Université de Montréal

Sex Differences in Cocaine Use in Rats

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Mémoire présenté à la Faculté des études supérieures
en vue de l'obtention du grade de Maître ès Sciences

février, 2019

Université de Montréal

Faculté des études supérieures

Ce mémoire intitulé:

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RÉSUMÉ

Les mâles et les femelles répondent différemment à la cocaïne. La transition vers la toxicomanie peut être plus rapide chez les femmes. En préclinique, les femelles sont plus vulnérables aux propriétés renforçantes et motivationnelles de la cocaïne. Les études rapportant des différences sexuelles sur la consommation de cocaïne ont majoritairement été menées avec un accès continu à la drogue [e.g., LgA (sessions de 6 h), Long Access ou ShA (sessions de 1-2 h), Short Access]. Ceci favorise des niveaux de cocaïne au cerveau soutenus pendant toute la session d'auto-administration. Or, les usagers les plus expérimentés de cocaïne consommeraient la drogue par intermittence au sein d'une session d'intoxication, ce qui produirait des pics de cocaïne au cerveau. Un nouveau modèle d'auto-administration de cocaïne chez le rat autorise cet accès intermittent (IntA) à la cocaïne. L'accès IntA, versus LgA, produit les changements neurobiologiques, psychologiques et comportementaux pertinents à la toxicomanie. Ici, nous avons comparé la consommation de cocaïne chez des mâles et des femelles ayant un accès quotidien IntA ou LgA à la drogue (10 sessions de 6 h, 0.25 mg/kg/infusion, i.v.). Les rats des deux sexes LgA ont consommé plus de cocaïne que les rats IntA, mais seules les femelles LgA ont escaladé leur consommation. La sensibilisation psychomotrice était uniquement vue chez les rats IntA, de façon plus importante chez les femelles. Cinq et 25 jours après la dernière session IntA ou LgA, la motivation pour la drogue sous ratio progressif (0.083-0.75 mg/kg/infusion) était similaire chez les rats IntA et LgA. Les femelles étaient plus motivées à avoir la drogue que les mâles, uniquement dans un contexte IntA. Ainsi, les conditions LgA et IntA pourraient être utiles à étudier les différences sexuelles dans la consommation de cocaïne ou dans l'état motivationnel des animaux pour la drogue, respectivement.

Mots-clés: Addiction, Auto administration, Cocaïne, Différences de sexe, Accès prolongé, Accès intermittent, sensibilisation psychomotrice, Motivation, Ratio progressif

ABSTRACT

In both humans and laboratory animals, females and males can respond differently to cocaine. Women can progress more rapidly from initial cocaine use to addiction. Studies in laboratory rodents have also demonstrated that females can be more vulnerable to the reinforcing and incentive motivational effects of cocaine. Most preclinical studies characterizing the effects of cocaine use in females and males have been conducted using continuous-access self-administration procedures [e.g., (6 h sessions), Long Access or ShA (1-2 h sessions), Short Access]. These procedures achieve produces high brain concentrations of drug. However, human addicts take cocaine intermittently during a bout of intoxication, and this would produce spiking brain cocaine levels. A recent intermittent-access (IntA) cocaine self-administration procedure models this in rats. Compared to LgA, IntA self-administration is uniquely effective in producing the neurobiological, psychological and behavioral changes that underlie the transition to cocaine addiction. Here, we compared cocaine use in female and male rats that self-administered the drug (0.25 mg/kg/ infusion, i.v.) during 10 daily, 6-h LgA or IntA sessions. LgA rats took more cocaine than IntA rats, and only female LgA rats escalated their intake. However, only IntA rats (both sexes) developed psychomotor sensitization and sensitization was greatest in the females. Five and 25 days after the last self-administration session, we quantified incentive motivation for cocaine by measuring breakpoints for the drug under progressive ratio under progressive ratio schedule. There were no significant sex differences on this measure in LgA rats. However, under IntA, females reached higher breakpoints for cocaine than males. Thus, LgA might be best suited to study sex differences in cocaine intake, while IntA might be best suited to study sex differences in incentive motivational processes in cocaine addiction.

Keywords: Sex differences, Addiction, Cocaine, self-administration, Long Access, Intermittent Access, Psychomotor sensitization, Motivation, Progressive Ratio

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LIST OF ABBREVIATIONS

BDNF   Brain-derived neurotrophic factor

BE   Benzoylecgonine

EME   Ecgonine methyl ester

DA   Dopamine

DZ   Dizygotic

DS   Dorsomedial striatum

FR.   Fixed ratio

h   Hour

HCl   *Hydrochloric acid*

IntA   IntA access

i.v.   Intravenous

LgA   Long access

MZ   Monozygotic

NAc   Nucleus accumbens

OFC   Orbitofrontal cortex

PR   Progressive ratio

S   Second

SA   Self-administration

ShA   Short access

WD   Withdrawal

ACKNOWLEDGEMENTS

Introduction

1. Addiction

1.1. Definitions and statistics

Drug addiction is a disorder in which compulsive drug-taking and drug-seeking behavior continues despite the negative effects associated with the consumption of the drugs. Addictive substances induce euphoria or relieve distress, with the main risk groups being alcohol, tobacco and prescribed and illicit drugs (Cami & Farre, 2003). Over the years, numerous researchers and research institutions have looked into the world-wide phenomenon of drug abuse, gathering statistics and other data on the types of drugs being abused, the users of these drugs, and information regarding drug-related deaths, injuries and disabilities (Peacock et al., 2018). According to data compiled by the United Nations Office Against Drugs and Crime, approximately 246 million people used psychoactive drugs in 2013 (Ouzir & Errami, 2016). In 2015, the estimated prevalence of drug use among the global adult population was 15.2% for daily tobacco smoking; 18.4% for heavy episodic alcohol use (in the past 30 days); and 0.35, 0.37, 0.77 and 3.8% for past-year cocaine, opioid, amphetamine and cannabis use, respectively (Peacock et al., 2018). The highest mortality rates were caused by tobacco smoking (110.7 deaths per 100,000 people), followed by alcohol consumption (33.0 deaths per 100,000 people), and illicit drugs (6.9 deaths per 100,000 people) (Peacock et al., 2018).

Drug addiction is considered by some experts to be a chronic medical illness (M. B. Walker, 1989). The addiction concept comes from definitions of addiction which focus on the extreme intake of substances (M. B. Walker, 1989). Some authors make an important distinction between addiction and psychological dependency (M. B. Walker, 1989). For

example, Ullmann & Krasner (1975, p. 444) define psychological dependency as "use of a foreign substance (drugs, alcohol) as a typical response to a variety of situations." Likewise, Davison & Neale (1974, p. 234) define addiction as "a physiological process by which the body responds to certain drugs." Hence, addiction is categorized by physiological responses which are tolerance and withdrawal (M. B. Walker, 1989), a phenomenon in which a progressive amount of the drug must be consumed to achieve the same responsiveness (Ouzir & Errami, 2016). This leads to dependence due to repeated compulsive drug-seeking behavior (Ouzir & Errami, 2016), in which cessation of drug use produces a syndrome of reactions in the body, referred to as withdrawal symptoms (Ouzir & Errami, 2016).

The Diagnostic and Statistical Manual of Mental Disorders (DSM) has handled behavioral addictions. In the DSM-IV, the term "dependence" is used to describe individuals showing a maladaptive pattern of substance use causing distress or significant impairment and which leads to difficulty in substance-taking behavior control, withdrawal symptoms and tolerance (Altman et al., 1996; Ouzir & Errami, 2016). However, withdrawal is not an essential criterion, according to the DSM-IV definition. This is confirmed by some clinical observations which have demonstrated that dependence can occur without the development of withdrawal or tolerance. (Altman et al., 1996).

Altman et al. (1996) reported definitions for a variety of addiction-related terms. They classify "addiction" as a psychopathological condition only when the drug use control is lost. Furthermore, "dependence" describes as the need to consume a drug or drugs to

function within regular limits the need to consume a drug within regular limits. "Drug abuse", according to the researchers, causes several problems, including behavioral psychopathology and loss of activity in society. Criminal acts could be also described as a problem that can occur due to drug abuse. The term "use" is related to taking any psychoactive drug for non-medical reasons (Altman et al., 1996). It is worth mentioning here that "abuse" and "use" do not refer to either the user's psychological or physiological state.

Compared to its predecessors, the DSM-5 changed the addictions chapter from "Substance-Related Disorders" to "Substance-Related and Addictive Disorders" to reflect the development of understanding on addiction (Grant & Chamberlain, 2016). As the field of addictions has undergone dramatic changes in recent years, the DSM-5 has tried to address whether addiction must be expanded to include other types of behavior beyond psychoactive substances. An important difference between the DSM-4 and DSM-5 is the relocating of gambling disorders to the Substance-Related and Addictive Disorders section, after listing it in the chapter on impulse control disorders (Grant, Potenza, Weinstein, & Gorelick, 2010). Despite some evidence pointing to problematic gambling behavior as being highly similar to clinical substance addiction, other research classifies this behavior under impulse control disorders and deals with it accordingly (J. E. Grant et al., 2010).

Which behaviors to include as behavioral addictions is still open for debate. A growing body of research provides sufficient proof to categorize behavioral addictions as

substance addictions for a variety of domains (Grant et al., 2010), given that emotional dysregulation could in fact cause cravings to occur not only in substance use but also behavioral disorders (de Castro, Fong, Rosenthal, & Tavares, 2007). The existence of varying degrees of "craving" intensity has been reported by numerous individuals with behavioral addictions, which is similar to the experience of those with substance use disorders. Moreover, the behaviors tend to lower anxiety levels and lead to an emotional "high" state not unlike that present during substance intoxication. On the other hand, this "high" is reported to reduce in intensity (i.e., tolerance-like effect) with the repetition of the same behavior of, for instance, kleptomania, pathological gambling, compulsive sexual encounters, etc., resulting in the perceived necessity to raise the amount or level of the behavior in order to acquire the desired mood (Blanco, Moreyra, Nunes, Saiz-Ruiz, & Ibanez, 2001; J. E. Grant & Potenza, 2008).

Individuals experiencing this form of behavioral addiction claim that a dysphoric state occurs if they scale back or abstain entirely from the behavior (i.e., withdrawal-like effect), but the "withdrawal" sensation falls well short of typical and severe substance withdrawal effects (Grant et al., 2010). Consequently, the DSM-5 only categorizes gambling as being in the same disorder classification as substance addiction, whereas other behaviors such as compulsive sexual behavior have not acquired sufficient research proof to include them in any disorder category (Grant & Chamberlain, 2016).

1.2. Brain systems and drug addiction

Human and animal studies show that repeated drug intake can have an effect on the brain, causing changes in complex and persistent ways (Robinson & Berridge, 1993). According to the incentive-sensitization theory, the desire to use or abuse a drug is based on the drug providing a sense of pleasure in the user, which then leads to the development of brain adaptations towards that pleasurable stimulus (Robinson & Berridge, 1993). The brain's reward system was developed over time by providing an impetus for people to engage in behaviors and activities that lead to species survival. Such activities can include eating specific foods and engaging in sexual activity. During these events, dopamine is released as a reward. Simultaneous with the development of the dopamine-based reward system for species-survival motives was the inclusion of negative behaviors, such as drug abuse, that were similarly pleasurably rewarded despite the danger these activities posed to the user (Ouzir & Errami, 2016).

Central dopaminergic neurotransmission features a number of different actions which occur at various levels in the mesocorticolimbic reward pathway (Berridge & Robinson, 1998). Although long thought by many neuroscientists to be a "pleasure" neurotransmitter, mesolimbic dopamine is currently undergoing a big change in understanding within the scientific community, with the "pleasure center" handle falling out of favour (Berridge & Kringelbach, 2008). Instead, mesolimbic dopamine neurons are now being viewed as being activated more by motivational or predictive properties (Schultz, 1997; Carelli, 2004; Redgrave & Gurney, 2006), considering that dopamine

systems can be triggered even by non-rewarding stimuli (Salamone, 1994; Horvitz, 2000;).

Furthermore, research indicates that dopamine signaling, whether in humans or in animals, provides no clear evidence between liking a thing compared to wanting it (Volkow et al., 2002; Leyton et al., 2002; Robinson, Sandstrom, Denenberg, & Palmiter, 2005). As psychological precepts, 'liking' and 'wanting' can be viewed as separate psychological components of reward (Robinson & Berridge, 1993; Berridge, 1996). According to Berridge and Robinson (1998), dopamine systems in humans and animals are not required for mediating hedonic pleasure connected to reinforcers or predictive associations related to hedonic reward learning. Dopamine could thus more likely be a factor in incentile salience in neural representations for reward-related stimuli, incentive salience being a factor in both reward and motivation. Following this line of thought, dopamine systems could be considered to play a role in 'wanting' incentiĺes, though not in 'liking' them; these systems could also play a role in acquiring novel 'likes' or 'dislikes'.

Research indicates that drug addiction causes heightened levels of dopamine (DA) transmission within the same portion of the brain as the other established reward system. When the drug reaches the brain, DA is released in increasingly larger amounts, essentially flooding the brain's reward system and reinforcing the negative behavior that caused its release (Kelley & Berridge, 2002). The nucleus accumbens (NAc) contributes to the prompting of behaviors and the signaling of their pleasure (Centonze et al., 2002), while the striatum plays a role in compulsive behaviors and the increased desire for more

drugs (Willuhn, Burgeno, Everitt, & Phillips, 2012). The involvement of DA in both the dorsal striatum (DS) and NAc has been shown to be critical to the onset of desiring or "craving", with NAc's endogenous opiates delivering a pleasurable sensation; however, although the pleasurable sensation is only temporary, the craving continues (Berridge, 1996).

The elevation of drug intake to constant and compulsive use from occasional use indicates a change in the neural activation pattern to the DS from the NAc (Willuhn et al., 2012). Hence, the addictive behavior denotes less DA activity in the NAc and more DA activity in the DS (Volkow, Fowler, Wang, & Swanson, 2004). The origin of the desire for a specific drug or cocktail of drugs can be found in the NAc's and dorsomedial striatum's initial activation, as it is here that the drug use earns its first rewards.

From the above, we can see that changes and adaptations in the brain cause changes in the motivation process, which in turn lead people into a cycle of addictive behavior during which they abuse drugs even while knowing their dangers (Foddy & Savulescu, 2010). Some research points to drug addiction being a disorder that affects a user's motivational, learning and decision-making abilities and behaviors, with DA affecting corticostriatal neurons, which in turn have an impact on the user's ability to plan actions or gauge values of specific outcomes related to drug use (Berke & Hyman, 2000; Hyman, 2005). Furthermore, drug use has been shown to change the orbitofrontal cortex (OFC) neuronal activity by hampering OF-dependent learning (Ouzir & Errami, 2016). In searching for ways to break the drug-addiction cycle, researchers discovered the

importance of suppressing medial orbitofrontal cortex reward signals to enable self-control (Hayashi, Ko, Strafella, & Dagher, 2013). Other researchers found that interactions among decision-making brain regions could be a contributory factor in developing addictive behaviors and thus could serve as target areas for moderating addictive-related behaviors (Ouzir & Errami, 2016).

Current research reportes that drug addiction involves more than just the DA system. Several recent articles have discussed other systems which might contribute to addiction, such as the oxytocin system and the serotonin system (McGregor & Bowen, 2012; Muller & Homberg, 2015; Ouzir & Errami, 2016).

1.3. Factors involved in drug abuse

Many studies have demonstrated the importance of psychology, biology, environment and social influences on drug abuse

1.3.1 Biological factors

Genetic basis for drug abuse

Changes in genes in a number of brain systems as well as several molecular neuroadaptations have been related to drug abuse (Hyman & Malenka, 2001). The strongest evidence for the genetic effect of drug use comes from studies conducted on twins. In the research, both monozygotic (MZ) and dizygotic (DZ) twins demonstrated heritability of more than 40% for addiction to alcohol and other substances (Uhl, 1999). (Note that MZ twins are genetically identical while DZ twins share 50% identical genes.) Studies that compared the prevalence of drug abuse in MZ twins with the prevalence in

DZ twins reported greater similarity in the former than in the latter, which is suggestive of a genetic influence.

Several genetic factors can prevent the development of drug addiction in individuals. For example, a mutation in alcohol dehydrogenase and production of an inactive aldehyde dehydrogenase enzyme in individuals is believed to lead to unpleasant symptoms after drinking, which decreases their risk of abusing alcohol (Thomasson, Crabb, Edenberg, & Li, 1993). In addition, in the promoter region, dynorphin may act to attenuate the dopamine (DA) level increases in the synaptic which is caused by cocaine. Alleles containing three or four 68-bp repeats (but not one or two) may act to exert a protection against cocaine abuse (Chen et al., 2002). Additionally, studies on smoking behavior have shown that individuals who carry the defective cytochrome P-450 2A6 alleles (* 2 and * 4) smoked fewer cigarettes than individuals with normal alleles (Rao et al., 2000).

Conversely, some genetic variations can make individuals more likely to use alcohol and drugs. For instance, the neuropeptide Y Leu7Pro allele is a risk for alcohol dependence (Lappalainen et al., 2002). As well, many studies have explored the genetic background of addiction using the genetic variants in single nucleotide polymorphisms (SNPs), demonstrating an important contribution of SNPs in the vulnerability to tobacco, alcohol, cannabis, cocaine and other substance dependencies (Ouzir & Errami, 2016). The corticotrophin-releasing factor (CRF) also plays a role in the development of drug-seeking behavior and the motivation of drug withdrawal and dependence. Further, it has

been suggested that there is interaction between norepinephrine systems and CRF in mediating the unwanted effects correlated with stress and increasing the rewarding effects of abused drugs (Ouzir & Errami, 2016).

