to be discussed, route of administration and found partially conflicting results. In 1988, Nomikos and Spyraki directly compared the routes of administration and assessed a number of other factors that could potentially affect cocaine-induced CPP. In contrast to IP injections, the other injection required surgery to implant a catheter into the jugular, to be used for intravenous (IV) drug delivery. CPP testing was conducted as described previously for Spyraki et al. 1982.

First, dose-response curves were generated using 0.5, 1.0, 2.5, 5.0 and 10 mg/kg IV or IP cocaine. Consistent with their previous result, all doses above 1.0 mg/kg IP produced a significant increase in time spent in the cocaine-paired chamber. Here, however, 10 mg/kg IP resulted in the greatest amount of time spent (more than half of the total test time) in the cocaine-paired chamber and was thus used in subsequent experiments. In contrast, only the three lower doses (0.5, 1.0, and 2.5) administered IV resulted in place preference. However, less than half of the total time was spent in the cocaine-paired side by rats conditioned with 2.5 mg/kg IV cocaine. The authors noted that the 2.5 mg/kg IV dose resulted in convulsions that are typical of high doses. These convulsions also sensitized with repeated administration and could have led to a place aversion. Nonetheless, the fact that the IV dose-response curve is shifted to the left

indicates greater sensitivity than IP injections, i.e. a smaller dose IV produces similar preference of that observed following a larger dose administered IP. As such, because the .5 mg/kg IV cocaine dose produced preference of a similar magnitude to 10 mg/kg IP cocaine, it was used in subsequent experiments.

Next, rats received a varying number of conditioning injections (1, 2, 3, or the traditional 4) to examine how this factor affected the strength of cocaine-induced (0.5 mg/kg IV or 10 mg/kg IP) CPP. After only two injections, rats administered IV cocaine developed preference for the cocaine-paired chamber, with each additional injection resulting in even greater time being spent in the cocaine-paired chamber. Rats administered IP cocaine only showed CPP after being conditioned with 4 injections. Although not significantly different, rats administered IP cocaine for two or more trials spent less time in the cocaine-paired chamber than those administered IV cocaine for the same number of trials. These findings provided additional support for their conclusion of increased sensitivity to IV compared to IP cocaine. Similarly, for morphine, it was also shown that fewer IV trials were required to produce CPP than SC trials (1 vs 3; Mucha et al. 1982; Mucha and Iversen 1984).

While several other experiments were conducted, the last to be described here assessed the persistence of CPP. Cocaine-induced CPP was again established following four conditioning trials of 0.5 mg/kg IV or 10 mg/kg IP cocaine. Following the initial post-conditioning test (day 1), chamber preference was again assessed 7 days later. Although the amount of total time spent decreased (possibly due to an extinction effect) from post-conditioning day 1, rats conditioned with either IV or IP cocaine still spent more time in the cocaine-paired chamber than controls on post-conditioning test day 7. Additionally, the total time spent in the cocaine-paired chamber on post-conditioning test day 7 did not differ in IV vs IP rats, and IP rats tested on post-conditioning

day 30 still preferred the cocaine-paired chamber. These results show additional similarities between the test doses, and suggests they could be used interchangeably.

However, IV drug administration is one of the fastest routes of delivery, resulting in peak drug levels in the brain in a matter of seconds (Wiggins et al. 1989; Pan et al. 1991; Shuster 1992). In contrast, drugs administered IP reach the brain far slower because they are first absorbed from the peritoneal cavity, then the portal system, followed by general circulation where it can finally reach the brain (Shuster, 1992). Given that both routes are effective, the first experiment presented in Chapter II incorporates IP injections. Note, however, that IV injections are the primary route of administration for all experiments presented in Chapters II and III.

Cross-Sensitization to Reinforcing Effects of Cocaine

Armed with the knowledge that the psychomotor activating effects of drugs, including cocaine, can cross-sensitize, Lett (1989)) assessed CPP following repeated cocaine administration and examined cross-sensitization of the reinforcing effects of morphine and cocaine. In this design, some rats were sensitized with repeated, intermittent injections of 20 mg/kg IP cocaine (10 total, spaced 24-72-h apart) prior to CPP. The other rats received saline injections. During CPP, a distinctive chamber was paired with cocaine administration (three 2.5 mg/kg IP injections total) in most rats and the other chamber was neutral (saline was not administered in this context). To serve as a baseline, some sensitized (cocaine) and non-sensitized (saline) rats were exposed to the distinctive chamber in the absence of cocaine, which was instead administered ~30-min or more after removal from the chamber (therefore unpaired). Consistent with Spyraki et al. (1982), the non-sensitized rats showed reliable place preference, but sensitized rats spent greater time in the cocaine-paired chamber relative to both non-sensitized and unpaired rats. If rats were instead pretreated with morphine and then assessed for

CPP with cocaine, morphine-sensitized rats spent the greatest amount of time in the cocaine-paired chamber, indicating cross-sensitization.

Pros and Cons of Conditioned Place Preference for Measuring Drug Reinforcement

The studies described above highlight some clear advantages of CPP, such as: (1) detection of drug reward and aversion; (2) sensitivity to low drug doses; (3) testing the animal in a drug-free state; (4) results following a single drug-pairing; and (5) can be used to concomitantly assess the psychomotor activating and reinforcing effects of drug. It is amazing that so much valuable information can come from such a simple model, but the most glaringly obvious limitation is that the experimenter controls drug delivery and administration is not a matter of choice. CPP cannot, therefore, measure an animal's willingness to work to obtain a drug (motivation) or even a drug-associated cue (reinstatement), both of which are considered addiction-like behavior.

There are additional limitations to CPP, but they are far more trivial in nature. For example, it is work-intensive to generate dose-effect curves and it is difficult to control for the fact that some animals have a side preference before any injections are administered. Given that rats are novelty-seekers, there is a potential confound on the test day because drug administration in one context may make it less familiar. However, the addition of an actual novel context (in addition to drug-paired and saline-paired) did not change the result: rats still preferred the drug-paired context (Mucha and Iversen 1984; Parker 1992). It is also important to note that drug-induced CPP has been observed in humans (Childs and de Wit 2009, 2016; Mayo et al. 2013; Childs and De Wit 2013), and continues to serve as a useful model, but is not employed in any of the studies reported here given our interest in addiction-like behavior.

Contingent Drug Administration in Rats

A Brief History

To no surprise, one of the first studies that allowed animals to self-administer drug examined opioids (Headlee et al. 1955). Seemingly inspired by the experimenter-administered drug literature, this procedure also involved restraining rats in a supine position to prevent body movement. The head of the rat, however, was allowed to move laterally in order to interrupt vertical infrared beams on each side. Similar to CPP, the side that the rats' head spent the least amount of time in was designated the drug side. As a result, all head motions to that side resulted in the delivery of an IP injection from an automatic apparatus. Prior to undergoing this self-administration test, all rats received daily injections of morphine or codeine for two weeks and then underwent 48 hours of withdrawal. Unlike saline controls, both morphine- and codeine-pretreated rats showed increased preference for the side that resulted in injection delivery (i.e., saline-pretreated rats self-administered saline, morphine-pretreated rats self-administered morphine, etc.). Here, the animals were willing to "work" to obtain additional injections of the drug, thus indicating that the drug was reinforcing and potentially addictive. While the authors concluded this method was worthy of further study, it was not adapted for widespread use.

A few years later, another method of self-administration was developed that allowed rats to administer drugs intravenously (Weeks 1962). Here again, morphine was the drug under investigation, but the animals were allowed to move freely. A lightweight saddle was strapped behind the forelegs and connected to an apparatus that allowed intravenous injections to pass through a cannula in the jugular vein. Physical dependence was first established using experimenter-administered injections of increasing doses and subsequent injections were administered by an automatic apparatus following a lever-press response from the rat (Weeks 1962). After a few chance lever-presses, rats regularly responded to obtain 10 mg/kg IV morphine. The response rate increased when the dose was reduced to 3.2 mg/kg two days later

and abrupt withdrawal resulted in a seven-fold increase in responses in the absence of overt abstinence symptoms.

In a separate experiment, the response requirement was also manipulated. Initially, the reinforcement ratio was 1:1 (continuous), i.e. one lever press injected one 3.2 mg/kg dose. After 12 to 24 hours, the ratio increased to 5:1 for 23 to 34 hours, followed by 10:1 for the same time frame. The 10:1 ratio was then repeated but the dose increased to 10 mg/kg. These various fixed-ratios (FR) increased the number of responses required to earn one drug injection. As such, the greatest amount of drug was consumed when the least amount of work was required (1:1). Daily intake decreased following the response requirement increase, with the total responses per hour increasing with the ratio and nearly doubling following the increase to 10:1 from 5:1. When the dose increased to 10 mg/kg, responses per hour decreased. This resulted in less daily intake than when the ratio was 1:1, but greater intake than the other two ratios. Collectively, these findings suggest that the rats were willing to engage in compensatory behavior to meet the changing demands (increasing FR) of the procedure, but inevitably fell short of the initial level of intake obtained under continuous reinforcement.

In 1968, Pickens and Thompson used similar procedures to assess lever-pressing for cocaine. Here instead, tubing of the jugular catheter extended over the shoulder subcutaneously and exited the back via an external portion of a shoulder harness that was largely subcutaneous. Additional tubing then extended from the harness to a swivel that was connected to an automated infusion pump programmed to deliver injections following operant responses. Although some of the details have changed over the years to improve the design (such as now using a nickel-sized piece of mesh as the base of the catheter instead of the stainless steel/fiberglass screen-based harness), conceptually the procedure remains the same till this day.

After surgical implantation of the catheter, the rats were housed in the chamber that allowed them to self-administer cocaine. Drug was available for 14 hours daily, during which time responses on a lever resulted in the delivery of a 0.5 mg/kg infusion of cocaine in a saline solution paired with illumination of a stimulus light to facilitate conditioning. The number of hourly infusions stabilized in approximately three days, after which time experimental manipulations started. The reinforcing effects of cocaine were demonstrated by the fact that rats: (1) lever-pressed to earn cocaine, but stopped responding when the same number of infusions were administered automatically (and thus rats were not accidentally hitting the lever, but instead did so deliberately); (2) initially increased responding when cocaine was either substituted by saline or omitted (i.e., only the stimulus light illuminated), but then stopped responding all together (therefore, neither saline nor the cue light were inherently reinforcing); (3) began responding on a previously non-drug paired lever when the contingency changed (e.g., the initial drug-paired lever became inactive (no-drug) and the control lever became active (drug-producing).

In addition, the effect of dose on cocaine-reinforced responding was also assessed by changing the concentration of the drug solution (as such all doses were administered over 50-sec). Each dose (those reported varied from .25 to 3.0 mg/kg) was available for at least two, 6-h intervals. The inverse relationship seen with morphine was also observed here: when the dose of cocaine increased, responding decreased. Additionally, if instead the FR requirement was varied and the dose remained constant (1 mg/kg), the frequency of responding increased enough so that a similar amount of intake was obtained at each ratio. Throughout testing (both experiments) a consistent pattern of administration was also observed, with injections being spaced out ("post-reinforcement pauses") and hourly intake being relatively constant.

By the late 1980s, it was determined that animals will voluntarily self-administer most drugs abused by humans (Yokel 1987). The many studies using drug self-administration procedures provided much information of the psychological and neurobiological basis of the reinforcing actions of drugs, but there was increasing realization that mere self-administration of a drug does not necessarily constitute 'addiction-like behavior' (for reviews, see Wise 1996; Panlilio and Goldberg 2007; Ahmed 2011, 2012). As a result, self-administration procedures became the gold standard with the goal of establishing paradigms that (1) closely resemble human drug taking patterns and (2) produce addiction relevant behaviors.

Animal Models of Addiction

In humans, addiction (i.e., SUD) is diagnosed according to the DSM-V that includes 11 criteria. Not surprisingly, both tolerance and withdrawal are included criteria (although these are not necessary nor sufficient criteria), as well as using drug in larger quantities or for longer durations than intended and unsuccessful attempts to reduce use. Additionally, drug craving and spending considerable amounts of time seeking, using, or recovering from the drug are criteria. Lastly, recurrent use in hazardous situations and despite recognition of problematic use, as well continued use at the expense of social, work, or recreational activities and/or recurrent failure to fulfil obligations and persistent social or interpersonal problems. Over the years, a number of self-administration procedures were developed in an attempt to model these aspects of addiction. Below, an early model is first described, followed by widely-adapted (to varying degrees) models shown to produce addiction-like behaviors.

One of the first attempts to model the transition to addiction allowed rats to choose between orally self-administering ethanol (ETOH) or tap water for several months, during which time the social environment was manipulated (Wolffgramm and Heyne 1991; for review, see

Wolffgramm and Heyne 1995). This model was developed based on clinical observations of controlled consumption (a stage that can be very long-lasting), which, in some individuals, later became less amendable to other influences (i.e., social) to the point that craving for the drug dominated behavior and a loss of control over intake was observed (Coper et al. 1990). Wolffgramm and Heyne (1991) found that during the first few months of free choice, stable drug intake was influenced by housing and dominance rank. Specifically, socially-isolated rats preferred higher concentrations and consumed more ethanol than group-housed rats. Similarly, when group-housed rats experienced short-term isolation or were mildly socially deprived (by being placed in single cages that still allowed contact), increased ETOH consumption was observed. Further, if housing conditions remained stable, socially-subordinate rats consumed nearly twice as much ETOH as socially-dominant rats, but if changed, only socially-dominate rats increased intake.

After approximately 6 months of continuous ETOH access, however, drug intake started to increase, with higher doses being consumed from week to week despite stable environmental conditions. The ETOH was withdrawn after 9 months of access and then withheld for 9 months before being made available again to retest drug preference. Compared to drug-naïve rats, continuous access rats consumed far more drug. This suggested that continuous access rats had developed "behavioral dependence", but Wolffgramm and Heyne (1991) suggested that a loss of control over drug taking was necessary to consider such dependence equivalent to addiction in humans.

Therefore, to determine if the rats had actually lost control over drug-taking, the influence of an aversive additive (quinine) was assessed and short-term isolation and dominance were re-tested. The addition of quinine to the ETOH solution decreased intake in rats with continuous

access, but intake still exceeded that of naïve controls. In addition, being placed in isolation for 24-h no longer increased ETOH intake in rats with continuous access, but led to a considerable increase in naïve controls. Lastly, while socially-subordinate rats initially consumed more ETOH than socially-dominant rats, this effect disappeared after 4 weeks and intake remained similar even when quinine was added. Collectively, these results suggested that neither gustatory nor social factors were able to influence drug intake in behaviorally dependent rats. Similar results were later observed following oral self-administration of an opioid and amphetamine (Heyne 1996; Heyne and Wolffgramm 1998). Nonetheless, the author concluded that such irreversible preference and loss of control over drug intake were evidence of an addicted-phenotype in rats. One short-coming of these studies using an opioid or amphetamine is that oral drug administration was used, which results in slow drug absorption, relative to the routes of administration preferred by people with a SUD, and the rate that drugs get to the brain is an important factor in the development of addiction (Samaha and Robinson 2005).

Long Access (LgA) Self-Administration

Soon after the amphetamine oral self-administration study was published (Heyne and Wolffgramm 1998), a cocaine IV self-administration procedure was introduced that explored a factor that might have contributed to increased drug intake (Ahmed and Koob 1998). Indeed, the earlier model suggested that drug intake increased after extended exposure to drug (months), but many of the earlier cocaine self-administration studies, which typically involved 1-2 hour test sessions, observed relatively stable patterns of consumption. As a result, Ahmed and Koob (1998) simply increased the duration rats were allowed to self-administer cocaine. The rats were first trained to a criterion requiring the self-administration of 15 infusions within a 2 hour access period. Next, rats were matched to experimental groups of either decreased or increased access.

The short access (ShA) group only had 1-h to self-administer cocaine each day and the long access (LgA) group had 6-hrs. Each rat had 1 session/day for 12 days. To no surprise, LgA rats consumed much more drug than ShA rats due to differences in drug availability. While both groups started off relatively stable, by the fifth session, LgA rats significantly increased their total intake, which continued to increase gradually with additional sessions.

For the sake of generality, the authors made minor changes to the experimental timeline (such as excluding the initial acquisition criterion) and attempted to reproduce the escalation of drug intake. The results were indeed consistent. In addition, dose-effect curves were generated and, similar to experiments described previously, the total number of infusions per hour decreased as the dose increased in both groups. Ultimately, this observation led to the conclusion that animals adaptively self-administer to a preferred level of intoxication, which they referred to as the "hedonic set point". As such, it was hypothesized that an increased hedonic set point maybe responsible for escalated drug intake. In support of this hypothesis, they found that LgA rats self-administered a greater number of infusions/hour at every dose available compared to ShA rats. They also noted that the dose-effect curve for LgA rats only shifted upwards, and thus no horizontal shifts to the left (indicating tolerance) or right (indicating sensitization). Even further, after a 35 day withdrawal period, LgA and ShA rats initially consumed a similar amount of cocaine. Within six sessions, however, LgA rats resumed an escalated level of intake and further increased intake in additional sessions. In contrast, ShA rats continued to administer a relatively stable amount of drug across sessions.

Nevertheless, the authors concluded that an increased hedonic set point is associated with the transition to addiction and that LgA was capable of modeling this transition because it produced an addiction-like behavior (escalated intake). Unlike with the earlier oral self-administration

model, the LgA model became widely-adapted, possibly because of the short experimental timeline and the use of a route of administration that gets drug to the brain faster (thus increasing addictive potential; for review, see Samaha and Robinson 2005). Indeed, LgA was later shown to produce several other addiction-like behaviors following the self-administration of various drugs of abuse (for review, see Edwards and Koob 2013). For example, Ferrario et al. (2005) showed that compared to ShA, LgA rats continued to seek-drug despite not being reinforced to greater extent. Such drug-seeking in rats is comparable to human compulsive drug users spending a considerable amount of time to obtain drug. Of particular interest here, however, is the effort put forth to obtain drug while it is actually available.

Motivation for Cocaine Following LgA vs ShA

Even though responding for cocaine under various FR schedules provides indication of how willing the animal is to work for a given dose, motivation for drug is better assessed using a progressive ratio (PR) schedule (Depoortere et al. 1993; for review, see Richardson and Roberts 1996). Under these conditions, the response requirement increases progressively following the delivery of each infusion within a single test session. For example, only one lever-press maybe required for the first infusion. The second infusion, however, will not be delivered until after the lever is pressed twice, and the next infusion after four lever-presses, and then eight lever-presses are required for the following infusion, and so on. The response ratio will continue to increase for each successive infusion and at some point, the animal will eventually stop responding. If, for example, the current ratio was 124:1 and the infusion was earned, but only 180 responses were made for the next ratio of 208:1, then 160 would be the highest ratio completed and deemed the "breakpoint". As such, rats with higher breakpoints are considered more motivated to work for drug than those with lower breakpoints.

In 2003, Paterson and Markou assessed motivation following LgA vs ShA experience using a PR schedule of reinforcement for multiple doses of cocaine. Each dose (0, 0.031, 0.063, 0.125, and .25 mg/kg) was available in a single session lasting up to 6-hrs. Here again, the LgA dose-effect curve was shifted upwards relative to ShA rats, indicating greater motivation. Specifically, higher breakpoints were obtained in LgA rats for 0, 0.031, and 0.125 mg/kg cocaine. It is interesting, however, that there was a group difference for saline, but not for the training dose (.25 mg/kg). The authors attributed greater responding for saline in the LgA rats to increased incentive motivational value of the cocaine-cue. While Wee et al. (2008) did not find a difference in breakpoint for saline in LgA and ShA rats, they did find a greater breakpoint in LgA compared to ShA rats for higher doses of cocaine (0.5 and 1.0 mg/kg). In contrast, Liu et al. (2005) did not find an increased breakpoint following LgA experience, and others later reported no difference in breakpoint following LgA vs ShA (Willuhn et al. 2014).

To further investigate differences in motivation following LgA vs ShA, Oleson and Roberts (2009) used behavioral economics, which is a branch of microeconomic theory that combines insights from multiple disciplines, including psychology and economics, to understand decision making in individuals. These concepts were applied to animal behavior in the 1990s by Hursh and others (Hursh 1991, 1993; Bickel et al. 2000; Winger et al. 2002). Here, economic "price" for animals was produced by instead systematically decreasing the dose of drug available each day (1120, 630, 350, 200, 110, 60, 30, 20, 10, 5, and 3 µg/kg cocaine per infusion; FR1 for each dose; 2-h session). This was done by manipulating the pump duration, rather than the drug concentration. The lowest dose at which daily intake was maintained is referred to as the threshold. In addition, the self-administration behavior observed as the price changed was analyzed by applying an exponential equation that generates a "demand-curve". The data is then

manipulated to get the best curve fit, which is used to produce demand metrics that describe the behavioral choices of the individual. The unit-price (FR1/unit dose) at which maximal responding occurs is referred to as Pmax (Hursh 1991), a metric considered theoretically related to breakpoint on a PR schedule. It is at this apex of the price-response function that the slope of the demand curve becomes elastic, e.g., consumption is relatively stable up to this point and then begins to fall.

Following 14 consecutive days of ShA or LgA to cocaine self-administration, rats were tested using the threshold procedure. Interestingly, ShA rats maintained greater responding at lower doses than LgA rats. At the two highest doses, LgA rats consumed more cocaine than ShA rats. However, once the dose reached 24 μg/kg cocaine per infusion, ShA rats consumed more drug than LgA rats. As a result, ShA rats also had a higher Pmax than LgA rats. This was interpreted to mean that the lower doses were less reinforcing in LgA rats due to tolerance. The opposite result, however, was found by Zimmer et al. (2012) using similar methods. They instead report a higher Pmax in LgA rats compared to ShA. The primary difference between the studies is that the former design tested each dose across days (between-sessions) and the latter experiment tested all doses within a single session (each dose was available for only 10-min, resulting in a 110-min session). As such, the authors concluded that the two threshold procedures assessed different aspects of motivation. The between-session version measured the max price animals were willing to pay to reach the "hedonic set point". In contrast, the within-session version measured the max price animals were willing to pay to maintain consumption at the preferred set point level.

Using the within-session threshold test, Bentzley et al. (2014) also found increased motivation following LgA experience. Here, however, a different behavioral economic metric

was reported. The "essential value" alpha was described as a measure of demand elasticity that captured how quickly consumption fail as the price increased (however, see Newman and Ferrario, 2020 for discussion on why this measure does *not* reflect demand elasticity). During testing, the first dose of drug is the largest, and thus requires very little effort to earn the preferred amount. Despite such a "free consumption" period, rats will stop self-administering once the desired concentration is reached and this is captured by the metric Q_0 (preferred level of consumption when price is negligible). As the dose decreases, however, more effort is required (i.e., the price is increasing). Maintaining Q_0 even when the price drastically increases indicates less behavioral flexibility. As such, demand for drug is more inelastic or price-insensitive and this results in a smaller alpha value. If consumption, however, decreases after relatively small increases in price, then demand for drug is more elastic and reflects less motivation to obtain the drug. Following LgA, they report an increase in Q_0 (and thus an elevated hedonic set point) and a decrease in alpha is interpreted as indicating greater motivation.

The decrease in alpha observed following LgA, however, appears transient. Even though Q_0 remains elevated with repeated testing on the threshold test, alpha increased and was no longer significantly different from baseline. The same effect was also later reported by James et al. (2019). In contrast, Kawa et al. (2019b) only report an increase in Q_0, but no significant change in alpha or Pmax (note, however, the trend of the changes indicated less motivation). Nonetheless, regardless of the test used, there is a lack of consistency regarding increased motivation following LgA and how it differs from motivation following ShA.

Psychomotor Activation Following LgA vs ShA Cocaine Self-Administration

In a similar fashion, the literature is also mixed regarding whether or not psychomotor sensitization is expressed following LgA experience. In an early experiment comparing

psychomotor activation 14 days after discontinuation of ShA vs LgA, Ben-Shahar et al. (2004) report greater locomotor activity following a self-administered .25 mg/kg IV infusion of cocaine, compared to baseline locomotor activity, in ShA rats only. This was interpreted as a sensitized locomotor response, but this conclusion is problematic for a number of reasons. First, the baseline locomotor activity test was conducted a day prior (withdrawal day; WD13), during which time the rats were allowed to roam freely but not permitted to self-administer. On the test day, all groups (including drug naïve controls) were allowed to self-administer a single, low-dose cocaine infusion. This dose failed to increase psychomotor activation in drug naïve controls and the level of activity observed did not significantly differ from ShA or LgA rats. It does appear that locomotor activity tended to be greater in the ShA group compared to the controls, but likely did not reach significance due to large variance in the ShA group. This comparison instead would suggest a sensitized locomotor response, rather than comparing back to a baseline that does not resemble the actual test.

In a second experiment (Ben-Shahar et al. 2005), many of the flaws described above were resolved. Here, the baseline test also included an injection (saline) and the dose of cocaine was larger (although administered IP). They reported that neither ShA nor LgA rats differed in locomotor activity from controls following the administration of 15 mg/kg IP cocaine, and this was interpreted as the absence of sensitization. Due to large variance during the baseline test, it does not appear that ShA rats engaged in greater locomotor activity following the cocaine injection. The LgA rats, however, more than doubled locomotor activity following the injection, but this was not interpreted as sensitization (as was the case for the first experiment). Regardless, locomotor activity did not differ between the ShA and LgA groups. In this design, additional groups of rats were also tested on WD60. The control and ShA rats had very similar elevated

levels of locomotor activity following the cocaine injection. In contrast, the response in LgA rats was reduced and this was thought to reflect tolerance to the psychomotor activating effects. Even though reduced locomotor activity might indicate the emergence of stereotypy, the authors considered this unlikely because doses larger than 15 mg/kg IP are typically required to induce stereotypy.

Interestingly, Ferrario et al. (2005) reported greater stereotyped behavior in LgA rats compared to ShA and control rats following administration of both 15 and 30 mg/kg IP cocaine on WD30. Their measure of locomotor activity revealed an inverted U-shape that prompted visual inspection of video records. It then became apparent that the rats were engaged in stereotypy and the behavior was quantified by an observer blind to treatment conditions. Nonetheless, these results highlight the important of utilizing multiple behavioral measures and doses to assess psychomotor sensitization (for discussion, see Flagel and Robinson 2007). There are, however, other studies that take these factors into consideration and still find no difference in psychomotor sensitization in LgA vs ShA (Ahmed and Cador 2007; Knackstedt and Kalivas 2007). In addition, there are also more recent reports finding tolerance to both the psychomotor activating and dopamine elevating effects of cocaine following LgA experience (Calipari et al. 2014a; Siciliano et al. 2016). Nonetheless, evidence for either tolerance or sensitization following LgA is mixed.

Despite all of the discrepancies described above, the LgA model continues to be the most widely accepted model of addiction. Over the years, however, additional models have emerged that focus on other factors (i.e., not solely high and escalated intake) that contribute to the subsequent development of addiction-like behavior. Two models with promising results are discussed in further detail below.

