The Importance of the Temporal Pattern of Drug Use in Addiction

By

Alexander Borg Kawa

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Doctoral Committee:

Professor Terry E. Robinson, Chair
Professor Kent C. Berridge
Associate Professor Shelly B. Flagel
Professor Robert T. Kennedy

Alexander B. Kawa

akawa@umich.edu

ORCID iD: 0000-0002-8870-0233

Dedication

To all those individuals that are suffering from addiction

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Abstract

Drug addiction affects tens of millions of people worldwide and yet there is no biological marker for diagnosis and available treatment options remain largely ineffective. Interestingly, of the individuals that recreationally use drugs of abuse only a subset eventually develop the problematic patterns of use that define addiction. The question to be addressed in this dissertation is how brain and psychological function change in susceptible individuals during the transition from recreational drug use to addiction. Preclinical models of addiction offer the opportunity to study specific aspects of addiction through manipulations that would not be possible in humans and are thus invaluable in the study of addiction. Currently, the most widely used preclinical self-administration models of addiction stress the *amount* of drug an individual consumes, and suggest that addiction can only occur following the consumption of large quantities of drug. While the amount of drug consumed is important in the development of addiction it is only one factor that contributes. The pharmacokinetics of drug use are also important in the development of addiction. This is especially important when considering that human drug use tends to be intermittent both between and within bouts of use during the transition from casual drug use to addiction. However, until recently intermittent patterns of drug intake have largely not been studied in preclinical self-administration models. In the studies presented here we developed a novel procedure for modeling the development of cocaine

addiction by combining a recently introduced intermittent access (IntA) self-administration procedure with an established prolonged access procedure. We found that this procedure produced remarkably robust addiction-like behavior when measured on a variety of tests.

In chapter 2 we used this procedure to investigate individual differences in the susceptibility to develop addiction-like behavior in rats that are especially prone to attribute incentive-salience to reward-paired cues (sign-trackers; STs) versus rats less prone to do so (goal-trackers; GTs). We found that STs were more motivated to self-administer cocaine prior to IntA experience, but following prolonged IntA, all individuals developed addiction-like behavior such that STs and GTs no longer differed. In chapter 3, we used a similar experimental design to study sex differences in the development of addiction-like behavior. We found that females were far more motivated to work for cocaine than males following prolonged IntA self-administration. The magnitude of this difference was larger than what is typically seen in studies examining both sexes, suggesting that females may be *particularly* susceptible to the effects of intermittent cocaine exposure. Finally, in chapter 4 we sought to elucidate the neural mechanisms that promote the development of addiction-like behavior following IntA, and how these processes may differ as a function of the temporal pattern of cocaine experience. We found that the temporal pattern of cocaine intake had a large impact on subsequent motivation for cocaine and the increased motivation observed following IntA was strongly correlated with sensitized DA release in the nucleus accumbens core in response to cocaine.

Taken collectively these studies show that consuming large amounts of cocaine is not necessary for the development of addiction-like behavior, and that intermittent cocaine experience is very effective at producing addiction-like behavior via a sensitized mesolimbic dopamine response to cocaine.

CHAPTER I

GENERAL INTRODUCTION

The Impact of Addiction

Drug addiction is a crippling disease and global health problem. In the majority of cases there is no cure for addiction and a person suffering from addiction must live with the disease for many years, if not the remainder of their life. In the United States, deaths attributed to overdose have been increasing, relative to population growth, since at least 1999 (NIDA, 2017). In 2016 alone there were more than 64,000 overdose deaths and approximately 10,000 of these were the result of cocaine use (NIDA, 2017). Worldwide the numbers are even more striking and depressing. 29.5 million people suffer from some form of use disorder and in 2015 the world lost an estimated 28 million years of "healthy" life as the result of drug use (UNODC, 2017).

The consequences of drug addiction extend far beyond the death toll. The practice of unsafe administration, particularly injection, leads to disease outbreak, which in turn leads to suffering and a substantial financial burden on the user and society as a whole. As the result of unsafe drug injection approximately 6.1 million people live with Hepatitis C and 1.6 million people live with HIV (UNODC, 2016). Further, drug users are at a 40X higher risk of contracting tuberculosis than the average non-drug user (UNODC, 2017). In addition, drug use

has led to mass incarceration, particularly in the United States. Incarceration has proven to be ineffective reducing drug use, but in fact exacerbates the problem. Approximately 33% of prisoners report drug use while in prison, with 16% reporting monthly use, and this use occurs in a setting that is less safe, and more conducive to the spread of disease, than street-use (Spohn and Holleran, 2002; Small et al., 2005; UNODC, 2016). Within the United States, both incarceration and drug addiction disproportionately affect low SES communities and ethnic minorities (Substance Use and Mental Health Administration, 2015).

In spite of wide recognition of the problem of drug addiction there remains a staggering treatment gap. In 2015 in the United States alone ~22.7 million people *required* treatment for drug addiction, but only 2.5 million received treatment (NIDA, 2017). This treatment gap exists for a number of reasons, but certainly one large contributing factor is the lack of time- and cost-effective treatment options. Another contributing factor is the difficulty in diagnosing addiction. No single biological marker exists for diagnosis, nor is there a consensus on how to define addiction. The NIDA website defines addiction as, "A primary, chronic disease of brain reward, motivation, memory and related circuitry," (NIDA, 2017). The Diagnostic and Statistical Manual of Mental Disorders (DSM) largely avoids the term addiction altogether in favor of "Substance Use Disorder", which they define as existing on a continuous spectrum depending on the number of 'criteria' a patient meets (American Psychiatric Association, 2013). Given the jarring public health impact of addiction and the lack of effective and efficient treatment, the impetus for basic research on the psychology and neurobiology underlying addiction is pressing.

The Importance of Preclinical Models of Addiction

The transition to drug addiction is a progressive shift from casual use towards compulsive use to the detriment of one's self and society (O'Brien et al., 2006; Saunders, 2006). Most people

use a potentially addictive drug at some point in their life and are able to control their use. However, in a minority of individuals, recreational drug use progresses to problematic use and addiction. Thus, one major question in addiction research concerns how, in susceptible individuals, drugs change the brain and psychological function in ways that promote the transition from casual drug use to addiction. This remains a difficult question to address in humans for a number of reasons. First, it is hard, if not impossible, to predict who will go on to develop addictive patterns of use following initial drug use. While there are a number of personality traits that correlate with increased susceptibility to addiction, no studies in humans have actually proven that personality prior to drug use can be predictive of the development of addiction. Further, it is unclear how much these personality traits are causes versus consequences of drug use. Second, once addiction has developed, and after drugs have been used for decades, it may be too late to determine how drug use changed neuropsychological function in the first place, leading to years of problematic use. Again, it is impossible to parse apart the many effects of long-term drug use from an underlying cause driving the addiction. Third, addicts have often experienced decades of poor nutrition/health, poly-drug use, stressful conditions, etc., all of which also change the brain. Fourth, the environmental context and cues associated with drug use powerfully modulate their effects (Badiani, 2013; Leyton and Vezina, 2014), and in humans it is difficult to study the neurobiological effects of drugs in the environmental context in which they are usually used. Relatedly, even studies conducted in labs using the latest brain imaging techniques are limited in spatial and temporal resolution. For these reasons, preclinical models are especially important for isolating drug-induced changes in neuropsychological function that contribute to the transition to addiction.

Drug Self-Administration and Theories of Addiction

The most widely accepted preclinical models of addiction involve the use of drug self-administration procedures. Much of the early self-administration work was directed towards identifying drugs that would be self-administered, and more broadly the question of whether non-human animals (referred to as animals hereafter) could be addicted to a drug. According to A.R. Lindesmith (1938), the prevailing viewpoint at the time was that "only those to whom the drug's effects can be explained can become addicts," and "Certainly from the point of view of social science it would be ridiculous to include animals and humans together in the concept of addiction," (Spragg, 1940). In hindsight it is informative to observe how these early researchers defined addiction. The prevailing theories of addiction at the time appeared to support "escape training"- or administering drugs to alleviate the aversive state of withdrawal (Plant and Pierce, 1928; Himmelsbach et al., 1935; Lindesmith, 1938; Spragg, 1940; Nichols et al., 1956). It was reasoned that if the drug user (human or lab animal) could not identify the drug as the source of withdrawal-induced discomfort, then they would not take the drug to alleviate this discomfort, and addiction would not develop. This theory was undoubtedly influenced by the prevalence of morphine addiction at the time, and the fallout of marketing over-the-counter heroin as a less-addictive alternative to morphine through the year 1910 (Himmelsbach et al., 1935).

Notably, early attempts to study addiction in animals were composed of administering non-contingent drug injections and then quantifying withdrawal symptoms as correlates of addiction (Plant and Pierce, 1928; Himmelsbach et al., 1935). However these studies were limited in scope as the experimenters could only test drugs for which the withdrawal symptoms (and addictive potential) in humans were already known and compare these symptoms to the symptoms induced in the animals. In order to study the addictive potential of newly developed

drugs, researchers started to ask whether animals would voluntarily administer drugs. Spragg (1940) showed that if chimpanzees were non-contingently injected with morphine by an experimenter to the point of inducing physical withdrawal then they would demonstrate several addiction-like behaviors beyond withdrawal symptoms. For example, the monkeys would work for experimenter administered injections of morphine and were documented pulling the experimenters towards them when they were deprived of morphine, a behavior not seen prior to morphine-dependence (Spragg, 1940). And while Spragg considered this evidence enough of morphine addiction in primates, he remained skeptical that addiction could occur in rodents, stating, "Animals such as the rat, for example, could probably never become addicted to morphine, simply because they are not capable of forming [drug-withdrawal] associations of this order," (p. 126).

The earliest studies of drug self-administration in rodents also included experimenters non-contingently injecting rats with a drug until withdrawal symptoms were evident and then allowing the animal to work for the drug. Typically the drug would be dissolved in solution and consumed orally (Nichols et al., 1956; Wikler et al., 1963). Rats were shown capable of learning to make an operant response (head-movement) to obtain a morphine or codeine solution injected intraperitoneally (Headlee et al., 1955) and would drink a morphine solution that they would normally avoid when they were tested during withdrawal (Nichols et al., 1956). Interestingly, Nichols et al., (1956) also showed that rats would prefer a morphine-solution up to 35 days into withdrawal. The authors acknowledge that physical dependence at this point would be extremely low but explain this finding as habitual conditioning and then proceed to argue that physical dependence and withdrawal are necessary for addiction to occur.

The study of drug self-administration exploded in 1962, when James Weeks published an article that described a procedure for intravenous (IV) morphine self-administration in a rat (Weeks, 1962). In this study rats were allowed to press a lever for varying doses of morphine and Weeks observed that the rate of responding varied with the dose of morphine. In line with the current state of addiction research at that time, these rats were pre-treated with experimenter-administered hourly injections of morphine to induce "addiction". However, with the introduction of an IV self-administration procedure it was now possible to test the positive reinforcing effects of drugs through a route of administration comparable to human abuse and without confounding sensory effects. Subsequent studies in monkeys (Deneau et al., 1969) and rats (Kumar et al., 1968) showed that physical dependence was not necessary for animals to self-administer drugs (for an early review of the literature see Schuster and Thompson, 1969). In addition, Deneau et al., (1969) showed that monkeys would self-administer morphine, codeine, cocaine, d-amphetamine, pentobarbital, ethanol, and caffeine without pre-treatment and despite the fact that symptoms of withdrawal were only evident in animals self-administering the depressants listed above- not the stimulants. From this the authors concluded that monkeys would self-administer most drugs that humans abused. The additional finding that monkeys did not self-administer drugs that humans did not commonly abuse such as nalorphine, chlorpromazine, or mescaline provided further validation for the use of IV self-administration in animals (Deneau et al., 1969).