Mental disorders

According to the published research, there appears to be a high concordance of drug use and mental illness. Psychiatric illnesses such as bipolar disorder, schizophrenia, anxiety, depression, phobias and attention deficit hyperactivity disorder are thought to be strongly influenced by the use of drugs and also increase the risk of drug abuse and addiction (B. F. Grant & Harford, 1995; Merikangas et al., 1998; Regier et al., 1990). As well, these illnesses can render the brain more vulnerable to addiction (Berg, Sentir, Cooley, Engleman, & Chambers, 2014; Ouzir & Errami, 2016). The World Health Organization (WHO) reports that individuals with psychiatric illnesses are three times more likely to develop substance abuse disorders than normal individuals (UNODC, 2010). Antipsychotic drugs induce hyper-sensitivity within the dopamine systems, but they are not categorized as drugs of abuse. Antipsychotics increase the actions of other drugs, including alcohol, cocaine, opioids and cannabis, and can also be used to counter the adverse effects of illicit drugs (Malekshahi, Tioleco, Ahmed, Campbell, & Haller, 2015; Samaha, 2014).

1.3.2 Environmental and social factors

Environmental and social factors play an important role in the development of drug-taking behavior.

Environmental stimuli

The environmental effects associated with a drug influences the ability to reuse this drug even after its discontinuation. For example, images of local drug injection sites and injection equipment (syringes, pipes, drug sachets, etc.) are all stimuli associated with drug abuse because they are associated with episodes of drug-use experienced in the past and serve as clues to drug availability (Childress et al., 1993; Childress, McLellan, Ehrman, & O'Brien, 1988). In addition, environmental stimuli such as events, sounds, lights, etc., which are present as cues during the drug administration can serve as conditional stimuli and develop the ability to evoke a response similar to the drug administration (S. Siegel, 2005; Stewart, de Wit, & Eikelboom, 1984).

Stress

Several studies have reported increased drug-taking following stress-inducing situations as opposed to non-stress situations (Sinha, 2001, 2008). In social drinkers, exposure to stressors can lead to increased alcohol consumption compared to drinking behavior in non-stressful situations (Sinha, 2001). Furthermore, there is little doubt that exposure to acute or chronic stress plays a significant role in drug addiction and relapse (Shaham, Rajabi, & Stewart, 1996). Exposure to acute behavioral stress can facilitate self-administration of amphetamines (Piazza, Deminiere, le Moal, & Simon, 1990), morphine (Alexander, Coambs, & Hadaway, 1978; Shaham & Stewart, 1994) and cocaine (Haney, Maccari, Le Moal, Simon, & Piazza, 1995; Miczek & Mutschler, 1996). Laboratory models propose that mood enhancement (positive reinforcement) or relief from stress (negative reinforcement) can enhance the vulnerability to abuse the drug.

Based on preclinical findings, Koob and Le Moal (1997) suggested a model which links positive and negative reinforcement properties. They suggest that changes in brain reward circuits due to stress lead to a greater sensitivity to the drugs and increase the motivation to obtain it. Therefore, "stress may act to 'prime' brain reward systems, thereby enhancing the reinforcing efficacy of drugs, particularly in those vulnerable to drug abuse" (Piazza & Le Moal, 1998). Additionally, the critical role of CRF in stress-induced drug-seeking behavior has been reported by studies using animal models (Badiani, Belin, Epstein, Calu, & Shaham, 2011; Shaham et al., 1996). It has thus been hypothesized that CRF has an important role in alcohol and drug dependence (Heilig & Koob, 2007).

1.3.3. Drug characteristics and speed of administration

In addition to biological, social and environmental factors, pharmacologic and physicochemical properties of drugs are important factors in drug consumption. Physicochemical properties of molecules, relative lipid solubility, degree of ionization, active transport, and blood flow at the target tissue influence which drugs cross biological membranes (Farre & Cami, 1991). Water-solubility facilitates the injection of a drug, while lipo-solubility increases the drug passage through the blood-brain barrier (Farre & Cami, 1991). Pharmacokinetic parameters, such as absorption, distribution, metabolism and elimination, have important effects on the action of the drug. If the drug is absorbed quickly, it will be transported to the brain and concentrated more quickly. The metabolites produced during the metabolism of the drug could be inactive metabolites or, conversely, more active than the original substance, which would change the effect of the drug.

Moreover, the rapid elimination of the drug consumed would be associated with more immediate withdrawal symptoms (Farre & Cami, 1991).

The speed at which the drug of abuse impacts certain targets in the brain is an important factor in the determination of its potential for abuse. This is seen in the differences in drug abuse belonging to the same family. For example, heroin enters the brain and activates its target receptors faster than methadone, which is why methadone is less addictive and could be used for treatment against heroin addiction (Oldendorf, Hyman, Braun, & Oldendorf, 1972). The drugs that are most often abused are administered via routes that ensure they rapidly reach high levels in the brain and produce the fastest effects. For example, cocaine administration by faster routes (e.g., intravenous injection or smoking of crack) is preferred and creates a greater risk of drug addiction comparing to slower pathways (e.g., intranasal administration) (Hatsukami & Fischman, 1996). Smoking a cigarette increases the risk of nicotine abuse compared to its consumption by chewing tobacco or using a transdermal patch (Benowitz, 1996). Furthermore, a short half-life produces stronger withdrawal syndromes than a long half-life (Farre & Cami, 1991).

1.4. Animal models used in drug abuse

1.4.1. Operant conditioning

Operant conditioning techniques are often used in addiction to measure the effects of abuse drugs on the individual or animal consumer (Meyer & Quenzer, 2005). Operant conditioning is a procedure based on associative learning, with learning defined as a

change in behavior as a response to environmental stimuli, and response defined as that which produces a consequence. The stronger the relationship between the stimuli and the response, the more the individual becomes conditioned to the response, and the more likely the response is to being repeated (Schuster & Thompson, 1969). Intracranial auto-stimulation and self-administration are the two operating conditioning techniques used most often in addiction studies (Meyer & Quenzer, 2005).

Intracranial auto-stimulation (ICSS)

This technique is not considered as a commonly used technique. In short, It was used first by Olds and Milner (1954) to study electrical stimulation in certain brain regions in animals. This stimulation produces a positive reinforcement which is similar to those which are produced by a primary reward such as water, food and sex (Olds & Milner, 1954). The technique allows the animal to receive an electrical current in a specific region of the brain through an implanted electrode directly in the study area (Meyer & Quenzer, 2005).

Self-administration

Self-administration, which started to be used in addiction studies in 1962, is a significant technique for studying the reinforcing properties of drugs. Weeks (1962) demonstrated in his study that rats are able to learn to press a lever and self-administer morphine that was delivered intravenously (Weeks, 1962). In this technique, the training and testing are done in standard operant cages, and a catheter is implanted in the animal's jugular vein to allow the direct access of the drug to the circulatory system. Different reinforcement schedules,

including the fixed ratio (FR) and the progressive ratio (PR), were used in the self-administration experiments. The FR schedule is characterized by the need to press the lever a fixed number of times in order to be rewarded by the drug infusion (Panlilio & Goldberg, 2007). This program is used in the preliminary examination of drugs with a high risk of abuse (Arnold & Roberts, 1997). In the PR schedule, the number of lever presses required to get a drug infusion increases exponentially with each successive infusion. The final ratio reached is called the "breakpoint". This is the point where the animal stops responding, and it is used to measure the motivation of the animal to obtain the drug (Panlilio & Goldberg, 2007; Richardson & Roberts, 1996). This reinforcement program can also be used to study the cue-induced (Crombag & Shaham, 2002), drug-induced (de Wit & Stewart, 1981, 1983) and stress-induced (Shaham & Stewart, 1995) reinstatement following an abstinence period.

1.4.2. Conditioned place preference test

This test has been used to examine the neural basis of drug abuse (Wise, 1989). The conditioned place preference test (CPP) is dependent on the classical conditioning principle between the effects of a drug and the drug user's environment (Meyer & Quenzer, 2005) and uses a two-compartment test chamber. First, the test drug is injected in the subjects who are restricted to one of the compartments. Following a certain number of sessions, both compartments are made available to the animals and they are again tested, this time without administration of the drug (Balster, 1991).

2. Cocaine

2.1. History and statistics

Cocaine is a natural stimulant drug found in the leaves of the coca plant, *Erythroxylon coca*. Since antiquity, the leaves of this shrub, which is native to western South America, have been chewed by Peruvian Indians and Bolivians for several different religious, medicinal and work-related reasons (NIDA 1999). The consumption of cocaine dates back more than 5000 years, when it was consumed by ingestion to increase energy, remove fatigue and decrease hunger. As a drug, cocaine belongs to the family of psychostimulants that causes increased alertness, arousal and excited behavior (Meyer & Quenzer, 2005) and is an alkaloid known as benzoyl-methyl-ecgonine. This tertiary amine contains three units: a lipophilic group, a hydrophilic group, and an aliphatic group (Ambre, Ruo, Nelson, & Belknap, 1988).

The use of native coca had generated considerable interest in Europe by the 19th century and many efforts were made to isolate the purified psychoactive component in the leaves. Isolating cocaine was successfully achieved, and the drug has since been explored for its manifold properties, including general stimulant properties, local anesthetic properties, and potential value as a tonic. The purified psychoactive ingredient isolated from the coca leaf was used as an anesthetic in the 1860s (Stolberg, 2011).

Indeed, the anesthetic use of this drug was particularly significant, since cocaine was found to be suitable to use in eye surgery, for which no prior drug had been appropriate. Furthermore, cocaine was used as a local anesthetic due to its action on the blood vessels

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in the anesthetized area. Because cocaine has the ability to constrict blood vessels and limit bleeding, it has become valuable as a local anesthetic in areas of the body richly supplied with blood vessels, such as the nose and throat. Using cocaine for certain medical reasons as a local anesthetic continues today, even though several therapeutic uses of the drug have been abandoned (Petersen 1977).

Starting in the 19th century, cocaine began to be added to many different products, such as wine, tea and Coca Cola, due to the stimulatory properties of the substance (Warner, 1993). It also began to appear outside its native South American region as an illicit drug. When marketed as such, cocaine is reduced to a white crystalline powder and often 'cut' or combined with other ingredients to increase seller profits. These added ingredients include sugars involving glucose and lactose, and some other local anesthetics such as tetracaine, procaine and lidocaine, all of which have a similar appearance and taste to cocaine (Petersen, 1977).

Because of its relatively high cost as an illicit drug as well as its desirable properties, cocaine is considered a status drug that is mostly marketed to well-heeled users (Petersen, 1977). However, cocaine is also the drug of choice for the less affluent. In fact, about 15.9 million people worldwide use cocaine. Of these, 1 million are diagnosed as drug addicts, according to the DSM-IV description (UNODC, 2010). The majority of cocaine users (6.2 million) are found in North America, with cocaine being responsible for a large number of deaths annually due to its consumption after heroin and other drugs from the family of opioids (UNODC, 2010). In addition, according to data from the 2011

Drug Abuse Warning Network (DAWN), about 1.3 million visits to emergency departments occur due to drug abuse, with cocaine being involved in 505,224 of the visits. Hence, cocaine is involved in over one in three (around 40 percent) of the drug-misuse related emergency department visits (CBHSQ, 2010).

2.2. Pharmacology of cocaine

2.2.1. Routes of administration

Packaging and routes of administration of cocaine are important because they influence the pharmacokinetics of the drug (Porrino, 1993). Drug speed and duration of action are modulated by how the drug is administrated; the more immediate the effects, the more likely the drug is to be abused (Hatsukami & Fischman, 1996). Oral and intranasal routes are slower than intravenous routes and inhalation (Hatsukami & Fischman, 1996; Jones, 1990) (Hatsukami and Fischman, 1996, Jones, 1990). Cocaine can be smoked, injected intravenously, or absorbed by mucous membranes.

Coca paste

The extraction of coca paste is done by mixing the extract with water, kerosene and sulfuric acid (Warner, 1993). This formulation is consumed by smoking it on its own or by mixing it with tobacco, marijuana or heroin, which allows for rapid transportation to the brain (Verebey & Gold, 1988).

Coca leaves

Coca leaves contain a low concentration of cocaine (0.5-1%), and in this form, the absorption of cocaine is slow (Verebey & Gold, 1988). Limited gastrointestinal absorption and low cocaine content in the leaves help to prevent the toxic effects associated with purified forms of cocaine (Warner, 1993).

Cocaine HCl (hydrochloric salt)

This form is either taken orally, intranasally, or injected intravenously after dissolving it in water (Meyer & Quenzer, 2005). Cocaine HCl is often mixed with other substances such as caffeine to mimic stimulating effects, lactose to give it volume, or procaine to produce an anesthetic effect (Warner, 1993). This formulation has a very low melting point, which means it is completely destroyed when burned (Warner, 1993).

The most common way to use cocaine is intranasally (Guindalini, Vallada, Breen, & Laranjeira, 2006). In humans, the maximum plasma concentration is reached slowly, about 30 minutes after taking the drug intranasally (Javaid, Fischman, Schuster, Dekirmenjian, & Davis, 1978). The physiological effects reach their maximum between 15-40 minutes after consumption and continue for at least one hour (Hatsukami & Fischman, 1996). Similar to the oral route, the bioavailability of cocaine taken intranasally is only 20-30%. However, the intranasal route has faster effects, shorter duration of action, and higher maximum plasma levels, which explains why this route of admine has a greater risk of abuse than oral administration (Verebey & Gold, 1988).

In contrast, the intravenous route of cocaine use has the greatest risk for abuse. This consumption method is characterized by the rapid injection of a large dose into the circulatory system, with the intention for the drug to reach the brain very quickly, unlike other pathways (Verebey & Gold, 1988). When cocaine is injected, the effects appear almost immediately (30 seconds). They then reach their maximum very quickly (5 minutes), after which they dissipate (within 30 minutes) (Hatsukami & Fischman, 1996).

Crack cocaine

Crack is made by heating a mixture of cocaine HCl, water and baking soda. This formulation makes it possible to produce high drug concentrations in the brain very quickly because it is usually taken by inhalation (Verebey and Gold, 1988). "Crack" is so named due to its bursting sound when heated (Warner, 1993). Compared to other routes of administration, smoking crack has many advantages. For example, it is non-invasive (Foltin & Fischman, 1992), cheaper, and the plasma and brain concentrations of cocaine rapidly increase and produce effects that are similar to the intravenous injection route (Jones, 1990). Moreover, smoking crack decreases the risk of infections (HIV) and other medical problems associated with intravenous injections (Foltin & Fischman, 1992).

2.2.2 Mechanism of action

The dopamine system in the brain is stimulated by different kinds of reinforcing stimuli including sex, food and several drugs of abuse (such as cocaine). Normally, dopamine (DA) is released into the synapse and binds to specialized proteins (DA receptors) on the neighboring neuron (Baik, 2013). DA works as an active messenger which carries a signal

from neuron to neuron. It is then removed from the synapse by another specialized protein (transporter) to be recycled for additional use (Baik, 2013).

Cocaine and some other drugs interfere with the normal communication process. By binding to the dopamine transporter, cocaine blocks the removal of DA from the synapse. This leads to an accumulation of DA in the synapse.

2.2.3 Metabolism and elimination

The metabolism and elimination of cocaine is very rapid, with a half-life of 0.5 to 1.5 hours and a clearance of about 2 liters per minute (Jatlow, 1988). Eighty-five to ninety percent of the dose is found in the urine (Jatlow, 1988). Esterase enzymes, which occur in plasma, liver and brain, are involved in the metabolism of cocaine. Cocaine is mainly metabolized to ecgonine methyl ester (EME) and benzoylecgonine (BE), but these metabolites are inactive and unable to block the recapture of dopamine at the pre-synaptic terminus (Jatlow, 1988). BE and EME metabolites have a longer half-life, about 7.5 hours for BE and 3.6 hours for EME (Ambre et al., 1988).

2.3. Effects of cocaine

2.3.1. Acute effects

There are several acute effects of cocaine as well as other psychostimulants characterized by behavioral stimulation (Meyer & Quenzer, 2005). The duration of euphoric effects by cocaine depends mainly on the route of administration. The main short-term effects include constricted blood vessels and increased blood pressure, heart

rate, and body temperature (Fonseca & Ferro, 2013). As well, cocaine increases energy, alertness, concentration and self-confidence, and also causes insomnia, sexual stimulation and enhanced locomotor activity. Some cocaine users report behaviors such as irritability, anxiety, aggression, fear and paranoia (R. K. Siegel, 1977). Users may also experience tremors, vertigo, and muscle twitches (R. K. Siegel, 1977).

Cocaine mainly has an effect on the sympathetic nervous system. Along with raising heart rate and blood pressure (Resnick, Kestenbaum, & Schwartz, 1977), it causes hyperthermia (Meyer & Quenzer, 2005) and also induces release of corticotropin-releasing hormone, corticotrophin and cortisol (Baumann et al., 1995; Heesch et al., 1995; Sarnyai, Shaham, & Heinrichs, 2001). Due to these effects of the drug, using higher doses could be associated with seizures, cerebrovascular accident, intracranial hemorrhage, and cardiac arrest (Meyer & Quenzer, 2005).

2.3.2. Chronic effects

Repeated and continuous use of cocaine lead to tolerance and sensitization. Repeated exposure to cocaine can cause the brain to adapt to the drug's presence, resulting in a decrease in the sensitivity of the reward pathway to natural reinforcers (Buttner, 2012; Wolf, 2010). At the same time, there is an increase in the sensitivity of circuits involved in stress, causing negative moods and displeasure when the drug is not being taken (withdrawal signs). Because of these effects, cocaine users usually focus more on taking the drug than on relationships and natural rewards like food (Riezzo et al., 2012). Consuming cocaine more frequently and at increasingly higher doses causes

restlessness, paranoia, panic attacks, and even full-blown psychosis. During these episodes, cocaine users can experience hallucinations and lose touch with reality. The risk of adverse effects (physiological and psychological) rises with increasing higher doses and with repeated use of the drug (Riezzo et al., 2012). In addition, users experience a great deal of fatigue when the effect of the drug dissipates (R. K. Siegel, 1977).

2.3.3. Toxic effects

The Drug Abuse Warning Network (DAWN) reported that, in the United States in 2009, of the approximately one million emergency visits due to illicit drugs use, cocaine was involved in about 422,896. Different medical problems may occur with cocaine use. The most common complications are neurological (seizures, headaches, strokes, coma), cardiovascular (disturbances in heart rhythm and heart attacks), and gastrointestinal (nausea and abdominal pain) (Riezzo et al., 2012). In rare instances, cocaine-related death can occur suddenly on the first day of cocaine use or unexpectedly thereafter. Deaths often occur due to seizures or cardiac arrest. Combining cocaine and alcohol can make cocaine particularly dangerous because the two substances react together and produce cocaethylene, which might induce toxic effects from the two drugs working on the heart (Pennings, Leccese, & Wolff, 2002).