Multi-Symptomatic Model

Keeping in mind that addiction typically develops after prolonged exposure in humans, rather than manipulate session duration, Deroche-Gamonet et al. (2004) instead focused on the number of IV self-administration sessions. Here, rats were allowed to self-administer cocaine for nearly three months. Each daily session alternated between periods of drug availability ("drug-periods"; 40-min each, 3 total) and "no-drug periods" (15-min each, 2 total) that were signaled by changes in chamber illumination. Throughout this experience, various addiction-like behaviors were repeatedly assessed, inclusive of responding when drug was not available; continued administration in the face of an adverse consequence (electric shock); and motivation to obtain cocaine under a PR schedule of reinforcement.

Following the prolonged self-administration period, animals underwent 5 days of withdrawal and were then non-contingently administered cocaine. During this test, responses for cocaine were not reinforced and therefore decreased over time. After a priming dose was administered, however, responding dose-dependently increased even in the absence of reinforcement. This is referred to as reinstatement of drug-seeking and resembles relapse in humans. Because addiction is a chronic relapsing disorder, with 90% of diagnosed individuals resuming drug use after attempting to abstain (Dejong 1994), rats were assigned to groups based on their propensity to reinstate drug-seeking. As such, rats with the greatest cocaine-induced drug-seeking (40% highest responding; HRein) were compared to those with the lowest drug-seeking (40% lowest, LRein).

After 13 sessions of self-administration, drug-seeking during the no-drug period was comparable in both groups. Following session 38, however, only the HRein rats increased drug-seeking, which more than doubled by self-administration session 54. Interestingly, after 32

sessions, the percent change from baseline self-infusions paired with electric shock was similar in both groups. Here again, compared to baseline, only the HRein rats increased self-infusions by session 74, indicating greater resistance to punishment. Lastly, following 35 self-administration sessions, HRein rats had a far higher breakpoint the LRein rats, and motivation for cocaine further increased after 52 sessions in HRein rats only. While it was clear that only the HRein rats were showing hallmarks of addiction after prolonged access, the authors took it a step further to formally "diagnose" all rats.

According to the DSM-V, no single criterion is necessary to be considered affected, but the presence of at least 2 criteria is diagnosed as a mild SUD, 4-5 is considered moderate, and 6 or more is severe. Here, they applied a similar diagnostic approach by examining the number of addiction-like behaviors present (3 max). For each criterion, only scores within the 66^{th} to 99^{th} percentile of the distribution counted towards the "diagnosis". As such, if a rat scored in the 80^{th} percentile for all addiction-like criteria, he would be positive for 3 criteria and considered "addicted". Remarkably, the percentage of rats positive for all 3 addiction-like criteria (17%) closely resembled that of the percentage of humans diagnosed with a SUD (Anthony et al. 1994). In addition, despite consuming a similar amount of drug, 3 criteria rats engaged in far greater non-reinforced drug-seeking, were more resistant to punishment, and reached a higher breakpoint (and were thus more motivated) than 0 criteria rats. In fact, the intensity of each addiction-like behavior was proportional to the number of positive criteria present.

Collectively, these findings highlight the importance of assessing individual differences in addiction susceptibility and provide a useful translational approach to assess addiction severity in rats. As such, others have assessed addiction severity using the diagnostic criteria approach and have found similar results (Kawa et al. 2016; Singer et al. 2018). Prolonged self-

administration, designs, however, are not as widespread in use, possibly due to issues such as equipment limitations and catheter failure. Furthermore, the extended training period serves the same purpose of exposing the animals to a large amount of drug, but instead uses shorter sessions across a far greater number of days. Using this model, differences in addiction-like behavior did not emerge until after 30+ days of self-administration. As a result, the authors concluded that the interaction between a long history of drug exposure and a vulnerable phenotype drives the development of addiction. Results from a newer model, however, suggest that high levels of drug exposure are not necessary to produce robust addiction-like behavior.

Intermittent Access (IntA) Self-Administration

Consistent with the temporal pattern associated with LgA models, early reports of human cocaine use patterns describe cycles of binge use (Gawin and Kleber 1985; Gawin 1989, 1991), during which time drug is taking repeatedly for an extended period of time. It was, however, later noted that "sustained high level use is rare" (Cohen and Sas 1994). In fact, cocaine use was observed to typically diminish over time and was laced with periods of abstinence, thus suggesting intermittency between bouts of use. In addition, cocaine users were reported to recognize the advantage of waiting between doses, but the compulsive nature of addiction makes this difficult (Ward et al. 1997). As a result, cocaine users typically engage in adjunctive behavior, such as smoking cigarettes, to assist with mediating delays between doses (Ward et al. 1997). Further, Beveridge et al. (2012), more recently reported that experienced human cocaine users typically waited over an hour between doses within a bout of use.

Extended delays between doses are advantageous because they allow brain-cocaine levels to fall prior to administration of the next dose. Once administered, the subsequent dose would then result in a rapid rise in brain-cocaine levels, resulting in the subjective "high". This

repetitive cycle of "spiking" was not observed in existing preclinical models due to animals loading up at the start of the session and then periodically responding throughout the session to maintain a relatively stable brain-cocaine concentration. As a result, in order to better model the intermittent pattern of use seen in humans, Zimmer et al. (2012) developed the intermittent access (IntA) model. With this procedure, brief Drug-Available periods (5-min) alternate with longer No Drug-Available periods (25-min), during which time brain-cocaine levels fall to near zero. This cycle repeated 12 times, resulting in the same session duration as LgA (6-h). The total time to self-administer, however, was the same as ShA (1-h). To date, only a handful of studies have directly compared these various models. The relevant results are summarized below in addition to IntA only findings.

Motivation for Cocaine Following IntA vs LgA

IntA was first assessed by determining if the model was capable of producing robust motivation for cocaine (Zimmer et al. 2012). As such, after 12 sessions of ShA, IntA, or LgA, all rats were tested using the within-session threshold procedure to quantify cocaine demand. Of note, IntA rats were found to self-administer a cluster of infusions during the first minute of drug availability. This resulted in a rapid peak in brain-cocaine concentration comparable to that of other groups, but instead of being maintained, the concentration gradually declined during the No Drug-Available period. As to be expected, LgA rats consumed far more drug than the other groups, which did not differ in total intake. Importantly, Pmax was significantly greater in LgA than ShA, as previously reported, but was even greater following IntA compared to LgA. This result suggested that the pattern of intake was more critical to the development of addiction-like behavior than total drug consumption. This conclusion was later collaborated by additional studies finding greater motivation (reported as a decrease in the behavioral economic measure

alpha), non-reinforced drug-seeking, cue-induced reinstatement, and resistance to punishment in IntA vs LgA rats (Nicolas et al. 2019; James et al. 2019; Kawa et al. 2019b; Garcia et al. 2020). To further explore the relationship between high and escalated intake and subsequent incentive motivation, Allain et al. (2018) allowed rats to self-administer under LgA or limited IntA conditions, followed by a PR schedule of reinforcement. With increasing IntA experience, rats also escalate drug intake (Kawa et al. 2016; Pitchers et al. 2017). As a result, to ensure that IntA rats had both low and non-escalated drug intake, only 2 infusions could be self-administered per Drug-Available period (IntA-Lim). Soon after 10 days of LgA or IntA-Lim, motivation was assessed. Despite having an eightfold lower cumulative intake, IntA-Lim rats reached higher breakpoints for cocaine than LgA rats. Further, breakpoints for IntA-Lim rats did not differ from IntA rats with higher and escalated intake. Even further, rats with extended (6-h; Long-IntA) and short (2-h; Short-IntA) IntA experience do not differ in incentive motivation (PR) or cocaine-primed reinstatement (Allain and Samaha 2018). These findings provide additional support for the notion that the temporal pattern of drug administration is far more critical to the development of robust addiction-like behaviors than total drug consumption.

Psychomotor Activation Following IntA Cocaine Self-Administration

The IntA-Lim procedure is also especially useful for assessing psychomotor sensitization because locomotor activity can be assessed during the No Drug-Available periods following administration of a fixed dose. In using this procedure, Allain et al. (2017) found greater locomotor activity during session 10 of IntA-Lim compared to session 1, indicating psychomotor sensitization. This same effect was also observed in rats with Short- and Long-IntA experience, with locomotor activity being greater during session 18 compared to 1 (Allain and Samaha, 2018). Similarly, in a recent report, regardless of whether or not rats escalated their intake during

IntA (escalator and non-escalators), psychomotor sensitization was expressed when tested 14 days after discontinuation of self-administration (Garcia et al. 2020).

Sex Differences in Psychomotor and Incentive Sensitization Following IntA vs LgA

As noted previously, females typically express greater psychomotor sensitization than males when intermittently administered cocaine non-contingently, but it was unknown if the same effect would be observed following cocaine self-administration. In addition, there were also reports of females being more motivated than males to self-administer cocaine (Roberts et al. 1989; Cummings et al. 2011; although, see Lynch and Taylor 2004), but none of these experiments involved LgA experience. In light of these unknowns, Algallal et al. (2019) exposed both females and males to either LgA or IntA and then assessed motivation for cocaine using PR. During the 10 sessions of IntA or LgA, locomotor activity was also examined to assess psychomotor sensitization.

As expected, LgA rats consumed more drug than IntA rats. However, within each condition, only LgA females consumed more drug than males. Locomotor activity in LgA males remained relatively stable across sessions and a slight decrease was observed in LgA females. Regardless of sex, IntA rats engaged in greater locomotor activity than LgA rats, but IntA females expressed greater psychomotor sensitization than IntA males. Following periods of withdrawal (5 and 25 days), the number of infusions administered under a PR schedule of reinforcement was greater in females than males across access conditions. This finding is consistent with an earlier report showing the IntA females were far more motivated (as assessed using behavioral economic measures) to work for cocaine than IntA males (Kawa and Robinson 2019). Here again, regardless of sex, IntA rats earned a greater number of infusions than LgA rats as early as WD5.

Relationship between Psychomotor and Incentive Sensitization Following IntA vs LgA

To even further explore the relationship between total drug consumption and motivation, correlations were examined between cumulative cocaine intake and number of infusions under PR in LgA and IntA males and females (Algallal et al. 2019). Interestingly, for LgA males, high drug intake was positively correlated with greater motivation. In LgA females, a very weak, non-significant positive correlation was also observed. In contrast, no relationship was found in IntA females or males. Additionally, the relationship between psychomotor sensitization and subsequent motivation was also assessed. In both IntA females and males, the extent of psychomotor sensitization positively predicted infusions earned under a PR schedule of reinforcement, consistent with a previous report using males only (Allain et al. 2017). Because there was no evidence of sensitization in LgA rats, this relationship could not be examined.

A similar relationship, however, was assessed previously in male rats with LgA experience and a negative correlation (trending, did not reach statistical significance) was found between the extent of psychomotor sensitization and the number of infusions earned during later LgA sessions (i.e., once intake stabilized following escalation; Ahmed and Cador 2006). Collectively, the findings from this study were interpreted to mean that psychomotor sensitization was not associated with the transition to escalated intake. But the new evidence discussed above suggests that this form of addiction-like behavior is less relevant to the transition to addiction. Instead, results from the IntA model with non-escalated intake now show that greater psychomotor sensitization is associated with more robust incentive motivation, a form of addiction-like behavior especially relevant to the transition to addiction.

Given the promising results of the IntA model, the studies presented here were designed to further explore psychomotor activation and incentive motivation following IntA experience.

Chapter II first directly compares psychomotor sensitization after acute and extended withdrawal from IntA or LgA, followed by an assessment of sex differences in psychomotor sensitization following IntA-Limited using a within and between subjects design, and finally, examination of cross-sensitization to d-Amphetamine following IntA to cocaine. In Chapter III, the relationship between psychomotor and incentive sensitization is explored in females and males using IntA-Limited and a novel within-session IntA-Demand procedure.

Chapter II

Intermittent Access Cocaine Self-Administration Produces Psychomotor Sensitization: Effects of Withdrawal, Sex, and Cross-Sensitization

Many drugs of abuse have psychomotor activating effects, indicated by an increase in locomotor activity and rearing at low doses, and at higher doses by focused stereotyped behaviors, such as repetitive head, limb and oral movements - effects thought to be largely due to their ability to increase dopamine (DA) neurotransmission in the dorsal and ventral striatum (Wise and Bozarth 1987, for review). Historically, interest in the psychomotor activating effects of drugs stems in part because such effects provide an indicator of DA activity, and in part because it has been argued that, "the reinforcing effects of drugs, and thus their addiction liability, can be predicted from their ability to induce psychomotor activation" (Wise and Bozarth 1987; p.474). Furthermore, when many drugs of abuse are administered repeatedly and intermittently there is a progressive increase (sensitization) in their psychomotor activating effects, which is associated with increased DA neurotransmission (Robinson and Becker 1986; Kalivas and Stewart 1991; Stewart and Badiani 1993; Vezina 2004). Such sensitization-related forms of neurobehavioral plasticity have been hypothesized to contribute to addiction (Robinson and Berridge 1993).

Many early studies of the phenomenon of psychomotor sensitization involved non-contingent drug injections given by an experimenter, and it became important, therefore, to determine if sensitization-related changes in brain and behavior are also induced when drugs are

self-administered. Indeed, there are many reports that drug (here we focus on cocaine) self-administration not only induces psychomotor sensitization (Hooks et al. 1994; Phillips and Di Ciano 1996; Zapata et al. 2003) but also sensitization to its motivational effects (Deroche et al. 1999; Vezina et al. 2002; Morgan et al. 2006; Lack et al. 2008) and sensitization of DA neurotransmission, especially when animals are tested after a period of withdrawal (Hooks et al. 1994; Zapata et al. 2003; Wiskerke et al. 2016). However, most of these early studies of sensitization produced by cocaine self-administration experience involved relatively limited or Short Access (ShA) conditions (although see Ferrario et al., 2005), and over the years it became increasingly apparent that such conditions are not especially effective in producing addiction-like behavior (Ahmed 2011). This led to considerable effort to develop more realistic preclinical models of addiction (Ahmed and Koob 1998; Deroche-Gamonet et al. 2004) that can be uniquely valuable for isolating persistent drug-induced changes in brain and behavior that may promote addiction, which is difficult to do in humans.

Currently, the most widely used rodent model of cocaine addiction utilizes long-access (LgA) intravenous (IV) self-administration procedures, introduced by Ahmed and Koob in 1998. This involves allowing rats to self-administer cocaine for 6-hrs or more in a single daily session. Relative to rats tested under more limited, or Short Access (ShA, 1-2 h/day) conditions, LgA experience is reported to produce a number of addiction-like behaviors, including escalation in drug-intake (Ahmed and Koob, 1998), high motivation for drug (Paterson and Markou 2003; Morgan et al. 2006; Wee et al. 2008; Ben-Shahar et al. 2008), continued drug-seeking in the face of an adverse consequence (Vanderschuren and Everitt 2004; Pelloux et al. 2007), and a high propensity to relapse (Mantsch et al. 2004; Kippin et al. 2006). LgA is also reported to reduce evoked DA release in the ventral striatum (Calipari et al. 2013, 2014a), and decrease the ability

of cocaine to induce striatal DA overflow and to inhibit DA uptake via the DAT (Ferris et al. 2011; Calipari et al. 2013, 2014a), all of which is accompanied by a decrease in cocaine's psychomotor activating effects – the opposite of sensitization (Calipari et al. 2013, 2014a). Thus, studies using LgA procedures have been cited in support of the view that in addiction, brain reward systems are rendered hyposensitive because of blunted DA neurotransmission. By this view drug-seeking and -taking are primarily motivated by a desire to overcome a deficiency in DA, and associated anhedonia (e.g., Koob and Volkow 2016; Volkow et al. 2016).

In contrast, studies using a more recently developed intermittent access (IntA) cocaine self-administration procedure paint a very different picture (Zimmer et al. 2012; for reviews, Allain et al. 2015; Kawa et al. 2019a). IntA involves the use of successive drug available periods, interspersed with periods when drug is not available, which produces a series of 'spikes' in brain cocaine concentrations within a session. This is thought to better reflect the temporal pattern of use in humans, especially during the transition to addiction (Ward et al. 1997; Beveridge et al. 2012). Importantly, IntA experience not only produces the addiction-like behaviors described above, but in many instances is more effective in doing so than LgA, despite much less total drug consumption (Kawa et al. 2016, 2019b; Allain et al. 2017, 2018; Allain and Samaha 2018; James et al. 2019b). Furthermore, IntA experience sensitizes striatal DA neurotransmission (Calipari et al. 2013, 2014b, 2015; Kawa et al. 2019b). Thus, studies using IntA procedures are more consistent with an incentive-sensitization view of addiction, which posits that, "addiction is caused primarily by drug-induced sensitization in the brain mesocorticolimbic systems [including DA] that attribute incentive salience to reward-associated stimuli" (Robinson and Berridge 2008, p. 3137). By this view, drug-seeking and taking in addiction is primarily motivated by a hyper-responsive DA system and sensitized "wanting" (Robinson and Berridge

1993).

The question addressed here is whether IntA experience also produces psychomotor sensitization. A few studies suggest yes, based on measures of locomotor activity within-subjects during IntA sessions (Allain et al. 2017; Allain and Samaha 2018; Algallal et al. 2019), but this phenomenon has not been well characterized. We asked, therefore, whether the psychomotor sensitization produced by IntA experience has similar characteristics to that described in many studies utilizing experimenter-administered drugs. Specifically, we asked whether, as with experimenter-administered drug, the psychomotor sensitization produced by IntA experience is expressed to a greater extent after long vs short periods of withdrawal (Robinson and Camp 1987; Paulson et al. 1991; Paulson and Robinson 1995; Grimm et al. 2001); whether females show greater sensitization than males (Glick and Hinds 1984; Robinson 1984; Van Haaren and Meyer 1991; for review, Becker et al. 2006); and whether treatment with one psychostimulant, cocaine, produces cross-sensitization to another, amphetamine (Akimoto et al. 1990; Schenk et al. 1991; Hirabayashi et al. 1991; Shanks et al. 2015).

General Materials and Methods

Subjects

Male and female Sprague-Dawley rats (Envigo, Haslett, MI), ~55 days old upon arrival, were housed individually in a climate-controlled colony room on a reverse 12-h light/12-h dark cycle. All training and testing were conducted during the dark period. Rats were given 1 week to acclimate to the colony room before any manipulation. Water and food were available *ad libitum* until 2 days before the first self-administration session. At this time, all rats were mildly food restricted to maintain a stable body weight. This was done to prevent excessive weight gain, which is unhealthy, particularly in adult male rats (Rowland 2007). All procedures were

approved by the University of Michigan Institutional Animal Care and Use Committee.

Intravenous catheter surgery

Intravenous (IV) catheters were surgically implanted as described previously (Crombag et al., 2000). Briefly, rats were anesthetized with ketamine hydrochloride (90 mg/kg, IP) and xylazine (10 mg/kg, IP), and an indwelling catheter was secured into the jugular vein. The catheter exited via a port situated just above the shoulder blades. All rats were given antisedan (1 mg/kg, IP) following completion of the surgery, to rapidly reverse the effects of anesthesia. The analgesic carprofen was given at the start of the surgery and again the two days following surgery (5mg/kg, SC). In addition, catheters were flushed daily with 0.2 ml sterile saline containing 5 mg/ml gentamicin sulfate (Vedco, MO) for the duration of the experiment, including all withdrawal periods. Catheter patency was tested prior to behavioral testing, and periodically throughout an experiment (e.g., if responding for cocaine suddenly declined), by administering an IV infusion of 0.1 mL of methohexital sodium (10 mg/ml in sterile water, JHP Pharmaceuticals). If a rat did not become ataxic within 10-sec of the infusion it was removed from the study.

Cocaine self-administration

All self-administration testing took place in Med Associates chambers (22 × 18 × 13 cm; St Albans, VT, USA) located within sound-attenuating cabinets. Each chamber was equipped with a ventilating fan, which masked background noise, a tone generator, and a red house light. For all studies, active responses resulted in an IV infusion of cocaine hydrochloride (NIDA, 0.4 mg/kg/infusion, weight of the salt, in 50 µl of sterile saline delivered over 2.6-sec), whereas inactive responses were recorded but had no consequence. In addition, each infusion was accompanied by a tone and/or light stimulus, which continued throughout

the time out period (see individual experimental design below for additional details). For all studies, infusions were given on a fixed ratio 1 schedule of reinforcement, and one session was conducted per day.

Acquisition of cocaine self-administration

To ensure that all rats received the same amount of drug and cue exposures during initial training, an infusion criteria (IC) procedure was used, as described previously (Saunders and Robinson 2010). During each IC session rats were allowed to take a fixed number of infusions (e.g., 10 infusions = IC10), with the number of infusions available increasing across days as each rat met the infusion criteria (e.g., IC10, IC20, IC40). During initial acquisition the time out period was 20-sec (including infusion time). Rats were removed from the chambers when the criterion was met, and thus session length varied from animal to animal.

Intermittent and Long Access cocaine self-administration

Intermittent access (IntA) procedures were similar to those previously described (Zimmer et al. 2012; Kawa et al. 2016). Briefly, each session consisted of alternating Drug-Available and No Drug-Available periods lasting 5-min and 25-min, respectively. The house light was on at the start of the session, and the first Drug-Available period was signaled by the house light turning off. During Long Access (LgA) sessions, drug was available throughout the entire 6-h session.

Assessing psychomotor activity

Depending on the experiment, psychomotor activity was assessed following experimenter-administered and/or self-administered cocaine. In all experiments involving experimenter-administered cocaine, behavior was recorded in test chambers with walls made of black, expanded PVC (68.58 x 33.02 x 66.04 cm), and grey wire mesh grid floors. Each chamber was equipped with a camera (CVC-130R, Speco Technologies, Amityville, NY, USA)

suspended ~18 cm above the center of each test chamber. To assess the behavioral response to self-administered cocaine *during* an IntA session, rats were allowed to administer three infusions of cocaine during each Drug-Available period within the self-administration chamber. In these studies, each self-administration chamber was equipped with a camera mounted on the back wall of the sound-attenuating chamber. Additional details regarding groups, habituation, and doses tested are given within each experiment below.

Cocaine and amphetamine both produce psychomotor activation, which can manifest as increases in locomotor activity, as well as increases in repetitive stereotyped behaviors (e.g., head movements), during which time locomotion may decrease (Lyon and Robbins 1975). Locomotor activity in the psychomotor test chambers was quantified using TopScan motion-tracking software (High-Throughput Option Version 3.00, Clever Sys Inc., Reston, Virginia, USA) or EthoVision XT software (Version 11.5, Noldus Information Technology b.v., Wageningen, The Netherlands). For these automated analyses (TopScan and EthoVision) user-defined areas were located at the far left and far right side of the chamber, and *crossovers* from one area to the other was counted using center-point detection, as an estimate of locomotor activity. Importantly, direct comparisons were only made between measures made using the same software and approach.

Locomotor activity in the self-administration chambers was scored by visual examination of videos. For this, the experimenter was blind to experimental conditions and the number of infusions available was capped to three so that all rats took the same amount of cocaine (see also Exp 2 below). Behavior was then quantified during each following 25-min No Drug-Available period. The chamber was divided into two equal halves and locomotor activity was determined by again counting the number of crossovers (center-point) from one side of the test chamber to

the other, in 5-min bins.

Stereotyped behavior was examined using two different measures. One, behavior was rated using a scale adapted from Ellinwood and Balster, 1974 and Robinson et al., 1988 (see Table 2.1). Two, a parametric measure of the intensity of stereotypy was obtained by quantifying the frequency of head movements during periods when the rat was 'in place', as described by Ferrario et al. (2005). Periods 'in place' were defined as periods when both back paws remained in the same place for a minimum of 2-sec. The frequency of head movements was then determined by dividing the total number of head movements by the total time spent in place. For both rating and frequency measures, one 30-sec sample of behavior was assessed every 5-min during the first 40-min following each cocaine injection, resulting in eight 30-sec samples/dose/rat.

Experiment 1: Does IntA and LgA cocaine self-administration experience produce psychomotor sensitization that varies as a function of withdrawal period?

The psychomotor response to experimenter-administered (IP) cocaine was assessed in male rats prior to any self-administration experience (Baseline) and then again on challenge test days conducted after 1 and 30 days of withdrawal (WD1, WD30) from IntA or LgA cocaine self-administration experience (see Timeline shown in Fig. 2.1a). Rats were first habituated to the psychomotor activity test chambers by placing them into the chambers for one hour on two consecutive days. Next, rats underwent IV catheter surgery followed by 7 days of recovery as described in General Methods above. Baseline cocaine-induced psychomotor activity was then assessed as follows. Rats were placed into the psychomotor test chambers and left undisturbed for 30-min. They were then removed and given an IP injection of saline, and placed back into the chamber for 30-min. This was followed by an IP injection of 7.5 mg/kg of cocaine, then 1-h later

15 mg/kg, and after an additional 1.5-h a 30 mg/kg injection, as described previously (Ferrario et al. 2005; Oginsky et al. 2016). Behavior was video-recorded throughout and testing was conducted in red light conditions.

Next, rats underwent acquisition for cocaine self-administration using the IC procedure described above. For this study, the self-administration chambers were equipped with one active and one inactive nose-poke port (right/left counter-balanced). Active responses resulted in an infusion of cocaine, the illumination of the active nose-poke port and presentation of a tone. The tone and nose-poke light remained on during the infusion and the 20-sec time out period. Each rat was given 2 sessions of IC10, 3 sessions of IC20, and 6 sessions of IC40. Rats were then assigned to IntA (n=9) or LgA (n=10) groups, counterbalanced by average session duration during the last three IC40 sessions. For both IntA and LgA the time out period was 7.6-sec. Importantly, each IntA session consisted of 12 Drug-Available and 12 No Drug-Available periods, resulting in a 6-h session length, and therefore, both LgA and IntA groups were exposed to the self-administration chambers for the same amount of time each day.

Experiment 2: Are there sex differences in the psychomotor sensitization produced by IntA cocaine self-administration experience?

Repeated, intermittent treatment with experimenter-administered psychomotor stimulant drugs produces greater psychomotor sensitization in female than male rats (Glick and Hinds 1984; Robinson 1984; Van Haaren and Meyer 1991; for review, Becker et al. 2006). The purpose of this experiment was to determine if this is also the case following IntA cocaine self-administration experience (Fig. 2.1b).

For this experiment, the self-administration chambers were equipped with an active and an inactive retractable lever, rather than nose poke ports, in part to prevent responding during No

Drug-Available periods, which could interfere with the quantification of drug-induced psychomotor activity (see below). In addition, because females often acquire cocaine self-administration more readily than males, rats in this study first underwent food self-administration training, in an attempt to reduce differences in acquisition between the sexes. For food self-administration training (30-min per session, 2-5 sessions total), a food cup was located in the center of the front wall of the chamber. Each response on the active lever resulted in the delivery of one food pellet (45 mg, banana flavored pellets; BioServe, #F0059, Frenchtown, NJ, USA), paired with illumination of the cue light above the active lever. Responses on the inactive lever had no consequence and active/inactive levers were right/left counterbalanced. During this time, rats were also habituated to the psychomotor test chambers (1-h/day, 2 consecutive days). After food training, rats underwent IV catheter surgery followed by recovery and baseline IV psychomotor testing as described below.