In light of these new findings, the prominent theories of addiction adapted to incorporate drug abuse in the absence of physical dependence (Seevers, 1968; Deneau et al., 1969). A distinct dissociation was made between drugs classified as stimulants and those classified as depressants based upon their ability to produce physical withdrawal symptoms. Along these lines

distinctions were made between primary psychological dependence and physical dependence which led to secondary psychological dependence. It was posited that primary psychological dependence was induced by the rewarding effects of drugs and only contingent on the user forming the association between the drug and its pleasant effects. Further, physical dependence only occurred for depressant drugs and followed primary psychological dependence and continued high dose usage. Physical dependence led to secondary psychological dependence via a desire to avoid the aversive state of withdrawal (Fig. 1.1). This shift in the view of addiction is summarized by Seevers (1968), "Primary psychological dependence is *all* that is needed to lead to uncontrollable compulsive abuse with any psychoactive drug in certain susceptible individuals," (p. 1263) and "While it is true that the person psychologically dependent on the stimulants may have a compulsion for more drugs, this is not related to any physical need but rather to the desire for reward," (p. 1265).

As IV self-administration was accepted as a viable means to study the reinforcing effects of drugs of abuse, studies were launched in a number of different directions, including but not limited to, predicting the abuse liability of drugs (e.g., van Ree et al., 1978; Collins et al., 1983), and attempts to identify the neurobiological mechanisms underlying drug reinforcement (for review see Wise, 1987). One of the critical developments to come from this research was that the reinforcing effects of drugs were due to activation of *endogenous* brain mechanisms via the drug, acting as an analogue to some endogenous transmitter. A number of studies suggested that dopamine was a potential mechanism for the reinforcing effects of all classes of drugs (for reviews see Wise, 1980; Wise and Bozarth, 1982). Dopamine was further implicated in drug use and abuse when it was discovered that a number of drugs abused by humans (opioids, ethanol, nicotine, amphetamine, and cocaine) all shared the ability to increase dopamine concentration in

the nucleus accumbens (Di Chiara and Imperato, 1988). Di Chiara et al., (1988) also showed that drugs that were not commonly abused by humans did not change dopamine concentration in the nucleus accumbens. Although dopamine's specific role in reinforcement was yet unknown it was becoming more clear that it played a critical role in addiction.

A series of studies on the psychomotor activating effects of drugs, and dopamine's role in this and other behaviors gave rise to new views of addiction. One unifying effect of all abused drugs including: psychomotor stimulants (Jerussi and Glick, 1974), opioids (Babbini et al., 1979; Iwamoto, 1984), nicotine (Iwamoto, 1984), ethanol (Friedman et al., 1980), and others is that at some dose they all produce psychomotor activation (for reviews see Wise and Bozarth, 1987; Robinson and Berridge, 1993). Psychomotor refers to motor behaviors such as locomotion, rearing, biting, various forms of stereotypy (i.e. repetitive head movements, gnawing, sniffing), and others that are under the control of mental activity. One theory that arose linked the psychomotor activating effects of drugs (stimulants in particular, but not exclusively) to the approach towards rewards or reward-paired cues and thus the reinforcing effects of drugs (Wise and Bozarth, 1987). According to this theory, the forward locomotion and psychomotor effects of a drug are tantamount to the reinforcing properties of that drug. It was further posited that psychomotor activity and positive reinforcement rely on a common, homologous biological system- the mesolimbic and mesocortical dopaminergic system (Glickman and Schiff, 1967; Wise and Bozarth, 1987).

In addition to simply causing psychomotor activation, a number of studies showed that one long lasting consequence of repeated drug use was psychomotor sensitization (for reviews see Robinson and Becker, 1986; Robinson and Berridge, 1993). Psychomotor sensitization describes an increase in the psychomotor activating effects of a drug as the result of past drug

experience. For example, repeated administration of a low dose of amphetamine results in a progressive increase in drug-induced locomotion and repeated administration of a moderate-to-high dose of amphetamine will come to elicit stereotyped behavior that is typically seen in response to acute administration of a higher dose of amphetamine (Wallach and Gershon, 1971; Robinson, 1984; Robinson and Becker, 1986). These results indicate a 'shift to the left' on the dose-response curve of the psychomotor activating effects of drugs. As mentioned above, these psychomotor activating effects of drugs of abuse are mediated by the midbrain dopamine system (e.g., Hamamura et al., 1991; for reviews see Wise, 1987; Kalivas and Stewart, 1991).

In 1993, Robinson and Berridge introduced the incentive-sensitization theory of addiction (Robinson and Berridge, 1993). The incentive-sensitization theory of addiction can be concisely (though not exclusively) summarized in 3 key points: 1) Drug use causes long-term changes to the brain, 2) these changes render the brain's motivation and reward system hyper-sensitive to drugs and drug cues, 3) the brain systems that are sensitized are responsible for attributing incentive-salience to stimuli, and explicitly not responsible for the pleasure or euphoria associated with drug use (Robinson and Berridge, 1993; Berridge and Robinson, 2016). Further, Robinson and Berridge expanded upon dopamine's role in addiction and brain function in general. They postulated that dopamine is responsible for the attribution of incentive-salience, or "wanting", and that the psychomotor activating effects of dopamine are a correlate of incentive-salience attribution (Berridge et al., 1989; Berridge and Valenstein, 1991; Robinson and Berridge, 1993).

This proposed role of dopamine within mesolimbic neural circuitry was initially born out of negative results in an experiment designed to test dopamine's role in sensorimotor arousal versus hedonia (Berridge et al., 1989). In this study Berridge et al. (1989) lesioned ascending

dopamine projections and found that both positive and negative taste reactions remained unchanged in the presence of oral infusions of taste stimuli. This prompted the authors to suggest that DA was not necessary for general stimulus-elicited taste responses or necessary selectively for ingestive (or hedonic) taste reactions. Follow-up studies confirmed that even when lesions destroyed ~99% of dopamine in the striatum they had no effect on taste reactivity, or "liking" (Berridge and Robinson, 1998). Instead it was proposed that dopamine was involved in the attribution of salience to stimuli causing these stimuli to become "wanted" (Berridge et al., 1989; Robinson and Berridge, 1993).

The incentive-sensitization theory of addiction was originally termed a 'neuroadaptationist model' and since 1993 a great deal of work has been done to further our understanding of how the brain changes in addiction and to identify systems that could be targets for treatment. However, one huge issue in the pre-clinical literature has been identifying neural changes that result from casual drug use versus those causal in the development of addiction. I will review the modern literature relevant to this issue below.

Modeling the Transition to Addiction

As mentioned above, most people use a potentially addictive substance at some point in their life and are able to control their use without issue. However, in a minority of individuals, drug use progresses from recreational to compulsive and these individuals suffer from addiction (Anthony et al., 1994; Piazza and Deroche-Gamonet, 2013). The percentage of all users who eventually suffer from addiction varies from study to study and depends on drug class and a number of other factors so estimates must be interpreted cautiously. That being said, estimates of initial users who will go on to develop addiction tend to center around 15% and usually range from 10-30% across drug classes (Anthony et al., 1994; DeJong, 1994; Hursh and Winger, 1995;

Nutt et al., 2007; Degenhardt et al., 2008). It remains an open area of research how individuals that develop addiction differ from individuals that are able to control their use, and how drugs change the brain differentially between these two populations. In order to study this in animals, we need animal models that attempt to reflect the clinical condition and produce addiction-like behavior. Put simply, self-administration of a drug is not the same as addictive drug use nor is it a sufficient model of addiction. As Hyman and Malenka wrote in 2001, "Although drug self-administration by rodents has provided important information, it is difficult to argue that it truly models compulsion, when the alternative to self-administration is solitude in a shoebox cage," (Hyman and Malenka, 2001).

Fortunately a great deal of research has been dedicated to identifying addiction-like behaviors in rats and developing self-administration procedures that better model addiction. Addiction-like behaviors that can be measured in rats are often drawn from the DSM criteria that are used to diagnose addiction in humans (Deroche-Gamonet et al., 2004; Ahmed, 2012; see Table 1.1). These behaviors include escalation of drug intake, high motivation for drug, high propensity to reinstate drug-seeking, drug seeking despite adverse consequences, drug-seeking when drug is not available, choice of drug vs. alternative reinforcers, and measures of withdrawal. Together these behaviors are more descriptive than simply measuring the amount of drug consumed and individually they provide insight into specific aspects of the multiple symptoms of addiction. Most often these addiction-like behaviors are measured at multiple points during an experiment to measure how they change as a function of increasing drug experience, or they can be used to compare the effects of different self-administration procedures or the effects of different treatments.

The self-administration procedure to be used in a given study depends on the objective of that study. For example, it may be advantageous to use a self-administration procedure that produces these addiction-like behaviors only in a subset of rats, or it may be ideal to use a procedure that produces the most robust addiction-like behaviors in the largest proportion of individuals. Here I review existing animal models of the transition to addiction and how they can produce very different, even opposite, outcomes. Needless to say, it is concerning if different "models of addiction" produce very different changes in brain and behavior. (For this section I will focus primarily on studies involving cocaine)

Existing animal self-administration models: Long Access Self-Administration (LgA)

Arguably, the most widely accepted model of addiction in use today involves the so-called 'Long Access' (LgA) self-administration procedure (Ahmed and Koob, 1998; Edwards and Koob, 2013). The defining characteristic of LgA is that it allows the animals to self-administer drugs for 6+ hours/day. Until the late 1990's most experiments on neural systems that mediate the reinforcing and motivational effects of drugs used self-administration procedures that lasted 1-2 hours- what would now be referred to as 'Short Access' (ShA) (although see, Heyne and Wolffgramm, 1998). This is because a 1-2 hour session is all that is required to assess how most manipulations affect self-administration behavior. However, it was also well known at that time, that under these conditions, rats show very stable levels of drug intake over many weeks. In 1998, Ahmed and Koob published a seminal paper in which they compared rats allowed to self-administer cocaine under ShA conditions with those allowed to self-administer for 6 hours/day (LgA) (Ahmed and Koob, 1998). The ShA group showed stable levels of intake over 22 days of testing, as expected, but the LgA group escalated their intake, and after a period of abstinence escalated intake even further. Ahmed and Koob (1998) concluded that LgA, "*may*

provide an animal model for studying the development of excessive drug intake and the basis of addiction".

In this particular study, Ahmed and Koob (1998) focused on one addiction-like behavior-escalation of intake. But there are now many studies reporting that, relative to ShA, LgA produces a number of other addiction-like behaviors (for review see Edwards and Koob, 2013; Table 1.1). Several studies have shown that LgA experience increased the amount of effort animals were willing to expend to self-administer cocaine (Paterson and Markou, 2003; Wee et al., 2008; Bentzley et al., 2014). LgA has also been reported to increase rats' willingness to self-administer cocaine in the face of adverse consequences (Xue et al., 2012; Bentzley et al., 2014). Interestingly, Ahmed (2011) reported that following extended training, LgA rats did not take more cocaine when a foot-shock was delivered contingently with the cocaine, but following punishment, their responding returned to pre-punishment baseline levels more rapidly than ShA animals. Another behavioral symptom of addiction is resistance to extinction or persistent responding despite the drug no longer being available. Following LgA, when extinction training was conducted relatively soon (24-72 hours) after the last self-administration session, animals generally did not exhibit resistance to extinction (Mantsch et al., 2004; Sorge and Stewart, 2005; Ahmed, 2011). However, when extinction was conducted 3 weeks after the last LgA self-administration session, LgA animals responded more than ShA animals (Ferrario et al., 2005). In addition, when compared to ShA rats, LgA rats generally showed greater cocaine-induced reinstatement (Mantsch et al., 2004; Ahmed and Cador, 2006), cue-induced reinstatement (Kippin et al., 2006), and stress-induced reinstatement (Mantsch et al., 2008).