In addition, many cocaine users also use heroin, which is another dangerous combination. Individuals use these two drugs together because the sedating effects of heroin offset the stimulating effects of cocaine. However, combining cocaine and heroin

can result in users taking too high a dose of heroin without realizing it. The effect of cocaine wearing off faster might also lead to an overdose of heroin, causing the slow-down or even stoppage of the user's respiration.

3. Sex differences

Biological sex is an important factor in cocaine addiction. Many studies have demonstrated that males and females respond differently to cocaine which is our focus in this study.

3.1. Sex differences in cocaine addiction in humans

3.1.1. Cocaine use and intake

Over the past few decades, the use of cocaine by women has increased to about 40% of regular cocaine users (Jackson, Robinson, & Becker, 2006), with women reporting taking more cocaine than men (Griffin, Weiss, Mirin, & Lange, 1989). Moreover, even though men continue to have a higher likelihood than women to abuse drugs and develop addictions (Brady & Randall, 1999), women escalate their cocaine consumption faster than men (Becker, 2016). Women also report greater difficulty controlling their consumption of cocaine and frequently consume the drug more than intended (Elman, Karlsgodt, & Gastfriend, 2001). Typically, both men and women use alcohol and marijuana more than cocaine, but women show a greater lifetime addiction on cocaine. As women appear to be more vulnerable to drugs than men, the increase in the percentage of cocaine use by women could potentially lead to a public health crisis (Jackson et al., 2006).

3.1.2. Craving after abstinence periods

The withdrawal symptoms experienced by women during abstinence periods are more unpleasant than those experienced by men (Hudson & Stamp, 2011; Kosten, Gawin, Kosten, & Rounsaville, 1993). Compared to men, women show greater cue-induced

craving when exposed to cocaine-associated cues. Further, they report more frequent cravings than men (Elman et al., 2001). Becker (2016) has also reported that women show higher levels of craving induced by cues during abstinence than men. These higher levels are suggestive of greater vulnerability in terms of treatment results.

3.1.3. Treatment

In women, the escalation of cocaine intake is faster, the time from the initial use of the drug to seeking treatment is shorter, and the total consumption when seeking treatment is higher compared to men (Becker & Hu, 2008; Griffin et al., 1989). Women progress more rapidly from initial cocaine use to entering treatment (McCance-Katz, Carroll, & Rounsaville, 1999), seek treatment more readily (Fiorentine, Anglin, Gil-Rivas, & Taylor, 1997), and enter treatment at a younger age (Kosten et al., 1993). Also, women present more severe drug problems and poorer psychological functioning when they enter treatment (Kosten et al., 1993). These study results indicate that, for women, cocaine use proceeds much like a "telescoping effect". Specifically, the transition from occasional drug use to addiction moves significantly faster in women than in men, and there is also a much briefer opportunity for medical intervention and treatment in women than in men (Brady & Randall, 1999; Kawa & Robinson, 2018, 2019).

3.2. Sex differences symptoms relevant to cocaine addiction in rodents

Cocaine self-administration experiments in rodents (particularly in rats) have inherent face validity for studying several different aspects of cocaine addiction, including sex differences.

In laboratory experiments, subjects are trained in an operant conditioning chamber to nose-poke or lever-press to receive a drug injection such as cocaine. Acquisition of cocaine self-administration is assessed by the number of days that an animal needs to reach a certain level of response to the drug. This is a useful animal model to identify and study factors that make individuals vulnerable to drug use (Lynch & Taylor, 2004; Reichel, Chan, Ghee, & See, 2012).

3.2.1. Cocaine consumption

In these lab experiments, female rats acquire cocaine self-administration faster than male rats, particularly with lower doses (Hu, Crombag, Robinson, & Becker, 2004; Roth & Carroll, 2004). However, female and male animals may differ in the rate at which they learn to self-administer cocaine (Kawa, Bentzley, & Robinson, 2016; Lynch & Taylor, 2004). On the other hand, Lynch and Taylor (2004) have also suggested that male and female animals may not differ in how they acquire cocaine self-administration when test conditions promote rapid acquisition.

Using this behavioral test, female animals escalate their drug consumption faster than male animals (Lynch & Taylor, 2004; Reichel et al., 2012). Under continuous access conditions, female rats take more cocaine than their male counterparts and are also more likely to increase their cocaine intake when shifted from short- (1h) to LgA (6h) self-administration sessions (Roth & Carroll, 2004). Recently, Kawa and Robinson (2018) also showed that under intermittent access conditions, female rats take more cocaine than male rats.

3.2.2. Psychomotor sensitization

The standard operant cages used in this study consist of four horizontally aligned infrared sensors to assess the locomotor activity of the rats. Compared to male rats, female rats are more likely to develop sensitization to the psychomotor activating effects of cocaine (Glick & Hinds, 1984; Hu & Becker, 2003). Psychomotor sensitization is thought to reflect neurobiological changes linked to the pathological desire for drugs (De Vries, Schoffelmeer, Binnekade, Mulder, & Vanderschuren, 1998; Lorrain, Arnold, & Vezina, 2000; T. E. Robinson & Berridge, 1993).

3.2.3. Relapse-like behavior

In the laboratory studies with rodents, animals have extinction periods to determine the abstinence effect on responding to cocaine. During these extinction sessions, the animals are placed in the operant cages, but no cocaine is delivered and no cues are presented when the animals indicate that they want the drug. The session continues until the animals decrease and then stop the drug seeking behavior.

The next step examines whether the animals will reinitiate self-administration (reinstatement) (Becker, 2016). Studies have demonstrated that female animals show greater drug- and cue-induced reinstatement for cocaine and morphine than male animals. Stress-induced reinstatement was also shown to be higher in females (Feltenstein, Henderson, & See, 2011; Fuchs, Evans, Mehta, Case, & See, 2005). Female rats are more susceptible to cocaine-primed relapse behavior (Lynch & Carroll, 2000) but less susceptible to cue-induced relapse (Fuchs et al., 2005).

3.2.4. Motivation

in experimental laboratories, to assess the incentive motivation for a drug, rats are tested using a progressive ratio (PR) schedule of reinforcement that rises with every successive delivered. The incentive motivation is assessed by measuring the breakpoints under this schedule, which continues until the rats stop and or do not complete the response requirement (Becker, 2016). Female animals reach higher breakpoints on a PR schedule than male rats (Cummings et al., 2011; Westenbroek, Perry, & Becker, 2013). Several other studies also reported sex differences in the motivation for cocaine (Lynch, 2006; Roberts, Bennett, & Vickers, 1989)

4. Previous data and objectives of the study

Previous data

Despite similarities to human drug-using behavior, drug self-administration which occurs in lab animals does not appear to lead to addiction in all cases. Over the past few years, however, test results are beginning to show some behaviors in lab animals normally reflective of addiction in human. Overall, around one-fifth of the male test rats showed any behaviors similar to addiction, which mirrors addiction percentages in humans (Becker, 2016). From these results, we can see that the drug self-administration test has inherent face validity for studying addiction.

A widely used animal model of cocaine self-administration involves giving animals continuous access to drugs during long daily sessions (called 'Long-access' or LgA). This produces continuously high brain concentrations of drug during each session (Ahmed & Koob, 1998). However, human addicts might take cocaine intermittently during a bout of intoxication, and this would produce intermittently spiking brain cocaine levels (Beveridge et al., 2012)

A recent intermittent-access (IntA) cocaine self-administration procedure was modeled in rats, with cocaine made available for 5-min phases, separated by 25-min no-cocaine phases (Zimmer, Dobrin, & Roberts, 2011; Zimmer, Oleson, & Roberts, 2012). Studies assessing addiction-like behaviors in rats using this modern access approach suggest that the pattern of intermittency more closely resembles the temporal pattern of cocaine intake in humans; furthermore, the IntA method is effective at producing

incentive-sensitization and other addiction-like behavior in male rats, although it produces less total drug intake than in LgA rats (F. Allain, Bouayad-Gervais, & Samaha, 2018; F. Allain, Roberts, Levesque, & Samaha, 2017; Kawa et al., 2016; Zimmer et al., 2011; Zimmer et al., 2012).

To date, tests using the IntA procedure have been conducted in male animals exclusively. However, studies in humans and those which use the traditional model in animals suggest that females and males respond differently to cocaine (Becker & Hu, 2008). Women report taking more cocaine and at closer time intervals than men (Griffin et al., 1989), are more vulnerable to relapse to cocaine use (Elman et al., 2001; McKay, Rutherford, Cacciola, Kabasakalian-McKay, & Alterman, 1996), and seek treatment more readily (Fiorentine et al., 1997). Studies in laboratory animals suggest that biological factors contribute to sex differences in the susceptibility to cocaine addiction. Compared to male rats, female rats take more drug (Roth & Carroll, 2004), escalate their drug intake to a greater degree, and work harder to get a single injection (Lynch & Taylor, 2004). Female rats also show more robust psychomotor sensitization (Glick & Hinds, 1984; Hu & Becker, 2003).

Gaps in knowledge

Comparing outcomes for IntA versus LgA cocaine tests is important because these results have implications for modeling in laboratory animals changes in the brain, psychological functioning and behaviour that drive the addiction process. Unfortunately, however, most of the studies comparing IntA and LgA have been exclusively conducted

in male animals. No study has yet been conducted assessing the sex differences as a function of access conditions.

Objectives of the study

Because comparisons of IntA and LgA models is challenging long-held beliefs in our field, and because there are important sex differences in the vulnerability to cocaine addiction, there is a need to conduct studies to compare female and male rats allowed to self-administer cocaine under IntA or LgA conditions. In our tests, we assessed consumption patterns, psychomotor sensitization, and incentive motivation to take cocaine under a progressive ratio schedule of the reinforcement, following short and long drug withdrawal periods.

Article

Sex differences in cocaine self-administration behaviour under Long Access versus Intermittent Access conditions

Running head: Biological sex and cocaine use in rats

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Abstract

Women can progress more rapidly from initial cocaine use to addiction. Similarly, female rats can be more vulnerable than male rats to the incentive motivational effects of cocaine. Most preclinical studies on this issue have used self-administration procedures that provide continuous cocaine access during each session ('Long-access' or LgA, and 'Short-access'). However, intermittent-access (IntA) cocaine self-administration better models the intermittency of human cocaine use. Here, we compared cocaine use in female and male rats that received 10 daily, 6-h LgA or IntA sessions. Cocaine intake was greatest under LgA, and female LgA rats escalated their intake. Only IntA rats (both sexes) developed locomotor sensitization to self-administered cocaine and sensitization was greatest in the females. Five and 25 days after the last self-administration session, we quantified responding for cocaine (0.083-0.75 mg/kg/infusion) under a progressive ratio (PR) schedule, a measure of motivation for drug. Across conditions, females earned more cocaine infusions than males under a PR schedule. Across sexes, IntA rats also earned more infusions than LgA rats, even though IntA rats had previously taken much less cocaine. Cumulative cocaine intake significantly predicted responding for cocaine under a PR schedule in male LgA rats only. In IntA rats, the extent of locomotor sensitization significantly predicted responding under a PR schedule. Thus, LgA might be best suited to study sex differences in cocaine intake, whereas IntA might be best suited to study sex differences in sensitization-related neuroadaptations involved in cocaine addiction. This has implications for modeling distinct features of cocaine addiction in animals.

Keywords: Sex differences, Cocaine self-administration, Long Access, Intermittent Access, Psychomotor sensitization, Progressive Ratio Schedule

Introduction

Females and males can respond differently to cocaine (Becker & Hu, 2008). Men are more likely to abuse drugs and to develop addiction (Brady & Randall, 1999). However, women report taking more cocaine (Griffin et al., 1989), and women can be more vulnerable to relapse to cocaine use after abstinence (Elman et al., 2001; McKay et al., 1996). Women also progress more rapidly from initial cocaine use to entering treatment (Griffin et al., 1989; McCance-Katz et al., 1999), and they enter treatment at a younger age (Kosten et al., 1993).

Biological factors contribute to sex differences in the susceptibility to cocaine addiction. Compared to male rats, female rats are more likely to develop psychomotor sensitization to cocaine (Glick & Hinds, 1984; Hu & Becker, 2003). Psychomotor sensitization is thought to reflect neurobiological changes linked to pathological drug wanting (De Vries et al., 1998; Lorrain et al., 2000; T. E. Robinson & Berridge, 1993). In agreement, compared to male rats, female rats can acquire intravenous (i.v.) cocaine self-administration sooner (Lynch & Carroll, 1999b), they take more cocaine throughout the circadian cycle (Lynch, 2006) and they work harder for cocaine under a progressive ratio schedule of drug reinforcement (PR) (Lynch, 2006; Roberts et al., 1989). Female rats are also more susceptible to cocaine-primed relapse behavior (Lynch & Carroll, 2000). Specifically under LgA conditions, where drug is available virtually continuously for 6 h+/session, female rats take more cocaine than male rats, and they are also more likely to increase their cocaine intake when shifted from short- (1-h) to longer (6-h; LgA) self-administration sessions (Roth & Carroll, 2004).

LgA self-administration procedures are a widely accepted approach to model the behavioural, psychological and neurobiological features of cocaine addiction, and to study sex differences in these features. LgA involves giving animals virtually continuous access to cocaine for extended sessions (6+h/session) (Ahmed & Koob, 1998). This achieves high and sustained levels of cocaine intake and brain concentrations of drug (F. Allain et al., 2018; Zimmer et al., 2012). However, in humans, cocaine intake is intermittent, both within and between bouts of intoxication [(Beveridge et al., 2012; Gawin & Kleber, 1986; Ward, Haney, Fischman, & Foltin, 1997), reviewed in F. Allain, Minogianis, Roberts, and Samaha (2015)]. This pattern of intake would produce repeated peaks and troughs in blood/brain concentrations of cocaine (Beveridge et al., 2012; Zimmer et al., 2012). To model this intermittency, Zimmer et al. (2011) developed an Intermittent-Access (IntA) self-administration procedure in rats, and it is uniquely effective in producing changes in brain and behaviour that mediate the transition to addiction. IntA involves signalled periods where drug is available, separated by signalled periods where drug is not available, and this achieves spikes and troughs in brain concentrations of drug (F. Allain et al., 2018; Zimmer et al., 2011; Zimmer et al., 2012).

IntA produces much less cocaine intake than LgA but it more effectively produces features of addiction. This includes increased motivation for the drug, as measured by behavioral economics measures (Zimmer et al., 2012) or responding under a progressive ratio schedule of cocaine reinforcement [PR; (F. Allain et al., 2018)]. Potentiated motivation for cocaine also lasts longer in IntA than in LgA rats, such that increases in behavioural economics metrics of motivation for drug persist for ≥ 50 days after IntA

experience, but they dissipate within ~7 days after LgA experience (James et al., 2018). IntA rats show decreased elasticity of the cocaine demand curve, an increased willingness to respond for cocaine despite electric foot shock, and more cue-induced relapse than generally seen in LgA rats (Kawa et al., 2016). Limited IntA experience is also sufficient to produce such addiction-relevant features (F. Allain & Samaha, 2018; Calipari, Siciliano, Zimmer, & Jones, 2015). Thus, beyond how much drug is taken, the temporal pattern of drug use is decisive in predicting outcome. This is challenging long-held beliefs about what constitutes a useful procedure to model features of cocaine addiction in laboratory animals (F. Allain et al., 2018; F. Allain et al., 2015; Kawa, Allain, Robinson, & Samaha, 2019; Kawa et al., 2016).

In a recently published study, it was found that compared to male rats given IntA to cocaine, female IntA rats take more cocaine and show a faster and greater increase in motivation for the drug, as measured by behavioural economics indices (Kawa & Robinson, 2019). Thus, under IntA conditions, females could be more vulnerable to cocaine-induced incentive sensitization, an effect thought to facilitate the transition to addiction (Kawa & Robinson, 2019; T. E. Robinson & Berridge, 1993). Here we directly compared female and male rats given IntA versus LgA cocaine experience. Doing so could have important implications for comparing and contrasting the sexes on distinct features of cocaine addiction in preclinical studies using laboratory animals. Thus, we assessed cocaine consumption patterns, the development of psychomotor sensitization and motivation for cocaine, as measured by responding for the drug under a PR schedule.

Materials and Methods

Animals

The animal care committee of the Université de Montréal approved all experimental procedures, and these followed the guidelines of the Canadian Council on Animal Care. Adult male (225-250 g) and female (150-175 g) Wistar rats (Charles River Laboratories Saint Constant, QC) were housed individually under a reverse 12h/12h dark/light cycle (lights off at 8:30 am). Female and male rats were housed in separate rooms. Experiments were conducted during the dark phase of the rats' circadian cycle. Water was available ad libitum. Food was restricted to 20 g/day for females and 25 g/day for males. This is not ad libitum food access, but it exceeds estimated dietary intake for adult rats (5-7 g /day/100 g of body weight) (NRC, 1995). In both females and males, mild food restriction produces healthier rats compared to ad libitum feeding, which promotes excessive fat deposition and obesity (Martin, Ji, Maudsley, & Mattson, 2010; Rowland, 2007). Here, all rats gained weight over days (Supplementary Figure 1; Main effect of week, $p < 0.0001$; main effect of sex, $p < 0.0001$. No other comparisons were significant), indicating that the food restriction regimen was mild. Similar feeding regimens are often used in drug self-administration studies to facilitate acquisition of initial food-reinforced responding (Ahmed & Koob, 1999; T. E. Robinson, Gorny, Mitton, & Kolb, 2001), and to also reduce the total amount of cocaine needed.