All rats underwent two days of habituation to the psychomotor test chambers and experimenter administered drug injection procedure. On these days the rats were first placed in the chambers for 30-min; they were then removed and placed into a square plastic holding cage. Their IV catheter was then attached to a length of PE20 tubing connected to a 1.0 ml syringe mounted on a Harvard Apparatus syringe pump (Holliston, MA). The syringe pump was then used to infuse 60 µl of saline over 5-sec (142 µl/min). The tubing was then detached from the catheter and animals were quickly placed back into the psychomotor test chambers for an additional 30-min. Three additional saline infusions were administered separated by 30-min, for a total of 4 saline infusions. On the baseline test day, rats were again placed in the chamber for 30-min followed by one saline infusion. Next, rats were given three infusions of increasing doses of cocaine (0.25, 0.5. and 1.0 mg/kg, IV), with 30-min between each infusion. The infusion itself

consisted of 30µl of saline, followed by 10µl of cocaine, and another 20µl of saline for a total infusion volume of 60µl. This ensured that the infusion volume was larger than the dead space of the IV catheter. The doses, infusion time, and protocol used were chosen because they were previously shown to produce psychomotor sensitization (Samaha et al. 2002; Ahmed and Cador 2006). Behavior was video-recorded throughout.

Following baseline psychomotor testing, rats underwent acquisition of cocaine self-administration using the IC procedure. Because they had already undergone food training, IC training was reduced to 2 sessions of IC10 followed by 5 sessions of IC20. Each infusion was accompanied by the illumination of a cue light located above the active lever. The cue light remained illuminated during the 20-sec time out period. In addition, the active lever was retracted during each time out in order to familiarize the rat with lever retraction.

Female rats have been reported to self-administer more cocaine, and are more motivated to do so, relative to males (Roberts et al. 1989; Lynch and Taylor 2004; Roth and Carroll 2004; Lynch et al. 2005; Cummings et al. 2011; Smith et al. 2011). Therefore, we next sought to determine if this were the case under our test conditions. To this end, rats were next trained to self-administer using a within-session threshold procedure, as described previously (Oleson et al. 2011; Bentzley et al. 2013). At the start of these 110-min sessions (signaled by the house light being off and insertion of the levers, which remained extended throughout the entire session), rats earned infusions on an FR-1 schedule and the dose of cocaine available decreased every 10-min on a quarter logarithmic scale (1.28, 0.72, 0.40, 0.23, 0.13, 0.072, 0.040, 0.023, 0.013, 0.007, 0.004 mg/kg/infusion). Dose was manipulated by changing the duration of the infusion, and the cue light remained illuminated during the duration of each infusion (7-10 sessions). There was no signaled time out period, but additional infusions could not be earned while an

infusion was being given.

Next, rats were assigned to IntA (males n=10, females n=9) or Control groups (males n=10, females n=9). Rats in the Control group did not receive any additional self-administration experience, but each day they were removed from their home cage and placed in holding chambers for the same amount of time as rats in the IntA group were in the self-administration chambers. In order to ensure that there were no differences in the total amount of cocaine consumed between males and females in the IntA group (which could itself produce differences in psychomotor sensitization), we used a modified IntA procedure similar to that described previously (Allain et al. 2018). Briefly, rats were limited to three infusions per 5-min Drug-Available period (IntA-Limited). The cue light was illuminated for the duration of each infusion (2.6-sec). The No Drug-Available period started as soon as the allotted infusions were self-administered, or after 5-min, whichever came first, and was signaled by illumination of the house light and retraction of both levers. Each IntA-Limited session consisted of 8 Drug-Available and 8 No Drug-Available periods. Locomotor activity in response to self-administered cocaine was evaluated during IntA-Limited sessions 1, 3, 7 and 10, within subjects. Finally, the psychomotor response to an experimenter-administered IV infusion of cocaine was evaluated 12 days after IntA-Limited testing, using a between-subjects design comparing IntA-Limited and Control groups. The same multi-dose procedure described for initial baseline testing was used (see also General Methods for details of psychomotor measures).

Experiment 3: Does IV cocaine produce cross-sensitization to IV amphetamine?

Experimenter-administered cocaine given IP induces cross-sensitization to IP amphetamines (Akimoto et al. 1990; Hirabayashi et al. 1991; Shanks et al. 2015), but similar cross-sensitization between IV cocaine and amphetamine had not been established. Therefore,

we first determined whether experimenter-administered *IV* infusions of cocaine would also produce cross-sensitization to subsequent IV amphetamine in females (Exp. 3a; see also Timeline Fig 2.1c). We then asked whether IntA cocaine self-administration experience would do so as well (Exp. 3b; see also Timeline Fig 2.1d).

Exp. 3a. Amphetamine cross-sensitization to experimenter-administered IV cocaine

Female rats were first habituated to the psychomotor activity test chambers (1-h per day, 2 days), and then underwent IV catheter surgery as described above. They were next habituated to the experimenter-administered IV infusion procedure (2 days, 30-min followed by a single IV infusion of saline; 60 µl over 5-sec) and returned to their home cage after an additional 30-min. Rats were then assigned to a saline- (n=10) or a cocaine-treated (n=11) group. On each treatment day, rats were placed into the chambers for 30-min, and then given an IV infusion of cocaine (1 mg/kg) or saline (60 µl delivered over 5-sec). Behavior post infusion was video recorded for 30-min before returning rats to their home cages. Infusions were given every other day for a total of 7 infusions. Rats then experienced 9 days of withdrawal and on WD10 the response to IV challenge infusions of *d*-amphetamine was examined.

On the amphetamine challenge day, rats were placed into the chamber for 30-min and then all rats (both Saline and Cocaine pretreated groups) were given an infusion of saline. After 30-min, all rats were given an IV infusion of 0.3 mg/kg of *d*-amphetamine. 2.5-h later rats received an infusion of 0.6 mg/kg of *d*-amphetamine (infusion volume: 60 µl delivered over 5-sec). Behavior was video-recorded throughout testing and recordings ended 3.5-h after the last infusion (see also General Methods).

Exp. 3b. Amphetamine cross-sensitization following IntA cocaine self-administration.

Here we asked whether IntA cocaine self-administration experience produces cross-

sensitization to IV amphetamine in male rats (see timeline Fig 2.1d). Rats were given food training followed by IV catheter surgery and recovery as described above for Exp. 2. They then underwent IC10 (2 sessions) and IC20 (5 sessions) cocaine self-administration training. Rats were randomly assigned to IntA (n=12) and Control (n=13) groups. The 15 IntA self-administration sessions were identical to those described in Exp. 2, except that rats could take an unlimited number of infusions during each of eight 5-min Drug-Available periods. Importantly, training was staggered such that IntA and Control groups both received their amphetamine challenge test 7 days after the last cocaine self-administration session (WD7). Habituation to the psychomotor testing and infusion procedure was conducted during WD4-7, as described for Exp 3a. The amphetamine challenge test was conducted similar to that described in Exp 3a, except that doses of 0.25 mg/kg and 0.5 mg/kg *d*-amphetamine were used (infusion volume: 60 µl delivered over 5-sec).

Statistics

Prism 8.0 (GraphPad Software) was used for all parametric statistical analyses. Differences across time, session/day, or dose in locomotor activity, percent time spent in place, frequency of head movements, and infusions were all analyzed using a two-way repeated-measures analyses of variance (ANOVA) based on the general linear model. The Geisser-Greenhouse correction was applied to all factors with three or more levels (such as time and dose) to mitigate violations of sphericity. Post-hoc multiple comparisons (and Sidak corrections) were used as appropriate. Group differences in cumulative cocaine intake was analyzed using an unpaired *t*-test. Wilcox Signed Ranks Test was used to analyze the non-parametric psychomotor rating scale data (SPSS v. 24).

Results

Experiment 1: Does IntA and/or LgA cocaine self-administration experience produce psychomotor sensitization that varies as a function of withdrawal period?

After being trained to self-administer cocaine using an infusion criteria (IC) procedure, rats were assigned to IntA or LgA groups matched for initial performance during acquisition. There were, therefore, no group differences in the acquisition of cocaine self-administration (data not shown). As expected, during the 10 day IntA or LgA self-administration period rats in the LgA group consumed about twice as much cocaine as rats in the IntA group (Fig. 2.2; $t(1,17)=7.265$, $p<0.001$).

The effects of LgA or IntA cocaine self-administration experience on psychomotor activity was then assessed 1 day after the last self-administration session (WD1). The psychomotor activating effect of increasing doses of IP cocaine were compared to that seen during the Baseline test, conducted prior to IntA or LgA experience (Fig. 2.3). Panels a-d in Fig. 2.3 show the time course of locomotor activity (i.e., crossovers). There was not a group difference in the increased frequency of crossovers with dose at baseline (data not shown; main effect of dose, $F(1.586, 26.97)= 6.501$, $p= 0.0079$; main effect of group $F(1,17)= 0.1040$, p=0.7510; dose X test day interaction; $F(3,51)= 0.09754$, p=0.9610), but only rats with IntA experience showed a sensitized locomotor response on WD1 relative to baseline. To simplify data presentation and analysis these data were collapsed across time (crossovers/min) and expressed as dose-effect functions (Panels e, f). Compared to Baseline, IntA rats showed an enhanced (sensitized) locomotor response on WD1 across all doses tested (Fig. 2.3e; main effect

of test day, $F(1, 8)= 11.47$, $p=0.0096$; main effect of dose, $F(2.383, 19.07)= 10.36$, $p=0.0006$). In contrast, in the LgA group cocaine-induced locomotor activity increased as a function of dose $F(2.193, 19.74)= 7.989$, $p=0.0023$), but there was no difference in locomotor activity between Baseline and WD1 (Fig. 2.3f; effect of test day; $F(1,9)= 1.855$, $p=0.2063$; dose X test day interaction; $F(1.860, 16.74)= 0.8166$, $p=0.4507$).

A second, non-automated measure of behavior, was derived from direct analysis of the video records. The percent of time spent in place during each test session (i.e., *not* locomoting) was calculated (Panels g and h). In the IntA group there was a dose-dependent decrease in time spent in place on both test sessions, that was greater on WD1 than at Baseline (Fig. 2.3g; effect of dose, $F(2.140, 17.12)= 4.598$, $p=0.0234$; effect of test day, $F(1,8)= 10.65$, $p=0.0115$), consistent with the sensitization indicated by an increase in crossovers captured by the automated measures. In the LgA group there was also a dose-dependent decrease in the time spent in place (Fig. 2.3h; effect of dose, $F(2.406, 21.65)= 3.183$, $p=0.0535$), but there was no difference between Baseline and WD1 (Fig. 2.3h; effect of test day, $F(1,9)= 0.02$, $p=0.8899$), again consistent with the automated assessment of locomotor activity.

The effect of LgA or IntA cocaine self-administration experience on psychomotor activity was next assessed on WD30 in the same rats (Fig. 2.4). Although at Baseline there was a dose-dependent increase in crossovers in both the IntA and LgA groups (Fig 2.4a-b open symbols), there was a decrease in crossovers in both groups on WD30 (Fig. 2.4a-b closed symbols). This was accompanied by an increase in the time spent in place (Fig. 2.4c-d). This pattern of behavior is inconsistent with cocaine-induced locomotor hyperactivity, but suggestive

of focused stereotyped behaviors. Indeed, visual observation of videos showed animals engaged in focused stereotyped behaviors, especially at the highest dose. For this reason, measures other than locomotion were required to assess drug effects on WD30. We used two measures: 1) a rating scale to assess the psychomotor activating effects of cocaine (Table 2.1; adopted from Ellinwood and Balster 1974 and Robinson et al. 1988); and 2) quantification of the frequency of stereotyped head movements while otherwise 'in place' (Ferrario et al. 2005).

In both the IntA and LgA groups cocaine produced higher stereotypy ratings on WD30 than at Baseline (IntA: Fig. 2.4e; Z=-2.668, p=0.008; LgA: Fig. 4f; Z=-2.803, p=0.005). The median ratings at Baseline following 30 mg/kg were 3.75-4.0, which corresponds to hyperactivity accompanied by normal looking head movements (see Table 2.1). This is consistent with the locomotor hyperactivity seen at this dose (Fig. 2.3e, f). However, on WD30 the median ratings were 7.1-8.0, which corresponds to discontinuous to continuous stereotyped head movements of high intensity and frequency (Table 2.1), and is consistent with the increased time in place shown in Fig. 2.4c, d.

A parametric measure of stereotyped behavior was obtained by counting the frequency of head movements while a rat was otherwise 'in place', that is, not locomoting (Fig. 2.4g, h). There was a dose-dependent increase in the frequency of head movements in both groups on both test sessions, that was greater on WD30 than at Baseline (IntA: Fig. 2.4g; effect of dose, $F(1.537, 12.30)= 47.95$, $p<0.0001$; effect of day, $F(1,8)= 36.78$, $p=0.0003$; day X dose interaction, $F(1.735,13.88)= 7.733$, $p=0.0069$; LgA: Fig. 2.4h; effect of dose, $F(1.679, 15.11)= 74.41$, $p<0.0001$; effect of day, $F(1,9)= 25.26$, $p=0.0007$; day X dose interaction,

$F(1.986,17.87)= 8.573$, $p=0.0025$). In summary, both rating scale and head movement measures of stereotyped behavior indicated that on WD30 rats with either IntA or LgA cocaine self-administration experience expressed especially robust psychomotor sensitization (also see Ferrario et al. 2005). Thus, psychomotor sensitization was expressed on WD1 and WD30 following IntA, but only on WD30 following LgA.

Experiment 2: Are there sex differences in the psychomotor sensitization produced by IntA cocaine self-administration experience?

There was no sex difference in days to criteria for food training (data not shown) or in the acquisition of self-administration behavior (data not shown). This is expected given that the IC procedure fixes the number of available infusions each day and rats must reach that number before moving to the next IC. During the threshold test females self-administered more cocaine than males at low doses (Fig. 2.5; effect of sex, $F(1, 36)= 9.197$, $p=0.0045$; effect of dose, $F(1.762, 63.43)= 44.26$, $p<0.0001$; sex X dose interaction, $F(10, 360)= 7.673$, $p<0.0001$), consistent with previous studies (Roberts et al. 1989; Cummings et al. 2011; Kawa and Robinson 2019). Therefore, in subsequent studies we used an IntA-*Limited* procedure to mitigate any effects of sex differences in total cocaine consumption on subsequent psychomotor sensitization.

Psychomotor sensitization: within-subjects analysis

During the IntA-Limited cocaine-self-administration sessions the number of infusions allowed during each Drug-Available period was capped at 3, resulting in a maximum of 24 infusions per session. All rats earned the maximum number of infusions, except during

Sessions 1 and 2 of IntA (a total of 3 rats earned 21-23 infusions), but there were no significant group differences in the amount of cocaine consumed during IntA (data not shown; $t(1, 17)=0.9459$, $p=0.3574$).

Fig. 2.6 shows the number of crossovers for the first three 5-min intervals during each No Drug-Available period on Sessions 1, 3, 7 and 10 of IntA-Limited testing. We examined the entire 25-min period and found that (1) group differences were confined to the first 5-10 min, and (2) activity returned to baseline within 15-25 min. Therefore, only the first 15-min are shown for the sake of clarity. There were no differences in cocaine-induced crossovers between males and females on Session 1 (Fig 2.6a). However, on Sessions 3, 7 and 10 self-administered cocaine produced greater psychomotor activation in females than males (Fig 2.6 b-d). To better display and analyze these data the number of crossovers during the first 5 min of each of the 8 No Drug-Available periods were averaged for Sessions 1, 3, 7 and 10. This captures peak locomotor activity for both males and females and is shown in Fig. 2.7. Both males and females showed an increase in locomotor activity across sessions (effect of session, $F(1.758, 28.13)=11.59$, $p=0.0003$). However, females showed a greater increase in locomotor activity across sessions than males, that is, greater psychomotor sensitization, as indicated by a significant sex X session interaction ($F(3, 48)=2.895$, $p=0.0447$).

Psychomotor sensitization: between-subjects analysis

Psychomotor sensitization can also be assessed by comparing the magnitude of locomotor activity between subjects, providing additional corroboration of effects. For this analysis male and female rats that had IntA-Limited experience were compared to their

respective Control groups. Animals in the Control groups received all the same treatments as the IntA-Limited groups, including surgery, acquisition of self-administration, and the threshold test, but no IntA-Limited experience. Twelve days after the last IntA-Limited self-administration session rats in both the Control and IntA-Limited groups received experimenter-administered IV challenge infusions of increasing doses of cocaine in the psychomotor test chambers (see Fig 2.1b for timeline). Note that all rats had been habituated to these test chambers, and the injection procedure, prior to acquisition of self-administration, and there were no group differences in locomotor activity at that time (data not shown). In the males, there was a dose dependent-increase in locomotor activity following IV cocaine (Fig. 2.8a; effect of dose, $F(2.285, 38.84)=26.21$, $p<0.0001$), but this was similar between IntA-Limited and Control groups. Females also showed a dose-dependent increase in locomotor activity (Fig. 2.8b; effect of dose, $F(1.716, 27.45)=18.56$, $p<0.0001$), but this was greater in the IntA than Control group (effect of group, $F(1, 16)=10.09$, $p=0.0059$; group X dose interaction, $F(3, 48)= 2.848$, $p=0.0472$). In summary, this between-subjects analysis showed that following IntA experience psychomotor sensitization was expressed in females, but not males. Taken together with within-subjects measures, these data show that IntA cocaine self-administration produces more robust psychomotor sensitization in females than males.

Experiment 3: Does cocaine produce cross-sensitization to amphetamine?

Previous studies have reported that experimenter-administered cocaine produces cross-sensitization to a subsequent injection of amphetamine, but these studies all involved IP

injections (Akimoto et al. 1990; Hirabayashi et al. 1991; Shanks et al. 2015). Therefore, we first determined whether experimenter-administered IV infusions of cocaine would also produce cross-sensitization (Exp 3a; Fig 2.1c). We then determined whether IntA cocaine self-administration experience produces cross-sensitization to amphetamine (Exp. 3b; Fig 2.1d).

Experiment 3a

Cocaine produced greater psychomotor activation (crossovers) on the last (seventh) day of IV cocaine treatment than the first, indicating sensitization (Fig. 2.9; effect of time, $F(3.045, 30.45)=63.15$, $p<0.0001$; effect of session, $F(1, 10)=15.6$, $p=0.0027$; session X time, $F(2.186, 21.86)=13.38$, $p=0.0001$). On the challenge test day infusions of 0.3 and 0.6 mg/kg *d*-amphetamine produced greater psychomotor activation in cocaine pretreated than control groups (Fig. 2.10a: 0.3 mg/kg, effect of group, $F(1, 19)=2.816$, $p=0.1097$, effect of time, $F(3.997, 75.94)=14.15$, $p<0.0001$, group X time, $F(6, 114)=3.103$, $p=0.0075$; Fig. 2.10b: 0.6 mg/kg, effect of group, $F(1, 19)=11.57$, $p=0.0030$, effect of time, $F(4.681, 88.93)=40.96$, $p<0.0001$, group X time interaction $F(6, 114)=2.579$, $p=0.0222$). Fig. 2.10c shows a summary of the dose-effect relationship (effect of group, $F(1, 19)=7.264$, $p=0.0143$; effect of dose, $F(1.729, 32.84)=42.37$, $p<0.0001$; group X dose interaction, $F(2, 38)=3.492$, $p=0.0405$).

Experiment 3b

The effect of challenge infusions of *d*-amphetamine on locomotor activity (crossovers) was compared between rats that had 15 days of IntA cocaine self-administration experience compared to controls that had surgery and underwent IC self-administration training, but had

no IntA experience (Fig. 2.1d). With increasing IntA experience rats escalated their intake during the first min of the Drug-Available periods, which was when they consumed nearly all drug (Fig. 2.11; effect of session, F (1.638, 18.02)=3.59, p=0.0564; effect of minute, F (1.233, 13.57)=192.3, p<0.0001; session X minute interaction (F (5.039, 55.43)=14.11 , p<0.0001; post-hoc analysis revealed this was driven by minute 1). This is consistent with previous reports (Kawa et al. 2016; Kawa et al. 2018a).

On the psychomotor test day the initial infusion of saline produced very little effect but the IntA group showed slightly greater activity than the control group, perhaps indicating conditioned hyperactivity (data not shown; main effect of group, F (1, 23)=5.118 , p=0.0334). A challenge infusion of 0.25 mg/kg of *d*-amphetamine produced slightly more activity in the IntA group than the Controls, but this was not statistically significant (Fig 2.12a; effect of group, F (1, 23)=2.104, p=0.1604; group X time interaction, F (14, 322)=1.648, p=0.0655). A challenge infusion of 0.5 mg/kg of *d*-amphetamine produced more crossovers in the IntA than the Control group (Fig. 2.12b; Effect of group, F (1, 23)=8.037, p=0.0094; group X time interaction, F (14, 322)=2.339, p=0.0043). Fig. 2.12c shows the dose-effect relationship (group X dose interaction, F (2, 46)=4.664, p=0.0143). In summary, IntA cocaine self-administration experience produced cross-sensitization to *d*-amphetamine.

Discussion

We asked whether IntA cocaine self-administration experience produces psychomotor sensitization, with similar characteristics to that produced by repeated, intermittent treatment with IP experimenter-administered drugs. It did. (1) Psychomotor sensitization was expressed

both early (one day; WD1) and late (30 days, WD30) after the discontinuation of IntA self-administration, but was more robust at WD30 than WD1. Interestingly, following LgA self-administration experience psychomotor sensitization was only evident at WD30. (2) IntA self-administration experience produced greater psychomotor sensitization in female than male rats. (3) IntA self-administration experience produced cross psychomotor sensitization to another psychomotor stimulant drug, *d*-amphetamine. These findings have a number of implications for thinking about how cocaine may change brain and behavior in ways that can promote the transition to addiction, as discussed below.

The effect of IntA vs LgA cocaine self-administration on the induction of psychomotor sensitization as a function of withdrawal period.

Intermittent Access

In many early studies psychomotor stimulant drugs were administered IP or SC by an experimenter, repeatedly and intermittently, and in such studies sensitization was often only apparent, or expressed more strongly, after a period of withdrawal (Robinson and Camp 1987; Paulson et al. 1991; Paulson and Robinson 1995; Grimm et al. 2001). Similar effects were seen here following IntA cocaine self-administration experience. At WD1 psychomotor sensitization was manifest by locomotor hyperactivity (Fig. 2.3). However, by WD30 locomotor hyperactivity was no longer evident, because of the emergence of focused stereotyped behaviors (Fig. 2.4). Of course, the latter reflects a greater drug effect (Lyon and Robbins 1975; Segal 1975), and therefore, is evidence of robust psychomotor sensitization. These results are similar to those seen after ShA cocaine self-administration experience (see Introduction for references), and during

IntA sessions, as reported by Samaha and colleagues (Allain et al. 2017; Allain and Samaha 2018; Algallal et al. 2019).

It is clear that IntA cocaine self-administration experience is capable of sensitizing brain circuitry that mediates the psychomotor activating effects of cocaine. Of course, the different psychomotor activating effects of psychostimulant drugs, including locomotor hyperactivity and stereotyped behaviors, are thought to be mediated largely by DA projections from the midbrain to both the dorsal and ventral striatum (Creese and Iversen 1975; Kelly et al. 1975; Pijnenburg et al. 1976; Vezina and Kim 1999; Vanderschuren and Kalivas 2000). The behavioral data suggest, therefore, that IntA would also produce DA sensitization. Indeed, following IntA cocaine self-administration experience a single self-administered infusion of cocaine produces a greater increase in DA in the core of the nucleus accumbens *in vivo*, than in control rats with more limited cocaine self-administration experience (Kawa et al. 2019b). Furthermore, IntA cocaine self-administration sensitizes electrically-evoked DA release measured in the nucleus accumbens *ex vivo* (Calipari et al. 2013) and sensitizes the ability of cocaine to inhibit DA uptake (Calipari et al. 2013, 2015). Thus, data to date show that IntA experience produces both psychomotor and DA sensitization, similar to the effects produced by intermittent, non-contingent injections of cocaine or amphetamine (Robinson and Becker 1986; Kalivas and Stewart 1991; Stewart and Badiani 1993; Vezina 2004).

Long Access

Data on the ability of LgA or high dose cocaine self-administration experience to induce psychomotor sensitization are mixed. When animals were tested soon after the discontinuation of

LgA self-administration there are reports that the psychomotor activating effects of cocaine are actually attenuated (i.e., show tolerance), consistent with reports that the ability of cocaine to increase extracellular DA *in vivo* is also decreased, as is its ability to inhibit DA uptake (Calipari et al. 2013, 2014a). On the other hand, there are also reports of no tolerance to either cocaine's psychomotor activating effects (Ahmed and Cador 2006), or effects on extracellular DA (Ahmed et al. 2004; Kawa et al. 2019b), following LgA experience, relative to ShA experience. When animals have been tested longer after the discontinuation of LgA experience (at least 14 days) studies are also mixed as to whether psychomotor sensitization is expressed. Some studies report that psychomotor sensitization is not evident, even after long periods of withdrawal (Ben-Shahar et al. 2004, 2005), and one study even reports evidence of tolerance to the psychomotor activating effects of cocaine at WD60 (Ben-Shahar et al. 2005). Others have reported that long after the discontinuation of LgA rats express similar sensitization to ShA rats (Knackstedt and Kalivas 2007), or even especially robust psychomotor sensitization (Ferrario et al. 2005), as was found here.

What might account for the very different effects of LgA experience, especially after a long period of withdrawal? One possibility is that in some studies animals were tested for psychomotor sensitization in a context where they had never experienced the drug. Under some circumstances the expression of psychomotor sensitization can be very context-specific (Badiani et al. 1995; Crombag et al. 2000), so this may account for some of the negative findings. Indeed, this may account for why in the current study males expressed sensitization within the self-administration chambers, but not when tested outside this environment (compare Figs. 2.7 and

2.8). Perhaps more important, however, is that some studies relied on a single measure of psychomotor activity, locomotion, and this can lead to spurious conclusions. As shown here, and by Ferrario et al (2005), at WD30 measures of locomotor activity did not reveal evidence of sensitization (Fig 2.4). But that was because of the emergence of focused stereotyped behaviors, which are indicative of a stronger (i.e., sensitized), not weaker, drug effect. Therefore, analysis of a range of behaviors (captured by the rating scale) and head movements (i.e., focused stereotypy) revealed that LgA experience had in fact produced very robust psychomotor sensitization. The dangers of relying solely on measures of locomotor activity in studies of psychomotor sensitization are discussed in detail by Ferrario et al. (2005) and Flagel & Robinson (2007), and they suggest that when only locomotor activity is assessed negative results have to be interpreted with great caution (also see Robinson and Berridge 2008).