As the LgA procedure became more accepted as a model of addiction, researchers began to focus on the neurobiological consequences of LgA experience. Early after the discontinuation

of LgA experience, or other high dose procedures (e.g., Calipari et al., 2014a), rats show a marked decrease (tolerance) in a number of measures of dopamine function. This includes decreases in cocaine-induced inhibition of dopamine uptake and electrically-evoked dopamine release measured using Fast Scan Cyclic Voltammetry in a slice preparation (Calipari et al., 2014a). It also decreased cocaine-induced dopamine overflow measured with microdialysis and some (but not all) measures of cocaine-induced psychomotor activation (Ferris et al., 2011; Calipari et al., 2013, 2014a). In addition, LgA experience can also decrease phasic dopamine release in the striatum during cocaine self-administration (Willuhn et al., 2014). Tolerance to cocaine-induced inhibition of dopamine uptake following LgA subsided after 14 and 60 days, but could be reinstated by a single re-exposure to the drug (Siciliano et al., 2016). This last piece of data is consistent with a number of studies that have shown that drug effects can change immensely as a function of the time since last drug use.

Generally, sensitization tends to be absent or minimal when tested immediately (~24 hours) after cessation of cocaine self-administration, and manifests over longer abstinence periods (~3 days-6 months), while tolerance follows the opposite time course (Weiss et al., 1992; Stacy Hooks et al., 1994; Robinson and Kolb, 1997; Ferrario et al., 2005). For example, when rats were allowed to self-administer cocaine using a ShA-like procedure and then tested for their response to an IP injection of cocaine 1 or 21 days later, they showed psychomotor sensitization, relative to drug-naïve controls, only after the 21-day abstinence period (Stacy Hooks et al., 1994). In the same study, cocaine-evoked DA release was only increased after 21 days of abstinence and not after 1 day of abstinence (Stacy Hooks et al., 1994) and similar results were obtained after 1-month of abstinence (Tran-Nguyen et al., 1998). Similarly, following ShA experience and a 21-day abstinence period, electrically-evoked DA release was increased in a

slice preparation of the NAc (Wiskerke et al., 2016). Further, in ShA rats, neuronal excitability in the NAc was markedly decreased on a number of measures when tested two days after the last self-administration session. However, when tested 3-4 weeks after cessation of self-administration an increase in neuronal excitability was observed (Ortinski et al., 2012). These changes could be due in part to long term potentiation of VTA excitatory synapses following ShA cocaine self-administration that lasts up to 3 months after the last cocaine experience (Chen et al., 2008).

Related to these time-dependent changes in behavior and brain function is the occurrence of 'incubation of craving' following drug self-administration. Incubation of craving refers to a progressive increase in cue-induced reinstatement of drug-seeking behavior during protracted abstinence (Grimm et al., 2003; Lu et al., 2004). Relative to ShA, LgA cocaine self-administration is especially effective in producing incubation of cocaine craving, which is seen both when abstinence is forced or voluntary (Venniro et al., 2016). A series of studies by Marina Wolf and her colleagues suggests that following LgA experience this time-dependent increase in the motivational properties of a cocaine cue is due to a time-dependent increase in the number of calcium-permeable AMPA receptors in the nucleus accumbens core (Wolf, 2016). Wolf (2016) states, "incubation of cocaine craving is accompanied by increased recruitment of NAc core neurons that fire in a manner that is time-locked to cue-induced cocaine-seeking" ... and concludes, "a probable cellular underpinning of this is the strengthening of excitatory synapses onto MSNs in the NAc core" (p. 3). Glutamate plasticity may also be related to the enhanced expression of psychomotor sensitization when rats are tested long after the discontinuation of LgA experience (Ferrario et al., 2005). In summary, although more research is required, the neurobiological (and behavioral) consequences of LgA experience may vary considerably as a

function of time following the discontinuation of self-administration, from those reflective of tolerance to those suggestive of hyperexcitability (sensitization).

Although there are exceptions to the reports of marked tolerance in dopamine neurotransmission after LgA (e.g., Ahmed et al., 2003), and there are clearly effects of the time since last drugs use, the evidence for tolerance after LgA has been interpreted as support for the view that addictive behavior is a consequence of a drug-induced *hypo*dopaminergic state, and that continued drug-seeking is motivated by a desire to overcome this dopamine deficiency (Dackis and Gold, 1985; Caprioli et al., 2014; Koob and Volkow, 2016; U.S. Department of Health and Human Services, 2016; Volkow et al., 2016).

Existing animal self-administration models: Prolonged Self-Administration

Another pre-clinical self-administration procedure that has proven effective at inducing addiction-like behavior in rats is prolonged self-administration exposure (Wolffgramm, 1991; Wolffgramm and Heyne, 1995; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004). The critical aspect of prolonged exposure is the number of self-administration sessions the rats are given. The method of drug administration can differ from oral intake to IV self-administration, but in these studies the animals are exposed to large amounts of drug over the course of many weeks or months. Prolonged access is especially effective for the study of individual differences in addiction vulnerability because following prolonged access only a small percentage of the rats tested develop addiction-like behavior (Deroche-Gamonet et al., 2004). Piazza and colleagues have shown in a series of studies that rats that eventually show addiction-like behavior do not differ on most measures of addiction from non-addict rats until they have had ~50-70 self-administration sessions (e.g., Deroche-Gamonet et al., 2004; Belin et al., 2009). The theoretical framing of this is that addiction occurs in three sequential steps: initial

recreational drug use, sustained and escalated drug use, and finally loss of control and addiction (Piazza and Deroche-Gamonet, 2013). Therefore only individuals who consume large quantities of drugs over extended periods of time are likely to become addicted. While prolonged exposure stresses the number of self-administration sessions and LgA stresses the length of each self-administration session, both procedures place critical emphasis on the *amount* of drug exposure.

Shortcomings of LgA and Prolonged Access and the Importance of Pharmacokinetics in Addiction

A fundamental assumption underlying the use of LgA and prolonged access procedures is that the *amount* of drug exposure is the critical factor responsible for the transition to addiction. This was put clearly by Ahmed (2012), who stated, "addiction-causing neuropathological processes could be set in motion only when rats can expose themselves sufficiently to cocaine to cross the 'threshold of addiction'—the minimum level of drug exposure required for inducing addiction. Conversely, below this critical level of cocaine exposure, there would be no drug-induced neuropathological changes, and drug use would remain under control, at least in the majority of drug-exposed individuals" (p. 110). Similarly, Edwards and Koob (2013) state, "excessive drug exposure likely remains an indispensable element driving the development of addiction" (p. 360). However, recent studies challenge this assumption.

Beyond the amount of drug exposure, two other pharmacokinetic factors might be as important, if not more important in predicting the transition to addiction- how fast drug reaches the brain, and how often- that is, the temporal pattern of drug use (Allain et al., 2015).

The importance of the speed with which a drug reaches the brain is especially important for drugs of abuse, such as cocaine, with multiple routes of administration (e.g., snorting, smoking, injecting) that affect how quickly the drugs reach the brain. This is important because

the addictive potential of a drug is tied to the rate at which it reaches the brain. Drugs and methods of administration that reach the brain the fastest are the most addictive. This is true in the clinical literature (e.g., Budney et al., 1993; Ferri and Gossop, 1999; for review see Hatsukami and Fischman, 1996) as well as the pre-clinical literature (Samaha et al., 2002, 2004; for review see Samaha and Robinson, 2005). In cocaine, this is presumably due in part to a strong correlation between rapid increases in brain cocaine concentration and the subjective, pleasurable effects of cocaine (Evans et al., 1996; Volkow et al., 2000). In addition, when cocaine reaches the brain more rapidly it more robustly engages the mesocorticolimbic brain circuits engaged in reward (Porrino, 1993; Samaha et al., 2004; Woolverton and Wang, 2004), promotes greater psychomotor sensitization (Samaha et al., 2002, 2004), and greater incentive sensitization (Samaha and Robinson, 2005; Wakabayashi et al., 2010; Bouayad-Gervais et al., 2014). Importantly in these studies the speed of cocaine delivery is manipulated but pharmacokinetic modeling predicts that this would not change peak brain cocaine concentrations (Samaha et al., 2002; Ferrario et al., 2008).

In addition to the speed with which a drug reaches the brain, the temporal pattern of drug use is also important. Especially in the present context, because the pharmacokinetics associated with LgA, and the majority of self-administration studies using fixed ratio schedules, do not reflect temporal patterns of drug use in humans. Most self-administration studies use schedules of reinforcement in which animals maintain continuously elevated brain levels of drug for the duration of daily sessions, be they 1-2 hour (ShA) or 6+ hour sessions (LgA; see Fig. 1.2). During a typical LgA session a rat will ‘load-up’ to a particular brain cocaine concentration early in the session and maintain a relatively stable level for the remainder of the 6-hour session (e.g., Ahmed and Koob, 1998). A shortcoming of this approach is that in humans, *intermittent* patterns

of cocaine use are the norm, both *between* and *within* bouts of use. Periods of cocaine use are frequently interspersed with periods of non-use (e.g., Cohen and Sas, 1994; Simon et al., 2002), and within a bout of use, cocaine users tend to take drug intermittently, with brain levels rising and then falling between 'hits' (Beveridge et al., 2012). As put by Ward et al. (1997), "Cocaine users … can clearly describe the advantages of waiting a long time between doses…" – cocaine users do not usually maintain uniformly high brain levels of drug for hours on end. This is presumably due to the pleasurable effects of cocaine being tightly tied to increases in brain cocaine concentration, as mentioned earlier. Furthermore, intermittent patterns of use are probably especially pronounced during the transition to addiction, prior to regular use, as no individuals (or very few) start taking a drug in an addictive pattern with the first use.

Successfully modeling the temporal pattern of cocaine use seen in humans is important because behavior can vary dramatically as the result of different temporal patterns of drug delivery. Pre-clinical studies using experimenter administered drugs have shown that intermittent injections, when compared to lower, sustained drug levels, are especially effective at producing CPP (Lett, 1989), psychomotor sensitization (Magos, 1969; Post, 1980; Robinson and Becker, 1986), the acquisition of self-administration (Horger et al., 1990; Piazza et al., 1990), and incentive-sensitization in the form of motivation to work for the drug (Mendrek et al., 1998; Vezina et al., 2002). Pattern of intake is also important for self-administered drugs (see below). Recently, Martin-Garcia et al., (2014) showed that when the temporal pattern of cocaine delivery during self-administration was manipulated via different inter infusion intervals (III) rats in the "high-frequency" group (i.e. smaller III) showed greater cocaine-induced reinstatement than rats in the "low-frequency" group (i.e. larger III) (Martín-García et al., 2014).

The temporal pattern of drug delivery also has a large impact on drug-induced neuroplasticity. Generally, intermittent doses of drugs tend to promote sensitization and longer-lasting, sustained doses of drugs tend to promote tolerance (Post, 1980). For example, once daily injections of cocaine increased cocaine-induced inhibition of striatal DA reuptake (Izenwasser and Cox, 1990), but continuous infusion of cocaine over 24-hours decreased cocaine-induced DA reuptake (Izenwasser and Cox, 1992). In addition, Unterwald et al., (2001) showed that when the same total dose of cocaine was injected once daily or given as two separate injections, increased D1-receptor binding in the striatum was only present in the animals given the cocaine over two injections. This led the authors to conclude, "These results demonstrate that the same total daily dose of cocaine administered in multiple small injections produces a greater effect on receptor regulation than a single larger injection. This suggests that the interval between cocaine injections is an important variable when studying the effects of cocaine on neurochemistry," (p. 103).