Apparatus

Rats were trained and tested in standard operant cages equipped with two retractable levers (Med Associates, St Albans, VT). Each cage also contained a discrete light above

each lever, a recessed port for food delivery and a 3.33-RPM syringe pump to deliver i.v.

cocaine infusions over 5 s. The rats' catheters were connected to the cocaine-containing

syringes via tubing protected by a stainless-steel spring, passed through a liquid swivel

set in a counterbalanced arm. This allowed i.v. infusions in freely-moving rats. Pressing

the active lever produced reinforcement (food pellet or intravenous cocaine), pressing the

inactive lever had no programmed consequences. At the start of each session, levers

were inserted into the cage and the house light was illuminated. During reward delivery

and the ensuing timeout period where applicable, both levers were retracted and the light

above the active lever was illuminated. The light was then extinguished, and the levers

were again inserted into the cage to indicate reward availability. Each cage also contained

four horizontally aligned photocell beams to measure locomotion during each self-

administration session. Locomotion was computed as photocell beam breaks/min.

Acquisition of food and cocaine self-administration

As shown in Figure 1 rats were first trained to lever-press for 45-mg banana-flavoured

food pellets (grain-based; VWR, Town of Mount-Royal, QC), under a fixed ratio 1

schedule of reinforcement (FR1) with a 20-s timeout period. Sessions lasted 1 h or until

100 pellets were self-administered. Once rats met this acquisition criterion, they were

switched to an FR3 schedule for at least 2 sessions. When rats reliably self-administered

food pellets (~ 25 pellets/session, on two consecutive sessions), they were and implanted

with catheters into the right jugular vein (Samaha, Minogianis, & Nachar, 2011; Weeks,

1962). Thereafter, catheters were flushed on alternate days with either saline or saline

containing 0.2 mg/ml of heparin (Sigma-Aldrich, Oakville, ON), and 2 mg/ml of Baytril (CDMV, St Hyacinthe, QC). Rats recovered for at least 5 days before any further behavioral testing. Following recovery, rats learned to self-administer cocaine. (0.25 mg/kg/infusion; Medisca Pharmaceutique, St-Laurent, QC; dissolved in 0.9% saline, delivered over 5 s, with a 20-s timeout, under FR3), as described in F. Allain et al. (2018). Next, half of the animals of each sex were given 6-h IntA sessions (IntA females; n = 10, IntA males; n = 12), and the other half was given 6-h LgA sessions (LgA females; n = 11, LgA males; n = 11).

IntA and LgA sessions

IntA or LgA sessions (0.25 mg/kg/infusion) were given 1/day, every other day, for 10 sessions. Each IntA-session consisted of twelve 5-min periods, where cocaine was available under FR3 without a timeout period (save for each 5-s infusion), intercalated with 25-min, no-cocaine periods where levers were retracted (Zimmer et al., 2012). During LgA-sessions, cocaine was available continuously under FR3, save for a 20-s timeout following each infusion.

Cocaine self-administration under a progressive ratio schedule of reinforcement (PR)

Five days following the last IntA or LgA session (WD5), we assessed responding for cocaine (0.083, 0.5 and 0.75 mg/kg/infusion, in counterbalanced order, 1 session/dose, every other day) under a PR schedule (Minogianis, Levesque, & Samaha, 2013;

Richardson & Roberts, 1996). Rats from each group were also tested again under a PR schedule, 25 days (WD25) following the last LgA or IntA session (IntA Females; n = 6, IntA Males; n = 5, LgA Females; n = 5, LgA Males; n = 7). After the PR tests given on WD5 and WD25, catheter patency was verified by giving the rats an i.v. infusion of Propofol (10 mg/mL; 0.1 mL/rat; CDMV, St-Hyacinthe, QC), a short-acting anaesthetic [T1/2 ~27 minutes in Wistar rats; (Dutta, Matsumoto, & Ebling, 1997)]. Rats that became ataxic within ≤ 10 s of the infusion were considered to have functional catheters. Only data from such rats were included for statistical analysis. Two rats were excluded because they did not meet criteria for acquisition of reliable cocaine self-administration behaviour and 6 were excluded because they lost catheter patency.

Modeling brain cocaine concentrations

Brain cocaine concentrations (µM) were estimated using self-administration data from the 10th IntA or LgA session, in representative male rats from each group, as in (F. Allain et al., 2018; Zimmer et al., 2011; Zimmer et al., 2012). We used a pharmacokinetic model developed and validated in male rats (Pan, Menacherry, & Justice, 1991).

Statistical Analysis

Three-way ANOVA was used to analyze both the number of self-administered infusions and locomotion during the 6-h sessions (Sex x Access (LgA or IntA) x Session; Session as a within-subjects variable). Two-way ANOVA was used to analyze cumulative cocaine intake following the ten 6-h sessions. Three-way ANOVA was used to analyze lever pressing behavior during the 6-h sessions (Sex x Lever type x Session; the latter two as

within-subjects variables) and number of cocaine infusions earned under a PR schedule (Sex x Access x Dose; Dose as a within-subjects variable). Significant interaction or main effects were investigated using two-way ANOVA. The statistical significance criterion was $p \leq 0.05$. Data were analyzed with GraphPad Prism (v. 7.0d) and SPSS (v. 20).

Results

Acquisition of food and cocaine self-administration behaviour is similar in male and female rats

Female and male rats acquired reliable food (Figure 2A; $p > 0.05$) and then cocaine (Figure 2B; $p > 0.05$) self-administration behaviour in a similar average number of days. The two sexes also took a similar amount of cocaine during the last two days of acquisition training (Figure 2C; $p > 0.05$).

Sex differences in the amount of cocaine taken under LgA, but not IntA

Figure 3A shows cocaine intake and estimated brain drug concentrations during the 10th self-administration session in a representative male LgA rat and male IntA rat. Brain cocaine concentrations would be continuously high during a LgA session (Figure 3A, black curve), but would follow a spiking pattern during an IntA session [Figure 3A , grey curve; see also (F. Allain et al., 2018; Zimmer et al., 2012).

Figures 3B-F show cocaine self-administration behaviour over the ten 6-hour sessions. Both LgA and IntA rats reliably discriminated between the two levers, pressing more on the active than on the inactive lever (main effect of Lever; LgA rats, $F_{1,21} = 100.74$;

Figure 3B; IntA rats, $F_{1,19} = 16.06$; Figure 3C; All P's ≤ 0.001). Female LgA rats pressed more on the active lever than male LgA rats (Lever type X Sex interaction effect; $F_{1,21} = 5.01$, $p < 0.05$; Figure 3B), and female LgA rats also significantly escalated their active lever presses (Lever type X Session interaction effect; $F_{9,21} = 5.37$, $p < 0.001$; Figure 3B). Under IntA, there was a trend for females to press more on the active lever than males, but this was not statistically significant (All P's > 0.05; Figure 3C). Under LgA, female rats took more cocaine over sessions than male rats (Sex x Session x Access interaction effect; $F_{9,40} = 2.96$, $p < 0.003$; Figures 3D and E; Main effect of Sex in LgA rats; $F_{1,21} = 6.57$, $p < 0.02$; Figure 3D). In addition, only LgA females escalated their intake over time (Sex x Session interaction effect; $F_{9,189} = 4.25$, $p < 0.001$; Figure 3D), such that from the 5th session onwards, they took more cocaine per session than on the 1st session (All P's < 0.05). Under IntA conditions, there was a tendency for female rats to take more cocaine than male rats (Main effect of Sex, $F_{1,19} = 3.43$, $p = 0.07$; Figure 3E). Cumulative cocaine intake was greater in LgA vs. IntA rats (main effect of Access condition, $F_{1,40} = 41.40$, $p < 0.001$; Figure 3F), and it was also greatest in females, across conditions (main effect of Sex; $p < 0.05$). In summary, LgA rats took more cocaine than IntA rats. In addition, only LgA produced significant sex differences in cocaine intake (0.25 mg/kg/infusion), where females took more cocaine and only females escalated their drug intake over time.

Sex differences in the pattern of cocaine intake over sessions under both LgA and IntA
As a qualitative measure of the pattern of cocaine intake in the LgA rats, we examined individual cumulative response records on the 1st, 5th and 10th sessions in all rats. Figure 4 shows the pattern of cocaine intake during the 1st, 5th and 10th LgA sessions in two

representative female rats (Figures 4A and B) and two representative male rats (Figures 4C and D). The female rats showed clear escalation of intake from the 1st to the 10th LgA session, while most male rats did not escalate. The female rats also showed two distinct patterns of intake. As illustrated in Figure 4A, some females (4/12) showed bouts of high-frequency cocaine intake followed by brief drug-free periods throughout the LgA session, in particular on the 10th session. As illustrated in Figure 4B, other females (8/12) took closely-spaced infusions continuously during the session, and they increased the frequency of cocaine intake over the 10 sessions. In contrast, as seen in Figures 4C-D, all males generally took closely-spaced infusions almost continuously during the session, and this pattern did not significantly change over sessions.

We also examined the pattern of cocaine intake in IntA rats. Male and female IntA rats took most of their cocaine infusions within the first 60 s of each drug-available period of the session—taking closely-spaced infusions in a burst-like pattern—and this loading effect can sensitize over sessions (F. Allain et al., 2018; Kawa & Robinson, 2019). We examined sex differences in this effect here. Figure 5 shows the pattern of cocaine intake during each 5-min drug period (i.e., 300 seconds) of the 1st (Figures 5A and D), 5th (Figures 5B and E) and 10th (Figures 5C and F) IntA sessions, in a representative rat from each sex. These data suggest that both female and male IntA rats took most of their cocaine infusions in the first 60 s of each 5-min drug period, and that this loading effect sensitized over sessions in females particularly. To analyse this further, Figures 5G-K show average number of cocaine infusions during each minute of the 5-min drug periods, over the ten IntA sessions. Across sessions, both IntA females and males took most of

their cocaine in the first minute of each 5-min drug-available period [Time (in 60-s bins) x Session interaction effect; Females; $F_{36,19}$ = 3.56; All P's < 0.05; Males; All P's < 0.05, except 0-60 s vs. 60-120 s, where p > 0.05; Figures 5G-K). Over the 10 IntA sessions, female rats also escalated the number of cocaine infusions they took in the first minute of each 5-min drug period (p = 0.007; Figure 5G), but the male rats did not (p > 0.05). To explore this 'loading' effect further, we analyzed episodes of burst-like cocaine intake across the 10 IntA-sessions (Figures 5L-P). An episode of burst-like intake was counted when a rat took ≥ 3 infusions/60 s (F. Allain et al., 2018; F. Allain & Samaha, 2018; Belin, Balado, Piazza, & Deroche-Gamonet, 2009). Both sexes showed most of their episodes of burst-like intake in the first 60-s of each 5-min cocaine period [Time (in 60-s bins) x IntA session interaction effect; $F_{36,19}$ = 3.58; Main effect of 60-s bin, Females, $F_{1,\ 18}$ = 25.98; Males, $F_{1,\ 20}$ = 6.01; all P's < 0.05; Figures 5L-P). However, only females escalated the number of these episodes in the first 60-s bin, such that from the 7th session on, the females showed more of these episodes than on the 1st session (all P's < 0.01). In summary, under IntA, both female and male rats took most of their cocaine in the first minute of each 5-min drug period and both sexes showed a burst-like pattern of cocaine intake during this first minute. However, only female rats escalated both their cocaine 'loading' effect and their episodes of burst-like intake across sessions.

IntA to cocaine promotes locomotor sensitization and this effect is enhanced in female rats

Figure 6A shows locomotor activity in LgA rats during the 10 self-administration sessions. Figure 6B shows locomotor activity in IntA rats, specifically during the 5-min cocaine-available periods of each session. There was no significant Session x Access condition x Sex interaction effect ($p > 0.05$; Figures 6A-B). However, cocaine-induced locomotion was greater in IntA rats than in LgA rats (main effect of Access; $F_{1,40} = 18.83$, $p < 0.0001$; Figures 6A-B), and it was also greater in female rats, across access conditions (main effect of Sex; $F_{1,40} = 14.07$, $p < 0.002$; Figures 6A-B). In addition, as can be seen by Figures 6A-B, locomotor activity remained stable or decreased over sessions in LgA rats, whereas locomotor activity increased over sessions in IntA rats. This is confirmed by a significant Session x Access condition interaction effect ($F_{9,40} = 2.26$, $p < 0.05$; Figures 6A-B). This suggested that IntA rats but not LgA rats, developed cocaine-induced locomotor sensitization. To examine this further, we analysed locomotor activity during the 25-min no-cocaine periods of the 1st versus 10th IntA session. During the no-cocaine periods, cocaine-induced locomotion is not confounded by lever-pressing behaviour (F. Allain, Roberts, et al., 2017). There was no significant Sex x Session x Time (min) interaction effect ($p > 0.05$; Figure 6C). However, across sessions, locomotion was greatest in female IntA rats (main effect of Sex; $F_{1,40} = 265.29$, $p < 0.0001$; Figure 6C). Across sexes, locomotion was also greater on the 10th versus 1st session, indicating that both female and male IntA rats developed locomotor sensitization to self-administered cocaine (main effect of Session; $F_{1,40} = 22.84$, $p < 0.0001$; Figure 6C). To assess potential sex differences in the extent of locomotor sensitization, we compared difference scores in locomotor behavior during the 25-min no-cocaine phases of the 10th versus 1st IntA sessions. This sensitization score was greatest in female rats ($p < 0.05$; Figure 6D). In

summary, we did not observe locomotor sensitization in LgA rats. In contrast, both female and male IntA rats showed significant sensitization, and sensitization was more pronounced in the females.

Across access conditions, female rats show more incentive motivation for cocaine than male rats, and IntA produces more incentive motivation for cocaine than LgA

Five and 25 days after the last 6-h self-administration session, we measured responding for cocaine (0.083-0.75 mg/kg/infusion) under a PR schedule (Figures 7A-D). At each withdrawal time, there was no significant Session x Access condition x Sex interaction effect ($p > 0.05$; Figures 7A-D). However, across access conditions, female rats earned more cocaine infusions than male rats (main effect of Sex; Withdrawal day 5, $F_{1,40} = 5.88$, Figures 7A-B; Withdrawal day 25, $F_{1,20} = 8.48$, Figures 7C-D; all *P's* < 0.02). Across sexes, IntA rats also earned more cocaine infusions than LgA rats early (5 days) after cocaine withdrawal (Main effect of Access; $F_{1,40} = 4.38$, $p < 0.05$; Figures 6A-B). On withdrawal day 5, rats also earned more infusions at higher cocaine doses (main effect of dose; $F_{2,40} = 11.86$, $p < 0.0001$; Figures 6A-B). No other comparisons were significant. Figures 7E-H show number of cocaine infusions (0.083-0.75 mg/kg/infusion) earned under a PR schedule by individual rats in each group, 5 and 25 days after cocaine withdrawal. These data show that there were no consistent changes in responding over the abstinence period. In summary, females showed more incentive motivation for cocaine than males, and across sexes, IntA experience produced more incentive motivation for cocaine than LgA experience.

The amount of cocaine taken in the past significantly predicts incentive motivation for cocaine only in male LgA rats

Figure 8 shows that there was a significant positive correlation between cumulative cocaine intake (total number of cocaine infusions taken over the ten 6-h sessions, multiplied by 0.25 mg/kg/infusion) and responding for cocaine (0.75 mg/kg/infusion; similar results were obtained at 0.083 or 0.25 mg/kg/infusion cocaine) under a PR schedule only in male LgA rats (r^2 = 0.77, p < 0.0001; Figure 8B). Thus, male LgA rats that took high amounts of cocaine in the past later showed high incentive motivation for the drug. There was no significant relationship between cumulative cocaine intake and subsequent responding for cocaine under a PR schedule in the other groups (All *P*'s > 0.05; Figure 8).

As mentioned above, during LgA sessions, some female rats took cocaine in distinct, high-frequency bouts. Others did not. The two phenotypes did not produce differences in responding for cocaine under a PR schedule (0.083-0.75 mg/kg/infusion; All *Ps* > 0.05; data not shown).

The extent of psychomotor sensitization to cocaine predicts incentive motivation for cocaine in female and male IntA rats

We determined whether the extent of psychomotor sensitization (the scores in Figure 6D) predicted responding for cocaine (0.75 mg/kg/infusion) under a PR schedule in the IntA rats (LgA rats did not show psychomotor sensitization). Because only a subset of the rats was tested under a PR schedule 25 days after cocaine withdrawal, we pooled the rats

across withdrawal times for this correlational analysis. Figures 8E and F show that higher levels of psychomotor sensitization predicted higher levels of incentive motivation for cocaine, in both sexes (Females, r^2 = 0.65, p < 0.0002, Figure 8E; Males, r^2 = 0.54, p < 0.002, Figure 8F).

Discussion

We assessed sex differences in cocaine self-administration behavior in rats given IntA versus LgA experience. Consistent with prior work (Becker & Koob, 2016; Lynch, 2018), females were more vulnerable to the reinforcing, psychomotor sensitizing and incentive motivational effects of cocaine than males. Importantly, drug access conditions (LgA vs. IntA) influenced sex differences in the response to chronic cocaine intake. Sex differences in the sensitivity to cocaine reinforcement were more readily observed with LgA. However, sex differences in locomotor sensitization to self-administered cocaine were more readily observed with IntA. Thus, the LgA procedure was more effective in producing sex differences in the amount of cocaine consumed, whereas the IntA procedure was more effective in producing sex differences in sensitization-related changes that are thought to contribute to the addiction process [see also (Kawa & Robinson, 2019; T. E. Robinson & Berridge, 1993). These findings can inform choices about how best to model distinct features of cocaine addiction in preclinical studies using female and male rats.

Female and male rats can differ in the rate at which they learn to self-administer cocaine, but we did not observe this here. This is consistent with a recent IntA study showing that female and male rats acquire at similar rates (Kawa & Robinson, 2019).

Using continuous-access procedures, some studies report that female rats can acquire cocaine self-administration more readily than male rats (Carroll, Morgan, Lynch, Campbell, & Dess, 2002; Hu et al., 2004). Others report that male rats can acquire more readily than females (Caine et al., 2004; Swalve, Smethells, & Carroll, 2016). Still, other findings suggest that the sexes might not differ in acquisition of cocaine self-administration behaviour when test conditions promote rapid acquisition [e.g., higher cocaine doses, food restriction, operant pretraining; (Lynch & Taylor, 2004) and references therein]. Some of these conditions resemble ours. However, fully assessing sex differences in the rate of acquisition of cocaine self-administration behaviour requires testing several cocaine doses and schedules of reinforcement.