In summary, some, but not all, studies are consistent with the idea that LgA cocaine self-administration may attenuate the psychomotor activating effects of cocaine when animals are tested soon after the discontinuation of self-administration, but when tested after a period of withdrawal very robust psychomotor sensitization is manifest (Ferrario et al. 2005; present study). This is consistent with the effects of repeated treatment with high doses of psychostimulant drugs administered by an experimenter, when sensitization may not be expressed early after the discontinuation of drug treatment, but become evident after a few weeks of withdrawal (Robinson and Camp 1987; Paulson et al. 1991; Paulson and Robinson 1995; Grimm et al. 2001). One reason the psychomotor effects may change over time in this way may be because both tolerance- and sensitization-related neuroadaptations can be present at the same

time (e.g., Izenwasser and French 2002), but tolerance-related adaptations can mask the expression of sensitization; that is, sensitization is expressed only as tolerance-related adaptations subside. This interpretation is supported by an interesting study by Dalia et al. (1998). Briefly, rats previously expressing psychomotor sensitization received implants of osmotic pumps that administered cocaine continuously for seven days. These rats expressed behavioral tolerance the day after removal of the pump, but by day 10 post-pump removal, the rats yet again expressed behavioral sensitization to cocaine, as tolerance waned.

Sex differences in the effects of IntA experience on psychomotor sensitization

Self-Administration

Both male and female rats were initially trained to self-administer cocaine using an "infusion criteria" procedure. Under these conditions there was no sex difference in the acquisition of self-administration, consistent with a previous study using the same procedure (Kawa and Robinson 2019). It should be noted, however, that when tested under free access conditions females have been reported to more readily acquire cocaine self-administration (Lynch and Carroll 1999; Hu et al. 2004; although see Algallal et al. 2019). Here, males and females consumed the same amount of drug during acquisition because each rat was allowed to take a predetermined and fixed number of injections. However, when tested using a 'threshold' procedure in which infusions were not capped, females consumed more cocaine than males at low doses (Fig. 2.5). This is consistent with reports that females are more motivated and/or consume more cocaine post-acquisition than males (Roberts et al. 1989; Cummings et al. 2011; Kawa and Robinson 2019). Therefore, in order to ensure that there were no group differences in

cocaine consumption during IntA (which could impact the degree of sensitization) both males and females were limited to 3 cocaine injections during each Drug-Available period of the IntA-Limited self-administration procedure used in Exp 2.

Psychomotor sensitization

For these studies, locomotor activity was monitored during IntA-Limited self-administration sessions and video was recorded throughout. Consistent with the absence of sex differences in cocaine consumption during IntA-Limited, there were no sex differences in the psychomotor activating effects of self-administered cocaine on the first day of IntA-Limited training. However, with increasing IntA-Limited experience a clear sex difference in the psychomotor activating effects of self-administered cocaine emerged, with females showing more robust psychomotor sensitization than males (Fig 2.7; also see, Algallal et al. 2019). This sex difference was also evident on a probe test when rats with IntA-Limited experience were compared to a control group that had acquired cocaine self-administration and underwent the 'threshold' test, but did not have IntA-Limited experience (Fig. 2.8). Thus, both within-subjects and between-subjects comparisons revealed that following IntA-Limited females expressed greater psychomotor sensitization than males.

These findings are consistent with many early studies reporting that experimenter-administered cocaine or amphetamine produces greater psychomotor sensitization in females than males (Glick and Hinds 1984; Robinson 1984; Van Haaren and Meyer 1991; for review, Becker et al. 2006). They are also consistent with a report that IntA experience produces more robust incentive-sensitization in females than males (Kawa and Robinson 2019). This greater

propensity for sensitization in females may contribute to the more rapid emergence of problematic drug use (the 'telescoping effect') in women, described in clinical studies (Anglin et al. 1987; Kosten et al. 1993; Brady and Randall 1999).

Behavioral variability

The estrous cycle was not monitored in this study, but several papers suggest that the phase of the cycle could affect our locomotor and motivation measures (Becker et al. 2012; Lynch 2018; Yoest et al. 2018). This could account for the somewhat larger variance observed in females at some of the doses tested during the within-session threshold procedure. However, a meta-analysis on this topic concludes that female behavior, even when tested regardless of estrous phase, is not more variable than male behavior (Becker et al. 2016). Thus, the role of the estrous cycle on sensitization produced by IntA experience could be a topic of future investigation, but we doubt whether consideration of this factor would alter the findings reported here.

IntA experience and cross-sensitization

There are a number of early studies reporting psychomotor cross-sensitization between the two prototypical psychomotor stimulant drugs, amphetamine and cocaine, when they were administered by an experimenter (Shuster et al. 1977; Akimoto et al. 1990; Schenk et al. 1991; Hirabayashi et al. 1991; Bonate et al. 1997; Shanks et al. 2015). However, all the studies reporting that treatment with cocaine induces psychomotor sensitization to amphetamine involved the use of IP injections (Akimoto et al. 1990; Hirabayashi et al. 1991; Shanks et al. 2015). We first asked, therefore, whether experimenter-administered *IV* injections of cocaine

would (1) induce psychomotor sensitization and (2) cross-sensitization to IV amphetamine. They did. We next asked whether IntA cocaine self-administration experience would also produce psychomotor cross-sensitization to IV amphetamine, and it did (Fig 2.12). This psychomotor cross-sensitization may be due to cross-sensitization of DA activity. IntA cocaine self-administration experience not only sensitizes the effects of cocaine at the DAT, but produces cross-sensitization to amphetamine's actions at the DAT as well. Indeed, IntA (but not LgA) cocaine self-administration experience increases the ability of *amphetamine* to inhibit DA uptake (Calipari et al. 2014b). Interestingly, the non-contingent administration of amphetamine, which produces psychomotor sensitization, also facilitates escalation of intake when rats were later allowed to self-administer cocaine (Ferrario and Robinson 2007). The phenomenon of cross-sensitization may help explain why the use of one drug of abuse increases the probability that others will be abused as well, and why polydrug use is so common in people with substance abuse disorders (Schenk 2002).

Conclusions and implications for theories of addiction

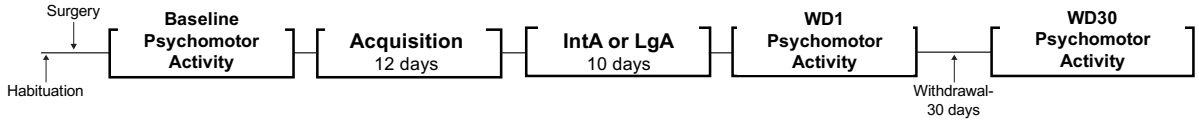
As mentioned in the Introduction, preclinical studies using LgA self-administration procedures have been cited in support of the idea that addiction is due, in part, to tolerance, leading to a drug-induced *hypo*dopaminergic state, and drug-seeking behavior is motivated to overcome this 'DA deficiency' (e.g., Koob and Volkow 2016; Volkow et al. 2016). Indeed, when tested soon after the discontinuation of LgA, or other high dose procedures, there may be tolerance to cocaine's psychomotor activating effects, and its ability to increase DA neurotransmission (Ferris et al. 2011; Calipari et al. 2013, 2014a), although evidence for this is

mixed (Ahmed et al. 2004; Kawa et al. 2019b and current results). However, after longer periods of withdrawal (30 days) robust psychomotor sensitization is evident (Ferrario et al. 2005; present study). We are not aware of any studies on the effects of LgA on DA neurotransmission after long periods of withdrawal. But, there are many studies showing that animals with prior LgA experience show enhanced glutamatergic transmission in the ventral striatum after long periods of withdrawal (Wolf 2016), when psychomotor sensitization is expressed, and when rats show especially robust drug-seeking behavior – so-called 'incubation of craving' (Grimm et al. 2001).

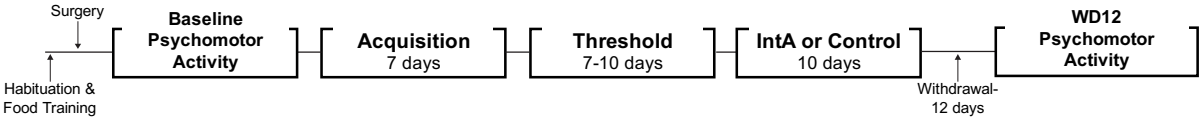
Most importantly, there is now considerable evidence that IntA cocaine self-administration, which is thought to better reflect human patterns of use, is more effective than LgA in producing addiction-like behavior, despite much lower levels of total drug consumption. IntA also produces robust psychomotor sensitization (present study), incentive-sensitization and DA sensitization (for reviews see, Allain et al. 2015; Kawa et al. 2019a), which is consistent with an incentive-sensitization view of addiction (Robinson and Berridge 1993). It will be interesting, therefore, to determine if IntA experience produces similar glutamatergic plasticity as LgA, and whether it is related to the expression of psychomotor sensitization and/or the robust reinstatement of drug-seeking that is evident even after short periods of withdrawal from IntA (Ferrario et al. 2010). Of course, it is also possible that the addiction-like behavior and psychomotor sensitization produced by IntA experience has a different neurobiological basis than that produced by LgA. If this is the case it would have important implications for thinking about how drugs may change brain and behavior in ways that promote a transition from casual drug use to the problematic patterns of use that define addiction.

In closing, the similarities in the sensitization produced by IntA cocaine self-administration experience and that produced by repeated, intermittent experimenter-administered drug treatments raise questions regarding the utility of the latter in studying the neurobiology of addiction. The dogma is that operant self-administration procedures are required to model drug-induced changes in brain that may promote the development of problematic patterns of drug use. But this may not be the case. Indeed, as argued by Robinson and Berridge (2008) over a decade ago, "both experimenter- and self-administered drugs can produce relevant outcomes, as long as they produce neural sensitization", and, "experimenter-administered drug administration procedures that produce robust sensitization may in some ways more effectively model addiction than self-administration procedures that fail to produce robust sensitization" (p. 3142).

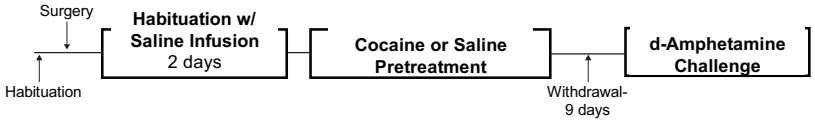
a. Exp. 1 Effect of withdrawal on psychomotor sensitization following IntA vs LgA:



b. Exp. 2 Sex differences in psychomotor sensitization following IntA-Limited:



c. Exp. 3a Effect of experimenter-administered cocaine on cross-sensitization w/ AMPH:



d. Exp. 3b Effect of IntA cocaine self-administration on cross-sensitization w/ AMPH:

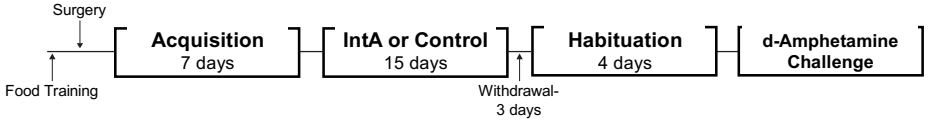


Figure 2.1. Experimental timeline. Flow diagrams for each experiment. Exp, Experiment; IntA, Intermittent Access; LgA, Long Access; Amph, *d*-Amphetamine.

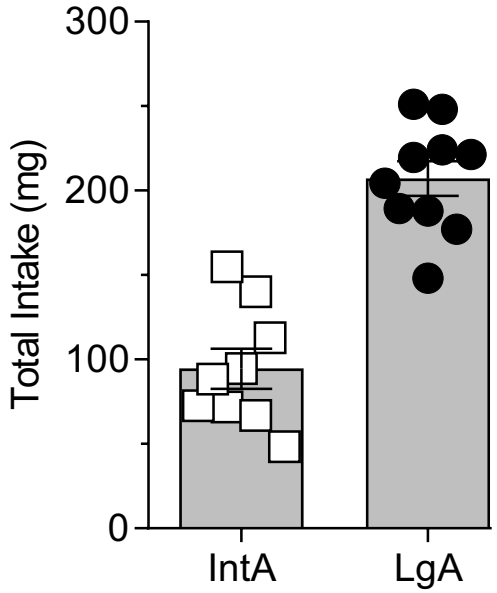


Figure 2.2. Consumption. Mean (± SEMs) total cocaine intake during 10 days of access to IntA or LgA. Circles and squares represent individual rats. Rats in the LgA group (n=10) rats consumed about twice as much cocaine than those in the IntA group (n=9).

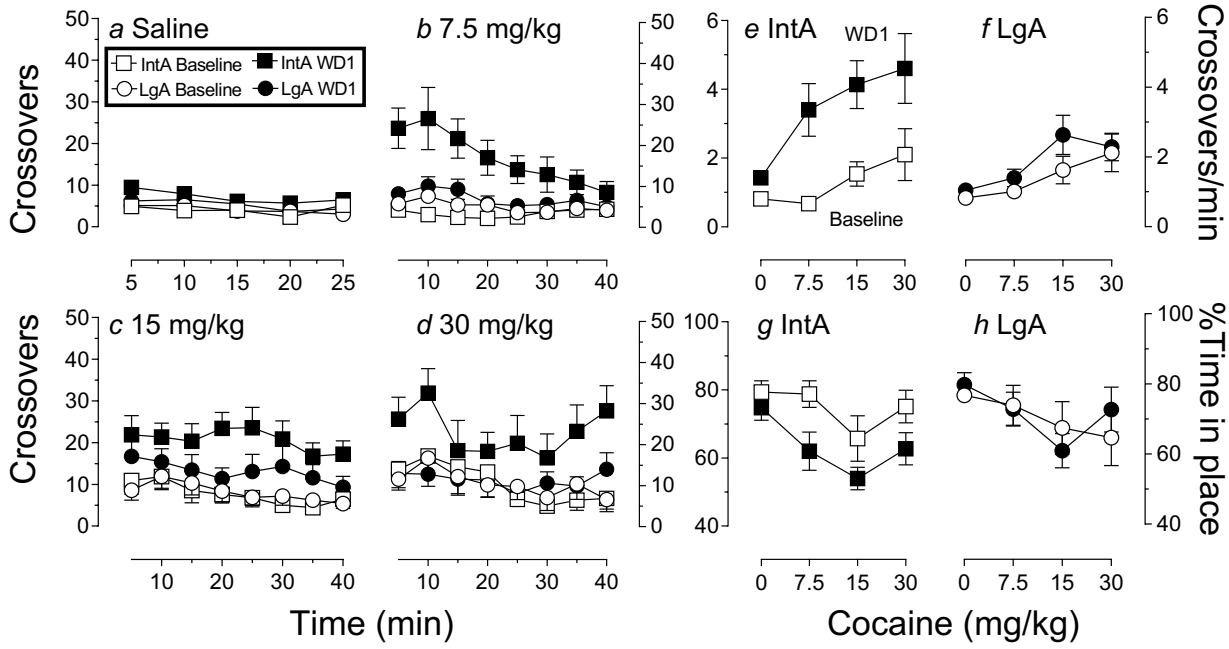


Figure 2.3. Withdrawal Day 1. Behavioral effects of cocaine at Baseline (prior to any self-administration experience) and 1 day after the discontinuation of IntA or LgA cocaine self-administration experience (WD1). Panels a-d show the time course of locomotor activity, as assessed by crossovers. Cocaine produced greater locomotor activity following IntA, but not LgA experience. Panels e & f show these same data collapsed across time to generate a dose-effect function, which clearly demonstrates that IntA, but not LgA experience produced psychomotor sensitization (see Results for all statistics). Panels g & h show the time animals spend 'in place' (i.e., not locomoting) as a function of group and dose. Consistent with the measure of locomotion, cocaine produced a greater dose-dependent decrease in time in place following IntA experience, relative to baseline, but there was no effect of session in the LgA group. All data represented as mean ± SEM.

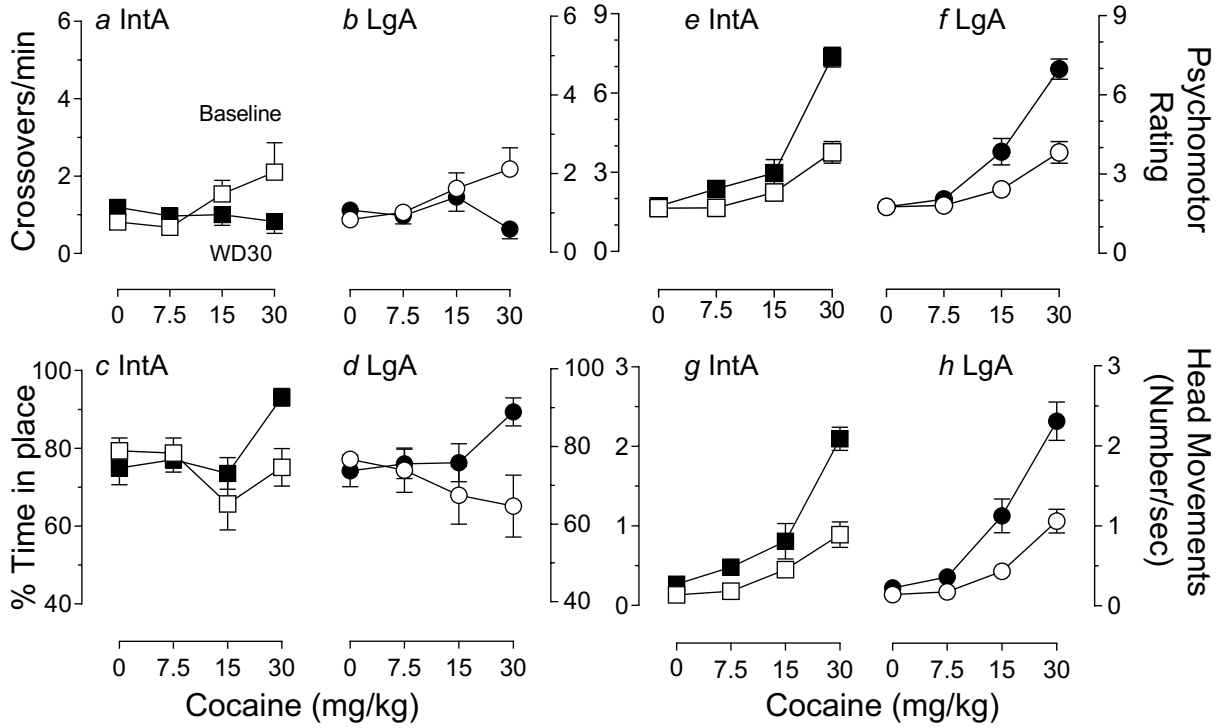


Figure 2.4. Withdrawal Day 30. Behavioral effects of cocaine at Baseline (prior to any self-administration experience) and then 30 days after the discontinuation of IntA or LgA cocaine self-administration experience (WD30). Panels a & b show that at Baseline there was a dose-dependent increase in crossovers, but this was not evident at WD30 and in fact on WD30 there was a dose-dependent increase in time in place in both groups (Panels c & d). This pattern of behavior suggests that on WD30 cocaine produced stereotyped behaviors in both groups, which was confirmed by inspection of videos. The degree of cocaine-induced stereotypy was assessed using a rating scale (Panels e & f) and by counting the frequency of stereotyped head movements (Panels g & h). Both measures showed that at this time psychomotor sensitization was evident in both the IntA and LgA groups. All data represented as mean ± SEM.

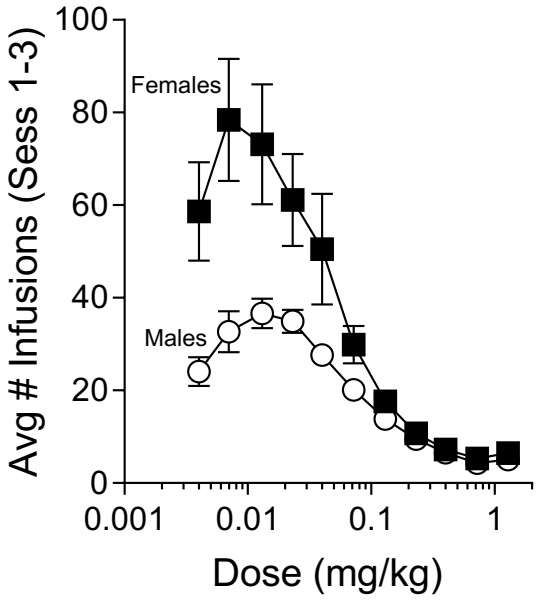


Figure 2.5. Within-Session Threshold. Motivation to obtain cocaine was assessed using a within-session threshold procedure after initial acquisition of cocaine self-administration. Females consumed more drug than males. All data represented as mean ± SEM.

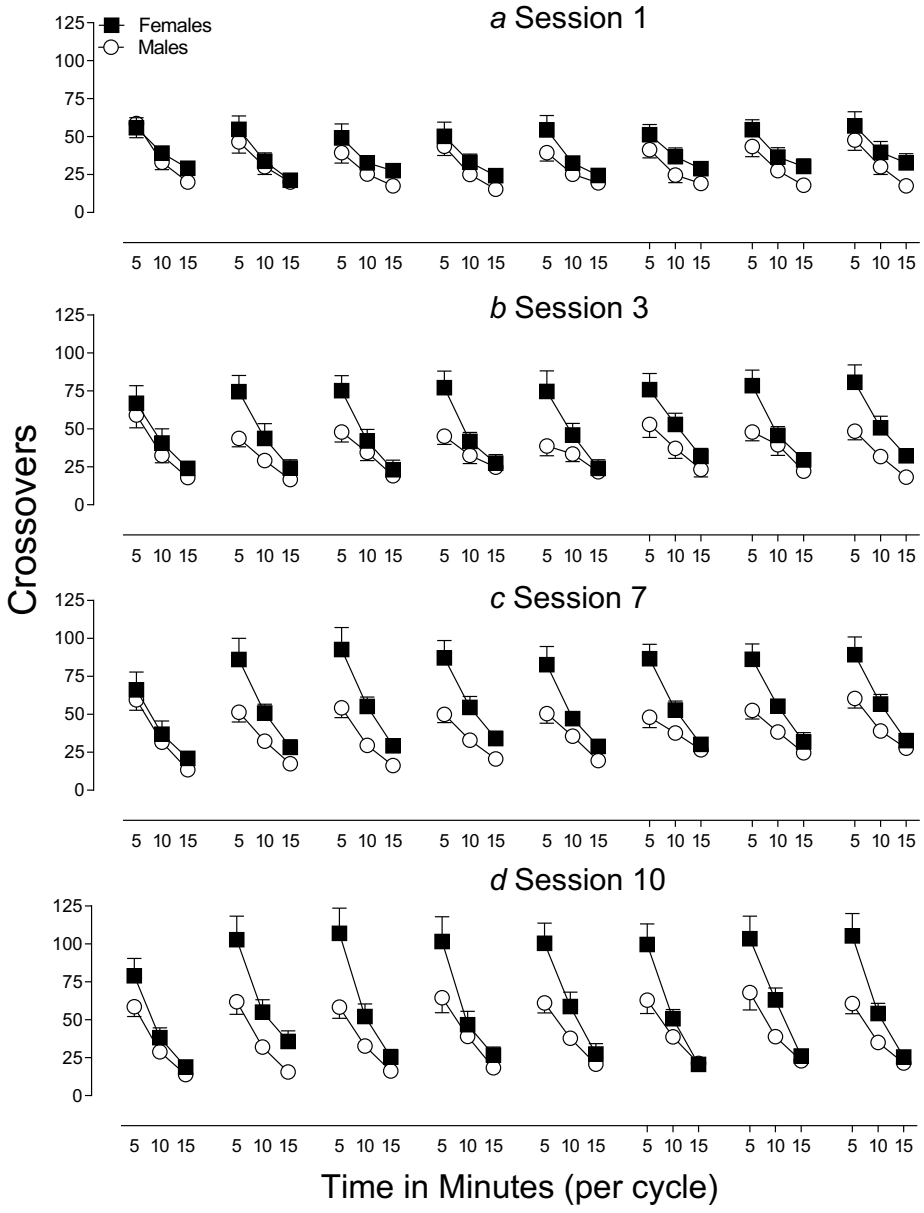


Figure 2.6. Psychomotor Activity During IntA. Mean (± SEM) crossovers for the first 15 min (5 min bins) after the self-administration of 3 infusions of cocaine, over 8 successive Drug-Available periods on Days 1, 3, 7 and 10 of IntA-Limited testing, in male and female rats. There was no sex difference in crossovers on Session 1 (a), but females showed greater psychomotor

activation on Sessions 3, 7 and 10 (Panels, b, c & d, respectively). See Fig. 2.7 for analysis of the effect of Session.

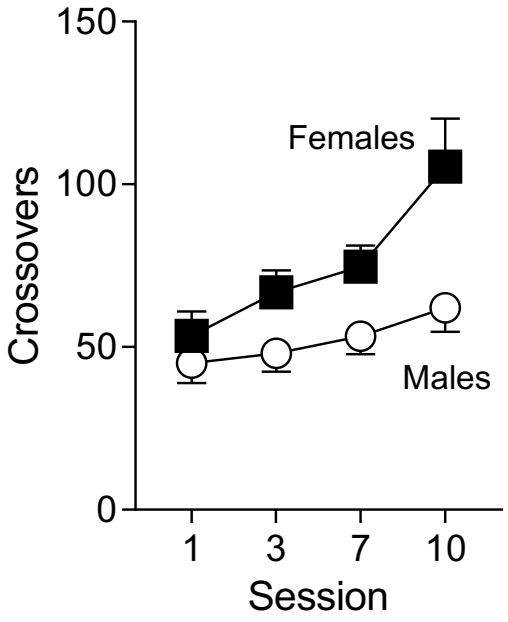


Figure 2.7. Within-Subject Psychomotor Activity. Mean (± SEM) crossovers during the first 5 min following the self-administration of 3 infusions of cocaine (averaged across the 8 daily Drug Not-Available cycles) as a function of session in male and female rats. There was an increase in crossovers as a function of session in both males and females, but the degree of psychomotor sensitization was greater in females than males.

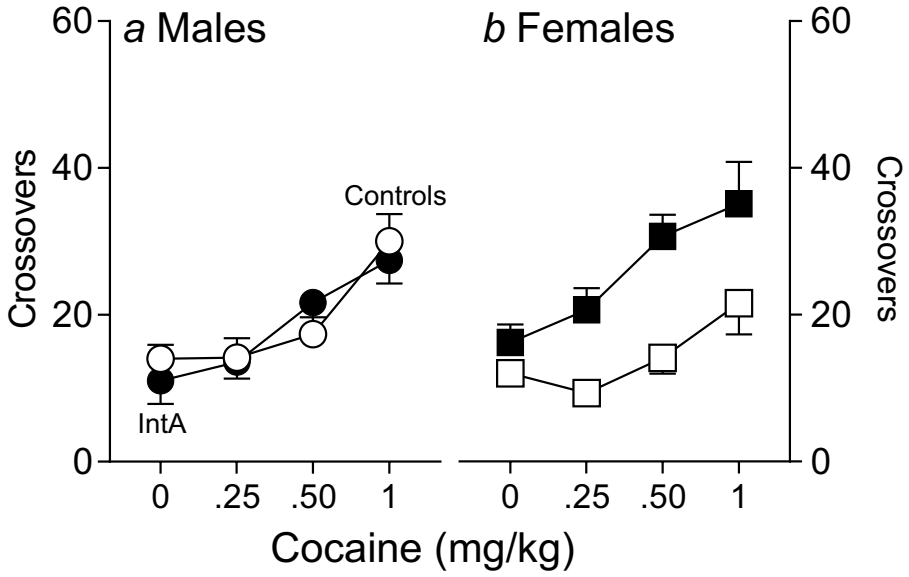


Figure 2.8. Between-Subject Psychomotor Activity. Mean (± SEM) crossovers produced by experimenter-administered IV cocaine challenge infusions in male and female rats with prior IntA cocaine self-administration experience, and Control rats that did not have IntA experience. Cocaine produced a greater dose-dependent increase in crossovers in the IntA group, relative to Controls (i.e., psychomotor sensitization), in females, but not males.