In conclusion, LgA and prolonged access self-administration procedures fail to capture the pharmacokinetics seen in human cocaine use, and a long line of research indicates that this is very important in the brain and behavior. One important question that remains is whether these results that were largely collected using experimenter administered cocaine generalize to self-administered cocaine and the development of addiction-like behaviors.

Intermittent Access Self-Administration (IntA)

To better model the intermittent pattern of cocaine use in humans, Benjamin Zimmer and colleagues, working in Dave Roberts' lab, developed what they called an 'Intermittent Access' (IntA) self-administration procedure. A typical IntA session consists of cycles of drug available

(5 min) and no drug available (25 min) periods (Zimmer et al., 2011, 2012). This results in repeated 'spikes' in brain cocaine concentrations (see Fig. 1.2).

Motivation

Zimmer and colleagues then asked how IntA experience influenced subsequent motivation for cocaine, relative to ShA and LgA experience, using a behavioral economic indicator of cocaine demand (see chapter 2). LgA resulted in much greater total cocaine intake than ShA, but the total intake during IntA did not differ from ShA (Fig. 1.2). LgA produced greater motivation for cocaine than ShA, consistent with previous reports, but importantly, despite much less cocaine consumption, IntA experience produced even *greater* motivation for cocaine than LgA experience (Zimmer et al., 2012). This confirmed that the temporal pattern of self-administered cocaine intake is important for subsequent motivation.

Neurobiology

Very little is known regarding the neurobiological consequences of IntA experience, however, the available evidence suggests that not only do IntA and LgA have very different effects, but in some instances produce opposite effects (see chapter 4). As mentioned above, LgA is reported to produce marked tolerance in dopamine neurotransmission, including a decrease in cocaine-induced inhibition of dopamine uptake (e.g., Calipari et al., 2014a). A series of studies from Dr. Sara Jones lab using *in vitro* voltammetry in the nucleus accumbens core suggests that in contrast to LgA, IntA experience *increases* cocaine-induced inhibition of dopamine uptake (Calipari et al., 2013; see chapter 4). This effect is even greater after a period of abstinence and it is also associated with increased motivation for drug (Calipari et al., 2015). Furthermore, IntA (but not LgA or ShA) increases electrically-evoked dopamine release (Calipari et al., 2013). Finally, IntA (but not LgA) cocaine self-administration experience produces cross-sensitization,

increasing both methylphenidate- and amphetamine-induced inhibition of the DAT in the nucleus accumbens core (Calipari et al., 2014b).

In conclusion, the available evidence suggests that in terms of dopamine function, IntA experience produces sensitization and LgA experience produces tolerance. These contrasting effects of IntA and LgA cocaine self-administration experience are consistent with a large literature involving the use of experimenter-administered psychomotor stimulant drugs. These studies show that intermittent injections produce psychomotor, incentive, and dopamine sensitization, whereas treatments that result in continuously high brain levels of drug produce tolerance (Post, 1980; Robinson and Becker, 1986; Robinson and Berridge, 1993).

In light of the unique ability of IntA self-administration to model human cocaine use patterns and its ability to produce sensitization it is interesting to consider how this procedure can be used to study individual variation in the susceptibility to develop drug-addiction.

Individual Differences in the Susceptibility to Develop Addiction

As has been mentioned throughout this introduction, recreational drug use is not the same as addiction, and only a small proportion of individuals that use drugs go on to develop addiction. This begs the question, what is unique about these individuals that leave them susceptible to addiction? A number of traits have been linked to an increased susceptibility to develop addiction, including but not limited to: impulsivity, novelty-seeking/risky behavior, and depression. In addition, the propensity to attribute reward-paired cues with motivational value has been suggested to be a risk-factor for the development of addiction. Data in support of this view has been reviewed extensively (Flagel et al., 2009; Saunders and Robinson, 2013) but I will summarize it below.

Everyday our ordinary proceedings are saturated with reward-associated cues- the flashing lights of a bar or the sign of a favorite fast food restaurant. Although most individuals are able to control their behavior in the presence of these cues, some experience strong cravings when they encounter cues that have previously been associated with a particularly salient reward (de Wit and Stewart, 1981; Jansen, 1998; O'Brien et al., 1998; Shaham et al., 2003; Panlilio et al., 2005; Flagel et al., 2009). Such is often the case when individuals suffering from addiction encounter cues that had previously been paired with the use of drugs (Leyton et al., 2007; Zijlstra et al., 2009). Indeed, one of the most frustrating clinical problems in the treatment and recovery from addiction is the propensity for addicts to relapse when they encounter drug-associated cues, even after prolonged periods of abstinence and a conscious desire to stay clean (DeJong, 1994).

Interestingly, there is considerable individual variation in the ability of a reward-paired cue to motivate behavior in humans (Schachter, 1968; Beaver et al., 2006; Mahler and de Wit, 2010; Garofalo and di Pellegrino, 2015) and animals (Flagel et al., 2009; Robinson and Flagel, 2009; Saunders and Robinson, 2010; Yager and Robinson, 2010; Yager et al., 2015). For example, if a discrete cue reliably predicts a food reward some animals will come to approach and vigorously engage with the cue when it appears (sign-trackers; STs) while other animals will not approach the cue, but when it appears will go the location where the food is soon to be delivered (goal-trackers; GTs) (Zener, 1937; Boakes, 1977; Robinson and Flagel, 2009). Further, only in some individuals does a discrete cue acquire the properties of an *incentive stimulus*. A reward-paired cue becomes an incentive-stimuli if it has: 1) the ability to attract attention and promote approach, 2) the ability to act as a conditioned reinforcer, that is, an individual will work for the presentation of the cue itself even in the absence of the primary reward, and 3) the ability to generate a conditioned motivational state and/or seeking for rewards (Bindra, 1978;

Berridge, 2001; Everitt et al., 2001; Cardinal et al., 2002; Milton and Everitt, 2010). There is considerable evidence that food-paired cues become incentive stimuli only in STs, and not GTs (Robinson and Flagel, 2009; Yager and Robinson, 2010; Flagel et al., 2011a). Interestingly, the attribution of incentive-salience to a food-paired cue predicts that attribution of incentive salience to a drug-paired cue. For example, a cocaine-paired cue elicits greater approach, is more desired (measured by the amount an animal is willing to work for its presentation), and spurs greater drug-seeking behavior in STs relative to GTs (Saunders and Robinson, 2010; Saunders et al., 2013). Additionally, this is not specific to food and cocaine cues as similar results have been found using an opioid-paired cue (Yager et al., 2015).

In addition to an increased propensity to attribute incentive salience to drug cues, a number of other findings have suggested that STs are at an increased risk of developing addiction relative to GTs. Even in the absence of discrete cues, STs are more motivated to work for the interoceptive effects of cocaine (Saunders and Robinson, 2011). The motivational effects of Pavlovian cues take longer to extinguish in STs than GTs (Beckmann and Chow, 2015; Ahrens et al., 2016). STs are more likely to choose cocaine over a food reward in a forced choice procedure (Tunstall and Kearns, 2015). Also, STs are more impulsive than GTs (Tomie et al., 2008; Lovic et al., 2011). And finally, STs are more susceptible than GTs to distractions during a cognitively demanding task (Paolone et al., 2013).

Neurobiologically, the ability of a cue to act as an incentive stimuli is associated with activation of a wide network of neural circuits involved in motivation and reinforcement (Cardinal et al., 2002; Milton and Everitt, 2010; Saunders and Robinson, 2013). It follows that there is also individual variation in the ability of a reward-associated cue to engage this "motivational circuit". For example, after Pavlovian training, when a lever has repeatedly been

paired with food delivery, phasic dopamine release in the nucleus accumbens core occurs in response to the lever presentation only in animals that have learned a sign-tracking conditioned response, even though all animals have learned the predictive value of the cue (Flagel et al., 2011b). Also, the presentation of food (Flagel et al., 2011a) and opioid cues (Yager et al., 2015) engages this motivational circuitry to a greater extent in STs than GTs. In addition, ST's increased susceptibility to distractions has been linked to unresponsive choline transporters in the prefrontal cortex that limits their ability to appropriately modulate acetylcholine transmission. This could lead to poor executive control over their behavior, and ultimately could contribute to difficulty resisting the temptations of cues (Paolone et al., 2013).

Taken together these findings establish that STs attribute greater incentive salience to drug-paired cues and therefore may be at greater risk to develop addiction and relapse after periods of abstinence. However, all of the previous studies on which this suggestion is based have used relatively limited, short access self-administration procedures. In chapter 2 I aimed to test whether STs and GTs differed in the development of several addiction-like behaviors using a novel prolonged, IntA self-administration procedure. This procedure was designed to better model human drug use and capture the changes in the brain that lead to addiction. The central hypothesis is that individuals that attribute greater incentive salience to Pavlovian cues will be more likely to develop addiction-like behaviors.

The remainder of this dissertation provides experimental data in which I attempt to explore the behavioral effects of IntA, and in particular how it interacts with individual differences in the development of addiction. Chapter 2 details the development of multiple addiction-like behaviors with increasing IntA experience in rats prone to attribute incentive-salience to reward-paired cues, and those less prone to do so. Chapter 3 describes how male and

female rats differ in the development of addiction-like behaviors following prolonged, IntA experience. And finally, in chapter 4, I attempt to elucidate how neural systems differ following ShA, LgA, and IntA, and the mechanisms that drive the development of addiction.