Relative to ad libitum fed rats, rats kept on a food-restricted regimen similar to the one we used can show potentiated cocaine self-administration, and more specifically, enhanced acquisition of cocaine self-administration behaviour (Campbell & Carroll, 2001). The question is whether food restriction has significantly contributed to differences in behaviour between males and females here. This is unlikely. Supposing that the food restriction was more significant in males, because they are larger than the females, one would expect both enhanced acquisition of food and cocaine self-administration behaviour in the males. However, females and males acquired food self-administration in a similar amount of time (~5 days), and throughout each experimental phase, females took either similar amounts or more cocaine than males.

Under LgA, female rats self-administered more cocaine than males, and only females escalated their intake. This is consistent with work showing that females can take more cocaine and they can also be more likely to escalate intake (Becker & Hu, 2008; Carroll et al., 2002; Lynch & Carroll, 1999b; Roberts et al., 1989; Roth & Carroll, 2004). Our male LgA rats did not escalate, similar to other LgA studies using lower cocaine doses [0.25-0.6 mg/kg/infusion; (Ferrario & Robinson, 2007; Kippin, Fuchs, & See, 2006; Mantsch, Yuferov, Mathieu-Kia, Ho, & Kreek, 2004; Minogianis et al., 2013)]. Under IntA, female and male rats took similar amounts of cocaine in the present study. However, when given access to higher cocaine doses for longer periods of time, IntA females can take more drug than IntA males (Kawa & Robinson, 2019). This suggests that sex differences in cocaine intake under IntA might emerge with a more extensive drug-taking history. Beyond the amount of drug taken, taking cocaine in a burst-like pattern is thought to contribute to the development of addiction-like features in rats (Belin et al., 2009; Martin-Garcia et al., 2014). Under IntA, we found that both sexes took most of their cocaine at the beginning of each cocaine-available period, taking closely-spaced infusions in a burst-like pattern (≥ 3 infusions/60 s) and this loading effect sensitized over sessions in females. This is generally consistent with prior findings in male (F. Allain et al., 2018; F. Allain & Samaha, 2018; Kawa et al., 2016) and female (Kawa & Robinson, 2019) IntA rats.

Our findings support the idea that intermittent cocaine use might more readily produce sensitization-related changes in brain motivation pathways in females, thus accelerating the addiction process (Kawa & Robinson, 2019). We found that female and male rats took similar amounts of cocaine under IntA, but females developed more robust psychomotor

sensitization to the drug. Psychomotor sensitization is thought to reflect brain changes that lead to sensitized drug wanting, thereby increasing the risk of addiction. IntA cocaine experience produces psychomotor sensitization [(F. Allain, Roberts, et al., 2017; F. Allain & Samaha, 2018) and present data], and the more an IntA rat is sensitized to cocaine the more it shows incentive motivation for the drug (F. Allain, Roberts, et al., 2017). This prior work was done in male rats only. Here we show that in both sexes, IntA cocaine experience promotes locomotor sensitization, and the extent of sensitization predicts responding for cocaine under a PR schedule, a measure of motivation for the drug. We found no evidence of sensitization in the LgA rats. However, we did not assess potential stereotyped movements or psychomotor sensitization after a withdrawal period, both of which can make a difference (Ferrario et al., 2005). The finding that psychomotor sensitization was greater in female versus male IntA rats also agrees with prior work using intermittent, experimenter-administered cocaine (Becker & Hu, 2008; Glick & Hinds, 1984; Hu & Becker, 2003; T. E. Robinson & Becker, 1986; Sell, Scalzitti, Thomas, & Cunningham, 2000). Thus, our findings bring together the literatures on sex differences in sensitization and cocaine self-administration and show that female rats might be more susceptible to sensitization-related neuroadaptations following voluntary IntA cocaine use [see also (Kawa & Robinson, 2019)]. This is reminiscent of clinical observations, where women enter treatment following a shorter period of cocaine use (Griffin et al., 1989; McCance-Katz et al., 1999), suggesting a more rapid course of addiction. Thus, it is possible that women transition faster to cocaine addiction because they are more vulnerable to sensitization-related neuroplasticity induced by the drug (Kawa & Robinson, 2019). However, it is not clear that this fast treatment seeking is specific for addiction

because in general women will seek treatment sooner in several kinds health-related issues.

Compared to LgA cocaine experience, IntA more effectively produces sensitization of incentive motivation for the drug, as indicated by both enhanced responding for cocaine under a PR schedule of cocaine reinforcement and increases in behavioural economics metrics of motivation to take cocaine (F. Allain et al., 2018; Zimmer et al., 2012). In agreement with this, we found that across sexes, LgA rats took twice more cocaine than IntA rats, but IntA rats later showed higher levels of motivation to take cocaine (as measured by infusions earned under a PR schedule). This concords with previous studies showing that compared to LgA, IntA produces more incentive motivation for cocaine (F. Allain et al., 2018; Zimmer et al., 2012). We found that responding for cocaine under a PR schedule was stable after 5 or 25 days of forced abstinence from the drug. This is in accord with James et al. (2018), but it is in contrast to Kawa and Robinson (2019), who found that motivation for cocaine increases after abstinence from IntA. Notably, Kawa and Robinson (2019) gave their rats 30 IntA sessions. This is significantly more than the 10 sessions used here or the 14 sessions used in James et al. (2018). These issues notwithstanding, our findings support the idea that IntA cocaine experience is uniquely effective in increasing incentive motivation for drug. These results are consistent with others showing that intermittent 'spikes' in brain cocaine concentrations are more effective than high and escalating brain concentrations in producing behavioural features relevant to cocaine addiction (F. Allain et al., 2018; Bentzley, Jhou, & Aston-Jones, 2014; James et al., 2018; Kawa et al., 2016; Zimmer et al., 2012).

There could be a number of explanations for the sex differences we observed. For instance, gonadal hormones have effects in the brain that can contribute to addiction (Becker, Perry, & Westenbroek, 2012; Lynch, 2018). We did not monitor estrous cycle here and estrous phase could influence our outcome measures. For example, females reach higher breakpoints for cocaine when estradiol levels are high (Roberts et al., 1989). The current findings, along with recently published work (Kawa & Robinson, 2019) are an initial step in characterizing the effects of IntA cocaine intake in female and male animals. As a first step, '..*inclusion of intact females, without regard to estrous cycle, and intact males is a valid approach to learn about females in neuroscience research*' (Becker, Prendergast, & Liang, 2016). In parallel, sex differences in the response to cocaine are also seen in the absence of gonadal hormones, suggesting that the brain systems that mediate cocaine's effects could also differ between the sexes (Hu & Becker, 2003; Hu et al., 2004). Of note, as seen in many preclinical and clinical neuroscience studies (Maney, 2016), our female and male animals also overlapped extensively on all behavioural measures. We illustrate this with individual values in some of the figures. Thus, in considering the amount and pattern of cocaine intake, psychomotor sensitization and incentive motivation for cocaine, there is not one phenotype typical of females and the other typical of males. This suggests that factors in addition to sex contribute to variation in the response to cocaine with IntA or LgA experience.

Conclusion

Comparing different addiction models and doing so in both female and male animals is needed to advance the field, because different models could allow us to probe the multiple neurobiological, psychological and behavioural mechanisms involved in addiction in both women and men. Our findings suggest that LgA procedures could be useful to model sex differences in the positive reinforcing effects of cocaine, as measured by drug intake under fixed ratio schedules of reinforcement. In parallel, IntA procedures could be better suited to study sex differences in sensitization-related neuroadapatations that are thought to contribute to pathological drug wanting and addiction. This has implications for the design of studies examining sex differences in the response to cocaine at different stages of the addiction process.

Author Contributions

ANS designed the experiments. HA and NAN performed the experiments with guidance from FA. HA and FA analyzed the data. HA and ANS wrote the manuscript with input from FA. All authors critically reviewed the content and approved the final version for publication.

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Figure legends

Figure 1. The sequence of experimental events. Male and female rats were trained to press a test lever for banana-flavored food pellets. Subsequently, they were implanted with intravenous catheters and allowed to self-administer cocaine (0.25 mg/kg/infusion, delivered intravenously over 5 s) during 1-h sessions. Rats of each sex were then assigned to self-administer cocaine during 6-h Long-Access sessions (LgA-rats) or 6-h Intermittent-Access sessions (IntA-rats). Five and 25 days following the last 6-h self-administration session, incentive motivation for cocaine was assessed by measuring breakpoints for the drug achieved under a progressive ratio schedule of reinforcement (PR).

Figure 2. Female and male rats do not differ in the acquisition of food or cocaine self-administration behavior. There were no sex differences in the number of days to acquire (A) food and (B) cocaine self-administration behavior, or in (C) cumulative cocaine intake over the last two days of acquisition training. Data are mean ± SEM. (n = 10 – 12/group).

Figure 3. Female rats take significantly more cocaine than male rats under Long-Access (LgA) self-administration conditions, but drug consumption is similar across sexes under Intermittent-Access (IntA). (A) Patterns of cocaine intake and estimated brain cocaine concentrations during the 10th session in representative male rats from the LgA and IntA groups. Under LgA, brain concentrations of cocaine would be continuously elevated. Under IntA, brain cocaine concentrations would follow a spiking pattern. Under LgA,

female rats escalated both (B) their active lever presses and (D) the number of self-administered infusions, but male LgA did not. Under IntA, (C) lever pressing behaviour and (E) the number of self-administered infusions were comparable across the sexes, and did not escalate over time. (F) female LgA rats had the highest levels of cumulative cocaine intake. AL – active lever; IL – inactive lever. #$p < 0.05$. *$p < 0.0001$, vs. the first LgA session in the female rats. &$p < 0.0001$, LgA vs. IntA. Data are mean ± SEM. n = 10 – 12/group.

Figure 4. Sex differences in the pattern of cocaine intake under Long-Access (LgA) conditions. Patterns of cocaine intake in representative (A and B) female and (C and D) male LgA rats during the 1st, 5th and 10th LgA-sessions. Each point represents one self-administered infusion. Female rats showed two different within-session patterns of cocaine intake. These are illustrated by data from female rat #1 and female rat #2. Female rat #1 consumed cocaine in distinct high-frequency bouts interspersed with brief drug-free periods, in particular on the 10th LgA session. Female rat #2 took closely-spaced infusions continuously during each LgA session, with the frequency of intake being highest on the 10th session. As illustrated by data from male rats #1 and #2, the males generally took closely-spaced infusions throughout each session, with no clear change across sessions. Finally, the female rats significantly escalated their cocaine use over sessions, whereas the males did not.

Figure 5. Sex differences in the pattern of cocaine intake under Intermittent-Access (IntA) conditions. Patterns of cocaine intake in a representative (A – C) female and (D –

F) male IntA rat during the 1st, 5th and 10th IntA-sessions. The Y-axis shows each 5-min cocaine available period of the 6-h IntA session, while the X-axis shows the 5-min drug available period in 60-second (s) bins. Each point represents one self-administered infusion. Both the female and male rat took most of their cocaine infusions in the first minute of each 5-min drug period. This is further analysed in (G – K), which show the number of cocaine infusions that females versus males took during (G) the first minute (0 – 60 s), (H) 2nd minute (60 – 120 s), (I) 3rd minute, (120 – 180 s), (J) 4th minute (180 – 240 s) and (K) 5th minute (240 – 300 s) of each cocaine available period across the ten-IntA sessions. (G) Both female and male rats took most of their cocaine infusions in the first minute of each 5-min drug available period, and this 'loading' effect significantly sensitized over sessions only in females. (L – P) burst-like events (≥ 3 infusions/60 secs) in females versus males during each minute of the 5-min drug available periods, across the ten-IntA sessions. (L) both female and male rats showed most of their burst-like events in the first minute of each 5-min drug available period, and this behavior sensitized significantly over sessions only in female rats. *$p < 0.05$, vs. 1st IntA-session in female rats. #$p < 0.05$. Data are mean ± SEM. n = 10 – 12/group.

Figure 6. Intermittent-Access (IntA), but not Long-Access (LgA) rats developed robust locomotor sensitization to self-administered cocaine, and sensitization was greatest in female IntA rats. (A) Locomotor activity (measured as beam breaks/min) in female versus male LgA rats over the 10 cocaine self-administration sessions. (B) Locomotor activity in female versus male IntA rats during the cocaine available periods of each IntA session. The female IntA rats showed a greater locomotor response to self-administered cocaine than the male IntA rats. (C) Time course of locomotor activity in female versus male rats during the no cocaine available periods of the 1st

and 10th IntA sessions. In both sexes, cocaine-induced locomotor activity was greater on the 10th versus the 1st IntA session, indicating psychomotor sensitization to self-administered cocaine. (D) The difference in the locomotor score during the no cocaine available periods of the 10th and 1st IntA sessions in female versus male rats. This score was higher in females vs. males, indicating greater psychomotor sensitization. $^{*}p < 0.05$. $^{\#}p < 0.001$. Data are mean ± SEM. n = 10 – 12/group.

Figure 7. Female rats earned more cocaine infusions under a PR schedule of drug reinforcement than male rats, and across sexes, Intermittent-Access (IntA) rats earned more cocaine infusions than Long-Access (LgA) rats. Number of infusions earned under a PR schedule of cocaine reinforcement in (A) LgA and (B) IntA female and male rats, 5 days after the last self-administration session. Number of infusions earned under a PR schedule of cocaine reinforcement in (C) LgA and (D) IntA female and male rats, 25 days after the last self-administration session. (E – H) show cocaine infusions earned under a PR schedule by individual rats from each group, tested on WD5 and again on WD25. Data are mean ± SEM. n = 10 – 12/group on withdrawal day 5; 5 – 7/group on withdrawal day 25.

Figure 8. The relationship between number of cocaine (0.75 mg/kg/infusion) infusions earned under a progressive ratio schedule and past cumulative cocaine intake or extent of psychomotor sensitization. (A – B) There was a significant positive correlation between previous cumulative cocaine intake and responding for cocaine under a PR schedule only in male LgA rats. There was no significant relationship between these variables in the

other groups. In both (E) female and (F) male IntA rats, there was a significant positive correlation between the degree of psychomotor sensitization (difference score in locomotor activity/min during the no-cocaine available periods of the 10th versus 1st IntA sessions) and responding for cocaine under a PR schedule. Data are mean ± SEM.