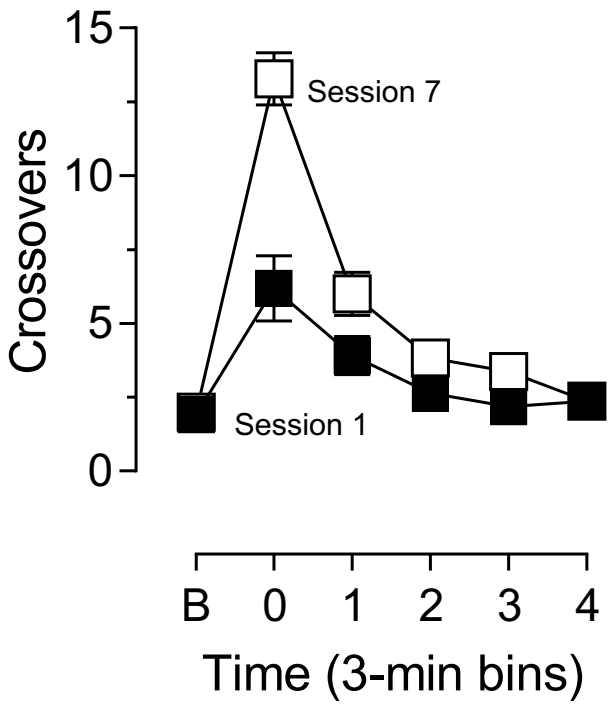


Figure 2.9. Cocaine Pretreatment. Mean (± SEM) crossovers in 3 min bins prior to (B, Baseline) and following the first and seventh infusion of experimenter-administered cocaine (1.0 mg/kg, IV; n=11). The behavioral response was greater after the seventh than the first session, indicating psychomotor sensitization.

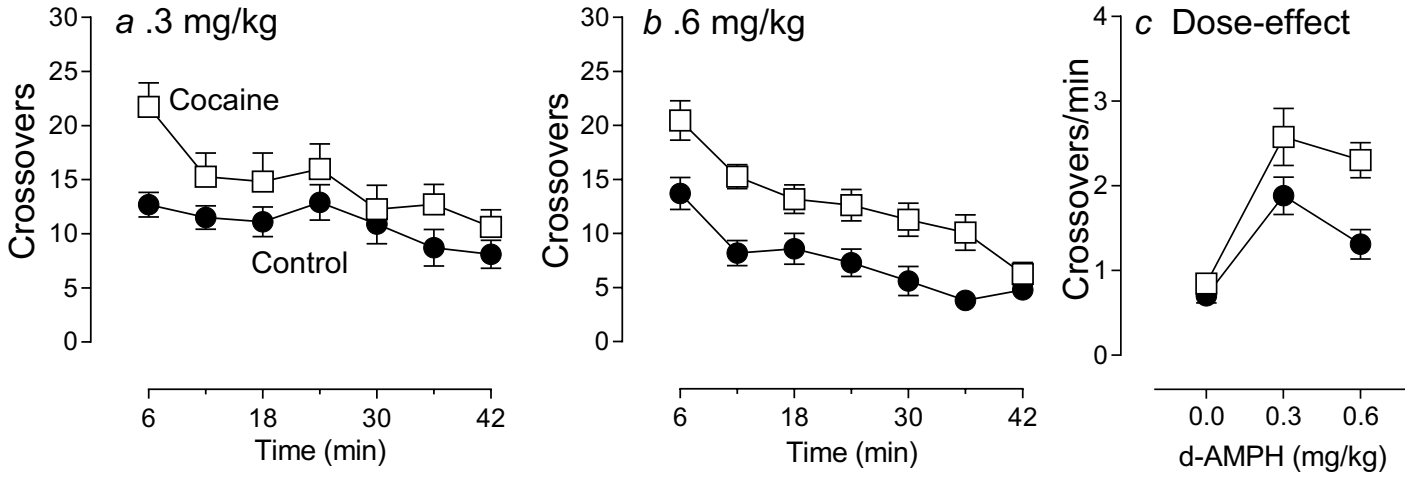


Figure 2.10. d-Amphetamine Challenge. Mean (± SEM) crossovers during the amphetamine challenge test day in rats pretreated with IV experimenter-administered cocaine (open symbols) or Saline-pretreated Controls (closed symbols). Panels a & b show the time course of the locomotor response to 0.3 and 0.6 mg/kg of amphetamine IV, respectively. Panel c shows the same data averaged across each dose as a dose-effect function. Psychomotor sensitization is indicated by the greater response in the Cocaine- relative to the Saline-pretreated Control group.

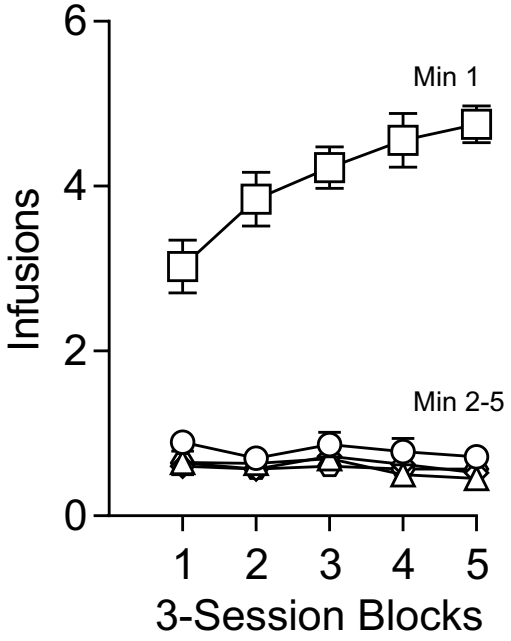


Figure 2.11. IntA Escalation. Mean (± SEM) number of infusions during each minute (Min) of the Drug-Available periods during IntA cocaine self-administration, averaged across 3 session blocks. It can be seen that (1) the rats took nearly all drug during the first minute of drug availability, and (2) that there was an increase in drug consumption with increasing IntA experience.

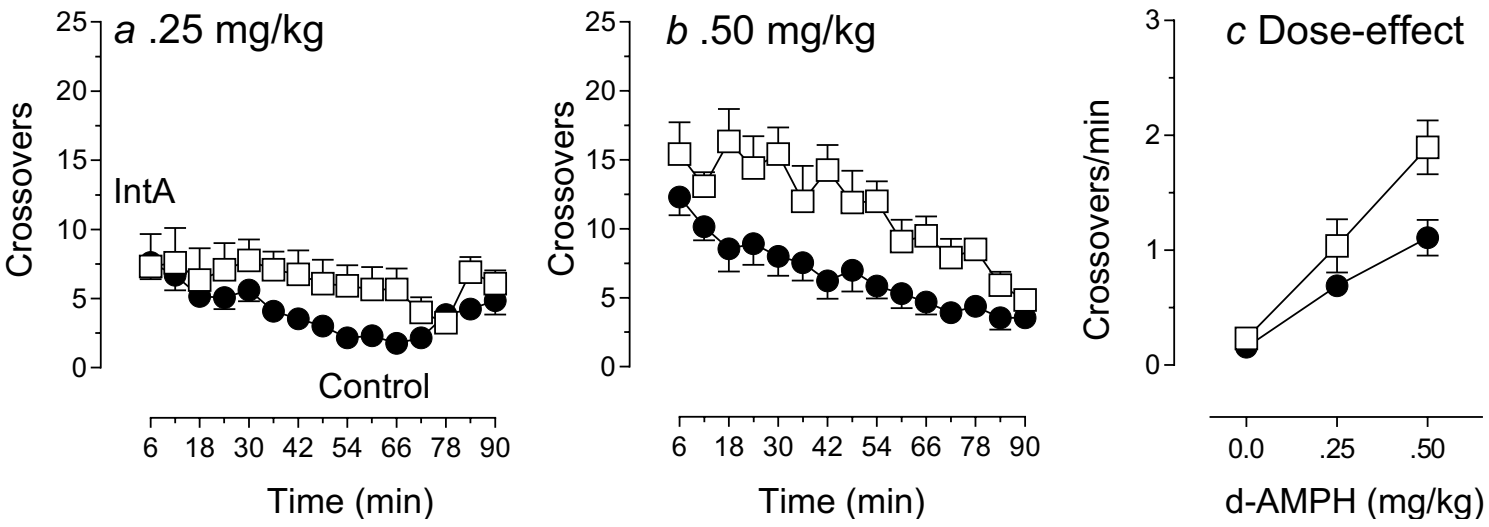


Figure 2.12. d-Amphetamine Challenge. Mean (± SEM) crossovers on the *d*-amphetamine challenge test day in rats with prior IntA cocaine self-administration experience, and Control rats that did not have any IntA experience. Panels a & b show the time course of the locomotor response to 0.25 and 0.5 mg/kg of *d*-amphetamine IV, respectively. Panel c shows the same data averaged across each dose as a dose-effect function. Psychomotor sensitization is indicated by the greater response in the IntA relative to the Control group after the higher dose of *d*-amphetamine.

Stereotypy Rating Scale
0 = Inactive—lying down, eyes open
1 = Inplace activities—normal grooming
2 = Normal, alert, active—moving about cage, sniffing, rearing
3 = Hyperactive—running movement characterized by rapid changes in position (jerky)
4 = Slow patterned—increased frequency of otherwise normal appearing head movements associated with repetitive exploration of the cage at normal level of activity; discontinuous; mild intensity
5 = Fast patterned—increased frequency of otherwise normal appearing head movements associated with repetitive exploration of the cage with *hyperactivity*; discontinuous; moderate intensity
6 = stereotyped (repetitive) up and down head movements and shuffling of the forepaws; *discontinuous*; moderate intensity and frequency
7 = stereotyped (repetitive) up and down head movements and shuffling of the forepaws; *discontinuous*; high intensity and frequency
8 = continuous, in place repetitive head and limb movements; moderate intensity and frequency
9 = continuous, in place repetitive head and limb movements; high intensity and frequency

Table 2.1. Stereotypy Rating Scale. Rating scale used to assess the psychomotor activating effects of cocaine. Adapted from Ellinwood and Balster, 1974 and Robinson et al. 1988.

Chapter III

Sex Differences in Incentive and Psychomotor Sensitization Following Limited Intermittent Access Cocaine Self-Administration

Repeated, intermittent exposure to many drugs of abuse, such as cocaine, results in a progressive increase in their psychomotor activating effects and in dopamine (DA) neurotransmission (for reviews see, Robinson and Becker 1986; Stewart and Badiani 1993). In animals, this form of neurobehavioral sensitization manifests as increased locomotor activity and rearing at low doses and in-place stereotyped head movements at high doses (Wise and Bozarth, 1987). Animals sensitized to cocaine also show an enhanced preference for a drug-associated environment and acquisition of drug self-administration (Lett 1989; Horger et al. 1990), both of which indicate increased incentive value. As such, psychomotor sensitization is thought to reflect sensitization-related changes in brain systems critical for incentive motivation (Robinson and Berridge 1993; De Vries et al. 1998; Lorrain et al. 2000). Sensitization to the incentive motivational effects of drugs is hypothesized to lead to the pathological drug wanting associated with addiction (Robinson and Berridge 1993). Given this, psychomotor sensitization has been extensively studied, and of importance here is the observation of greater expression in females compared to males (Robinson 1984; Algallal et al. 2019; for review, Becker et al. 2016).

More severe addiction-like behavior is also generally observed in female rats than

males. If given a choice between a palatable food reward and cocaine, more females than males develop a cocaine preference (Kerstetter et al. 2012; Perry et al. 2013, 2015). Females also escalate drug intake more rapidly (Roth and Carroll 2004; Smith et al. 2011) and are more motivated to obtain cocaine than males, both in a test that manipulates the response requirement (progressive ratio; Roberts et al. 1989; Cummings et al. 2011; Algallal et. 2019) and another that manipulates the available dose (behavioral economics; Kawa and Robinson 2019). Motivation for cocaine, as measured by progressive ratio, has been correlated with the extent of psychomotor sensitization in both female and male rats (Algallal et al. 2019), supporting the notion that increased psychomotor activation reflects changes within incentive motivation systems in the brain (Robinson and Berridge 1993; De Vries et al 1998; Lorrain et al. 2000).

The extent to which total drug consumption affects motivation is currently unclear. Studies using the long access (LgA; 6 hours) model that is characterized by high levels of drug consumption report greater motivation than rats with lesser consumption due to a shorter access period (Paterson and Markou 2003; Zimmer et al. 2012). In contrast, studies using the novel intermittent access (IntA) model, which more closely resembles human drug-taking patterns, report greater incentive sensitization than that seen following LgA, despite less drug intake (Zimmer et al. 2012; James et al. 2019; Kawa et al. 2019b; Minogianis and Samaha 2020). It is possible, therefore, that the especially robust incentive sensitization (as measured by behavioral economics) observed in females compared to males following IntA experience is due to the greater amount of drug consumed (Kawa and Robinson 2019). However, Kawa and Robinson (2019) did not find a relationship between consumption during IntA and probe motivation

(assessed after 30 sessions of IntA). In addition, Algallal et al. (2019) found that females self-administered more infusions under a progressive ratio schedule of reinforcement than males, despite similar drug intake during IntA. Here we sought to extend these findings by determining if cocaine demand assessed using a behavioral economic approach would be greater in females compared to males in the absence of a sex difference in cocaine intake during IntA.

To prevent a sex difference in consumption, the present study utilized an IntA procedure with infusion limitations (IntA-Limited) and assessed changes in both motivation and psychomotor activation. Demand for cocaine was assessed pre and post IntA-Limited using a modified within-session threshold test. Each descending dose of cocaine was available for 5-min and separated by a 25-min period during which drug was not available (opposed to uninterrupted drug availability). This change allowed us to 1) maintain intermittency throughout testing and 2) measure psychomotor activity during and following periods of unlimited cocaine access. In addition, psychomotor activation was also assessed using a single-session test and during IntA-Limited. We hypothesized that females would be more motivated than males despite similar consumption during IntA and that the behavioral economic measure of motivation, nPmax, would be positively correlated with the extent of psychomotor sensitization.

Materials and Methods

Subjects

A total of 64 (28 males; 36 females) Sprague-Dawley rats (Envigo, Haslett, MI), ~55 days old upon arrival, were housed individually in a climate-controlled colony room on a

reverse 12-h light/12-h dark cycle. Male and female rats were housed in cages located on opposite walls within the same room. All testing was conducted during the 12-h dark period. Rats were given 1 week to acclimate to the colony room before undergoing surgery. Water and food were available *ad libitum* throughout this time and during recovery (~7 days). To prevent excessive, unhealthy weight gain, mild food restriction began 2 days prior to the first self-administration session. This was done by maintaining the post-operative body weight, resulting in healthier male and female rats, relative to *ad libitum* feeding (Rowland 2007). All procedures were approved by the University of Michigan Institutional Animal Care and Use Committee.

Apparatus

All behavioral testing took place in Med Associates chambers (22 × 18 × 13 cm; St Albans, VT, USA) located inside sound-attenuating cabinets equipped with a ventilating fan to mask background noise. An infusion pump was mounted on the outside of the cabinet, on the right door. Infusions were delivered via a length of polyethylene tubing (PE20) inside a steel protective cover that connected the intravenous (IV) catheter to a liquid swivel mounted on a counter-balanced arm located on the top of the test chamber, allowing free movement in the chamber. Within each chamber, two retractable levers were located on the front wall, 6 cm above the floor, and a white cue light was situated 3 cm above the lever designated active (counterbalanced by side). All training was conducted under a fixed ratio 1 scheduled of reinforcement (FR1), with each response on the active lever resulting in the delivery of one reward and illumination of the cue light. Inactive responses had no programmed consequence. A red house light was located at the top and centered on the back wall, opposite the levers. The

start of each session was signaled by illumination of the house light and insertion of the levers into the chamber.

Assessing psychomotor activity

Within the same cabinets described above, a camera (CVC-130R, Speco Technologies, Amityville, NY, USA) was mounted on the back wall, angled towards the center of the test chamber. Additionally, the floor grid of each test chamber was equipped with two pairs of horizontal infrared photobeams (each consisting of a source and detector). One photobeam pair was located on the front end of the chamber near the levers and the other pair was located towards the back end. Once the signal between the source and detector was interrupted for a given pair (i.e., a beam break), additional beam breaks for that pair were not recorded until after the animal moved to the opposite side of the chamber, interrupting the signal of the other pair, and then back again. As such, this configuration should capture crossovers, a commonly used measure of locomotor activity. To confirm that beam breaks are indeed comparable to crossovers, video recordings of IntA-Limited (described below) sessions 1, 9, and 11 were scored for crossovers as described previously (Carr et al. 2020). Briefly, the chamber was divided into two equal halves and crossovers were scored by counting each time the center-point of the rat's body crossed from one side of the test chamber to the other. Behavior was quantified in 3-min bins during 4 of the 8 (odd cycles) 25-min No Drug-Available periods. This analysis revealed a strong positive correlation between automated beam breams and hand scored crossovers (Fig. 3.1; R^2= 0.88, p<0.0001), suggesting that these measures can be used interchangeably.

General outline of training and testing

As shown in Fig. 3.2, rats were trained to self-administer food prior to undergoing intravenous catheter surgery. Following recovery, rats acquired the cocaine self-administration behavior necessary to complete a single-session baseline psychomotor activity test and intermittent access procedures. Animals were first trained using IntA-Demand to assess baseline motivation, followed by IntA-Limited to prevent sex differences in consumption. Both the single-session psychomotor activity test and IntA-Demand were again administered to assess change following IntA-Limited experience. Details of each component of training and testing follow.

Food self-administration

All rats first learned to lever press for a palatable food reward to minimize sex differences in the acquisition of cocaine self-administration. For this training only, a food receptacle was located in the center of the front wall 3 cm above the floor and the attached pellet dispenser was located outside of the test chamber. Responses on the active lever resulted in the delivery of a banana-flavored pellet (45 mg; BioServe, #F0059, Frenchtown, NJ, USA), paired with illumination of the cue light and retraction of both levers for 2.6-sec. This time-out period was imposed to acclimate animals to retraction of the lever. Each rat had one 30-min session per day, for 2-5 days, as previously described (Carr et al. 2020).

Intravenous catheter surgery

Once rats earned at least 25 pellets/session on two consecutive sessions, indwelling catheters were implanted into the right jugular vein as described previously (Crombag et al.

2000; Carr et al. 2020). Rats were anesthetized with ketamine hydrochloride (90 mg/kg, IP) and xylazine (10 mg/kg, IP), the effects of which were rapidly reversed by antisedan (1 mg/kg, IP) following completion of the surgery. The analgesic carprofen (5mg/kg, SC) was administered immediately prior to and for two days following surgery. After implantation, catheters were flushed daily with 0.2 ml sterile saline containing 5 mg/ml gentamicin sulfate (Vedco, MO) or 3.5 mg/ml heparin (Sigma-Aldrich, MO) for the entirety of the experiment. Catheter patency was tested prior to behavioral testing, as needed throughout the experiment (i.e., if responding for cocaine suddenly declined), and upon completion of the experiment by administering an IV injection of 0.1 mL of methohexital sodium (10 mg/ml in sterile water, JHP Pharmaceuticals). If a rat did not become ataxic within 10-sec of the injection it was excluded from the study. One rat was excluded following surgery complications.

Acquisition of cocaine self-administration

After at least 7 days of recovery from surgery, all rats began training to self-administer cocaine. Each response on the lever designated active resulted in an IV infusion of cocaine hydrochloride (NIDA) dissolved in 0.9% sterile saline (0.4 mg/kg/infusion, weight of the salt, in 50 µl), delivered over 2.6-sec. Each infusion was paired with illumination of the cue light for a 20-sec time-out period (including infusion time), when lever deflections were recorded but had no consequences. An infusion criteria (IC) procedure was imposed during this initial training to ensure that all rats received the same amount of drug and cue exposures, as described previously (Saunders and Robinson 2010; Carr et al. 2020). Rats were first allowed to administer 10 infusions (IC10) for two consecutive days. If the criterion was met,

the fixed number of infusions increased to 20 (IC20) for five consecutive days. Regardless of criteria, two hours were allotted to earn all infusions. The session terminated following administration of the last infusion, thus resulting in varied session durations across rats and days. A total of 3 rats (2 males, 1 female) were excluded for failing to reach the infusion criteria and 1 female was excluded during this time due to a catheter malfunction.

Single-session psychomotor activity test

After the acquisition of self-administration behavior, baseline psychomotor activity was assessed following increasing doses of self-administered cocaine. The start of the session was signaled by the illumination of the house light after 30-min, to allow for habituation. The levers were then inserted for the first dose of 0.5 mg/kg. After this dose was administered, the levers retracted for 20-min. This cycle then repeated for 1 and 1.5 mg/kg cocaine. All infusions were paired with illumination of the cue light above the active lever for 10.5-sec to match the pump duration for the largest infusion. If the rats failed to make a response within 5-min, the levers retracted for a 1-min time-out. Following this period, the levers were re-inserted, accompanied by illumination of the cue light for 10-sec. If the rat failed to make a response again within 5-min, the appropriate dose was administered by the program.

IntA-Demand

The day after baseline psychomotor activity testing, rats were trained to self-administer using a within-session threshold procedure, similar to that described previously (Oleson et al. 2011; Bentzley et al. 2013). The traditional procedure allows animals to maintain a relatively high level of cocaine consumption across multiple consecutive doses

and days, a pattern of which is associated with tolerance-related neuroadaptations. Here instead we wanted to promote sensitization-related neuroplasticity and this is most pronounced in animals with intermittent drug exposure. To this end, we both decreased the time that each dose was available (from 10- to 5-min) and allowed brain cocaine concentration levels to repeatedly return to near zero by incorporating a 25-min No Drug-Available period between doses. Consistent with the traditional procedure, the dose of cocaine decreased on a quarter logarithmic scale (1.28, 0.72, 0.40, 0.23, 0.13, 0.072, 0.040, 0.023, 0.013, 0.007, 0.004, 0.002 mg/kg/infusion) by changing the pump duration. The cue light also remained illuminated during the duration of each infusion. In addition, there was no signaled time-out period following an infusion, but an additional infusion could not be earned while that infusion was being administered. Each rat underwent one 6-hr IntA-Demand session/day for 5-7 consecutive days. Subsequent testing (probe) was reduced to 3 sessions as previous experiments have shown rapid stability in responding following initial training (data not shown). During sessions 2, 4, 6, 8, and 10, locomotor activity was collected. A total of 13 rats (5 male, 8 female) were excluded during this procedure due to catheter failure, all during probe testing, except for 1 male.

Fits to the observed demand curves were generated from the IntA-Demand threshold procedure using a new and improved analysis (Newman and Ferrario 2020) instead of the previously described focused-fitting approach (Oleson et al. 2011; Bentzley et al. 2013). The incorporation of No Drug-Available periods between doses resulted in a less pronounced "loading" phase. As a result, all data points were included in the fit analysis, including those

following the point at which brain cocaine concentration was no longer relatively stable. Demand curves were fit using a new form developed by Newman and Ferrario and code written for use in Python to specifically analyze IntA-Demand and other within-session threshold procedures (Newman and Ferrario 2020) that produces four metrics: (1) the preferred level of consumption when minimal effort is required (Q0); (2) maximal effort output in responses (Rmax); (3) price (1/dose) at which the maximum response occurs (Pmax); (4) a normalized metric of motivation that takes into account the preferred level of consumption (nPmax).

IntA-Limited

Following completion of baseline IntA-Demand, rats continued to self-administer cocaine intermittently, but under limited conditions (IntA-Limited) to circumvent a sex difference in consumption that could potentially affect incentive motivation. As described previously (Carr et al. 2020), animals were limited to three infusions per 5-min Drug-Available period. The cue light was illuminated throughout the duration of each infusion (2.6-sec), during which time additional infusions could not be earned but otherwise there was not a programmed time-out period. The No Drug-Available period started once the last allotted infusion was self-administered or after 5-min, whichever came first, and was signaled by retraction of the levers. Each IntA-Limited session consisted of *8 Drug-Available and 8 No Drug-Available periods*, but session lengths varied slightly because the allotted 3 infusions were typically self-administered in the first minute of drug availability (sessions typically ended after ~210-min). Each rat underwent one IntA-Limited session/day for 5 consecutive

days, followed by 2 days off. This cycle repeated twice, followed by 2 additional days of testing, resulting in a total of 12 IntA-Limited sessions. During sessions 1, 3, 5, 7, 9, and 11, locomotor activity was also collected via beam breaks to assess psychomotor sensitization. A total of 17 rats (7 males, 10 females) were excluded during IntA-Limited testing due to catheter failure. In addition, 2 male rats were excluded for administering less than 85% of the infusion total (288), resulting in final n's of 14 males and 15 females.

Statistics

Prism 8.0 (GraphPad Software) was used for all statistical analyses. All repeated measures data, with the exception of threshold data, were analyzed using a two-way repeated-measures analyses of variance (ANOVA) based on the general linear model. The Geisser-Greenhouse correction was applied to all factors with three or more levels (such as time and dose) to mitigate violations of sphericity. Post-hoc multiple comparisons (and Sidak corrections) were used as appropriate. Given we predicted the direction of sex differences based on our previous studies (Kawa and Robinson, 2019; Carr et al. 2020) showing that females were more motivated at baseline and probe than males, all threshold data was analyzed using a standard two-sample, one-tailed *t* tests.