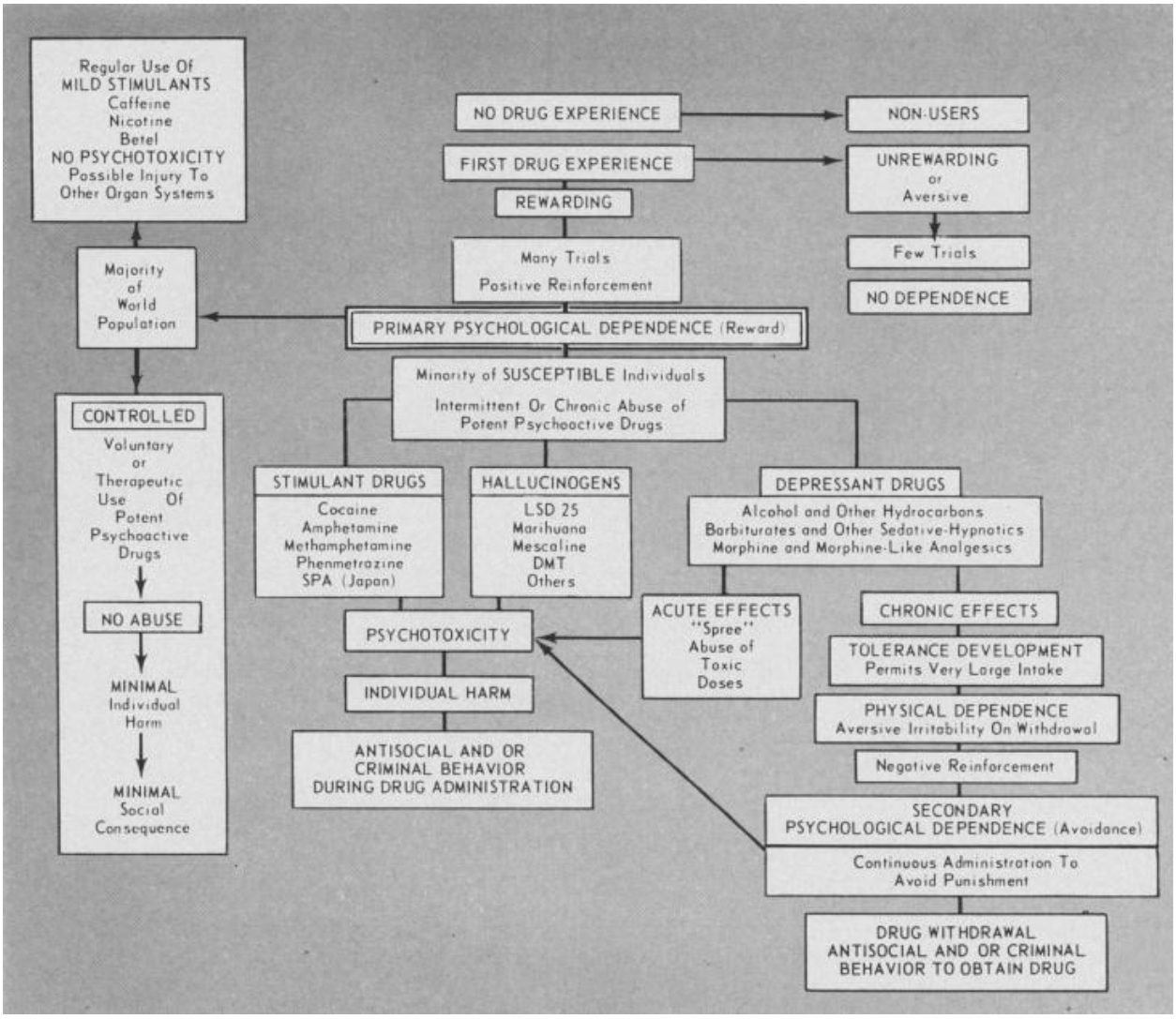
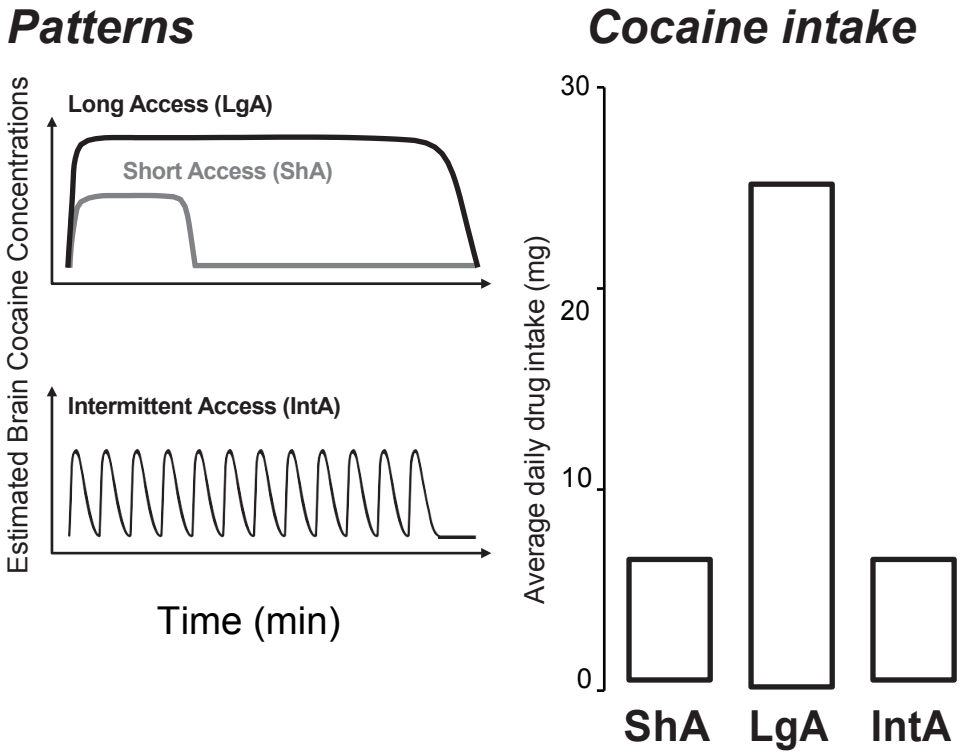


Figure 1.1. The steps leading to addiction. Used and adapted from (Seevers, 1968) under the "Fair Use principle". The progression from first drug use to addiction involves a number of steps. Importantly, in this model only primary psychological dependence (reward) was necessary for addiction to occur, representing a shift from the widely held theories of drug dependence and addiction at the time.



	Motivation for cocaine (Zimmer et al., 2012)	↑	↑↑	↑↑↑
Addiction-like behavior	*Escalation of intake* (Ahmed and Koob, 1998)	No	Yes	?
	Continued drug-seeking in the face of adverse consequences (Xue et al., 2012)	No	Yes	?
	Continued drug-seeking when drug is not available (Ferrario et al., 2005)	Yes	Yes	?
	Hedonic set point - Q_0 (Bentzley et al., 2014)	↔	↑	?
	Cocaine-induced DAT inhibition (Calipari et al., 2013, 2014, 2015)	↔	↓	↑

Figure 1.2. Comparison of self-administration procedures. The top left panel shows typical patterns of intake within a session using Long Access (LgA), Short Access (ShA), and Intermittent Access (IntA) self-administration procedures. The top right panel shows the average daily intake using these procedures. The bottom table shows addiction-like behaviors produced by these self-administration procedures.

DSM Criteria	Related Concept in Lab Animals	Example Measures
1) Persistent desire or unsuccessful effort to cut down on substance 2) Craving	-High motivation for drug -High propensity for 'relapse' or 'reinstatement'	-Break-point on a Progressive Ratio schedule of reinforcement -Behavioral economic metrics: P_{max} and α -Reinstatement – The extent to which a stimuli triggers drug-seeking behavior
3) Recurrent failure to fulfill role obligations 4) Recurrent substance use in hazardous situations 5) Continued substance use despite social problems 6) Continued use despite recognition of problems resulting from use 7) Important social, work, or recreational activities given up because of use	-Continued drug-seeking despite adverse consequences -Choice between drug and an alternative reinforcer (social interaction or food/sucrose)	-Behavioral economic metric: Max Charge -Drug-seeking despite receiving an electric shock -Preference for drug in a forced choice task
8) Considerable time spent in obtaining the substance	-Continued drug-seeking when drug is not present -Continued drug-seeking during signaled drug non-available periods	-Drug-seeking behaviors under 'extinction' conditions -'Extinction' sessions required to reduce drug-seeking behaviors -Responses during No-Drug periods of IntA sessions
9) The substance is often used longer or in larger amounts than intended	-Consumption when drug is available	-Escalation of intake – increased consumption across sessions
10) Tolerance 11) Withdrawal	-Taking more drug to get the same effect -Discomfort associated with the cessation of drug use	-Behavioral economic metric: Q_0 -Physiological signs of withdrawal: e.g. "wet-dog shakes"

Table 1.1. Addiction-like behaviors. The left column shows the DSM criteria used to diagnose Substance Use Disorders in humans, grouped loosely by related criteria. The middle column shows how these DSM criteria could be conceptualized in animal models. The right column shows tests used in animal behavior in an attempt to model the clinical criteria.

CHAPTER II

PROLONGED INTERMITTENT ACCESS COCAINE SELF-ADMINISTRATION PRODUCES INCENTIVE-SENSITIZATION AND ADDICTION-LIKE BEHAVIOR

INTRODUCTION

Drug self-administration in non-human animals is considered the best method for modeling drug use and addiction in humans. However, limited self-administration experience may not fully capture the changes in brain and behavior associated with the transition from casual drug use to addiction (Vanderschuren and Everitt, 2004; Ahmed, 2012; Piazza and Deroche-Gamonet, 2013). It is widely thought this transition requires the use of either 'long access' (LgA; i.e., sessions lasting 6-hr or more; Ahmed and Koob, 1998) or 'prolonged access' procedures (i.e., 1-2 hr sessions, but for more than ~30-40 days; Deroche-Gamonet et al., 2004). It is presumed that LgA or prolonged access lead to the development of addiction-like behavior because they are uniquely effective in altering brain reward systems (Kasanetz et al., 2010; Ahmed, 2012; Edwards and Koob, 2013).

But the *amount* of drug consumption is only one factor important in the development of addiction. Another critical factor concerns the temporal dynamics of drug delivery; i.e., pharmacokinetics (Hatsukami and Fischman, 1996; Zimmer et al., 2012; Allain et al., 2015). In

addicts, cocaine use is characterized by *intermittency*, both between and within bouts of use (Allain et al., 2015 for review). To model this, Zimmer et al. (2012) developed an Intermittent Access (IntA) self-administration procedure that produces repeated spikes in brain cocaine concentrations. Motivation for cocaine is higher after experience with IntA than LgA, even though far less drug is consumed (Zimmer et al., 2012). However, how motivation *changes* over time with IntA experience has not been studied. One goal of the present study was to do this, using behavioral-economic indicators of cocaine demand to quantify changes in motivation (Hursh and Silberberg, 2008; Oleson and Roberts, 2009; Bentzley et al., 2013). Behavioral-economic indicators provide especially unambiguous measures of motivation and of the preferred level of consumption when cost is low.

In addition, we previously hypothesized that rats prone to attribute incentive salience to discrete reward cues are more susceptible to addiction (i.e., sign-trackers [STs] > goal-trackers [GTs]) (Flagel et al., 2009; Saunders and Robinson, 2013), because STs are more attracted to drug cues (Saunders and Robinson, 2010), are more motivated to work for cocaine (Saunders and Robinson, 2011), and show more robust drug- and cue-induced reinstatement of drug-seeking behavior (Flagel et al., 2009; Saunders and Robinson, 2010, 2011; Saunders et al., 2013). However, all of these previous studies used procedures that involved only relatively limited exposure to drugs. We asked, therefore, whether STs are more susceptible than GTs to develop addiction-like behavior when allowed prolonged access to self-administered cocaine (approx. 70 days in total) using the IntA procedure.

MATERIALS AND METHODS

A total of 102 male Sprague-Dawley rats (Harlan, Haslett, MI and Charles River, Raleigh, NC) weighing 250-275 g on arrival were housed individually on a reverse 12-h light/12-h dark cycle (lights on at 20:00) in a climate-controlled colony room. All testing was conducted during the 12-hour lights off period. After arrival, rats were given 1 week to acclimate to the colony room before testing began. Water and food were available *ad libitum* until 2 days before the first day of self-administration, at which point the animals were mildly food restricted to maintain a stable body weight throughout testing. Note that the animals were not food *deprived*, but food *restricted*. That is, we did not reduce body weight but just maintained body weight. Male rats that are fed *ad lib* gain an inordinate amount of weight, especially in long duration studies, such as this one, and this is unhealthy. There is evidence that maintaining body weight at a stable level, in adult male rats, is the more healthy approach (e.g. Rowland, 2007). All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Apparatus

Behavioral testing was conducted in standard (22x18x13 cm) test chambers (Med Associates, St Albans, VT, USA) located inside sound-attenuating cabinets. A ventilating fan masked background noise. For Pavlovian training each chamber had a food cup located in the center of one wall, 3 cm above a stainless steel grid floor. Head entries into the food cup were recorded by breaks of an infrared photo beam located inside the magazine. A retractable lever illuminated from behind was located 2.5 cm to the left or right of the food cup, ~6 cm above the floor. The location of the lever relative to the food cup was counterbalanced across rats. A red house light that remained illuminated throughout all Pavlovian training sessions was located on

the wall opposite the food cup. For self-administration sessions, the food cup and lever were removed and two nose poke ports were added 3 cm above the floor on the left and right side of the wall opposite the house light. A nose poke into the active port was detected by an infrared photo beam inside the hole and resulted in an intravenous cocaine infusion, delivered by a pump mounted outside the sound attenuating chamber, through a tube connected to the rat's catheter back port. The infusion tube was suspended into the chamber via a swivel mechanism, allowing the rat free movement. All measures were recorded using Med Associates software.