Supplementary Figure 1. Female and male rats gained weight at a comparable rate over time. Data are mean ± SEM. (n = 22/group).
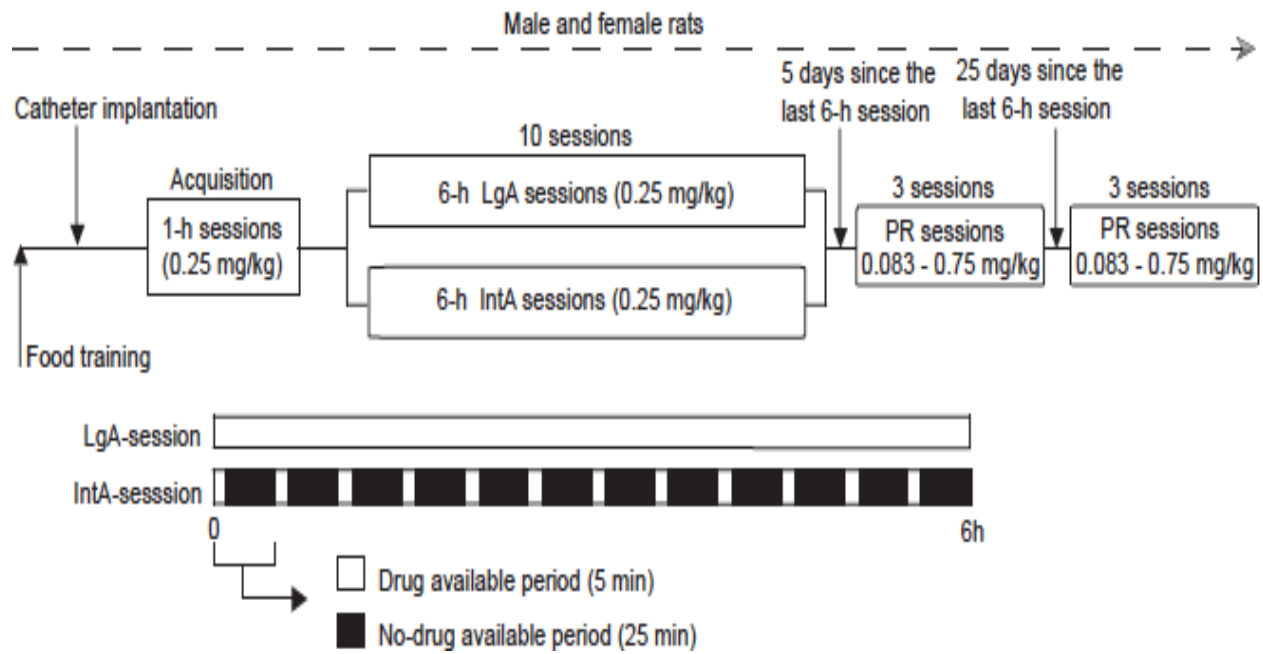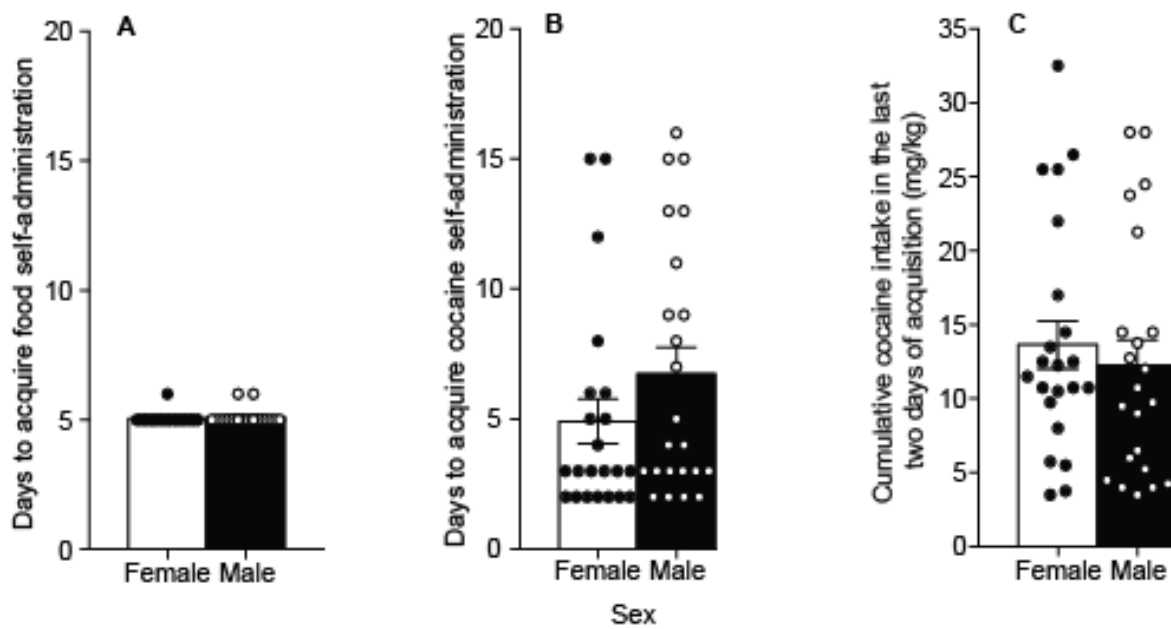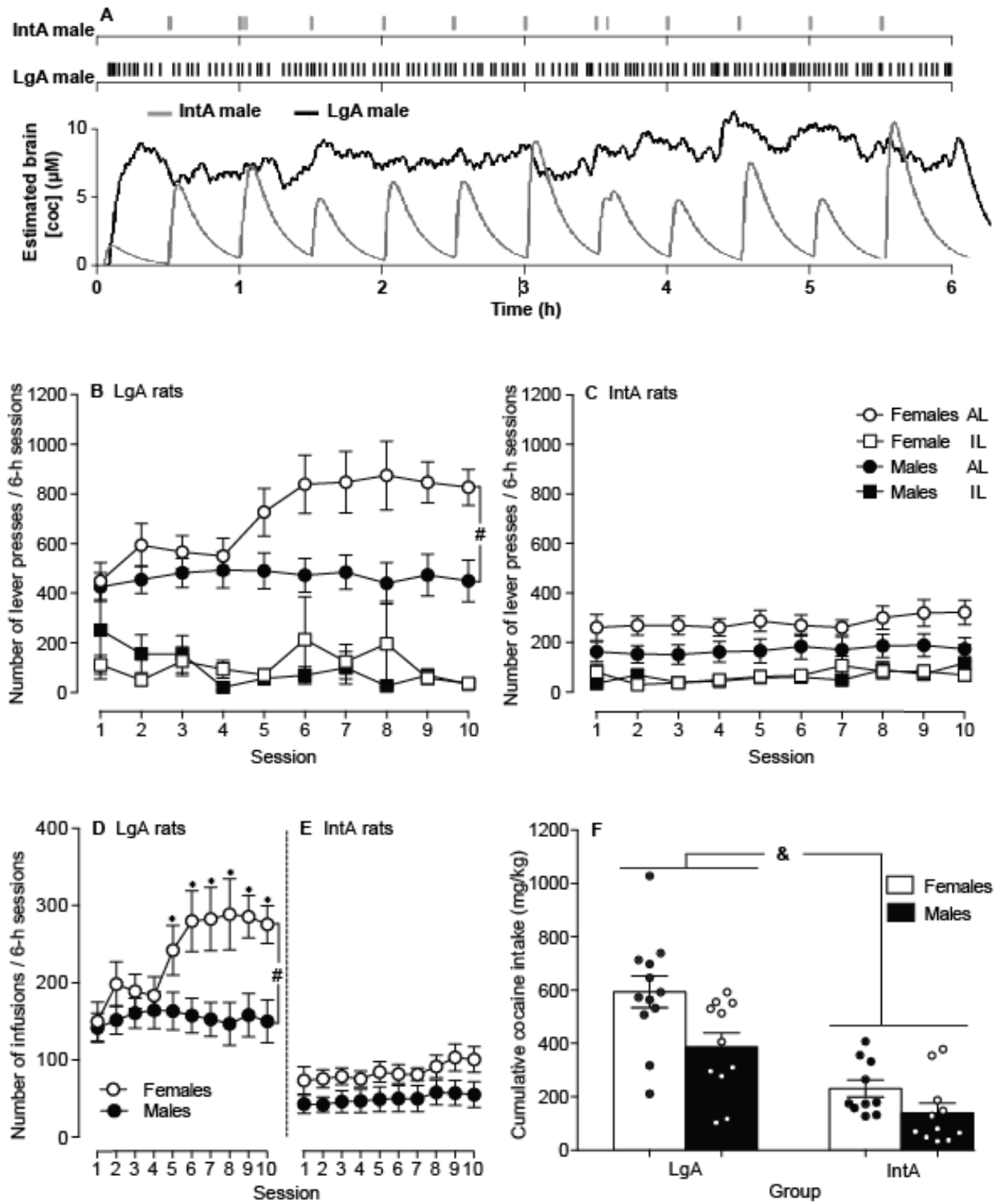
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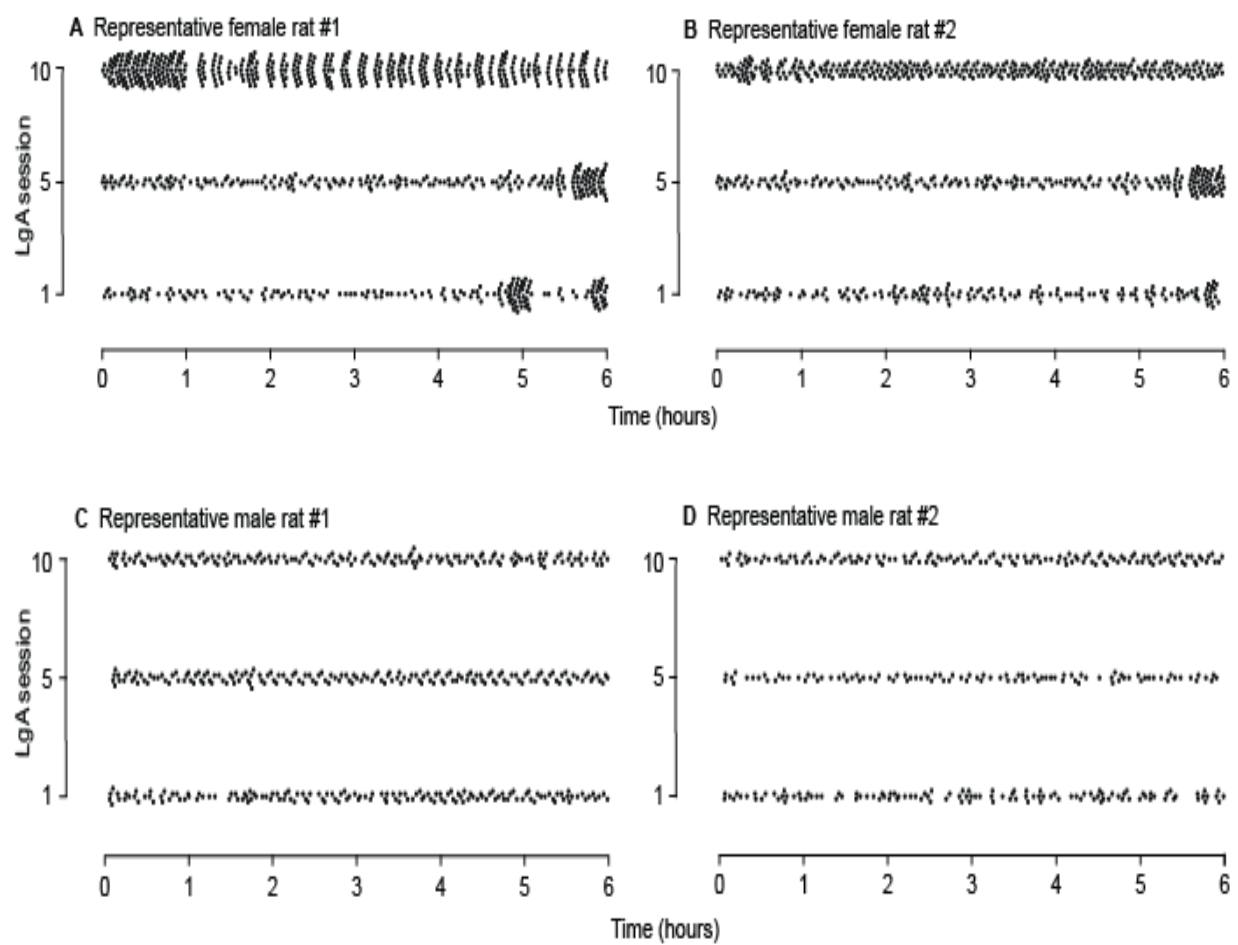
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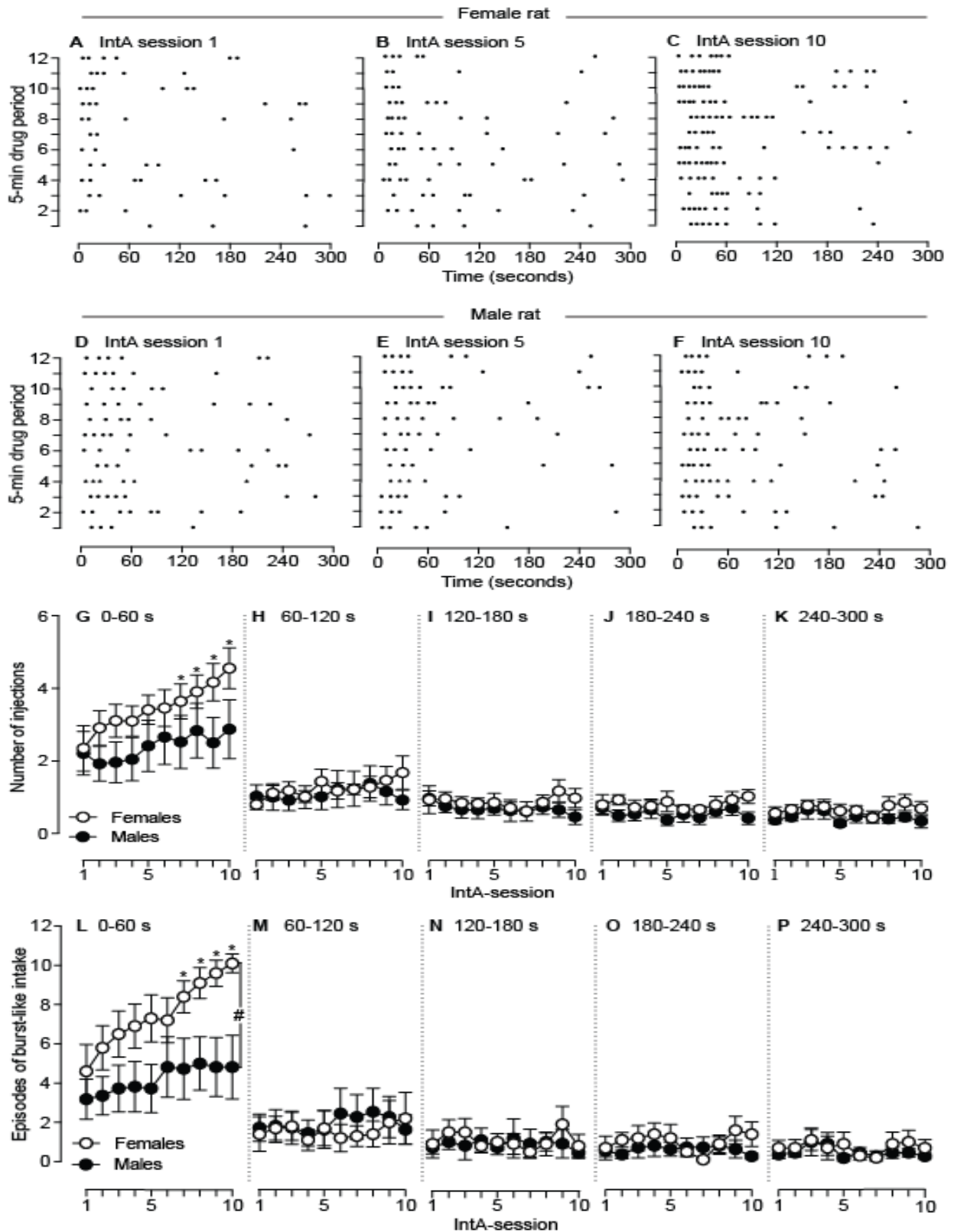
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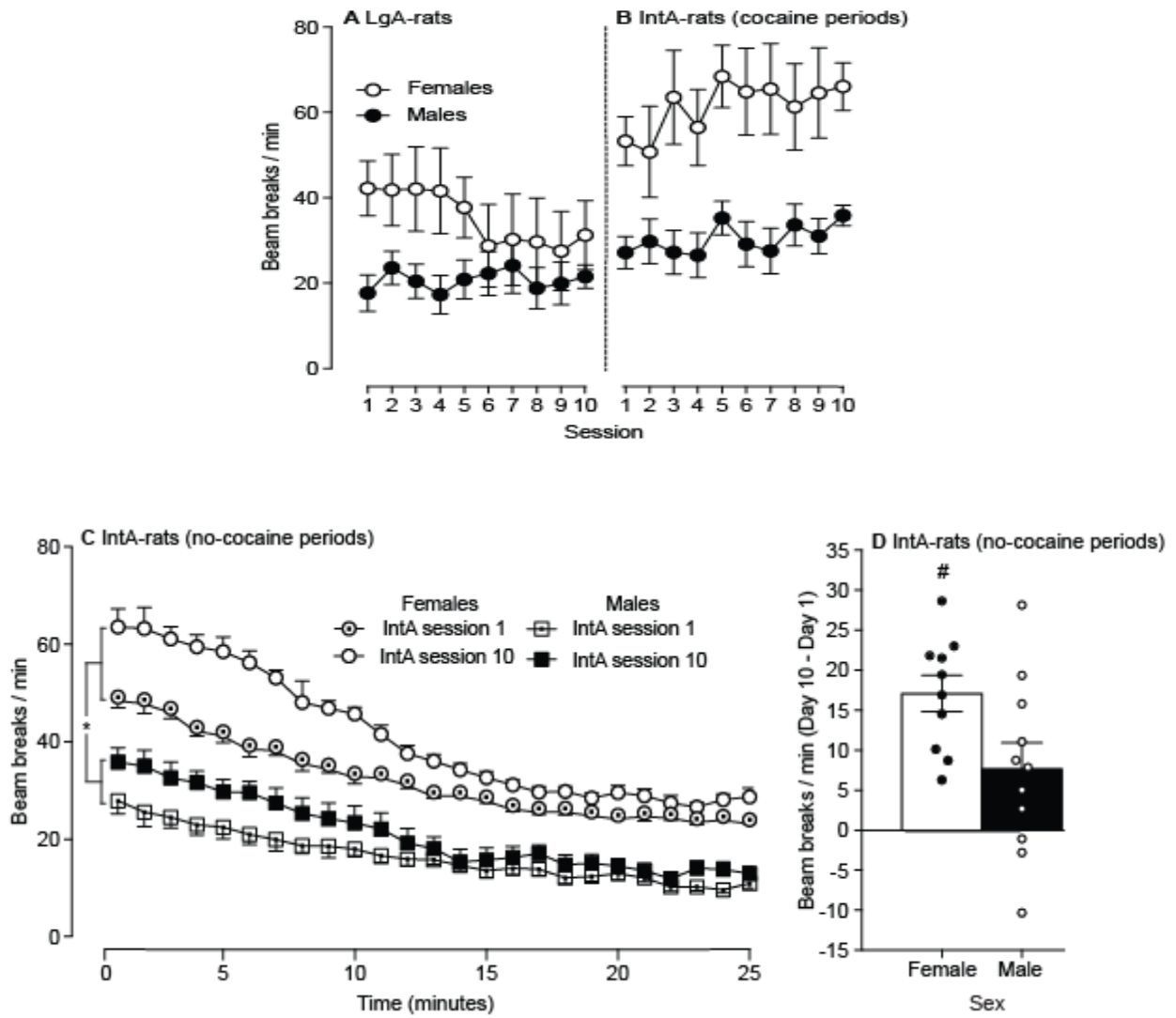
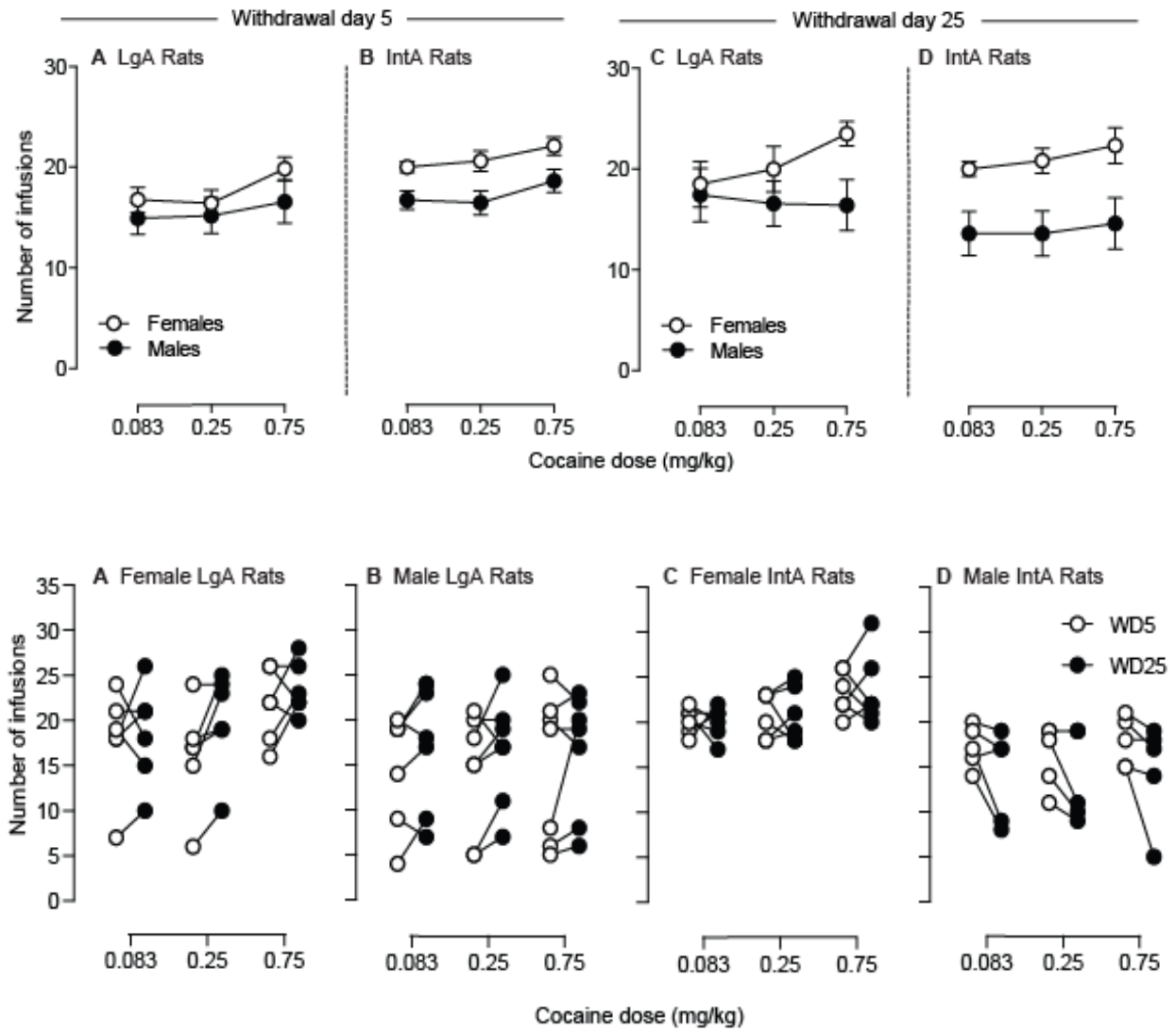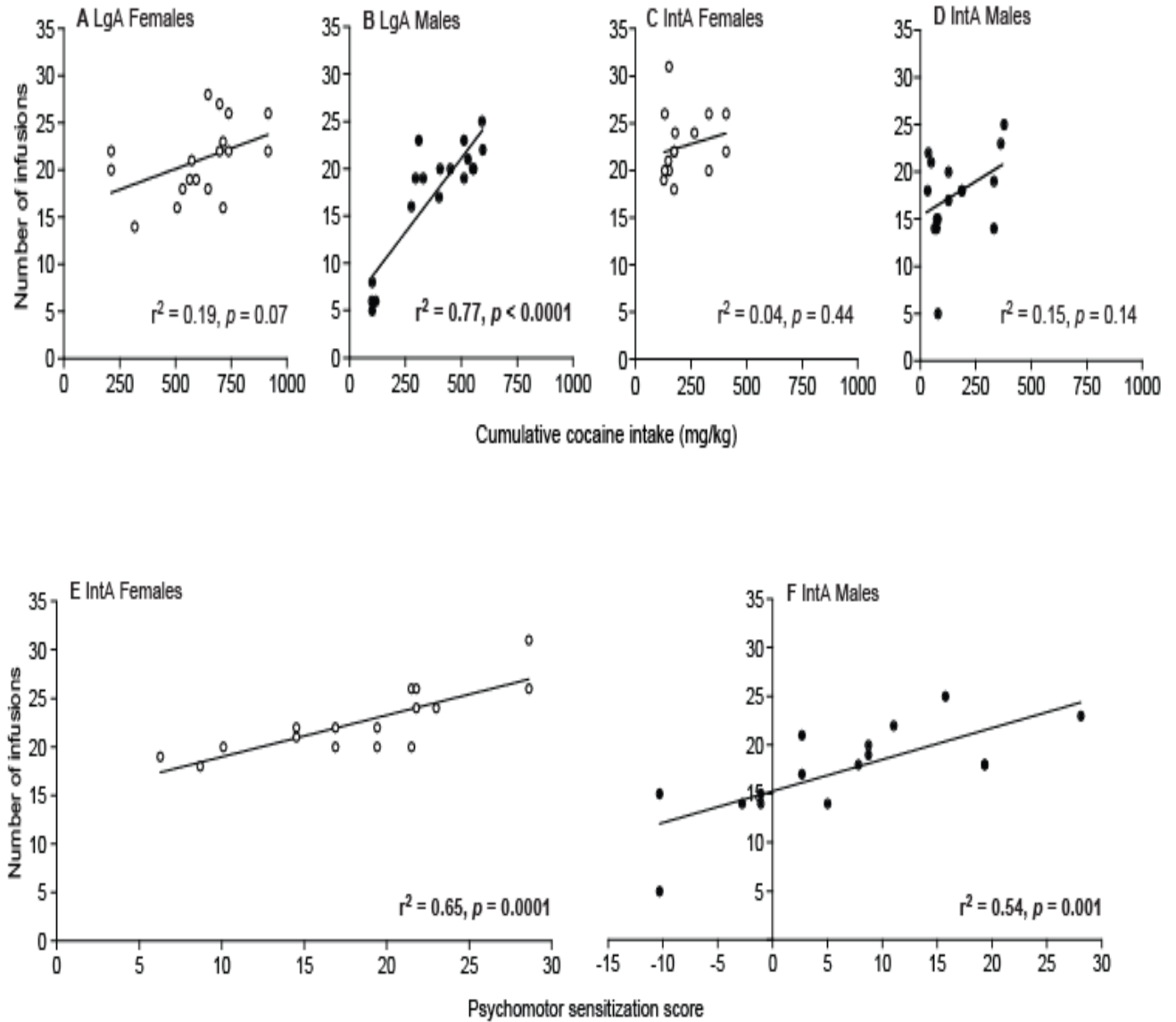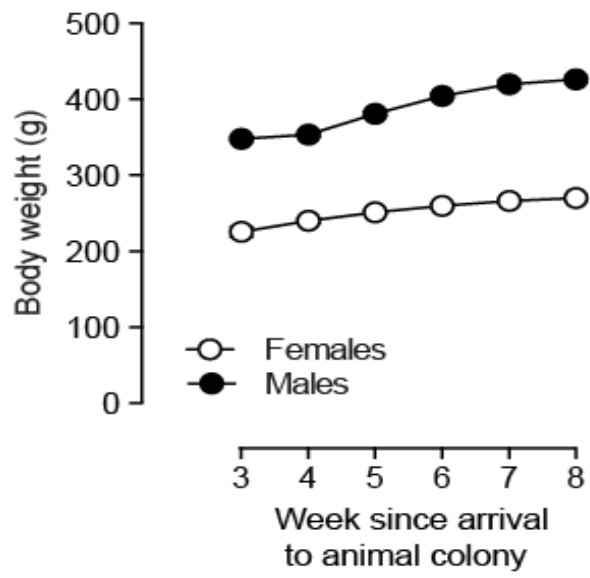## Figure 7