Results

Sex differences in the acute psychomotor activating and incentive properties of cocaine

All rats were first trained to self-administer food, followed by cocaine using a criterion (see Methods) and no sex differences were observed (data not shown), consistent with our previous study (Carr et al. 2020). Following acquisition of cocaine self-

administration behavior, the psychomotor activating effect of increasing doses of self-administered IV cocaine was assessed (Fig. 3.3). The total time course of this locomotor activity (Fig. 3.3a-c) was collapsed (beam breaks/min) and expressed as a dose-effect function (Fig. 3.3d). Compared to males, females showed a greater locomotor response across all doses tested (Fig. 3.3d; main effect of sex, $F(1, 25)= 23.51$, $p<0.0001$; main effect of dose, $F(2.222, 55.55)= 120.1$, $p<0.0001$; sex X dose interaction, $F(3, 75)= 8.372$, $p<0.0001$). In a similar fashion, locomotor activity was assessed during and following the first three doses of IntA-Demand session 2, and was found to be greater in females compared to males (Fig. 3.4d; main effect of sex, $F(1, 25)= 21.98$, $p<0.0001$; main effect of time, $F(2.722, 68.06)= 20.17$, $p<0.0001$; sex X time interaction, $F(29, 725)= 4.370$, $p<0.0001$). Females also showed greater cocaine-induced locomotor activity during the No Drug-Available period of the first session of IntA-Limited relative to males (Fig. 3.4b; main effect of sex, $F(1, 27)= 5.150$, $p=0.0314$; main effect of time, $F(3.019, 81.52)= 89.14$, $p<0.0001$; sex X time interaction, $F(24, 648)= 11.10$, $p<0.0001$).

The incentive motivational effects of cocaine were first assessed following the baseline psychomotor activity test. During the IntA-Demand procedure females worked harder to obtain cocaine, demonstrated by a greater Rmax (Fig. 3.3a; $t(1, 27)=2.751$, $p=0.0052$), and were more motivated to obtain cocaine, indicated by a higher nPmax (Fig. 3.3b; $t(1,27)=1.706$, $p=0.0497$), despite a similar preferred level of consumption (Fig. 3.3c; $t(1,27)=0.0557$, $p=0.478$); Fig. 3d; $t(1,17)=7.265$, $p<0.001$). These findings are consistent with previous studies (Roberts et al. 1989; Kawa and Robinson 2019; Carr et al. 2020).

Sex differences in psychomotor sensitization

Locomotor activity was examined under three different conditions to assess change with increasing intermittent cocaine self-administration experience. During IntA-Limited, animals were allotted up to 5-min to self-administer 3 infusions per Drug-Available Period. Given that the vast majority of animals administer all 3 injections in less than one minute, we were only able to assess locomotor activity during the No Drug-Available period (Fig. 3.6). The time course for the entire 25-min No Drug-Available periods on sessions 1, 9, and 11, are shown in Panels 3.6 a-b. Due to equipment limitations, we were only able to measure beam breaks on odd days of testing and include both sessions 9 and 11 because the latter session occurred after two days of withdrawal. For simplification, the 25-min time course was collapsed and analyzed for changes in psychomotor activation during IntA-Limited experience. Compared to session 1, females showed greater beam breaks/min during session 9, but not session 11(Fig. 3.6c; sess 9 vs sess 1, $t(1 ,14)=2.412$, $p=0.0151$; sess 11 vs sess 1, $t(1 ,14)=0.1495$, $p=0.4417$). In males, there was a trend for greater locomotor activity during session 11, but not session 9 compared to session 1 (Fig. 3.6d; sess 9 vs sess 1, $t(1 ,13)=1.121$, $p=0.141$; sess 11 vs sess 1, $t(1 ,13)=1.682$, $p=0.058$).

When comparisons of the magnitude of change from session 1 to 9 or 11 were made between sexes (Fig. 3.6e), data show that males expressed slightly larger locomotor sensitization across session 1 to session 11 compared to females (Fig. 3.6e; sess 9 % sess 1, $t(1 ,27)=0.331$, $p=0.372$; sess 11 % sess 1, $t(1, 27)=1.813$, $p=0.041$). This finding is inconsistent with previous studies showing greater psychomotor sensitization in females than

males (Algallal et al. 2019; Carr et al. 2020). We suspected this discrepancy might be due to the emergence of stereotypy. While inspection of video recordings did indeed reveal some stereotyped behaviors, the low intensity and frequency observed did not warrant quantification.

Given the restrictions of IntA-Limited, we also assessed locomotor activity during IntA-Demand. This allowed us to measure locomotor activity during and after the 5-min period drug was freely available (i.e., no infusion cap). Unlike the traditional within-session threshold procedure, here we also included a 25-min No Drug-Available period to maintain intermittent drug taking and promote sensitization-related neurobehavioral plasticity. As such, we examined beam breaks/min during early training sessions (session 2), and during later training (second to last session prior to IntA-Limited) given that behavioral stability on the program typically requires 5-7 days (Fig. 3.7). The time course of locomotor activity during this period reveals that both females and males were sensitized during IntA-Demand baseline training, evidenced by greater beam breaks/min during the first minute of the late session compared to the early session (Fig. 3.7a; females, main effect of time, $F(1.704, 22.16)= 14.25$, $p=0.0002$; main effect of session, $F(1.737, 22.58)= 4.969$, $p=0.0198$; time X session interaction, $F(4.420, 57.46)= 8.249$, $p<0.0001$; Fig. 3.7b; males, main effect of time, $F(1.408, 16.90)= 15.74$, $p=0.0004$; main effect of session, $F(1.585, 19.01)= 2.733$, $p=0.1001$; time X session interaction, $F(2.175, 26.10)= 6.021$, $p=0.0060$). In addition, males further increased beam breaks/min during the first minute of probe testing (final session after IntA-Limited), while females instead showed decreased beam breaks/min following the first

minute of Drug Availability on the probe test compared to baseline (early and late). This result suggests that females are indeed engaging in greater stereotyped behavior following IntA-Limited.

Above we noted that females engaged in greater beam breaks/per min than males early in IntA-Demand testing (Fig. 3.4b). This is also true later in baseline IntA-Demand training, but no longer true during probe testing (Fig. 3.7c; main effect of sex, $F(1, 25)= 12.84$, $p=0.0014$; main effect of session, $F(1.784, 44.60)= 2.354$, $p=0.1121$; sex X session interaction, $F(2, 50)= 6.212$, $p=0.0039$). This is not surprising given females marked decrease in locomotor activity during the probe test. Our final measurement of locomotor sensitization involved a single-session 3 dose test and no changes were detected in either sex (data not shown).

IntA-Limited produced a similar increase in incentive motivation for cocaine in females and males

The day after the last session of IntA-Limited, incentive motivation for cocaine was again assessed using the IntA-Demand procedure (Fig. 3.8). Relative to baseline, males did not change their preferred level of consumption (Q_0), while in females there was a trend for an increase in their preferred level of consumption (Fig. 3.8a; females, $t(1 ,14)=1.623$, $p=0.064$; males, $t(1, 13)=1.326$, $p=0.1038$). Females did not, however, show increases in the max price (Pmax) that they were willing to pay to obtain cocaine, but males did (Fig. 3.8b; females, $t(1 ,14)=1.306$, $p=0.106$; males, $t(1, 13)=2.133$, $p=0.026$). Interestingly, when Pmax is normalized based on Q_0 (nPmax), both females and males show increased incentive

motivation (Fig. 3.8c; females, $t(1, 14)=1.915$, $p=0.038$; males, $t(1, 13)=2.201$, $p=0.023$). To our surprise, females did not show a change in Rmax (Fig. 3.7d, $t(1, 14)=0.02$, $p=0.492$). However, males not only showed a significant increase in Rmax (Fig. 3.7d; $t(1, 13)=2.058$, $p=0.03$), but also changed to a greater extent than that of females (Fig. 3.7d; $t(1, 27)=2.026$, $p=0.026$), which is also apparent in the response curves (Fig. 3.7e; females, main effect of session, $F(1, 14)= 0.38$, $p=.548$; main effect of dose, $F(2.350, 32.90)= 33.83$, $p<0.0001$; session X dose interaction, $F(2.816, 39.42)= 0.434$, $p=0.718$; Fig. 3.7f; males, main effect of session, $F(1, 13)= 8.49$, $p=0.012$; main effect of dose, $F(11, 143)= 12.96$, $p<0.0001$; session X dose interaction, $F(11, 143)= 4.136$, $p<0.0001$).

The extent of locomotor sensitization, but not cocaine intake during IntA, predicts incentive motivation for cocaine in females and males

Psychomotor sensitization scores derived from traditional IntA experience have been reported to predict responding for cocaine under a progressive ratio schedule in both sexes (Algallal et al. 2019). Here we sought to determine whether the extent of locomotor sensitization produced by IntA-Limited (% change from session 1 to 11; Fig. 3.5e) correlated with the change in motivation (% baseline) represented by the behavioral economic metric nPmax. A higher locomotor sensitization score was associated with a greater increase in motivation for all animals, with the exception of one female (Fig. 3.9). As a result, we assessed this correlation both with (Panels a-c) and without (Panels d-e) the outlier. Fig. 3.9a shows a strong positive correlation between psychomotor and incentive sensitization when all males and all females except the outlier are pooled ($R^2= 0.53$, $p<0.0001$). When separated by

sex, the relationship remains positively correlated in both females and males (Fig. 3.9b, females, R^2= 0.39, p=0.016; Fig. 3.9c, males, R^2= 0.56, p=0.002). Even when the female outlier is included, the pooled analysis maintained the positive correlation between greater psychomotor sensitization and greater incentive sensitization (Fig. 3.9d; R^2= 0.21, p=0.013). In contrast, the inclusion of the outlier in the female only analysis obscured the relationship between psychomotor and incentive sensitization (Fig. 3.9e; R^2= 0.00006, p=0.978). It is possible that the one female outlier was engaging in far greater stereotypy, which, despite representing greater psychomotor sensitization (Lyon and Robbins 1975; Segal 1975), results in a lower locomotor sensitization score.

The cocaine demand assessment following IntA-Limited experience suggests that females require unlimited access, and thus greater consumption, to develop robust incentive sensitization. However, it has been previously reported that greater motivation is not correlated with total consumption during IntA-Unlimited (Kawa and Robinson, 2019). As expected, there were no sex differences in the total amount of cocaine consumed during IntA-Limited (data not shown; t(1 ,27)=0.3483, p=0.3652), which resulted in very little variance. Because IntA-Demand allowed unlimited consumption, we summed the total drug consumption during baseline IntA-Demand and IntA-Limited to test whether total intermittent drug exposure was correlated with probe motivation (nPmax). We found no correlation in females (Fig. 3.10a; R^2= 0.04, p=0.467) or males (Fig. 3.10b; R^2= 0.04, p=0.512), suggesting that total intake is not the critical factor impacting future motivation.

Discussion

The purpose of the current study was two-fold: to determine whether limited cocaine consumption under intermittent access conditions differentially affected motivation in males and females, and whether changes in nPmax (% baseline) were predicted by the extent of change in locomotor activity. We found increased motivation in both sexes following IntA-Limited, but the magnitude of change was not greater in females as hypothesized (Fig. 3.8c). The extent of locomotor sensitization was also positively correlated with the behavioral economic metric nPmax, but not total consumption under intermittent access conditions (Fig. 3.9, 3.10). These findings support the notion that intermittent access models of self-administration readily produce behavioral sensitization that is associated with the transition to addiction. The IntA-Limited model, in particular, appears especially useful for studying the "telescoping" phenomena observed in women (Randall et al. 1999), given that, under these conditions, incentive sensitization did not develop more rapidly in female rats compared to males.

Sex differences in the acute locomotor response and demand for cocaine

The psychomotor activating effects of cocaine were examined using three different procedures that produced a consistent result: females showed greater locomotor activity, measured as beam breaks/min, than males following initial exposure to self-administered cocaine (Fig. 3.3-4). This finding is inconsistent with our previous study (Carr et al. 2020). We believe this discrepancy is likely due to procedural differences. Here, our first assessment of locomotor activity required the self-administration of increasing doses of cocaine, and was thus conducted following the acquisition of cocaine self-administration. Previously, baseline

psychomotor activity was assessed prior to the acquisition of cocaine self-administration (i.e., drug naïve). As such, the current finding suggests that the minimal, although comparable, level of cocaine exposure during acquisition had readily sensitized the female rats compared to males.

Following the baseline psychomotor activity test, motivation was assessed using a within-session demand procedure. While the total number of training sessions were similar in the current and previous study, each decreasing dose of cocaine was available for a shorter period of time (5- vs 10-min) and was followed by a 25-min No Drug-Available period in the current study. This change was necessary to maintain the repeated "spikes" in brain cocaine concentrations shown to promote neurobehavioral sensitization and also allowed us to assess locomotor activity during and after periods of unlimited drug consumption. During session 2 of IntA-Demand training, females also made more beam breaks/min than males during both the period when cocaine was and was not available (Fig. 3.4a). Beam breaks/ min for both males and females further increased during later IntA-Demand baseline testing, indicating further locomotor sensitization (Fig. 3.7a-b). Sensitization during IntA-Demand is one possible reason we found greater locomotor activity in females compared to males on session 1 of IntA-Limited here, but not previously. In addition, the analysis of behavioral responding for cocaine during IntA-Demand revealed that females were more motivated to obtain cocaine than males following acute drug experience (Fig. 3.5b), consistent with previous reports (Kawa and Robinson 2019; Carr et al. 2020).

Sex differences in the locomotor response to cocaine following IntA Experience

The expression of locomotor sensitization was first assessed by comparing beam breaks/min on session 1 of IntA-Limited to sessions 9 and 11. We also compared locomotor activity levels during baseline IntA-Demand training to those observed during demand probe testing. Lastly, baseline vs probe beam breaks/min during the psychomotor activity test were also examined. To our surprise, different results were obtained from these various procedures. Collectively, however, these findings do reveal locomotor sensitization in both females and males, as described below.

In females, locomotor activity was greater on session 9 of IntA-Limited compared to session 1 (Fig. 3.6c). However, activity levels were comparable during sessions 1 and 11. This unexpected decrease in locomotor activity from session 9 to session 11 was observed following a 2 day withdrawal period. Generally, psychomotor sensitization is expressed to a greater extent following a period of withdrawal (Robinson and Camp 1987; Paulson et al. 1991; Paulson and Robinson 1995; Grimm et al. 2001). In males, psychomotor sensitization following 1 day of withdrawal from IntA-Unlimited manifests as locomotor hyperactivity, whereas after 30 days of withdrawal, a greater percentage of time is spent in-place performing stereotyped head movements (Carr et al. 2020). The current study is the first to assess psychomotor sensitization following acute withdrawal from IntA-Limited in females, and our results suggest an earlier emergence of stereotyped behaviors. This interpretation is further supported by marked decreases in beam breaks/min during the IntA-Demand probe, compared to baseline (Fig. 3.7a). Consistent with IntA-Limited session 1 vs 11, locomotor activity during the psychomotor activity test probe did not differ from baseline

(data not shown). Of note, a larger degree of variance was observed during the probe test. This suggests that some female behavior may have been predominated by locomotor hyperactivity, while others likely engaged in more stereotyped behaviors that reflect an even greater drug effect.

As for males, compared to IntA-Limited session 1, beam breaks/min were not greater on session 9, but further increased during session 11 (Fig. 3.6d). Given the expression of psychomotor sensitization in males in our previous study (Carr et al. 2020), we expected significantly greater locomotor activity on both later sessions. One possible explanation for this discrepant result is that locomotor activity during IntA-Limited session 1 was unusually high due to baseline IntA-Demand training. Across this training, males increased locomotor activity during the first minute, and thus were sensitized (Fig 3.7b). This higher baseline possibly made it more difficult to detect sensitization, which is not surprising considering that the magnitude of locomotor change in males is typically relatively small. We were, however, able to detect locomotor sensitization during the Drug-Available period of IntA-Demand. Compared to baseline (early and late), males made greater beam breaks/min during the first minute of the probe test. As with females, probe activity levels did not differ from baseline during the psychomotor activity test and far greater variance was observed during the probe test. Given that in the current study males were not only sensitized sooner, but also had a longer history of IntA experience, it is possible that more males were also performing stereotyped head movements soon after discontinuation of IntA-Limited self-administration.

Here we do not report greater psychomotor sensitization in females compared to

males because our analysis is based solely on a measure of locomotor activity. The results described above suggests that relative to males, females engaged in far more stereotyped behaviors, which is characterized by a decrease in locomotor activity due to in-place activities such as repetitive head movements. This might explain why the locomotor sensitization score produced using percent change in beam breaks/min from session 1 to 11 of IntA-Limited was greater in males compared to females (Fig. 3.6e).

Sex differences in motivation for cocaine following IntA-Limited Experience

Incentive sensitization was examined by determining the percent change in the behavioral economic measure nPmax from baseline to probe. While females started out more motivated than males, the magnitude of change did not differ in males and females following IntA-Limited experience (Fig. 3.5, 3.8). This finding was surprising given that females have been shown to express greater incentive sensitization than males following IntA-Unlimited (Kawa and Robinson 2019; Algallal et al. 2019). Below we speculate as to why the current study differs from previous studies.

While it is not always the case, female rats typically consume more cocaine than males (Lynch and Taylor 2004; Lynch et al. 2004; Kawa and Robinson 2019). However, despite this observation, the preferred level of consumption (Q_0) does not differ between females and males and Q_0 does not change with increasing IntA-Unlimited experience (Kawa and Robinson 2019). Here we also found a similar Q_0 in females and males, but consumption preference slightly increased in females following IntA-Limited experience (Fig. 3.8a). In humans, cocaine use is also similar in men and women, but women report using more drug

than intended (Robbins et al. 1999; Elman et al. 2001; Kennedy et al. 2013). As such, it is possible that females unintentionally consumed more drug when again allowed free-access to cocaine because they were unable to self-administer their preferred level throughout IntA-Limited. During IntA-Demand, the average preferred level of consumption was ~3.5 mg/kg in females and males. In contrast, only 1.2 mg/kg of cocaine could be administered per Drug-Available period during IntA-Limited.

Given that the amount of drug consumed during IntA-Unlimited is positively correlated with Q_0 (Kawa and Robinson 2019), it is also possible that consumption below the preferred level is why our females were not more motivated than the males. Interestingly, however, male rats allowed unlimited consumption during IntA are not more motivated (assessed by progressive ratio) than males with IntA-Limited experience (Allain et al. 2018). It has yet to be determined if females with IntA-Unlimited experience are more motivated than females with IntA-Limited experience. If this is indeed the case, the argument that females are more susceptible to incentive sensitization would still hold true, under the condition that females are able to consume as much drug as desired. This interpretation is supported by the fact that even though females did not consume more drug than males during IntA-Unlimited, they were still able to consume as much drug as they wanted and later self-administered more infusions than males under a PR schedule of enforcement (Algallal et al. 2019).

Throughout testing, we did not monitor the estrous cycle in females, and it is, therefore, possible that the phase of the cycle influenced our probe results (Becker et al. 2012;

Lynch 2018; Yoest et al. 2018). If, for example, our females were in the diestrus phase (cocaine is less reinforcing during this phase relative to the estrous phase) during probe testing, this might explain the relatively small increase in motivation following IntA-Limited experience. It is, however, also possible that females require greater drug exposure to induce the long-term compensatory changes in D1 and D2 DA receptor signaling in striatal circuitry associated with the transition to addiction. The current results suggest that the limited drug exposure allowed during IntA failed to drastically enhance D1 DA receptor signaling and diminish D2 DA receptors signaling in females relative to males.

We previously showed that females made more responses during baseline demand testing than males (Carr et al. 2020), and our current study is consistent with that finding. However, after IntA-Limited experience, the magnitude of the change in maximal response (Rmax) was greater in males than females (Fig. 3.8d). In fact, in females Rmax during the probe test did not differ from baseline. While Rmax can be interpreted as a measure of motivation, there is caution because high responding at a "cheaper price" does not reflect greater motivation than high responding at a "higher price". Here, maximum responding occurred at the same price in females and males (Fig 3.8e-f), but it is obvious that males responded far more during the probe test, compared to baseline. Females did express incentive sensitization, but their responding did not change from baseline to probe. One possible explanation for this is a ceiling effect. Responding in males essentially increased to the level of baseline responding of females, which in this case means that probe responding did not differ between males and females. Compared to the traditional within-session

threshold demand test, even greater responding is required with each decreasing dose because the addition of a No Drug-Available period brings brain cocaine concentrations level to near zero. In contrast, the continuous version of the test allows cocaine levels to remain elevated, that is up until the point the animal is no longer willing to defend Q_0. In other words, it is easier to maintain Q_0 when the dose decreases if drug is still on board compared to when it is not. As such, even though females are indeed more motivated following IntA-Limited experience, Rmax may not capture this under IntA-Demand conditions because of physical exhaustion.

Relationship between psychomotor and incentive sensitization

In both females and males, greater psychomotor sensitization expressed during IntA-Unlimited was correlated with greater incentive motivation under a PR schedule (Allain et al. 2017; Algallal et al. 2019). The current results are consistent with that finding. We report that the extent of psychomotor sensitization (based on percent change of beam breaks/ min during IntA-Limited from session 1 to 11) is positively correlated with the extent of incentive sensitization, as assessed by percent baseline change in the behavioral economic metric nPmax. There was an outlier female, but the correlation remained significant when included regardless if the locomotor score was calculated based on session 9 (greater locomotor activity; data not shown), session 11 (greater stereotyped behavior), or IntA-Demand (greater stereotyped behavior; data not shown). The consistency across IntA-Limited and IntA-Demand suggests the latter may be an ideal test to further explore how this relationship differs between the two purported models of addiction (IntA vs LgA). Unlike with the LgA

model, we also report that males with greater intermittent drug consumption were not more motivated (Allain et al. 2017; Algallal et al. 2019), a relationship that is also true for females (Kawa and Robinson 2019; Algallal et al. 2019). These findings add additional support for the notion that the expression of psychomotor sensitization is a reflection of neuroplasticity associated with the pathological drug wanting characteristic of addiction.

Conclusions

During baseline testing, females showed both greater psychomotor activation and incentive motivation than males, after acquiring cocaine self-administration behavior. However, following IntA-Limited experience, incentive sensitization was comparable in females and males, the extent of which was highly correlated with psychomotor sensitization in both sexes. However, it still may be the case that females are more susceptible to incentive sensitization, and the current results suggest that unintentionally exceeding the preferred level of consumption may contribute to the "telescoping" phenomena observed in women. This pattern of escalated intake under intermittent conditions is thought to promote and accelerate the development of sensitization and thus the transition to addiction.

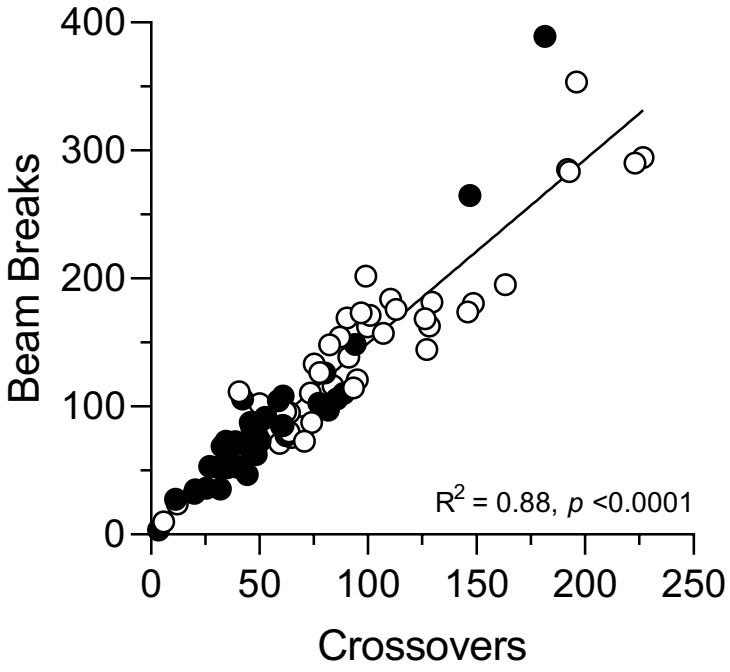


Figure 3.1. Correlation Between Automated Beam Breaks and Hand-Scored Crossovers. Relationship between an automated (beam breaks) and manually scored (crossovers) measure of locomotor activity during the No Drug-Available period of IntA-Limited sessions 1, 9, and 11. A strong positive correlation was found between these measures in females (open circles) and males (closed circles). All data are represented as the mean ± SEM.

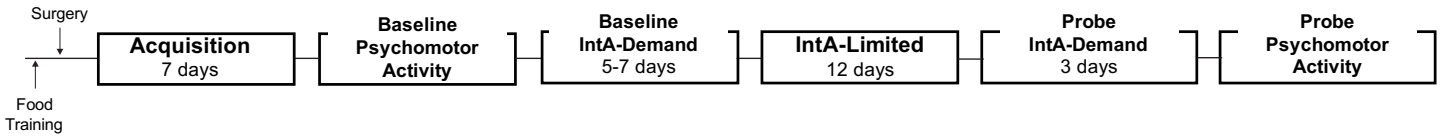


Figure 3.2. Experimental Timeline. Flow diagram of experimental events.

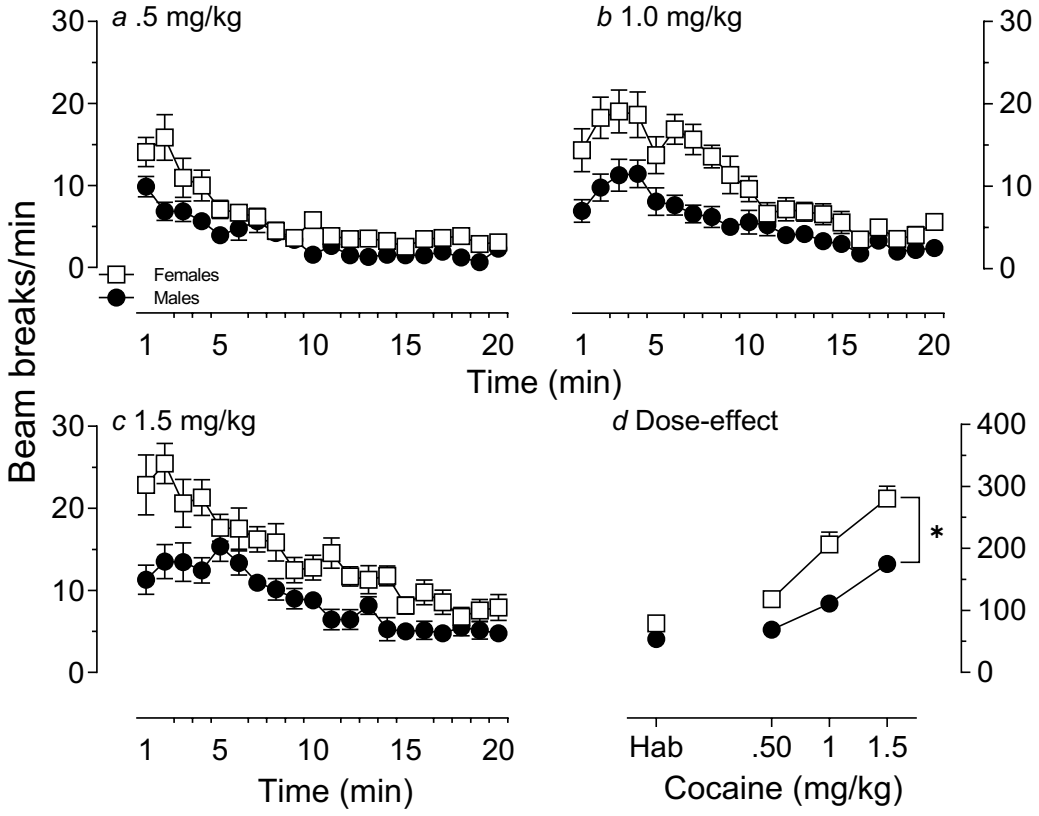


Figure 3.3. Sex Difference in Baseline Locomotor Activity. Baseline psychomotor activation following acute cocaine exposure (acquisition). Panels a-c show the time course of locomotor activity, as assessed by beam breaks/min. Increasing doses of self-administered cocaine produced greater locomotor activity in females compared to males. These data were collapsed across time to generate a dose-effect function as shown in Panel d. Note that females and males did not differ in beam breaks/min during the habituation period prior to the first infusion (time course not shown). All data are represented as the mean ± SEM.