Pavlovian conditioned approach training

Rats were first trained using a Pavlovian conditioned approach procedure described previously (Flagel et al., 2007). Briefly, rats were first familiarized with banana-flavored food pellets, and trained to retrieve pellets delivered into the food cup on a variable time 30-second schedule. The day after pre-training all rats began 5 consecutive days of Pavlovian approach training consisting of 25 trials, over 35-40 minutes. An individual trial commenced with the insertion of the illuminated lever (conditioned stimulus, CS) into the chamber for 8 seconds. The lever was then retracted and coincident with this, a single food pellet (unconditioned stimulus, US) was delivered into the food cup. The CS was presented on a variable time 90-second schedule. Lever deflections, food cup entries, latency to lever deflection, and latency to food cup entry during CS presentation were measured.

The averaged data from days 4 and 5 of training were used to calculate a Pavlovian Conditioned Approach (PCA) index score that quantifies each individual's propensity to approach the lever-CS vs the food magazine during the CS period (sign-tracking vs goal-tracking; see Meyer et al., 2012). A score of +1.0 indicates an animal that made a sign-tracking response on every trial, a -1.0 indicates an animal that made a goal-tracking response on every

trial, and a 0.0 indicates an animal with a 50:50 distribution of behavior towards the lever-CS and food cup. For the purpose of classification, rats with a PCA index of -0.5 or less were defined as GTs (n=37) and animals with a PCA index of 0.5 or greater were defined as STs (n=37). All animals with a PCA index between -0.5 and 0.5 were defined as intermediates (n=28). For this study, we were interested in comparing rats that clearly differed in their propensity to attribute incentive salience to reward cues, and therefore intermediates were excluded from further testing.

Intravenous catheter surgery

Next, ST and GT rats underwent intravenous catheter surgery as described previously (Crombag et al., 2000). Briefly, rats were anesthetized using ketamine hydrochloride (100 mg/kg i.p.) and xylazine (10mg/kg i.p.) and a catheter was inserted into the right jugular vein and tubing was run subcutaneously to a port located on the rat's back. Following surgery, catheters were flushed daily with 0.2 ml sterile saline containing 5 mg/ml gentamicin sulfate (Vedco, MO). Catheter patency was tested periodically with intravenous injection of 0.1 ml methohexital sodium (10 mg/ml in sterile water, JHP Pharmaceuticals). If a rat did not become ataxic within 10 seconds of the injection, the catheter was considered not patent and the animal was removed from the study.

Self-administration: acquisition

Rats were given ~7 days to recover from the catheter surgery, after which time self-administration training commenced. When the rats were placed in the chamber the house light was initially illuminated and the beginning of each session was signaled by the house light being extinguished. At that time a nose poke into the active port resulted in an intravenous infusion of cocaine hydrochloride (NIDA) dissolved in 0.9% sterile saline (0.4mg/kg/infusion in 50 µl

delivered over 2.6 seconds) on a fixed ratio 1 schedule. Each infusion was paired with the illumination of a cue light in the nose port for 20 seconds. Nose pokes during this time were recorded but had no consequences. An inactive port was also present at all times, and pokes there had no consequences. To ensure that during initial training all animals received the same amount of drug exposure, and CS-US pairings, an infusion criteria (IC) procedure was imposed on self-administration sessions, as described previously (Saunders and Robinson, 2010). IC session length was determined by how long it took each rat to reach the predetermined number of infusions, not by an explicit time limit. Each rat had 2 sessions at IC10, 3 sessions at IC20, and 4-6 sessions at IC40. A total of 2 rats (1 ST, 1 GT) were excluded during acquisition training because they failed to discriminate between the active nose port and the inactive nose port.

Self-administration: within-session threshold procedure

The day after acquisition of self-administration, rats were trained on a within-session threshold procedure, as described previously (Oleson and Roberts, 2009; Oleson et al., 2011; Bentzley et al., 2013). During this 110-minute session, rats received access to decreasing doses of cocaine in successive 10-minute intervals on a quarter logarithmic scale (383.5, 215.6, 121.3, 68.2, 38.3, 21.6, 12.1, 6.8, 3.8, 2.2 and 1.2 μg/infusion), achieved by decreasing the pump infusion duration (8175, 4597, 2585, 1454, 818, 460, 259, 145, 82, 46 and 26 ms). Also, during the threshold procedure, the nose port cue light was illuminated for the duration of each infusion. Importantly, there was *no timeout period* following each infusion. As during acquisition, the house light was illuminated when the rats were placed in the chambers and the beginning of the session was signaled by the house light being extinguished.

Demand curve fitting. Demand curves generated from the threshold procedure were fit using a focused-fitting approach, using procedures described in detail elsewhere (Bentzley et al., 2013). Briefly, each animal's brain cocaine concentration was calculated to determine relative stability during a session. Demand data points that failed to meet stability criteria were truncated before demand curves were fit by standard techniques (Bentzley et al., 2013). This typically resulted in elimination of the data point from the first 10-min bin, during which the subject 'loaded' on cocaine (Oleson et al., 2011), and elimination of all data points that occurred more than 20-min (two data points) after P_{max}, when the brain cocaine concentration had dropped significantly (Bentzley et al., 2013). Using this focused-fit approach, the values α and Q_0 in the exponential demand equation (Hursh and Silberberg, 2008) were manipulated to minimize the residual sum of squares, i.e., the square of the difference between the logarithm of the experimentally measured demand and the logarithm of the demand predicted by the exponential demand equation was found for each price and then summed across all prices.

This procedure yields values for a number of metrics. $\mathbf{Q_0}$ is a theoretical measure of consumption when no effort is required; that is, an inherent extrapolation of the animal's consumption at very low prices (Hursh and Silberberg, 2008; Oleson et al., 2011; Bentzley et al., 2013). $\mathbf{P_{max}}$ is defined as the price that elicits maximum responding; i.e., the maximum price (in effort) an animal is willing to pay to maintain Q_0 (Hursh, 1991; Bentzley et al., 2013). Consumption remains relatively stable at prices lower than P_{max} but falls rapidly at prices higher than P_{max}. Finally, $\boldsymbol{\alpha}$ is a measure of normalized demand elasticity and is equivalent to the slope of the demand curve – it is often taken to reflect the "essential value" of a commodity (Hursh and Silberberg, 2008; Bentzley et al., 2013). α is a uniquely unambiguous measure of motivation because it is normalized with respect to Q_0. Thus, changes in motivation that are accompanied by

changes in Q_0 (Bentzley et al., 2014), can be determined with greater confidence than by just P_{max}, or even breakpoint on a progressive ratio schedule (Hursh and Silberberg, 2008; Bentzley et al., 2013, 2014). Motivation is inversely proportional to α, meaning a larger α value corresponds to lower essential value.

For the baseline test, each rat was tested daily using the threshold procedure for a minimum of five sessions and until it produced three consecutive sessions with less than +/-25% variation in α. For baseline data analysis, P_{max}, α, and Q_0 values were averaged over the last 3 sessions for each rat. Each probe test that followed the baseline test consisted of testing each rat for two days using the threshold procedure. Data (not shown) from other experiments have shown that after initial training the rats no longer require multiple days for their behavior to stabilize. For probe test data analysis, P_{max}, α, and Q_0 values were averaged over the 2 sessions for each rat. A total of 7 rats (4 STs, 3 GTs) were excluded during the baseline threshold procedure because their behavior failed to stabilize, or their catheters failed.

Self-administration: within-session punishment procedure

After the rats exhibited stable performance on the threshold procedure they were tested using a similar procedure that manipulated cost by increasing the aversive consequences of self-administration (footshock), as described previously (Bentzley et al., 2014). In this test drug dose remained constant (38.3 μg/infusion) but cost was increased by increasing the intensity of a 0.5-sec contingent footshock that accompanied infusions. After 20-min of self-administration without punishment, the current increased in successive 10-min intervals (0.10, 0.13, 0.16, 0.20, 0.25, 0.32, 0.40, 0.50, 0.63, 0.79 milliamps, mA). Results were normalized for individual variation in Q_0 by defining punishment resistance as the maximum electrical charge (max charge) an animal was willing to endure in a bin to defend its shock-free preferred level of

cocaine consumption. For the baseline test, each rat was tested daily on the punishment procedure for a minimum of four sessions and until it produced three consecutive sessions with less than +/-25% variation in max charge. Each probe test that followed the baseline test consisted of testing each rat for two days on the punishment procedure. Data (not shown) from other experiments established that after initial training the rats no longer require multiple days for their behavior to stabilize.

Self-Administration: intermittent access procedure (IntA)

After completion of the baseline punishment test the rats were allowed to continue to self-administer cocaine, but now using an intermittent access (IntA) procedure, similar to that described previously (Zimmer et al., 2012). Briefly, the rats were placed into the chamber with the house light illuminated. The beginning of the first 5-min Drug-Available period started 2 minutes after the rats were placed into the chamber and was signaled by extinguishing the house light. During the Drug-Available period a nose poke into the active port resulted in an intravenous infusion of cocaine hydrochloride (NIDA) dissolved in 0.9% sterile saline (0.4mg/kg/infusion in 50 µl delivered over 2.6-sec) on a fixed ratio 1 schedule. Each infusion was paired with the illumination of a cue light in the nose port for the duration of the infusion. Pokes that were made during the 2.6-sec infusion period were recorded but not additionally reinforced. It is important to note that there was *no timeout period* following the infusion, so the rats could earn another infusion as soon as the preceding infusion ended. After the 5-min Drug-Available period, the house light turned on and signaled a 25-min No-Drug Available period. During the No-Drug Available period nose pokes were recorded but had no consequences. After 25-min the house light was again extinguished and another 5-min Drug-Available period began. Each IntA session consisted of *8 Drug-Available and 8 No-Drug Available periods*, resulting in a

4-hr session. This procedure results in a series of spikes in brain cocaine concentrations, rapidly rising to a peak, and falling to baseline prior to the next Drug-Available period (see Fig. 1 in Zimmer et al., 2012 for an illustration of changes in brain cocaine levels when using this vs. other self-administration procedures). An inactive port was also present at all times and pokes here had no consequences.

Each rat underwent one IntA session/day, an average of 5 days/week. We varied the number and pattern of days off each week to accentuate the intermittency - for example, one week animals may have had only 1 day off and then the next week the animals may have had 3 days off. However, animals were never given the day directly before a probe test off. The rats were given a total of 36 IntA sessions and underwent probe tests, using both the threshold procedure and the punishment procedure described above, after the 12^{th}, 24^{th}, and 36^{th} IntA sessions (see Fig. 2.3a). A total of 65 rats began IntA testing, but 20 (12 STs, 8 GTs) lost catheter patency before the reinstatement tests, and therefore the N is lower for those later tests. In all, the rats self-administered cocaine for a total of approximately 70 days, combining acquisition (mean of 9 days), threshold testing (mean of 26 days), and IntA (36 days).

Cocaine-induced reinstatement test

Following 36 IntA sessions, and the final threshold and punishment probe tests (P3), rats were tested for cocaine-induced reinstatement using procedures similar to those described previously (Deroche et al., 1999). On the first day of this 2 day test, rats were placed in the self-administration chambers with the house light illuminated. When the session started 2-min later, the house light was extinguished. All nose pokes during this test were recorded but had no consequences (that is, neither drug nor cue was presented). After a 90-min extinction period the rats received four IV saline infusions (20, 40, 80, 160 µl), each separated by 30-min. The

following day the rats were tested using the same procedure, except the saline was replaced by a cocaine solution (0.2, 0.4, 0.8, 1.6 mg/kg).

Extinction and cue-Induced reinstatement test

After the rats completed the cocaine-induced reinstatement test they underwent 2-hr extinction sessions each day for at least 5 days, until they made less than 20 active nose pokes in one session. The rats were placed into the chamber with the house light on and when the session started the house light was extinguished and stayed extinguished for the duration of the extinction session. Responses into the nose ports during these sessions were recorded but had no consequences. The day after a rat met the extinction criterion it underwent an additional day of testing identical to extinction except on this day pokes in the active port were reinforced by the illumination of the cue light for 2.6-sec. A total of 3 rats (1 ST, 2 GTs) were excluded for failing to extinguish responding at the previously active nose port.