Figure 8

Supplementary Figure 1

Discussion

5. Summary and implications of results

As already noted, females and males respond differently to cocaine (Becker & Hu, 2008). Understanding the sexual dimorphism in cocaine-taking and cocaine-seeking behaviors would facilitate better prevention and/or treatment strategies for cocaine addiction. Consistent with prior work (Becker & Koob, 2016; Lynch, 2018), the present study found that female rats were more vulnerable to the reinforcing, psychomotor sensitization and incentive motivational effects of cocaine than male rats. It was also noted here that drug access conditions (LgA vs. IntA) determine the ability to detect sex differences in some of the behavioral and psychological effects of chronic cocaine intake.

Sex differences in regards to sensitivity to cocaine reinforcement were more readily observed with LgA. Specifically, when tested under LgA, females self-administered more cocaine than males and only female rats escalated their intake over time. In contrast, female and male rats took similar amounts of cocaine under IntA conditions. However, sex differences in both psychomotor sensitization and incentive motivation for cocaine were more readily observed with IntA. Only IntA rats developed psychomotor sensitization to self-administered cocaine, and female IntA rats showed robust sensitization than male IntA rats. Female IntA rats also showed more incentive motivation for cocaine than male IntA rats, as measured under PR, both at early and late withdrawal periods.

Thus, the LgA procedure could be useful in investigating sex differences in the positive reinforcing value of cocaine. On the other hand, the IntA procedure could be better suited to investigate sex differences in the psychomotor sensitization and incentive motivational

properties of a drug. The findings of this study have implications for the design of studies examining sex differences in the neurobiological, psychological and behavioral response to cocaine at different stages of the addiction process.

6. Cocaine self-administration access conditions

One of the major susceptibility factors of cocaine addiction is whether a given individual continues to take drug using routes of administration, doses, and patterns of use that produce neuroadaptations, facilitating incentive-sensitization (F. Allain et al., 2015). Thus, the pattern of cocaine self-administration appears to be a critical determinant of cocaine addiction.

A commonly used rodent model in cocaine self-administration studies involves comparing rats given continuous long access to the drug (LgA, typically 6-hour daily sessions) with those given relatively short access to cocaine (ShA, typically 1-2 daily sessions) (Ahmed & Koob, 1998). Relative to ShA, the LgA procedure can promote robust escalation of cocaine consumption over days (Ahmed & Koob, 1998; Mandt, Copenhagen, Zahniser, & Allen, 2015; Mantsch et al., 2004), intense motivation to take cocaine (Hao, Martin-Fardon, & Weiss, 2010; Paterson & Markou, 2003), and greater cocaine-induced relapse after abstinence (Knackstedt & Kalivas, 2007; Mantsch et al., 2004). Such results led to the suggestion that consuming large amounts of drugs continuously in long access sessions is required for developing addiction symptoms (Ahmed & Koob, 1998).

Recent findings, however, have challenged this belief. Cocaine users reportedly consume cocaine intermittently, both within and between bouts of intoxication (F. Allain et al. (2015)). High intensity binges—lasting hours to days—are interspersed with days where little or no drug is used (Gawin & Kleber, 1986). These abstinence periods can be

used to mobilize resources needed to obtain the next dose (Simon et al., 2002; Ward et al., 1997). Experienced cocaine users can also experience numerous episodes of euphoria within a bout of intake (Gawin, 1991). Survey data suggests that compared to people who have used cocaine for fewer years (4-9 years), more experienced cocaine users (25-32 years) consume their drug in fewer intervals within a bout of intoxication (Beveridge et al., 2012). This would produce repeated peaks in blood/brain concentrations of cocaine during the bout of drug intake, rather than continuous high concentrations (Beveridge et al., 2012).

Zimmer et al. (2011) developed a self-administration procedure in rats to model the intermittent pattern of cocaine use reported in experienced human cocaine users. This intermittent-access (IntA) procedure involves giving animals cocaine access for 5-min bins, separated by 25-min epochs during which drug is not available. The IntA-sessions are also extended (4-6 hours), similar to LgA-sessions. In contrast to traditional self-administration procedures (i.e., long-access [LgA; 6h/session] or short-access [1-3h/session] procedures), where drug access is continuous during each session and blood/brain concentrations of cocaine are continuously high, IntA achieves spikes and troughs in blood/brain concentrations of drug (Zimmer et al., 2011; Zimmer et al., 2012).

Compared to continuous-access procedures, in particular LgA, studies found that IntA more effectively produces changes in the brain and in behavior that are relevant to addiction (Calipari, Ferris, Siciliano, Zimmer, & Jones, 2014; Calipari, Ferris, Zimmer, Roberts, & Jones, 2013). Rats with IntA experience are sensitized to cocaine-,

methylphenidate- and methamphetamine-induced blockades of the dopamine transporter in the nucleus accumbens, whereas rats with LgA experience show tolerance to cocaine-induced inhibition of the transporter (Calipari et al., 2014; Calipari et al., 2013). IntA rats also take much less drug than LgA rats, but IntA rats are more likely to show binge-like, high-frequency cocaine use, strong psychomotor sensitization, enhanced incentive motivation for cocaine, decreased elasticity of the cocaine demand curve, an increased willingness to respond for cocaine despite electric foot shock, and more cue-induced relapse than generally seen in LgA rats (F. Allain et al., 2018; F. Allain, Roberts, et al., 2017; Kawa et al., 2016; Zimmer et al., 2011; Zimmer et al., 2012). Several studies have demonstrated that addiction-like behaviors only occur after extensive experience with cocaine self-administration (Belin *et al.*, 2016; Piazza and Deroche-Gamonet, 2014). However, the recent intermittent access condition was shown to promote compulsive cocaine self-administration, despite the animals having consumed much less drug compared to the extended access condition (Kawa et al., 2016). The pharmacokinetics associated with IntA may be more effective in producing neuroadaptations that lead to pathological motivation for cocaine than other self-administration models (LgA and prolonged ShA) and may also better match patterns of use in humans (F. Allain et al., 2015; Zimmer et al., 2012).

These findings are challenging dogma in the addiction literature about what constitutes a good animal model of drug addiction (F. Allain, Bouayad-Gervais, & Samaha, 2017; F. Allain et al., 2015; Kawa et al., 2016), following on the mantra: "*All models are wrong but some models are useful*" (Box *et al.*, 2005). As it is important to determine which animal

models of addiction are more useful and for what purpose, comparisons of female and male cocaine intake as a function of cocaine access conditions were made in this study, such that rats either had 'Long Access' to cocaine (continuous access to drug for 6 h/session) or 'Intermittent Access' to cocaine (intermittent drug access for 6 h/session). The objectives of this study follow recent data showing that IntA is uniquely effective in producing the neurobiological, psychological and behavioral changes that underlie the transition to cocaine addiction.

7. Brain cocaine concentration model

Brain cocaine concentrations (µM) were estimated using the following formula derived by Pan et al. (1991):

$$C = dA.\left(e^{-\beta t} - e^{-\alpha t}\right) \ with \ A = \frac{k}{v.(\alpha - \beta)}$$

where d is the dose of cocaine (0.25 mg/kg/injection) and A is a constant (9.63 µM.mg^{-1}.kg) that integrates the rate constant 'k' for transfer of cocaine from blood to brain, the apparent volume 'v' of brain distribution and two constants and 'α' (0.6419 min^{-1}) and 'β' (0.0971 min^{-1}), which accounts for removal of cocaine from the system via redistribution or elimination. Finally, t in minutes is the time elapsed since the last self-administered infusion. In the experiment, a 5-s time resolution was applied for all estimates, and Dr. David C. S. Roberts kindly provided the Python script used to model brain cocaine concentrations.

using self-administration data from the tenth session, we showed representative response patterns that generated by each of the two self-administration procedures (LgA and IntA) and the corresponding modeled brain levels. The right axis is the cumulative dose consumed throughout the 10th session, while the left axis is the mathematically modeled brain-cocaine concentration. Only data from male rats were used because the pharmacokinetic model employed here was developed and validated in male rats only (Pan et al., 1991). This mathematical model has been used extensively to estimate brain cocaine concentrations following self- and experimenter-administered i.v. injections (Nicola & Deadwyler, 2000; Samaha, Li, & Robinson, 2002; Wise et al., 1995; Zimmer et al., 2011; Zimmer et al., 2012).

IntA rats had access to cocaine under FR3 during 5-min phases intercalated with 25-min no-cocaine phases. In contrast, LgA male rats had continuous access to cocaine under FR3 with a 20-s timeout period after each infusion. In this study, findings showing continuously high or spiking patterns of brain cocaine concentrations in the LgA and IntA sessions, respectively, are in agreement with those of previous work by others (F. Allain et al., 2018; Zimmer et al., 2011).

8. Cocaine self-administration behavior under the two access conditions

Humans and animals show dramatic sex differences in their responses to patterns of cocaine intake (Lukas et al., 1996; Russo et al., 2003), with women reportedly taking more cocaine than men (Geiffin *et al.*, 1989). Several results from pre-clinical self-administration tests performed on rats have been published. The findings indicate a higher level of drug self-administration in females than in males if the females are granted non-stop 24-hour cocaine access (Lynch & Taylor, 2004). Females also consume more when female and male rats have access to the drug using LgA conditions (Anker, Zlebnik, Navin, & Carroll, 2011; Carroll et al., 2002; Lynch & Carroll, 1999a; Roberts et al., 1989; Roth & Carroll, 2004). The findings of the present study, which show that female rats self-administer more cocaine than do male rats under the traditional self-administration procedure, are consistent with larger bodies of literature describing sex differences in cocaine consumption. The present results also demonstrate that females under LgA conditions increase their lever presses more rapidly in order to receive progressively higher amounts of cocaine and escalate their intake over the 10 sessions. In contrast, male rats in the study did not show any escalation in their intake. The data compiled on male LgA rats in the present study is similar to data from other LgA studies which used lower cocaine doses (Ferrario & Robinson, 2007; Kippin et al., 2006; Mantsch et al., 2004; Minogianis et al., 2013).

The only study that compared females and males under IntA conditions reported that female rats consume more cocaine than males during 30 days of IntA sessions (Kawa & Robinson, 2019). In comparison, female IntA rats in the present study did not consume

significantly more cocaine than IntA male rats. However, there was still a tendency for females IntA rats to consume more cocaine. It is plausible that the greater number of IntA sessions prior to PR testing and usage of higher cocaine dosages (Kawa & Robinson, 2019) may have contributed to the differences in these observations. These findings also suggest that sex differences in cocaine intake under IntA might require more extensive drug exposure. Additionally, the study design here incorporated testing of only one dose of cocaine during IntA and LgA sessions. Thus, further work is required to determine how sex differences in cocaine consumption might interact with drug dose and/or number of self-administration sessions.