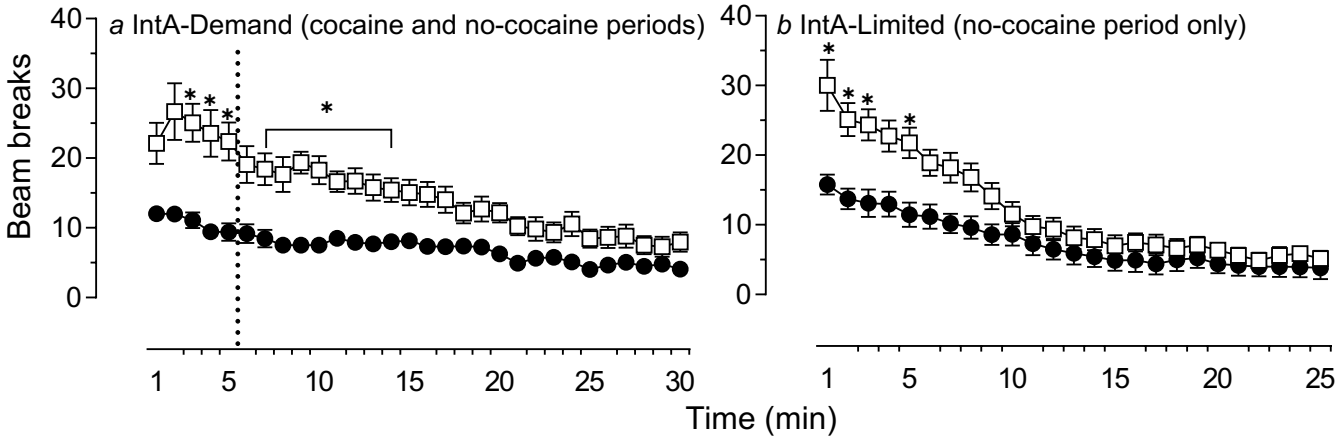


Figure 3.4. Sex Difference in Locomotor Activity During Early IntA Experiences. Time course of beam breaks/min during the Drug-Available and/or No Drug-Available period of IntA-Demand (baseline) and –Limited (session 1). Panel a shows that cocaine consumed during a 5-min free access period produced greater beam breaks/min in females compared to males. Once drug was no longer available (indicated by the dashed vertical line), females continued to engage in greater beam breaks/min than males. After IntA-Demand training, all rats transitioned to IntA-Limited. Panel b shows greater beam breaks/min in females compared to males following the self-administration of 3 infusions of cocaine. All data are represented as the mean ± SEM.

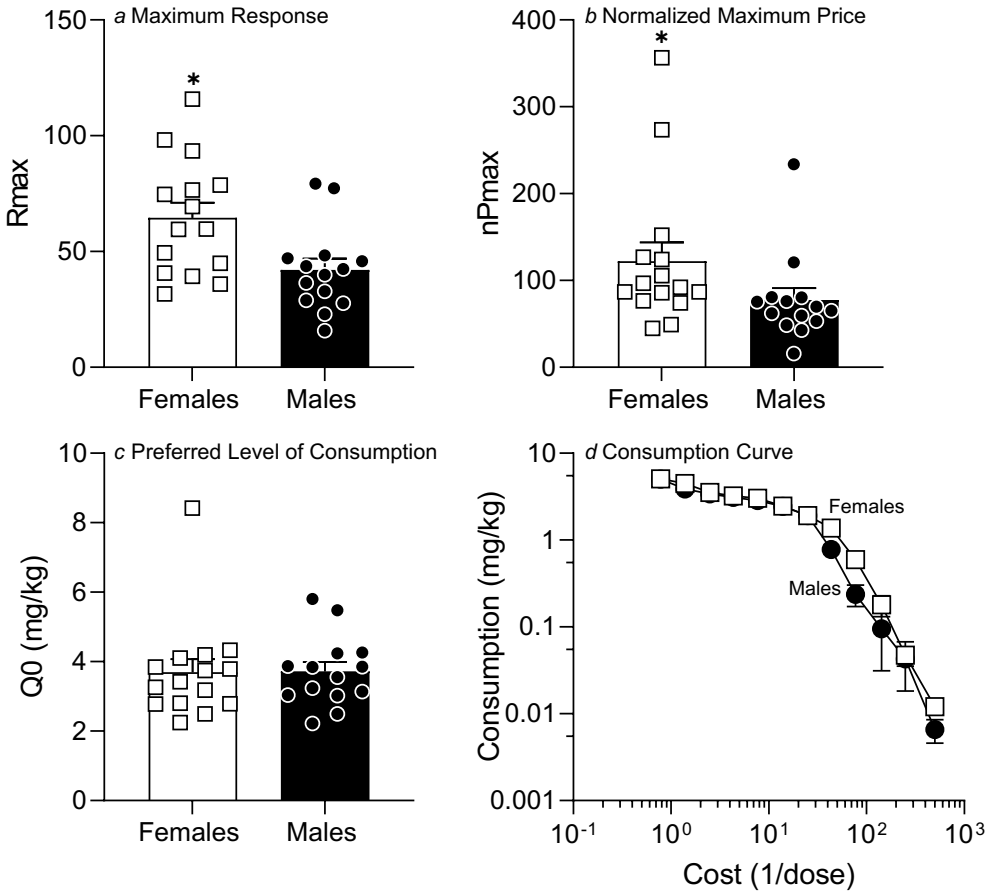


Figure 3.5. Sex Difference in Baseline IntA-Demand prior to IntA Experiences. Motivation to obtain cocaine was first assessed using IntA-Demand after initial acquisition of cocaine self-administration. Females made more responses to obtain cocaine (a) and were more motivated (b), but had a similar preferred level of consumption as males (symbols indicate individual rats; Panels c and d). All data are represented as the mean ± SEM.

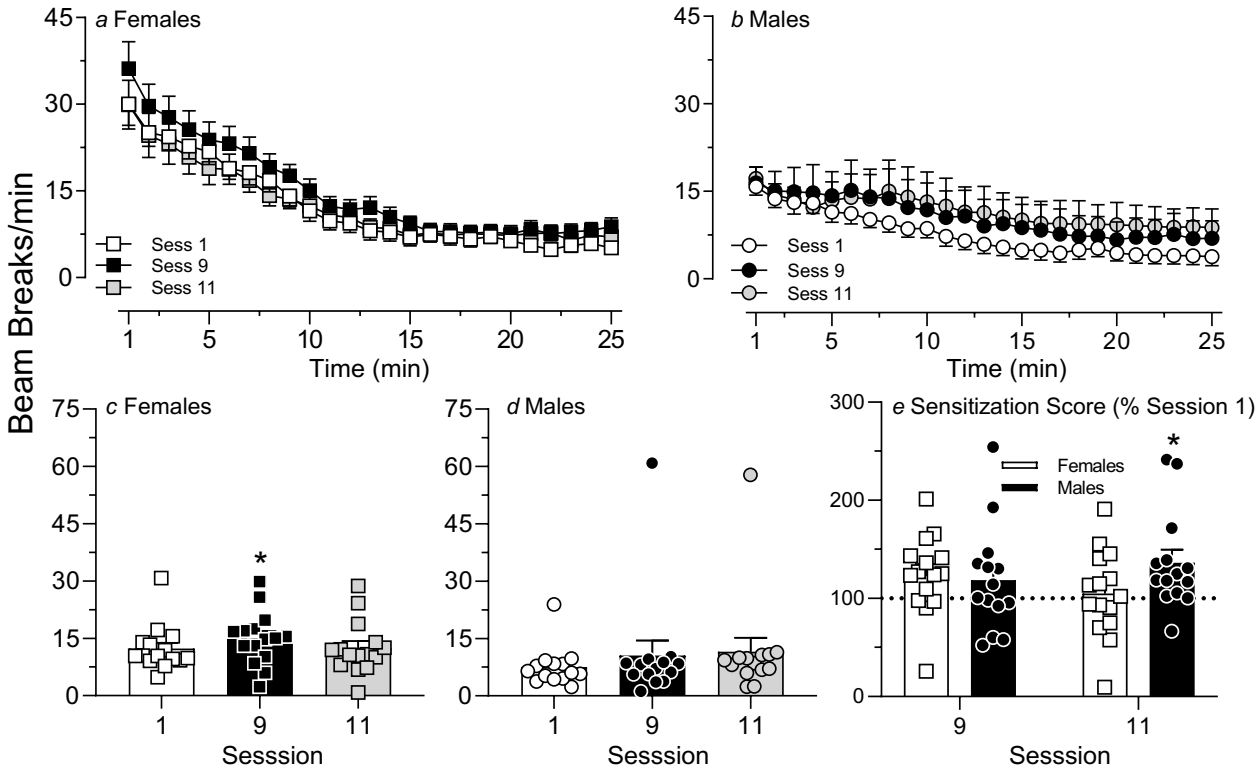


Figure 3.6. Change in Locomotor Activity During No-Cocaine Available Periods of IntA-Limited. Beam breaks/min during the No Drug-Available period of early and late IntA-Limited sessions. Panels a and b show the time course of beam breaks/min during sessions 1, 9, and 11 in females and males, respectively. The summed total of these data is shown in Panels c and d for simplification. Compared to session 1, females showed greater beam breaks/min on session 9 but not session 11 (c). In males, beam breaks/min increased slightly across sessions, but was not statistically significant (d). Psychomotor sensitization scores were calculated as a percent increase from session 1 (indicated by the dashed horizontal line) to session 9 and 11, as shown in Panel e. The extent of sensitization from session 1 to 9 did not differ in females and males, but the magnitude of change from session 1 to 11 was greater in males than females. Symbols indicate individual rats and all data are represented as the mean ± SEM.

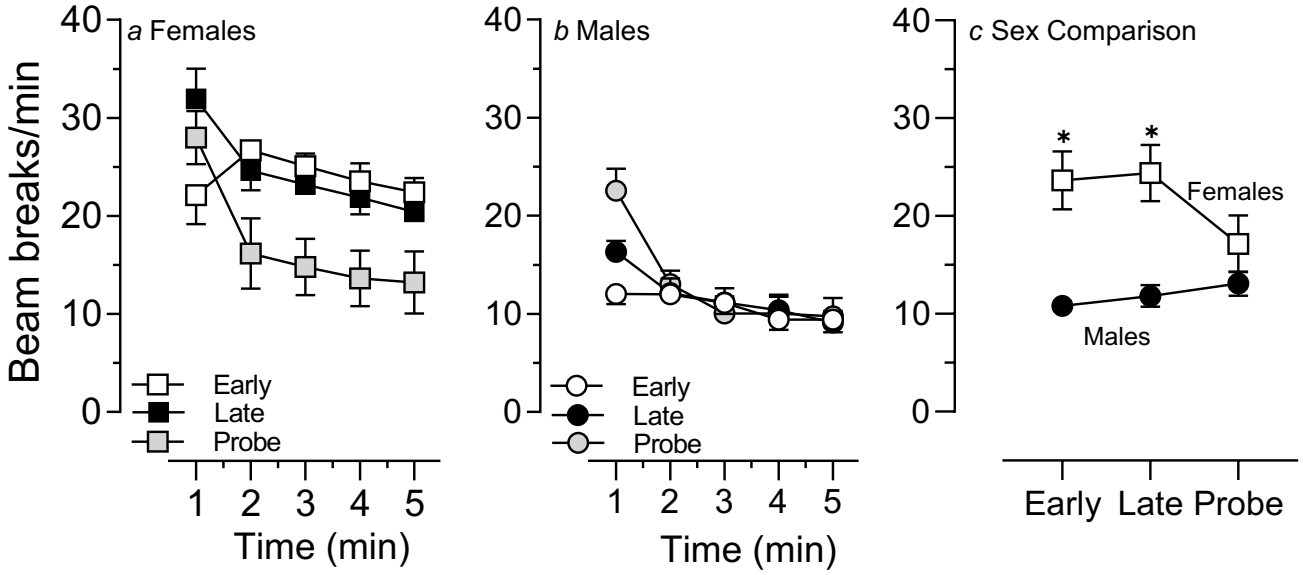


Figure 3.5. Change in Locomotor Activity During Cocaine Available Periods of IntA-Demand. Beam breaks/min during the Drug-Available period of IntA-Demand during baseline (early and late) and probe testing. Panels a and b show the time course of beam breaks/min for each phase of testing separated by sex and panel c shows total activity as a function of testing phase in males and females. During the first minute of drug availability, both females and males showed an increase in beam breaks/min later in training compared to early training, but females engaged in greater beam breaks/min than males throughout baseline testing. Following IntA-Limited training, motivation was assessed again (probe). Compared to baseline beam breaks/min, males increased activity levels while females decreased activity levels. All data are represented as the mean ± SEM.

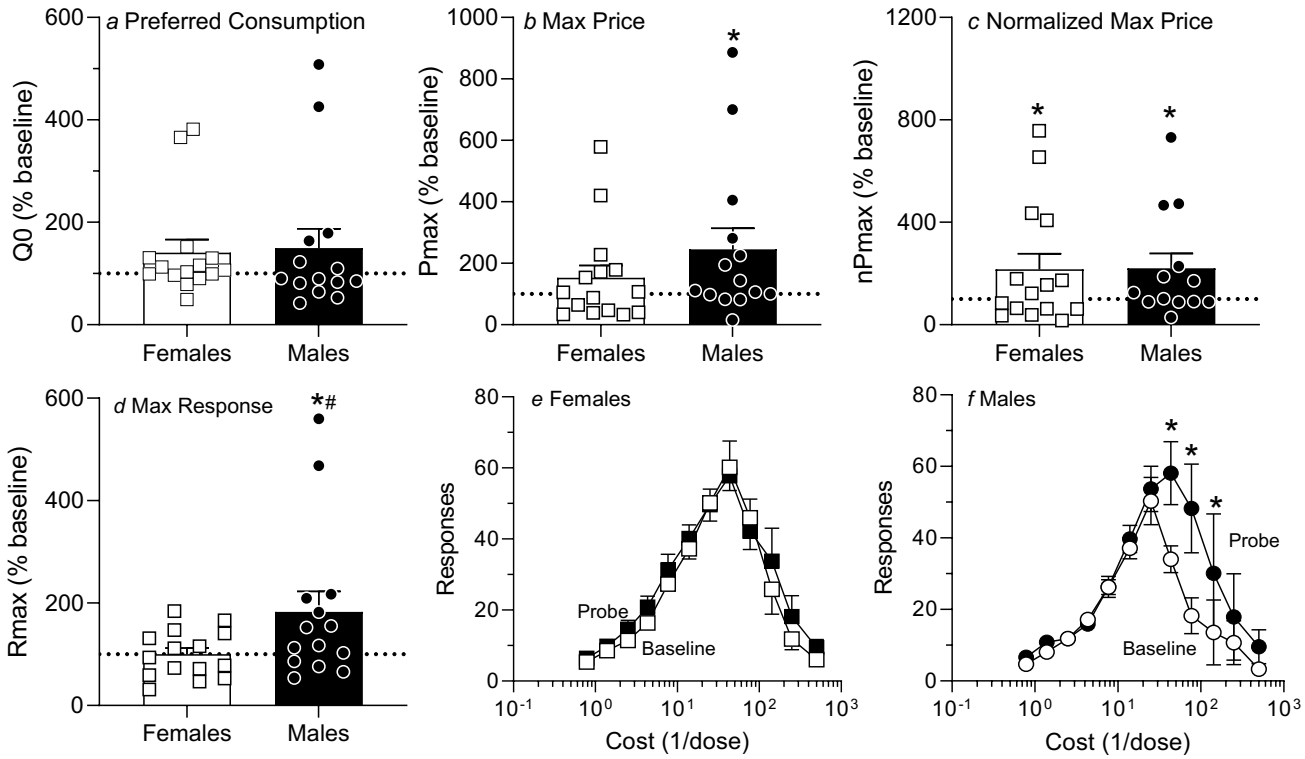


Figure 3.8. Change in Cocaine Demand Following IntA-Limited Experience. Percent change from baseline in behavioral economic metrics following IntA-Limited experience. The preferred level of consumption did not change significantly in males but there was a trend for an increased Q_0 in females (a). Females did not increase the maximum price they were willing to "pay" to earn cocaine, but males did (b). However, when the maximum price was normalized based on preferred level of consumption, both females and males showed increased motivation (c). Only males increased the number of responses made to earn cocaine, and this change in effort from baseline was greater in males than females (Panels d-f). Symbols indicate individual rats and all data are represented as the mean ± SEM.

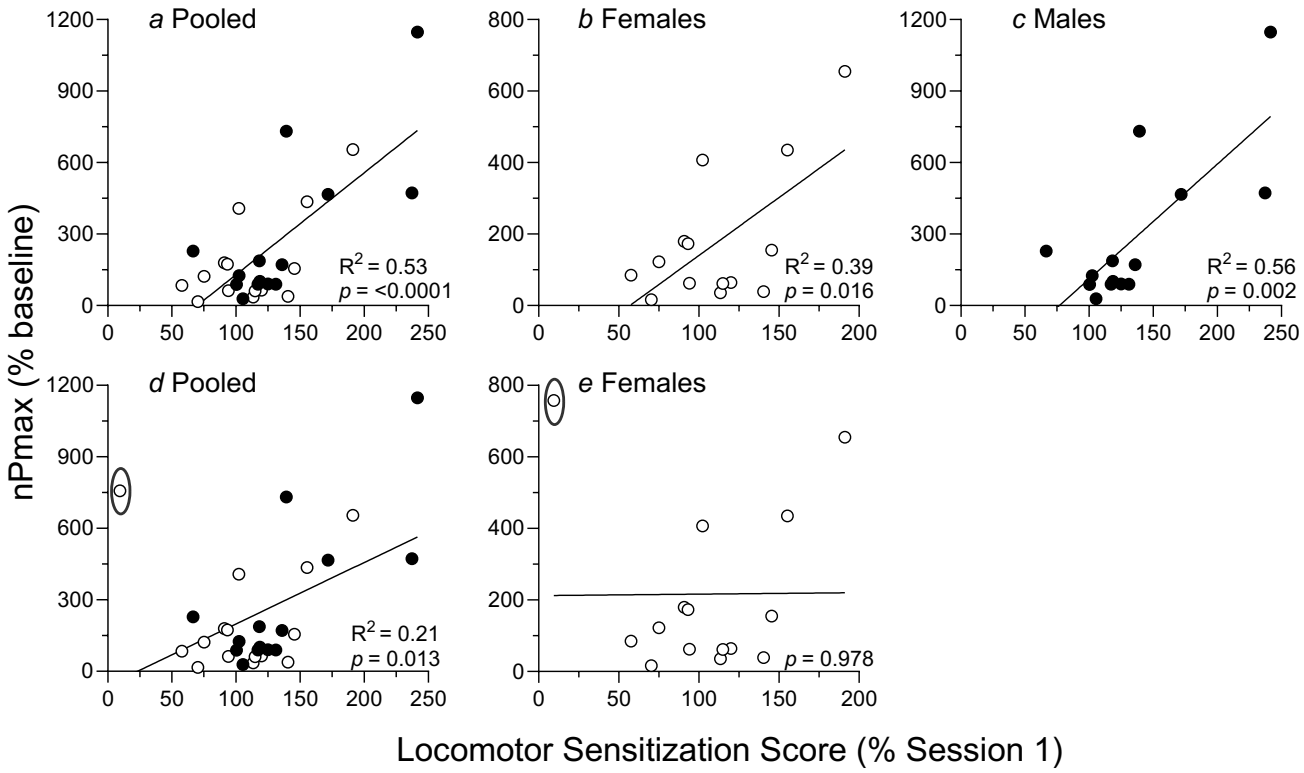


Figure 3.9. Correlations Between Psychomotor and Incentive Sensitization. Relationship between percent change from baseline in motivation (nPmax) and extent of locomotor sensitization (% change from session 1 to 11). When all animals were pooled, with the exception of an outlier, a significant positive correlation was found (a). This relationship remained consistent when females and males were separated (Panels b-c). When the single female outlier was included in the pooled analysis, a significant relationship between the extent of locomotor sensitization and motivation remained, but this relationship did not remain significant in the female only analysis (Panels d-e). All data are represented as the mean ± SEM

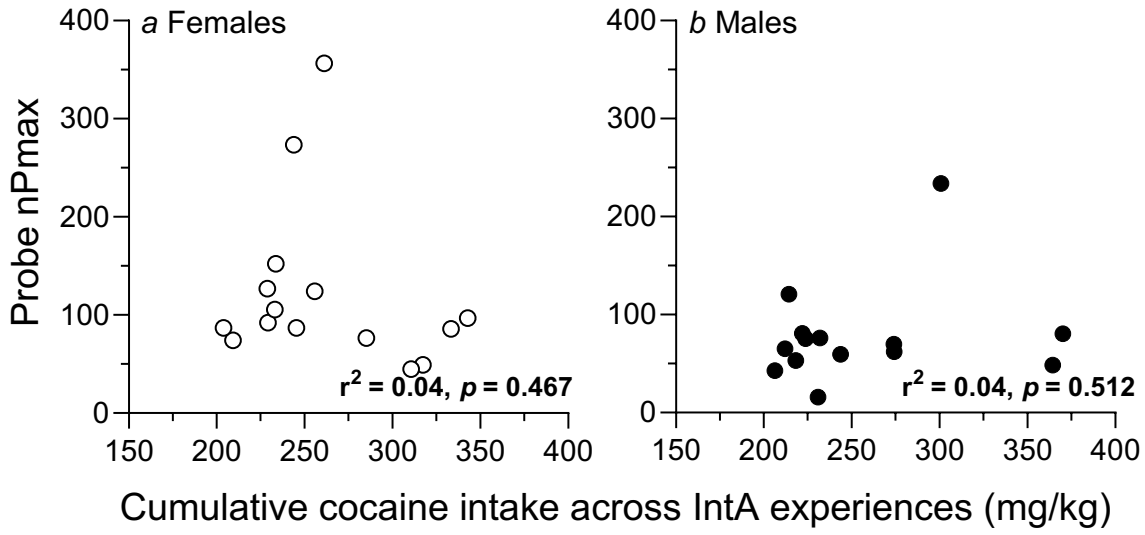


Figure 3.10. Correlations Between nPmax and IntA Consumption. Relationship between probe motivation (nPmax) and cumulative cocaine intake during IntA (both –Limited and –Demand). A significant relationship was not observed between these variables in females or males (Panels a-b, respectively). All data are represented as the mean ± SEM

Chapter IV

General Discussion

Summary of Results

Although only introduced in 2012, IntA self-administration has been utilized in at least 18 studies. Several groups have reported especially robust addiction-like behaviors following IntA experience, but less is known about the ability of IntA experience to produce psychomotor sensitization and how IntA experiences differentially affect female and male rats. To better address these questions, the experiments in Chapter II characterized psychomotor sensitization produced by IntA to cocaine, specifically examining persistence, sex differences, and cross-sensitization. We then further explored sex differences in psychomotor and incentive sensitization following IntA-Limited experience using new approaches (Chapter III). While many of the results are consistent with the literature (i.e., IntA produces incentive and psychomotor sensitization), the manipulations employed here also resulted in new and interesting findings. For example, we found that systematically decreasing the dose during IntA (IntA-Demand) is an effective procedure to measure both psychomotor and incentive sensitization within a single session. In addition, we found that preventing female rats from consuming their preferred level of cocaine during IntA (IntA-Limited) results in comparable, rather than greater, incentive sensitization as males. Given that females with IntA-Unlimited experience show

especially robust incentive sensitization and females with IntA-Limited experience show reduced incentive sensitization susceptibility, it is possible that comparison studies of females with IntA-Unlimited vs IntA-Limited may provide insights into the underlying neurobiological mechanisms of increased incentive sensitization in females. For example, it may be the case that the greater drug exposure allowed during IntA-Unlimited (i.e., self-administering Q_0) results in a greater enhancement of D1 DA receptor mediated signaling in striatal circuitry. To date, the growing literature regarding IntA suggests that it has a number of distinct advantages in modeling the transition to addiction, over other preclinical procedures, and it also has a number of important theoretical implications, all of which will be discussed below.

Modeling the Transition to Addiction: LgA vs IntA

In a seminal paper, Ahmed and Koob (1998) found that simply increasing the length of time allotted to self-administer IV cocaine from 1-hr (ShA) to 6-hrs (LgA) had a profound effect: escalation of drug intake (see Chapter I for discussion of earlier models). This showed that LgA experience was especially effective in producing an addiction-like behavior, and thus led to many subsequent studies using this procedure to model the development of addiction-like behavior. It was soon discovered that LgA self-administration of a number of drugs produced several addiction-like behaviors, resulting in LgA ultimately becoming one of the most prominent models of the transition to addiction (for review, Edwards and Koob 2013). Importantly, a major assumption underlying the LgA model is that high and sustained drug exposure is necessary to progress from casual to compulsive drug use. This assumption is problematic, however, because this pattern of use is rare in human cocaine users (Cohen 1994).

Within a bout of use, human users typically wait enough time between doses to allow the drug effects to wear off before administering the next dose. This is done to repeatedly experience the rapid rise in brain cocaine levels associated with the "high" (Beveridge et al. 2012).

To better model this intermittent pattern of use seen in humans, Zimmer et al. (2012) developed a self-administration procedure whereby each 5-min Drug-Available period was followed by a 25-min No Drug-Available period (IntA), resulting is repeated "spikes" in brain cocaine concentrations. Interestingly, IntA rats were actually more motivated to obtain cocaine than LgA rats, despite consuming far less drug; this result has since been replicated by several groups (Kawa et al. 2019b; James et al. 2019; Algallal et al. 2019; Minogianis and Samaha 2020) and is complementary to our finding of the expression of robust psychomotor sensitization in IntA, but not LgA, animals soon after the discontinuation of self-administration experience. While the literature regarding the ability of LgA to increase motivation for cocaine is mixed, results from IntA are remarkably consistent (see, Oleson and Roberts 2009; Bentzley et al. 2014; Kawa et al. 2019b; James et al. 2019). In fact, one study reports increased motivation after only 3 sessions of IntA (Calipari et al. 2015) and others report increased motivation even when only 2 or 4 infusions can be administered per cycle (Allain et al. 2018) and when the session duration is reduced to only 2 hours (Allain and Samaha 2018).