Addiction Criteria

Rats were classified as meeting 1-3 "addiction criteria" as described previously (Deroche-Gamonet et al., 2004). A rat was classed as positive for an addiction criterion if its performance on a given test of addiction-like behavior was in the top third of the sample. The tests used to classify animals were the P_{max} and Max Charge values on the third threshold probe test, and the average number of responses during the No Drug Available periods on the last 3 days of IntA self-administration, following Deroche-Gamonet et al. (2004). To determine the degree of incentive-sensitization we compared values from the Baseline threshold test with those on the last threshold test, as well as changes in self-administration behavior between the first and last 3 sessions of IntA self-administration. Rats meeting 2 or 3 addiction criteria (top third) were pooled because there were only 3 animals that were in the top third for all three tests. This

yielded 9 “2/3 criteria rats” (5 GTs, 4 STs), which were compared with 11 “0 criteria rats” (6 GTs, 5 STs).

Statistical analysis

Linear mixed-models (LMM) analyses were used for all repeated measures data. The best-fitting model of repeated measures covariance was determined by the lower Akaike information criterion score (West et al., 2007). Depending on the model selected, the degrees of freedom may have been adjusted to a non-integer value. A standard 2 sample t-test was used to compare within-session threshold and punishment data obtained from the baseline probe test. For these tests, we used a one-tailed test, because we predicted the direction of the group difference based on our previous study showing that, after limited experience, STs were more motivated to self-administer cocaine than GTs, based on higher breakpoints on a progressive ration schedule (Saunders and Robinson, 2011). Data for the α measure was not normally distributed and therefore all statistical tests involving α were run on log transformed data, consistent with previous reports (Bentzley et al., 2014). Statistical significance was set at $p<0.05$.

RESULTS

Individual variation in Pavlovian conditioned approach behavior

Figure 2.1 shows approach behavior as a function of Pavlovian training session for all STs and GTs. As described previously (Flagel et al., 2007; Saunders and Robinson, 2010), with training, STs showed an increase in the probability and vigor (number of contacts) with which they engaged the lever-CS, and a decrease in the latency to approach it (Fig. 2.1a-c). On the other hand, GTs showed an increase in the probability and vigor with which they engaged the food cup during the lever-CS period, and a decrease in the latency to approach it (Fig. 2.1d-f).

No group differences in the acquisition of cocaine self-administration

Due to the nature of the Infusion Criteria (IC) procedure used to train rats to self-administer cocaine (Saunders and Robinson, 2010) differences in acquisition would be evident in the time it took to reach the IC or the number of active responses made. The number of active responses increased across training (effect of IC, $F(2,138.9)=10.385$, $p<0.001$), and there were no group differences in the number of active nose pokes at any infusion criteria ($F(1,82.8)=.016$, $p=0.9$; Fig. 2.2a). The relatively low number of inactive nose pokes did not change ($F(2,128.8)=2.81$, $p=0.064$) across training in either group, which did not differ from one another ($F(1,70.1)=1.53$, $p=.221$) (data not shown). There were also no group differences in session length at any infusion criteria (no effect of group, $F(1,72.3)=.978$, $p=0.33$; Fig. 2.2b). Further, there was very little variation in the number of sessions required to reach our acquisition criteria because all but 3 animals (2 STs, 1 GT) only required the minimum of 9 sessions. Thus, using this procedure there were no group differences in the acquisition of cocaine self-administration behavior, as reported previously (Saunders and Robinson, 2010)

After limited drug experience STs are more motivated to self-administer cocaine

After limited cocaine self-administration experience STs have been reported to be more motivated to self-administer cocaine than GTs, as indicated by breakpoint on a progressive ratio schedule (Saunders and Robinson, 2011). Therefore, data obtained from the baseline within-session threshold test was analyzed separately, to determine if measures of cocaine demand would yield similar results.

There were no group differences in the number of sessions required for behavior to stabilize on the threshold procedure. STs had a higher P_{max} (planned one-tailed t-test; $t(1,61)=5.95$, $p=0.009$; Fig. 2.3a), and a lower α ($t(1,61)=2.14$, $p=0.019$; Figure 3b) than GTs,

indicating they were more willing to expend effort to obtain cocaine as cost increased. In contrast, STs and GTs did not differ on Q_0 ($t(1,61)=.838$, $p=0.182$; Fig. 2.3c), indicating that when the cost was low they both preferred the same level of cocaine consumption. Interestingly, STs and GTs did not differ on the within-session punishment procedure as measured by the Maximum Charge self-administered in any one 10-minute bin ($t(1,59)=.303$, $p=0.292$) or the total amount of charge self-administered throughout the 110-minute session ($t(1,59)=.469$, $p=0.248$; data not shown). Baseline demand curves generated during the threshold procedure for a representative ST and GT, after only limited drug experience, are shown in Fig. 2.3, Panels e and f, respectively.

Drug intake escalates with IntA cocaine experience in both STs and GTs

After the baseline behavioral-economic tests, animals transitioned to the IntA procedure for 36 additional self-administration sessions. With increasing IntA experience, there was a progressive increase in drug intake during the Drug Available periods (Fig. 2.4a; effect of session, $F(35,89.2)=3.426$, $p<0.001$), and this effect was evident in both STs and GTs, which did not differ (Fig. 2.4b; effect of session, $F(35,175.0)=1.651$, $p=0.019$; effect of group, $F(1,75.6)=0.598$, $p=0.442$; interaction $F(35,175.0)=1.056$, $p=0.394$). Figure 2.4c and d show that within each 5-minute Drug Available period both STs and GTs took most of their infusions during the first minute, consistent with Zimmer et al. (2012). Additionally, a progressive increase in drug intake was evident during the first minute of the Drug Available periods (effect of session, $F(35, 504.0)=9.099$, $p<0.001$; note the Log_2 scale, which makes it difficult to visualize the escalation in intake during the first minute). When introduced to the IntA schedule both STs and GTs quickly learned (within a few days) to discriminate between the alternating Drug Available and No Drug Available periods, which were signaled by changes in chamber

illumination (Fig. 2.4a-c). Figure 2.4d shows that with prolonged IntA experience responding during the first 5-min of the No Drug Available periods dropped to very low levels ($t(1,38)=4.25$, $p<0.001$), but responding remained high (and even slightly increased) during the last 5-min of the No Drug Available periods. This presumably indicates anticipation of the next Drug Available period, even though the animals had learned drug was not yet available.

The IntA procedure results in much less total drug consumption than LgA

Table 2.1 compares the average daily total drug consumption by the end of testing in the present study using IntA, and in the Zimmer et al. (2012) study using IntA, to selected studies using long (LgA) and short access (ShA) procedures. It can be seen that total daily drug consumption is much less using IntA than LgA, and comparable to that with ShA.

Motivation for cocaine increases (sensitizes) with IntA experience

Rats were given a probe test using the within-session threshold and punishment procedures after every 12 IntA sessions. To assess changes in cocaine demand as a function of IntA experience we first analyzed the data with the ST and GT groups pooled (Fig. 2.6a-d). With increasing IntA experience there was a progressive increase in cocaine demand as indicated by an increase in P_{max} (Fig. 2.6a; effect of session, $F(3,75.6)=8.56$, $p<0.001$), a decrease in α (Fig. 2.6b; effect of session, $F(3, 79.3)=12.94$, $p<0.001$) and an increase in Max Charge (Fig. 2.6c; effect of session, $F(3,81.1)=8.87$, $p<0.001$). Interestingly, there was no change in Q_0 (Fig. 2.6d; effect of session, $F(3,120.7)=2.13$, $p>0.1$). Thus, motivation for cocaine increased, indicating incentive-sensitization, when cost was manipulated by either increasing the effort required to defend the preferred level of consumption, or by increasing the aversive consequences for doing so. When the influence of increasing IntA experience was assessed for STs and GTs separately there were no group differences on any of these metrics (Fig. 2.6e-h). Notably, the groups did not

differ on any of the measures: P_{max} (effect of group, F(1,51.7)=0.098, p=0.756; group X session interaction, F(3,49.5)=0.71, p=0.553), α (effect of group, F(1, 51.1)=1.72, p=0.195; group X session interaction, F(3,57.4)=2.18, p=0.101), Max Charge (effect of group, F(1,51.7)=.259, p=0.613; group X session interaction, F(3,45.2)=0.21, p=0.889), and Q_0 (effect of group, F(1, 41.4)=0.019, p=0.891; group X session interaction, F(3,47.1)=0.484, p=0.695).

Figure 2.6i shows the demand curve from one representative rat (a ST) at baseline, and then again from the same rat after 36 days of IntA drug experience (Fig. 2.6j).

Both STs and GTs show robust drug- and cue-induced reinstatement

Following the final behavioral-economic tests, and 2-days of extinction, rats were tested for cocaine-induced reinstatement. STs and GTs did not differ in their rate of instrumental extinction prior to the drug prime reinstatement test (Fig. 2.7a; effect of group p-value>0.1). The cocaine-priming injections dose-dependently reinstated drug-seeking behavior to a comparable degree in STs and GTs, as measured by responses in the previously active nose port (Fig. 2.7b; effect of dose, F(4,40.9)=10.9, p<0.001), but not at the previously inactive nose port (p=0.116). Although there appears to be a difference between STs and GTs at the highest dose, this was not statistically significant (effect of group, F(1,47.7)=2.93, p=0.093; group X dose interaction effect, F(4,40.9)=1.69, p=0.17). The last 30 minutes of the drug-free extinction period was used for the 0.0 mg/kg dose.

Following the drug-induced reinstatement test, rats underwent additional extinction training for at least 5 days, followed by a test for cue-induced reinstatement of cocaine-seeking behavior (conditioned reinforcement). Again, STs and GTs did not differ in either the number of responses made during extinction or the number of sessions required to reach extinction criteria (Fig. 2.7c; effect of group p-value>0.1), as we have reported previously (Ahrens et al., 2015).

Both STs and GTs showed robust cue-induced reinstatement, compared to the last day of extinction (Fig. 2.7d; effect of session, $F(1,33)=61.5$, $p<0.001$; session X nose port [active vs. inactive] interaction, $F(1,33)=6.83$, $p=0.013$), and the two groups did not differ (effect of group, $F(1,33)=0.48$, $p=0.493$; session X group interaction, $F(1,33)=0.74$, $p=0.395$).

Table 2.2 compares the magnitude of cue-induced reinstatement of cocaine-seeking in the present study, to that seen in a number of selected studies (Grimm et al., 2003; Kippin et al., 2006; Saunders and Robinson, 2010; Yager and Robinson, 2013). It can be seen that the magnitude of cue-induced reinstatement in the present study was indeed robust, relative to that obtained after only limited experience with cocaine, and comparable to that seen in 'high reinstating' rats after prolonged assess to cocaine (Deroche-Gamonet et al., 2004).