Taking cocaine in a burst-like pattern is thought to contribute to the development of addiction-like symptoms in rats (Belin et al., 2009; Martin-Garcia et al., 2014). Developing burst-like patterns under the IntA procedure is indicated by multiple episodes of high-frequency drug intake during the session, where animals load up on the drug each time it becomes available again. My findings showed that both female and male IntA rats took most of their cocaine at the beginning of each cocaine-available period, taking very closely-spaced infusions in a burst-like pattern (taking ≥ 3 infusions/60 s). This loading effect sensitized significantly over sessions only in females, which is consistent with prior findings in male (F. Allain et al., 2018; F. Allain & Samaha, 2018; Kawa et al., 2016) and female (Kawa & Robinson, 2019) IntA rats, suggesting that the distinct spiking pattern in brain concentrations of cocaine produced by the IntA condition promotes intermittent episodes of high-frequency drug consumption.

IntA rats in the present study (male or female) did not significantly escalate their cocaine intake over time. This concords with the notion that the development of a burst-like pattern of cocaine use and the escalation of intake are dissociable phenomena (F. Allain et al., 2018). However, it remains to be determined whether the two phenomena are linked under LgA conditions. During an LgA-session, brain concentrations of cocaine would stay high because the access to the drug is continuous (F. Allain et al., 2018; F. Allain, Roberts, et al., 2017; Kawa et al., 2016; Zimmer et al., 2011; Zimmer et al., 2012), which might not evoke multiple episodes of burst-like pattern during the session (F. Allain & Samaha, 2018). In the present study, cumulative response records of all LgA rats were visually inspected. The inspections revealed that some LgA females developed a burst-like pattern of cocaine use over time, but no males did. This parallels the escalation effect, which was observed in LgA females in the study, but not in LgA males. All males generally had the same pattern of cocaine intake. They took closely-spaced infusions almost continuously during the session, and this pattern did not significantly change over sessions. The next section will be talk about sex differences in acquisition of cocaine self-administration behavior.

The experiment in the present research work was not designed to systematically assess the sex differences in the rate of acquisition of cocaine self-administration behavior. Doing so would require testing several cocaine doses and schedules of reinforcement. However, it was interesting to analyze the available data and assess whether female and male rats showed differences in acquisition at the dose used in the study. Criteria for acquisition of cocaine self-administration behavior were self-

administration of ≥ 6 infusions/session, pressing at least twice more on the active versus the inactive lever, and taking cocaine in a regular pattern throughout the session. Female and male rats took the same average number of days to meet these criteria, and they also took a similar amount of cocaine during the last two days of cocaine self-administration training. These results showed that female and male rats did not differ in acquisition of cocaine self-administration behavior.

Previous work by others, both in support and in contradiction to this notion, have been reported in the literature (Lynch & Taylor, 2004). Some groups have also reported that females acquire self-administration of cocaine faster than males (Becker & Koob, 2016; Hu et al., 2004; Lynch, 2008, 2018). Methodological differences could explain these discrepancies. For instance, Lynch and Taylor (2004), suggest that females and males might not show differences in the acquisition of cocaine self-administration behavior under experimental conditions that promote rapid acquisition (e.g., higher cocaine doses, food restriction, operant pre-training). These conditions resemble ours. However, under conditions that can slow acquisition (e.g., low doses, *ad libitum* feeding), females might acquire at faster rates than males.

9. Development of psychomotor sensitization

Psychomotor sensitization is a long-lasting increase in drug-induced psychomotor activity in response to repeated exposure to drug (T. E. Robinson & Berridge, 1993). It has attracted attention of many researchers because it is thought to reflect brain changes that lead to pathological drug-wanting (De Vries et al., 1998; Lorrain et al., 2000; T. E. Robinson & Berridge, 1993). In mammals, psychomotor and dopaminergic sensitization is consistently observed after limited drug experience in drug-naive individuals. This sensitization also was associated with enhanced reinforcing and rewarding effects of drugs (Bradberry, 2007).

Within the dopamine system, the LgA experience produces tolerance-related effects, while the IntA experience produces sensitization-related effects. Specifically, phasic dopamine release in both the dorsal and ventral striatum progressively decreases over time during LgA cocaine self-administration (Panlilio & Goldberg, 2007); (Ahmed, Lin, Koob, & Parsons, 2003). In the ventral striatum, IntA experience increases electrically-stimulated dopamine release and produces sensitization to cocaine-, methylphenidate- and methamphetamine-induced blockade of the dopamine transporter in the nucleus accumbens, whereas the LgA experience produces tolerance (Calipari et al., 2014; Calipari et al., 2013).

In the present study, LgA rats did not develop psychomotor sensitization to self-administered cocaine even though they showed incentive motivation for the drug, as measured by responding for the drug under PR. It should also be noted that the present study did not assess psychomotor sensitization after a withdrawal period, and this can

make a difference (Ferrario et al., 2005). Rats with prior LgA experience have shown marked psychomotor sensitization even after a month of abstinence (Ferrario et al., 2005). Thus, "the neurobiological effects of LgA may change as a function of time following the discontinuation of drug use, consistent with reports that sensitization is sometimes only apparent after a period of abstinence" (Kawa et al., 2016). In contrast, intermittent access to cocaine evokes robust psychomotor sensitization as measured by increasing in locomotion over 10 to 18 self-administration sessions (F. Allain, Roberts, et al., 2017; F. Allain & Samaha, 2018).

In support of this notion, intermittent access to cocaine in the present study promoted the development of psychomotor sensitization, and this effect was more pronounced in female than male rats. Consistent with these findings, other studies have also shown greater locomotor behaviors in female rats relative to male rats after acute or chronic cocaine administration (Festa & Quinones-Jenab, 2004; Perrotti et al., 2001; Russo et al., 2003; Q. D. Walker et al., 2001). As well, female rats have been shown to require lower doses of cocaine to achieve responses similar to those of male rats, and their cocaine-induced behavioral responses have also been shown to persist longer (Hu et al., 2004; Russo et al., 2003).

Studies have also demonstrated a correlation between the psychomotor sensitization and the incentive motivation. Specifically, the psychomotor sensitization has been shown to predict incentive motivation to cocaine in IntA male rats (F. Allain, Roberts, et al., 2017). The present study replicated these findings and further showed that this correlation is

applicable to female IntA rats as well. These results also support the notion that the brain changes which underlie sensitization to the incentive motivation for drugs also underlie psychomotor sensitization (De Vries et al., 1998; Lorrain et al., 2000; T. E. Robinson & Berridge, 1993). However, we did not specifically measured drug induce brain changes.

10. Assessing the motivation to obtain cocaine

In the present study, the effects of sex and access on the motivation to obtain cocaine were examined. The incentive motivation for cocaine in female and male rats was examined by determining breakpoints for cocaine (0.083, 0.5 and 0.75 mg/kg/i infusion, in counterbalanced order, 1 session/dose) under PR schedule after five days since the last IntA or LgA session. With the PR schedule protocol, the number of active presses required to obtain the next successive infusion increased exponentially with each infusion, until the animal stops pressing the lever. The last ratio reached prior to this point is the breakpoint, which is a measure of incentive motivation for drug (Richardson & Roberts, 1996). To assess the incentive motivation for cocaine following more extended abstinence from the drug, a subset of the rats in each group was once again tested under PR, 25 days following the last LgA or IntA session.

Overall, the findings of the present study show that female rats have greater incentive motivation for cocaine than male rats during both early (5-day) and late (25-day) withdrawal times. Previous studies have also reported that female rats are more motivated than make rats. Following a history of LgA self-administration, show higher motivation than male rats for cocaine (Hu & Becker, 2003; Hu et al., 2004; Lynch & Taylor, 2004; Roberts et al., 1989; Roth & Carroll, 2004). Furthermore, recently Kawa and Robinson (2019) have demonstrated this sex differences in the motivation under IntA conditions too. Female rats show greater incentive motivation to consume cocaine than male rats throughout the IntA experience and after an abstinence period (Kawa & Robinson, 2019).

The study also proposed that intermittent cocaine use may more readily produce sensitization-related changes in brain motivation pathways of females, and thus may accelerate the addiction process. In agreement with those of previous works (F. Allain et al., 2018; Kawa & Robinson, 2018, 2019), the findings from the present study showed no correlation between IntA intake and motivation. There is no significant relationship between the amount of past cocaine intake (total number of cocaine infusions taken over the ten 6-h sessions, multiplied by 0.25 mg/kg/infusion) and responding for cocaine under PR schedule.

It is well-documented that an IntA cocaine experience more effectively produces sensitization of incentive motivation for the drug, and this is thought to involve the distinct pharmacokinetic profiles achieved by the two procedures – namely, continuously high brain cocaine levels during an LgA session vs. intermittently spiking levels during an IntA session (F. S. A. Allain, 2018; Zimmer et al., 2012). The findings in the present study exhibit this significant difference in the motivation for cocaine between the two access conditions at WD5. IntA rats reached significantly higher levels of motivation to take cocaine comparing to LgA rats after short abstinence periods. We found that responding for cocaine under a PR schedule was stable after 5 or 25 days of forced abstinence from the drug

The present findings are in support of the notion that the IntA cocaine condition is more effective in increasing the incentive motivation for cocaine. Even though LgA rats took 2 to 3 times more cocaine than IntA rats over the ten self-administration sessions,

consumption of cocaine intermittently in the past led to greater (imotivation for the drug compared to a history of continuous consumption.

It has been previously shown that IntA rats prevented from escalating their intake still develop strong psychomotor sensitization (F. Allain, Roberts, et al., 2017). Despite the IntA rats in this study not escalating their intake over the ten-self-administration sessions, they reached very high levels of motivation and developing psychomotor sensitization. The present findings are consistent with those of previous studies showing that addiction-relevant symptoms can develop without escalation of intake (F. S. A. Allain, 2018; Minogianis et al., 2013; Zimmer et al., 2012); however, the present study's findings challenge the assumption that high and escalating levels of cocaine intake are necessary to increase the motivation for the drug (Ahmed & Koob, 1998; Hao et al., 2010; Paterson & Markou, 2003). Thus, the results in this study are consistent with those showing that less is more, and that spikes in brain concentrations of cocaine (i.e., IntA) are more efficient than high and escalating brain concentrations (i.e., LgA) in producing cocaine addiction symptoms (F. Allain et al., 2018; Bentzley et al., 2014; James et al., 2018; Kawa et al., 2016; Zimmer et al., 2012).

11. Explanations for sex differences

A possible explanation for sex differences in the response to cocaine could be that, with each self-administered infusion, more drug gets to the brain in females than in males. The present study did not measure brain concentration levels. However, pharmacokinetic studies in humans indicate minimal differences in the pharmacokinetics of both intranasal and smoked cocaine between men and women or across the menstrual cycle (Collins, Evans, Foltin, & Haney, 2007; Evans, Haney, & Foltin, 2002; Mendelson, Mello, & Negus, 1999; Mendelson, Mello, Sholar, et al., 1999; Sofuoglu, Dudish-Poulsen, Nelson, Pentel, & Hatsukami, 1999). Likewise, the brain concentrations of cocaine self-administrated intraperitoneally did not differ between female and male non-human primates (Bowman et al., 1999; Festa & Quinones-Jenab, 2004; Mendelson, Mello, & Negus, 1999). Previous studies have also shown minimal sex differences in the pharmacokinetics of cocaine after intravenous administration in monkeys (Mello et al., 1993; Mendelson, Mello, Sholar, et al., 1999). Thus, the existing data in the literature contraindicate the role for cocaine pharmacokinetics in mediating sexual dimorphism of cocaine addiction.

The role of gonadal hormones in the sexual dimorphism of cocaine addiction is well-documented. A large body of literature has elucidated how circulating hormones interact with the brain and has reported the role this plays in addiction (see (Becker et al., 2012; Lynch, 2018). Several studies (e.g., (Hu et al., 2004; Lynch, Roth, Mickelberg, & Carroll, 2001; Roberts et al., 1989) have shown that estradiol facilitates cocaine self-administration. It has also been reported that estradiol enhances the behavioral response to the drug, as shown by greater locomotion, stereotypy and rotational behavior after

treating OVX rats with estradiol (Becker, 1990; Peris, Decambre, Coleman-Hardee, & Simpkins, 1991). As well, estradiol enhances acquisition, escalation, reinstatement and motivation of drug-taking behavior in females (Becker, 2016).

Some studies suggest that the swift acquisition of cocaine-taking behavior in females, as well as their preference to consume more cocaine than male users, might be the result of a reduced rise of DA in the NAc compared to that in males. Larger amounts of the drug are required to obtain comparable increases in DA. The literature explains that when a user habitualizes drug-taking, higher and higher amounts of DA are then released within the DLS, followed by attenuated NAc DA release. Thus, the balance differentiations encoded in male and female users' neural systems could possibly provide the mechanism which causes diverse addiction behaviors in males and females (Becker, 2016). It is worth noting that glutamate function changes linked with cocaine addiction are similar in both males and females (Doyle et al., 2014).

Furthermore, DA activity can be affected by estradiol in those portions of the brain which play crucial roles in drug reward delivery (Jackson et al., 2006). Specifically, estradiol is known to boost AMPH-stimulated DA being released through the striatum region of the brain (Becker & Rudick, 1999; Castner, Xiao, & Becker, 1993), after which it attenuates the DA reuptake for the NAc (Thompson, 1999). It would perhaps be of interest to know whether estradiol has a similar effect on males as well. In fact, despite having in females an impact on DA neurotransmission and DA-mediated behaviors, a similar effect of estradiol in males does not seem to occur (Jackson et al., 2006). In a

well-known study, OVX females and CAST males were given an estradiol treatment 30 min before being permitted a cocaine self-administration session. It was found, in these test subjects, that the treatment enhanced the cocaine acquisition only in the females. From this, we can see a clear sex-based differentiation in brains with regard to estradiol response (Jackson et al., 2006).

Previous studies on humans have reported differences in the subjective effects of cocaine across menstrual cycles (Lynch, 2008). Specifically, women report higher subjective response to cocaine in the estradiol predominant follicular phase, compared to the luteal phase, where both estradiol and progesterone are raised (Jackson et al., 2006). Exogenous administration of progesterone in women during the follicular phase has been shown to attenuate the subjective effects of cocaine (Evans & Foltin, 2006; Sofuoglu, Mitchell, & Kosten, 2004), suggesting that progesterone may counter some of estrogen's effects (Lynch, 2008).

Likewise, cocaine-related effects have also been shown to vary across estrous cycles in rodents (Lynch & Carroll, 2000). Female rats have been shown to reach higher breaking points to obtain cocaine during the estrus phase when estradiol and progesterone levels show a sharp decline from peak levels compared with other phases of the estrous cycle (Carroll et al., 2002; Lynch, 2008; Roberts et al., 1989). Cocaine-seeking behavior in female rats has also been shown to be high during the estrus phase (low progesterone), compared to the proestrus phase (high progesterone) (Feltenstein and See (2007)). In support of this notion, studies have demonstrated that the acquisition rate of cocaine self-

administration was significantly higher in ovariectomized (OVX) rats treated with both estradiol and progesterone, relative to OVX rats treated only with estradiol (Jackson et al., 2006).

On the other hand, OVX female rats have also been shown to have higher cocaine intake and greater motivation than males even without treatment with estradiol (Hu et al., 2004; Russo et al., 2003), suggesting existence of sexually dimorphic neural systems mediating cocaine addiction. Thus, both intrinsic sex differences in brain organization and the actions of circulating estradiol contribute to increased vulnerability for cocaine use in female subjects (Hu et al., 2004). The pharmacological actions of circulating estradiol might enhance the rate of acquisition of cocaine habit in females, while the differences in brain organization might render females vulnerable to cocaine addiction (Jackson et al., 2006).

12. Benefits, limitations and future directions

The present study design incorporated the use of different reinforcement programs (i.e., fixed ratio or progressive ratio), allowing for a better mimic of the real environment where cocaine is not always available and obtaining the drug requires investment of time and effort (Panlilio & Goldberg, 2007). The present study is reminiscent of previous works by others which indicated that females are more vulnerable than males in certain aspects of cocaine addiction. In addition to these findings, the present study also demonstrates that access conditions play an important role and would facilitate better understanding of the sex differences underlying cocaine addiction. These several findings are important because they promote a better understanding of sex differences in relation to cocaine use under both traditional and more recent self-administration procedures. The findings also highlight that the IntA procedure could be better suited to study sex differences in the sensitization-related neuroadapatations that lead to increased incentive motivation for cocaine, as measured by appetitive responding for the drug.

This research investigation studied the effects of sex and access in cocaine self-administration. However, the present study design did not involve monitoring the estrous cycle, even though certain estrous phases could influence the study outcomes. For example, females may have reached higher breakpoints for cocaine when estradiol levels were high, as previously reported (Roberts et al., 1989). Our first step was to study sex differences as a function of cocaine access conditions without monitoring the cycle, because one of our criteria was the inclusion of intact males and intact females, without regard to the females' estrous cycle, as such inclusion is "a valid approach to learn about

females in neuroscience research" (Becker et al., 2016). However, in future work, we believe it is warranted to investigate the sex differences of conditioned cocaine-seeking under the two access conditions by monitoring the estrous cycle in female rats. In addition, future work could also involve assessment of brain-derived neurotrophic factor (BDNF) protein concentrations in mesocorticolimbic regions to determine if IntA female rats also show time-dependent increases in BDNF concentrations in the prelimbic cortex, nucleus accumbens core and ventral tegmental area after cocaine withdrawal, as our laboratory has recently shown the same in IntA male rats.

Conclusions

The present study determined sex differences in cocaine self-administration behavior in rats given the LgA vs. the IntA experience. In agreement with previous studies (Becker & Koob, 2016; Lynch, 2018), females were more vulnerable to the reinforcing, psychomotor sensitizing and incentive motivational effects of cocaine than males. This suggests that increased vulnerability to sensitization-related neuroplasticity could contribute to the faster transition to cocaine addiction in women. Importantly, drug access conditions (LgA vs. IntA) influenced sex differences in the response to chronic cocaine intake. The findings of this study show that across cocaine access conditions, female and male animals overlapped on many behavioral measures. However, there were also significant sex differences in outcomes, and this interacted with cocaine access conditions. Specifically, the LgA procedure was more effective in producing sex differences in the amount of cocaine taken, while the IntA procedure was more effective in producing sex differences in sensitization to the psychomotor activating and incentive motivational effects of cocaine.

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