In addition to greater motivation for cocaine, IntA has been shown to produce more robust cue-induced reinstatement than LgA (Kawa et al. 2019b; James et al. 2019; Nicolas et al. 2019; Garcia et al. 2020). Rats with IntA experience also escalate their intake (Kawa et al., 2016, Pitchers et al. 2017; Singer et al. 2018; Allain et al. 2018; Allain and Samaha 2018; James et al.

2019) and continue to seek drug both in the face of an adverse consequence (e.g., footshock) and when drug is not available (Kawa et al. 2016; Singer et al. 2018; James et al. 2019; Allain and Samaha 2018). Importantly, even though the vast majority of the studies utilizing the IntA model have investigated cocaine, there is an emerging literature for opioids (Fragale et al. 2020; O'Neal et al. 2020). Similar to cocaine, a transient increase in motivation was observed following LgA to fentanyl, whereas very robust incentive sensitization was observed following IntA to fentanyl (Fragale et al. 2020). In addition, greater cue-induced reinstatement was also found in IntA vs LgA rats. It seems clear, therefore, that the IntA model is capable of producing especially robust addiction-like behaviors across drug classes without high and sustained drug intake. Thus, results from the IntA model suggest that the temporal pattern of use, rather than the amount of exposure, is especially effective in promoting the transition to addiction, and thus warranted the additional investigations described below.

Characteristics of Psychomotor Sensitization

Similar to the literature regarding motivation, results are mixed on the expression of psychomotor sensitization following LgA experience (Ben-Shahar et al. 2004, 2005; Ferrario et al. 2005; Ahmed and Cador 2007; Knackstedt and Kalivas 2007; Calipari et al. 2014a; Sicilliano et al. 2016) but consistent following IntA experience (Allain et al. 2017; Allain and Samaha 2018; Algallal et al, 2019; Garcia et al. 2020; Allain et al. 2020). Indeed, some of the discrepancies concerning the effects of self-administered cocaine on psychomotor sensitization may be due to the inadequate assessment of stereotyped head movements (see Ferrario et al. 2005; Flagel and Robinson 2007). Rats exposed to a sensitizing regime of drug may show initial

increases in locomotor activity but later transition to stereotyped head movements (Segal and Mandell 1974; Segal 1975; Lyons and Robbins 1975; also, see Chapter I "Effects of Repeated Cocaine on Psychomotor Activation"). And because robust stereotyped head movements are performed in-place for extended periods, decreased locomotor activity is suggestive of this transition, but the emergence of stereotypy is not always investigated.

For example, Ben-Shahar et al. (2005) found decreased locomotor activity in LgA rats after administering 15 mg/kg IP cocaine after 60 days of withdrawal (WD60). This was interpreted as tolerance to the psychomotor activating effects of cocaine because they assumed 15 mg/kg was too low of a dose to produce stereotypy, and therefore, that reduced locomotor activity was indicative of a reduced response to cocaine. However, Ferrario et al. (2005) later reported reduced locomotor activity (e.g., an inverted u-shape dose response curve) that was accompanied by increased head movements following both 15 and 30 mg/kg IP cocaine administered on WD30. The primary difference between these studies is that when Ferrario et al. (2005) observed decreased locomotor activity, video records of the test were reviewed and it was obvious that the rats were engaged in stereotyped behavior. As a result, a detailed analysis was conducted that involved visual quantification of head movements. This analysis revealed a greater frequency of stereotyped head movements in LgA vs ShA rats, and thus very robust psychomotor sensitization. Interestingly, had Ferrario et al. (2005) relied solely on an automated measure of locomotor activity, they too would have reported no difference in the expression of psychomotor sensitization in LgA and ShA rats (Ahmed and Cador 2007; Knackstedt and Kalivas 2007). Nonetheless, this early study highlights that over-reliance on automated measures

can be problematic, and stresses the potential need to assess a range of behaviors when assessing psychomotor sensitization (for greater discussion, see Flagel and Robinson 2007).

The first experiment presented in Chapter II was designed to further explore the ability of LgA and IntA to induce psychomotor sensitization. Drug naïve male rats were first exposed to increasing doses of experimenter-administered IP cocaine (7.5, 15, 30 mg/kg) and placed in psychomotor activity chambers for a baseline assessment of psychomotor activation. Following IntA or LgA to cocaine self-administration, the cocaine challenge was again administered on WD1 and WD30 (see Fig. 2.1 for experimental timeline). We found that only IntA rats expressed a sensitized locomotor response following acute withdrawal (see Fig. 2.3). Our study is the first to assess behavioral sensitization following acute withdrawal from IntA, but this finding is similar to studies reporting increased locomotor activity from the first to last IntA session (Allain et al. 2017; Allain and Samaha 2018). There are, however, several reports of neural sensitization soon after the discontinuation of IntA, which is especially relevant considering psychomotor activation is thought to be largely mediated by DA projections (Creese and Iversen 1975; Kelly et al. 1975; Pijnenburg et al. 1976; Vezina and Kim 1999; Vanderschuren and Kalivas 2000). When evoked DA release was assessed following acute withdrawal from LgA or IntA, no change was found in LgA rats and increased release was found in IntA rats (Calipari et al. 2013), consistent with our finding. In addition, Calipari et al. 2013, also found that LgA experience decreased cocaine potency at the dopamine transporter (DAT), indicating neurochemical tolerance, whereas IntA increased potency, thus indicating sensitization.

Following extended withdrawal, both IntA and LgA rats showed robust psychomotor

sensitization, and to our surprise, the magnitude was not greater in IntA rats. To date, no other studies have examined behavioral or neural sensitization following extended withdrawal from IntA. However, using a similar excessive intake procedure (40 infusions of 1.5 mg/kg for 5 days), Siciliano et al. (2016) also found a decreased ability of cocaine to inhibit the DAT soon after discontinuation of self-administration, but this tolerance was reversed following extended periods of abstinence (14 and 60 days). However, if allowed to self-administer a single IV infusion of cocaine following the period of abstinence, decreased cocaine potency at the DAT was again observed. In addition, decreased locomotor activity was observed following administration of 15 mg/kg IP on WD60, but there was no mention of investigating the possible emergence of stereotypy. Collectively, these results suggest that the contingency of the cocaine challenge may influence whether sensitization- or tolerance-related neurobehavioral plasticity is observed following extended withdrawal from LgA. It is clear, however, that the initial tolerance observed is reversible, but the extent to which and under what conditions requires further investigation, especially in comparison to IntA.

Once we determined that IntA produces robust and persistent psychomotor sensitization in males, the second experiment presented in Chapter II was designed to examine the ability of IntA to produce psychomotor sensitization in females compared to males. Similar to above, drug naïve animals were first exposed to increasing doses of experimenter-administered cocaine (.25, 5, and 1 mg/kg IV) and then placed in psychomotor activity chambers to obtain baseline psychomotor activation. Following acquisition of self-administration behavior and baseline demand testing, a portion of each sex was allowed to self-administer cocaine under IntA-Limited

conditions and remaining animals served as controls. Twelve days after the last session of IntA-Limited, psychomotor activation was reassessed as described above (see Fig. 2.1 for experimental timeline). Consistent with the literature in which experimenter-administered drugs were used, we found that IntA-Limited experience produced greater psychomotor sensitization in females than males (also see, Algallal et al. 2019).

Compared to sex-matched controls with less self-administration experience (i.e., no IntA training), IntA-Limited females showed locomotor sensitization outside of the self-administration context, while males did not (see Fig. 2.8). Reports from the early literature show that the expression of psychomotor sensitization can be context-specific (Badiani et al. 1995; Crombag et al. 2000) and our between-subject analysis provides additional support for this notion. It is important to note, however, that relative to their baseline, IntA-Limited males still expressed psychomotor sensitization outside the SA context, consistent with our first study that instead used IP injections. Given that psychomotor sensitization is shown to be enhanced by a novel environment (Badiani et al. 1995), it is possible that the use of a different route of administration made the non-self-administration context more novel for males in the first study (and thus resulted in more robust sensitization), whereas the administration of an IV infusion outside of the self-administration context decreased novelty for males in the second study (and thus resulted in less robust sensitization).

Because both of the studies described above used experimenter-administered cocaine challenges, we decided to instead utilize a self-administered psychomotor activity test in the sex difference experiment described in Chapter III. Following this baseline assessment, animals were

allowed to self-administer cocaine under IntA-Demand conditions (baseline demand; results described in following section), followed by IntA-Limited training. Psychomotor activity was recorded during all three procedures and probe psychomotor activity and IntA-Demand tests were conducted after 12 sessions of IntA-Limited (see Fig. 3.1 for experimental timeline). Importantly, for this study, we also utilized a new automated measure (beam breaks) of locomotor activity and found it to be highly correlated with manually scored crossovers (see Fig 3.1). To our initial surprise, we did not observe robust locomotor sensitization in females or males across all of our measures. A thorough analysis of the data set, however, revealed higher than expected baselines and an early emergence of stereotypy that may have made it more difficult to detect locomotor sensitization, as described below.

For the psychomotor activity test, the animals first needed to acquire self-administration behavior, resulting in baseline activity being assessed after 7 days of repeated drug exposure. Consequently, we found that females engaged in greater locomotor activity than males during the baseline test (see Fig. 3.3). This finding is inconsistent with our sex difference study described in Chapter II that did not find a baseline sex difference in locomotor activity when tested prior to any self-administration experience (data not shown). It thus appears that drug exposure during acquisition may have sensitized the rats, with stronger effects in females than males. Relative to baseline, locomotor activity during the probe test did not differ in females or males, but greater variance was observed during the probe in both sexes. It is, therefore, possible that the early sensitization masked our ability to detect subsequent changes in behavior and that the rats were engaged in greater stereotyped behavior following over two weeks of intermittent drug exposure.

To circumvent sensitization- or tolerance-related neurobehavioral adaptations that may affect baseline differences in locomotor activity, it may be advantageous to conduct the baseline psychomotor activity test soon after (i.e., same day) the first session of acquisition.

During the novel IntA-Demand baseline training that occurred immediately prior to starting IntA-Limited, the rats were further sensitized (see Fig. 3.7). As a result, we also found greater locomotor activity in females than males during IntA-Limited session 1 (see Fig. 3.4). Here again, this finding is inconsistent with our sex difference experiment described in Chapter II that instead used a demand test that allowed cocaine to be continuously available throughout the entire session (see Fig. 2.6). In general, the magnitude of psychomotor sensitization in males is relatively small and because session 1 locomotor activity was higher than expected, this may be the reason we observed greater locomotor activity on session 9 and 11, but neither differed significantly from session 1 (see Fig. 3.6). During the IntA-Demand procedure, however, locomotor activity significantly increased from baseline (prior to IntA-Limited) to probe (after IntA-Limited), indicating that the males were also further sensitized by IntA-Limited experience (see Fig. 3.7). In contrast, we were able to detect greater locomotor activity in females rats on session 9 compared to session 1 (and thus psychomotor sensitization), but locomotor activity during session 11 did not differ from session 1. During demand probe testing, however, females significantly decreased locomotor activity compared to baseline, and this suggests the early emergence of stereotypy. Nonetheless, IntA-Limited sensitized both males and females, and although not quantified, the data suggests greater stereotypy in females than males, and thus more robust psychomotor sensitization.

Even though the results from IntA-Demand and IntA-Limited were not entirely consistent, psychomotor sensitization scores derived from the baseline change during both of these procedures predicted future motivation for cocaine, consistent with other IntA procedures (Allain et al. 2017; Algallal et al. 2019). These findings suggest that IntA-Demand is useful for assessing incentive and psychomotor sensitization in a single session and maybe be an ideal procedure for future studies comparing these behaviors after IntA vs LgA.

With our many procedural changes (e.g., IV, IP, self-admin context, outside the self-admin context, continuous demand, IntA-Demand) we gained greater insight regarding ways to best assess psychomotor sensitization. However, overall we found IntA readily produces robust psychomotor sensitization in females and males. Further, in both sexes, we also found that intermittent cocaine exposure (females experimenter-administered; males traditional IntA) resulted in an enhanced locomotor response to an amphetamine challenge (see Figs. 2.10 and 2.12), thus producing behavioral cross-sensitization that is consistent with a report of neural cross-sensitization following IntA (Calipari et al. 2014b).

Sex Differences in Cocaine Demand

Although there are some conflicting reports, female rats typically consume more drug, are more motivated to obtain drug, and appear more susceptible to relapse (Roberts et al. 1989; Lynch and Carroll 2000; Lynch and Taylor 2004; Roth and Carroll 2004; Lynch et al. 2005; Kippin et al. 2005; Kerstetter et al. 2008; Cummings et al. 2011; Smith et al. 2011; Nicolas et al. 2019). To date, two published studies have examined sex differences in motivation to obtain cocaine following IntA experience. In the first study, compared to males, females both consumed

more drug and were more motivated to obtain drug as measured by behavioral economic approaches and "demand" testing (Kawa and Robinson 2019). A large increase in motivation was observed after 12 sessions of IntA experience, and progressively increased with additional experience and following withdrawal. In the second study, consumption was similar in females and males, but females administered more infusions under a PR schedule of reinforcement (Algallal et al. 2019).

In our sex difference study described in Chapter II, females made more responses than males during the baseline within-session demand test and this same effect was observed during the sex difference experiment described in Chapter III (see Figs. 2.5 and 3.5). The demand test, however, was modified in Chapter III to maintain intermittency throughout the design (IntA-Demand) and a new and improved curve fitting approach was used to evaluate demand data (Newman and Ferrario 2020). During baseline testing, we also found a greater nPmax (metric of motivation) in females compared to males (see Fig. 3.5). Rats were then allowed to self-administer cocaine under IntA-Limited conditions for 12 sessions. The day after the last session, demand for cocaine was reassessed (see Fig. 3.1 for experimental timeline).

We found incentive sensitization in both females and males, but to our surprise, females were not more motivated than males (see Fig. 3.8). While our experimental timeline was very similar to Kawa and Robinson (2019), we purposefully limited consumption during IntA to prevent a sex difference in consumption. The current results suggest that total drug consumption may have been a factor in the greater motivation observed in females previously. However, similar to Kawa and Robinson (2019), we did not find any relationship between total drug intake

under IntA conditions (i.e, baseline IntA-Demand and IntA-Limited) and probe motivation (see Fig. 3.10). Thus, even though the primary difference in the designs was consumption level, this does not seem to account for the difference in results (see next paragraph for alternative explanation).

Behavioral economic procedures have gained increased popularity alongside IntA (which is the procedure that has most commonly been used in IntA designs), in-part because it provides other meaningful behavioral metrics, such as the animals preferred level of drug consumption. While it does not appear that total intake matters, the ability of the animal to self-administer to its preferred level (Q_0) appears crucial, particularly for females. For example, male rats with IntA-Limited and IntA-Unlimited experience do not differ in motivation (Allain et al. 2018). A similar study has yet to be conducted in females, but we would hypothesize, based on the current results, that females with IntA-Unlimited experience would be more motivated than females with IntA-Limited experience. In support of this is the fact that females with similar consumption as males during IntA-Unlimited are still more motivated to obtain cocaine than males (as assessed by PR). The critical part is that these females were able to self-administer their desired amount of drug (i.e., the preferred level of consumption, Q_0), which is typically unchanged with increasing IntA experience.

In our study, however, we found that females increased their preferred level of consumption following IntA-Limited. This is not thought to reflect a change in the "hedonic set point", but rather a rebound effect due to drug being freely available again. In humans, females report using more drug than intended (Robbins et al. 1999; Elam et al. 2001; Kennedy et al.

2013) and our finding suggests in rats, that unintentional excessive use might occur when drug availability goes from being limited to freely available. Nonetheless, it appears that IntA-Limited may have affected motivation in females by preventing them from consuming their preferred level of cocaine. Under these conditions, females are not more susceptible to incentive sensitization, and for this reason, females with IntA-Limited experience may serve as a useful control for comparison to females with IntA-Unlimited experience that appear more susceptible to incentive sensitization. Together, these models may provide useful insights into the "telescoping" phenomena observed in women.

Implications of the Results for Theories of Addiction

This section begins with a brief historical introduction to theories of addiction and then describes two prominent theories relevant to the experiments presented in this dissertation. First, the anhedonia view of addiction is described with particular attention to evidence in support of this view, including findings from the LgA model. A similar discussion of the incentive sensitization theory of addiction follows, which is supported by studies using the IntA model. It will become even more apparent that both LgA and IntA cannot model the transition to addiction, but both models and views provide useful insights into the addiction process. Note, several neurotransmitter systems have been implicated in the addiction process, but the discussion below is limited to dopamine neurotransmission.

Early theories of addiction were largely influenced by the physical distress syndrome associated with withdrawal from depressant drugs such as opioids, alcohol, and barbiturates. Prior to the realization that cocaine could produce "true addiction", it was noted that

“In a psychically dependent person each self-administered dose of the drug is said to alter in a "pleasurable" direction, of course, an unpleasant mood state that was the consequence, not of the effects of previous doses of the drug, but of antecedent anxiety, depression, boredom, and the like…In the tolerant and physically dependent user, however, each dose of the drug is said merely to stave off or suppress the "pain and suffering" associated with abstinence phenomena” (Wikler, 1973, p. 611).

Soon after, however, it was discovered that withdrawal from psychostimulant drugs also resulted in negative emotions, such as anxiety and irritability. As such, this component of withdrawal common to all abused drugs then became a focal point, with continued drug-use thought to be motivated by the desire to alleviate this negative affective state (Koob and Le Moal 1997). According to this anhedonia view, during initial use, the drug results in a largely positive hedonic state (i.e., a-process) that is then followed by a relatively mild negative hedonic state (i.e., the opponent b-process that emerges after the drug effects diminish). Repeated drug use, however, results in incremental reductions in positive affect due to tolerance-related neuroadaptations and this is accompanied by an increasingly negative hedonic state (i.e., “the dark side”).

The transition from a dominate a-process (binge/intoxication stage characterized by positive reinforcement) to b-process (withdrawal/negative affect stage characterized by negative reinforcement) is thought to reflect a change in the hedonic set point that contributes to a state of spiraling distress that drives the addiction process. In support of this view, early studies employing a threshold procedure for intracranial self-stimulation (ICSS, measure of reward circuitry functioning) found increased reward thresholds (interpreted as a decrease in reward value) during withdrawal from chronic cocaine self-administration (Markou and Koob 1991). The same effect was observed after chronic ethanol exposure (Schulteis et al. 1995) and in

morphine-dependent animals exposed to naloxone to precipitate withdrawal (Schaefer and Michael 1986; Schulteis et al. 1994). At the neurochemical level, reductions in the acute reinforcing effects of drugs have been associated with decreased dopamine transmission in the brain reward system, specifically within the nucleus accumbens (Weiss et al. 1992). For example, animals administered several doses of a dopamine antagonist in the accumbens shell drastically increase cocaine self-administration (Caine et al. 1995). In effect, this reduced dopamine transmission was thought to reflect a reduced a-process that ultimately led to these animals working harder to obtain cocaine, even though the dose was unchanged.

The escalated cocaine intake seen with the LgA model is also associated with progressive increases in reward thresholds (Ahmed et al. 2002) and decreased dopamine neurotransmission (Ferris et al. 2011; Calipari et al. 2013, 2014a; Willuhn et al. 2014; Sicilliano et al. 2016; but also see Ahmed et al. 2003; Kawa et al. 2019b). In humans, detoxified alcoholics show decreased subjective ratings of drug reward ("high") compared to controls (Volkow et al. 2007) and brain imaging studies show reduced drug-induced striatal dopamine responses and decreased dopamine D_2 receptor availability in both detoxified and active drug abusers (Volkow et al. 1997, 2007, 2014; Martinez et al. 2004, 2007; for review, see Volkow et al. 2009). As such, according to this view, drug-seeking is driven by a desire to overcome a DA deficiency (i.e., a *hypo*dopamingergic state) and the associated anhedonia, both of which contributes to the transition to addiction (Koob and Volkow 2016).

Incentive Sensitization Theory of Addiction

In contrast to the ideas presented above, the incentive sensitization theory maintains that

a *hyper*dopaminergic state results in pathological drug "wanting" that motivates continued drug-seeking and contributes to the transition to addiction. This theory was first introduced in 1993 by Robinson and Berridge to address key features of addiction that were not adequately explained by existing theories based on negative or positive reinforcement. According to the incentive sensitization theory, in some individuals, repeated drug use results in progressive, and rather persistent, sensitization-related neuroadaptations within the mesocorticolimbic system that is involved in the attribution of incentive salience. Once this system is rendered hypersensitive (sensitized), drugs and drug-associated stimuli exert greater control over behavior due to excessive "wanting" (craving) that manifests as compulsive drug-seeking and –taking. Thus, it is concluded that abusers crave drug (the first described key feature of addictive behavior) due to sensitization of the incentive motivational properties of both the drug and its associated cues, which is mediated, at least in part, by increased dopamine neurotransmission in the mesocorticolimbic system.

Also critical to understanding the development of addiction is why drug use is continued despite reports of reduced subjective pleasure and persistent craving despite protracted abstinence. The former question presents as one of the many shortcomings of positive reinforcement views of addiction, but a rather simple answer is put forth by the incentive-sensitization theory: drug "wanting" (i.e., craving) and "liking" (i.e., subjective pleasure) are dissociable processes mediated by different systems. As such, the authors propose that the mescocorticolimbic system involved in the attribution of incentive salience is rendered hypersensitive (not the neural system involved in "liking"), resulting in increased drug "wanting"

which can be dissociated from any changes in drug "liking". The latter question presents as one of the many shortcomings of negative reinforcement views of addiction (including the anhedonia view described above) given the occurrence of relapse long after symptoms of withdrawal have subsided. According to the incentive-sensitization theory, cravings still persist because drug-induced sensitization-related neuroadaptations are long-lasting, possibly permanent, with drug and drug-associated cues remaining highly salient and capable of precipitating relapse in "recovered" abusers.

While sensitization to the incentive motivational properties of drugs is central to this theory, sensitization to the psychomotor activating effects of drugs is thought to reflect changes within reward circuitry and is often used as indirect evidence for incentive sensitization theory. As such, much of the initial support for the incentive sensitization theory of addiction involved intermittent, non-contingent drug pretreatment to produce psychomotor sensitization (which is associated with increased dopamine neurotransmission; for review see, Robinson and Becker 1986; Stewart and Badiani 1993). Sensitizing regimens of drug pretreatment enhance CPP, facilitate acquisition of self-administration, accelerate intake escalation, and result in greater motivation assessed by PR (Lett 1989; Vezina 2004; Ward et al. 2006; Ferrario and Robinson 2007; Nordquist et al. 2007).

More recently, additional support for this view has come from the IntA model of self-administration, which has consistently produced especially robust incentive, psychomotor, and dopamine sensitization (Zimmer et al. 2012; Calipari et al. 2013, 2014b, 2015; Allain et al. 2017, 2018; Allain and Samaha 2018; Kawa et al. 2019b; James et al. 2019; Algallal et al. 2019; Carr

et al. 2020; Allain et al, 2020; Minogianis and Samaha 2020). Most compellingly, Kawa et al. (2019b) showed that a single self-administered infusion of cocaine following IntA experience resulted in increased dopamine release that was associated with robust incentive sensitization. Even further, the extent of psychomotor sensitization produced by IntA experience has been shown to predict future motivation for cocaine (Allain et al. 2017, Algallal et al. 2019; data presented in Chapter III). Historically, the absence of robust psychomotor sensitization following self-administered drug (especially under LgA conditions; exception, Ferrario et al. 2005) was used as evidence against the incentive sensitization theory, but the experiments presented in this dissertation show that when cocaine is administered in a pattern that more closely resembles human drug taking (i.e., intermittently), especially robust psychomotor sensitization is produced with similar characteristics described in the experimenter-administered literature.

There are also several reports of behavioral and neural sensitization in humans (Leyton 2007). For example, following repeated intermittent amphetamine administration, elevated mood, motor activity/energy ratings, eye-blink responses, speech amount and rate, and dopamine release are all increased (Strakowski et al. 1996; Strakowski and Sax 1998; Boileau et al. 2006). In addition, eye-tracking data has shown that abusers attention is biased towards visual drug-associated cues (Wiers and Stacy 2006), possibly due to the attribution of incentive salience that makes them more attractive, and drug-associated cues also cause an enhanced dopamine response (Boileau et al. 2007). Interestingly, there is also compelling support from Parkinson's patients that abuse dopaminergic drugs and meet the criteria for dopamine dysregulation syndrome (Evans et al. 2006). In these patients, enhanced drug-induced dopamine release is

observed and is associated with increased self-reported drug "wanting" but not "liking" and a form of stereotypy referred to a "punding" (Evans et al. 2006).

There is a great deal of evidence in support of the theories described above. There is, however, a major conflict: essentially opposite mechanisms have been proposed to underlie the transition to addiction. The LgA model is associated with a hypodopamingergic state and has resulted in behavioral tolerance (exception, Ferrario et al. 2005) that is interpreted as support for the anhedonia view, while the IntA model is associated with a hyperdopaminergic state and produces behavioral sensitization that favors an incentive sensitization view. As noted above, there are a number of behavioral inconsistencies observed following LgA experience and many of the assumptions underlying this model (such as the need for high levels of drug intake) have been challenged by strikingly consistent results from the IntA model. In addition to producing especially robust addiction-like behavior, the IntA model also more closely resembles human drug-taking patterns which suggests it is indeed a better model of the transition to addiction. Of note, we did not find especially robust incentive sensitization in females compared to males following IntA-Limited experience. This suggests that both the temporal pattern of use (a critical component for proponents of incentive sensitization theory) and the amount of drug consumed (a critical component for proponents of anhedonia theory) play an important role in females apparent increased susceptibility to transition to addiction. It is, therefore, necessary to further explore how use patterns interact with circulating hormones to promote the development of addiction-like behaviors in females.

Conclusions

The experiments presented in this dissertation add to the growing literature showing IntA produces especially robust incentive and psychomotor sensitization. Specifically, we now show that several variations of IntA self-administered cocaine is capable of producing psychomotor sensitization with characteristics similar to those observed in the early experimenter-administered literature and that the extent of psychomotor sensitization predicts future incentive sensitization. Interestingly, we also report that females are not more motivated than males when infusion limitations during IntA (IntA-Limited) prevent the self-administration of preferred levels of cocaine. As such, further studies are necessary to examine how consumption restrictions affect neurobehavioral plasticity in females to better understand the "telescoping" phenomena. In addition, there is also a need for studies assessing the neurobiological consequences of extended withdrawal from IntA and LgA. While IntA has typically shown more robust addiction relevant behavior soon after discontinuation of self-administration, this was not the case when psychomotor sensitization was assessed on WD30. Indeed, it may be the case that the expression of neurobehavioral sensitization persists far longer after IntA. Our promising results from IntA-Demand suggests that this model may be ideal for future studies comparing psychomotor and incentive sensitization following IntA and LgA experience. All in all, it is our hope that the results reported here will renew interest in psychomotor sensitization as we have shown that it is highly replicable following self-administered cocaine and provided a number of measures to do so effectively.

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