Addiction criteria

Finally, rats were classified as meeting either 0 or 2-3 criteria for addiction based on P_{max}, Max Charge values, and number of responses during the No Drug Available periods, as described previously (Deroche-Gamonet et al., 2004). Of course, 2/3 criteria rats would have the highest values on those tests for which they were selected, so the question we were interested in was whether 2/3 and 0 criteria animals differed in the degree to which they *changed* (sensitized) as a function of IntA experience (i.e., between the first (baseline) and last threshold tests), which would be indicated by significant interaction effects. The extent to which behavior *changed* differently in 2/3 criteria vs 0 criteria rats depended on the measure (Fig. 2.8). The 2/3 criteria rats did show a greater change than 0 criteria rats in P_{max} between the baseline and last threshold tests (Fig. 2.8a; effect of test session, $F(1,34)=18.5$, $p<0.001$; session X group interaction, $F(1,34)=5.14$, $p=0.03$). Thus, on this measure the 2/3 criteria rats appeared to show greater incentive sensitization. For α**,** the 2/3 criteria rats had overall lower values, consistent with being

selected based on P_{max}, which is highly correlated with α. However, in contrast to P_{max}, α decreased to the same extent in the 0 and 2/3 criteria rats, as indicated by a non-significant interaction effect (Fig. 2.8e; effect of test session, $F(1,34)=16.0$, $p<0.001$; session X group interaction, $F(1,34)=2.00$, $p=0.166$). Similarly, for Max Charge, both groups increased their willingness to continue to self-administer in the face of an adverse consequence as a function of IntA experience, but there was no difference between 0 and 2/3 criteria rats in the magnitude of the *change*, again, as indicated by a non-significant interaction effect (Fig. 2.8b; effect of test session, $F(1,34)=4.37$, $p=0.044$; session X group interaction, $F(1,34)=3.04$, $p=0.266$). Similar results were obtained for the number of responses during the No Drug Available period (Fig. 2.8c; session X group interaction, $F(1,34)=1.39$, $p=0.247$), and for the degree of escalation of intake. Both 2/3 criteria and 0 criteria rats escalated their intake with IntA experience, but they did so to the same extent (Fig. 2.8d; effect of test session, $F(1,34)=14.8$, $p=0.001$; session X group interaction, $F(1,34)=0.05$, $p=0.824$). Finally, 2/3 criteria rats did show greater drug-induced reinstatement of drug-seeking behavior than 0 criteria rats (Fig. 2.8f; $t(1,8)=3.41$, $p=0.014$), but these groups did not differ in the magnitude of cue-induced reinstatement (Fig. 2.8g; $t(1,8)=0.26$, $p=0.804$).

DISCUSSION

In addition to dose, the temporal patterns by which drugs reach the brain (pharmacokinetics) can powerfully influence their ability to change brain and behavior (Robinson and Becker, 1986; Allain et al., 2015). We asked, therefore, how prolonged experience with a newly developed intermittent access self-administration procedure (IntA; Zimmer et al., 2012), which produces repeated spikes in brain cocaine concentrations, changes

motivation for cocaine, as assessed using behavioral-economic indicators of cocaine demand (Hursh and Silberberg, 2008; Bentzley et al., 2013). IntA self-administration experience produced a marked and progressive increase in motivation for cocaine (incentive-sensitization), and other addiction-like behavior. This was indicated by: (1) a progressive escalation in drug consumption; (2) a progressive increase in P_{max}, i.e., the maximum price an animal is willing to pay (in effort) to maintain their preferred brain cocaine concentration; (3) a progressive decrease in α, which is a normalized measure of elasticity of the demand curve, or, how readily demand decreases as price increases; (4) an increase in Max Charge, i.e., the willingness to self-administer cocaine in the face of an adverse consequence; (5) continued anticipatory responding towards the end of No Drug Available periods, despite learning drug is not available; and (6) very robust drug- and cue-induced reinstatement of drug-seeking behavior following extinction. Interestingly, there was no change in Q_0, an index of the preferred brain cocaine concentration when cost is negligible.

We also separately assessed the degree to which demand for cocaine changed in STs and GTs. After limited drug experience, STs were more motivated to take cocaine than GTs (higher P_{max} and lower α), consistent with a previous study using a progressive ratio schedule (Saunders and Robinson, 2011). However, prolonged IntA self-administration experience produced marked incentive-sensitization for cocaine in both STs and GTs, such that after this experience motivation for cocaine was equally high in STs and GTs, and they no longer differed on any measure of addiction-like behavior. We discuss each of these findings in turn.

Escalation

A model of addiction that has become popular in recent years involves comparing rats given relatively short access to cocaine (ShA; 1-2 hr daily sessions) with those given long access (LgA;

typically 6 or more hr sessions) (Ahmed and Koob, 1998; Ahmed, 2011, 2012). LgA, unlike ShA, is reported to produce an escalation in drug intake and other forms of addiction-like behavior (Ahmed and Koob, 1998; Ahmed, 2011, 2012; Edwards and Koob, 2013), including an increase in P_{max} and Q_0, and a decrease in α (Bentzley et al., 2014). It has been hypothesized that LgA better models addiction than ShA because,

> "addiction-causing neuropathological processes could be set in motion only when rats can expose themselves sufficiently to cocaine to cross the 'threshold of addiction'—the minimum level of drug exposure required for inducing addiction" (Ahmed, 2012, p. 110).

That is, it is assumed that the *amount* of drug intake is the critical factor in producing addiction-like behavior, including escalation. However, the present results show that rats do *not* have to consume the large amounts of cocaine taken under LgA conditions to produce escalation of intake, or other addiction-like behavior, including increased motivation for drug (also see Zimmer et al., 2012). Total cocaine consumption produced by the IntA procedure used here is comparable to that seen under ShA conditions, and is far less than with LgA (Zimmer et al., 2012; Calipari et al., 2013; see Table 2.1). Indeed, it has been suggested previously that the high level of consumption produced by LgA is not required to produce escalation (Goeders et al., 2009; Beckmann et al., 2012; Mandt et al., 2012). It is worth noting that Zimmer et al. (2012) did not explicitly report escalation in their IntA groups. However, their analysis of intake over 14 days of self-administration resulted in a significant effect of day but no group X day interaction, so individual groups were not examined on this variable. Nevertheless, the escalation reported here does appear to be more robust, which could be related to procedural differences, such as our inclusion of the initial behavioral economic probe tests, which resulted in animals having more self-administration experience prior to IntA experience.

It is known that LgA vs. IntA experience results in different neurobiological adaptations (see below), and it is interesting to speculate that either can cause escalation of intake, but for different reasons. Perhaps because of the different pharmacokinetic profiles involved, the same apparent outcome (escalation) is due to tolerance to hedonic effects in the case of LgA, as previously suggested (Ahmed, 2012; Calipari et al., 2014a; Edwards and Koob, 2013- their Fig. 1) but to sensitization of drug "wanting" (incentive-sensitization) in the case of IntA (see discussion below). This notion is consistent with reports that Q_0 is increased after LgA (Christensen et al., 2008; Bentzley et al., 2014), but not after IntA (present study). Interestingly, when tested 1-2 days after LgA cocaine self-administration experience rats show a decrease in the ability of cocaine to elevate DA in the nucleus accumbens and to produce psychomotor activation (Calipari et al., 2013), but after a month of abstinence animals with prior LgA experience express marked psychomotor sensitization (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 (e.g., Paulson et al., 1991; Paulson and Robinson, 1995).

Motivation

Another defining feature of addiction, beyond escalation of intake, is an increase in motivation to take cocaine. Motivation is often measured by the willingness to bear increasing costs, either by imposing an increase in the effort required to procure cocaine or by imposing an adverse consequence (such as footshock) for doing so, and observing the extent to which an individual persists in consumption. Prolonged experience with IntA cocaine self-administration progressively increased motivation for cocaine by both of these measures, in both STs and GTs, as indicated by an increase in P_{max}, a decrease in α, and an increase in the maximum electrical

charge they would endure. Continued responding when drug is not available has also been reported to be a addiction-like behavior (Deroche-Gamonet et al., 2004), and here, rats continued to show anticipatory responding towards the end of the No Drug Available period, long after they learned that drug was not available. Although this is the first report of a progressive increase in motivation for cocaine with prolonged IntA experience, Zimmer et al (2012) previously reported that rats with a history of IntA cocaine self-administration had a significantly higher P_{max} than those with LgA experience, despite consuming much less drug (they did not report α values). Collectively, these findings are consistent with the notion that, "an intermittent pattern of use, more than the amount of drug used," (Allain et al., 2015, p. 175) may be especially important in the development of addiction.

Interestingly, there was no change in free consumption (Q_0) with IntA experience. Although they did not calculate Q_0, Zimmer et al. (2012) also found no difference in free consumption between groups with ShA, LgA or IntA self-administration experience during the initial portion of the threshold test, when cost was low, although others have found LgA experience does increase Q_0 (Christensen et al., 2008; Bentzley et al., 2014). It is difficult to know exactly what psychological process determines Q_0. Although there are alternative interpretations, one is that it reflects a balance between the positive and aversive effects of the drug, i.e., hedonic value (e.g.,Bentzley et al., 2013). Of course, it is impossible to know if Q_0 truly reflects the hedonic effects of cocaine in non-human animals, but if it did, prolonged IntA drug experience may dissociate motivation for drug and its hedonic consequences (see Oleson et al., 2011), consistent with Incentive-Sensitization Theory (Robinson and Berridge, 1993). This notion is also consistent with reports that, at least for psychostimulants, α better predicts addiction-like behavior than Q_0 (Bentzley et al., 2014).

Reinstatement

Both long and prolonged access to cocaine are reported to increase the propensity to reinstate drug-seeking behavior (Deroche et al., 1999; Ahmed, 2012), and here, prolonged IntA experience also produced robust drug- and cue-induced reinstatement of drug-seeking behavior. Although a comparison with other studies requires caution, Table 2.2 shows that the magnitude of reinstatement seen here was indeed robust. For example, the average number of active nose pokes in 1-hr during the cue reinstatement test (~200) was the same as reported in high reinstating rats (highest 40%) described by Deroche et al. (2004) and much higher than we have seen in rats with only limited drug experience (~60-80 responses; Saunders and Robinson, 2010), suggesting that prolonged IntA leaves animals especially prone to reinstate drug-seeking behavior. Interestingly, after limited drug experience STs show greater drug- and cue-induced reinstatement than GTs (Saunders and Robinson, 2010, 2011; Saunders et al., 2013), but after IntA experience they no longer differed, consistent with the changes in motivation for cocaine. However, it remains to be determined if this will also be true for other precipitators of relapse (e.g., context and stress).

Mechanisms

The temporal pattern by which stimuli impinge on the nervous system has a large effect on their ability to produce brain plasticity. The classic example is the influence of spaced vs. massed trials on learning. Another is the effectiveness of different patterns of stimulation to produce LTP (e.g., Larson et al., 1986) or LTD (e.g., Bear and Malenka, 1994), or dopamine-induced synaptic plasticity (e.g., Wieland et al., 2015). Temporal factors are equally important for *drug* experience-dependent plasticity (Allain et al., 2015 for review). For example, spaced injections are much more effective in producing behavioral and neural sensitization than massed

injections, and if blood levels of drug are maintained at elevated levels continuously (as with traditional self-administration procedures), tolerance rather than sensitization may result (Post, 1980; Robinson and Becker, 1986; Vezina, 2004). In addition, sensitization may become more evident after a period of abstinence (e.g., Paulson et al., 1991; Paulson and Robinson, 1995), perhaps contributing to 'incubation of craving' effects (Pickens et al., 2011 for review).

Thus, the greater effectiveness of IntA to increase motivation for drug, relative to either ShA or LgA experience (Zimmer et al., 2012), may be because a 'spiking' temporal pattern of consumption is more effective in producing incentive-sensitization and associated neuroadaptations relevant to addiction (Allain et al., 2015). Indeed, it is important to note that IntA and LgA are reported to have *opposite effects* on dopamine transmission when tests are conducted soon after discontinuing self-administration, the former producing sensitization and the latter tolerance (Calipari *et al*, 2013, 2014a, 2014b, 2015; c.f., Ferrario *et al*, 2005). It should be concerning that two porported 'models of addiction' (LgA and IntA) produce opposite neurobiological effects. It will be critical to determine, therefore, which better reflects the changes in brain associated with the transition from casual drug use to addiction. If the pharmacokinetics associated with IntA are more effective in producing changes in brain that lead to the development of addiction-like behavior, or produce qualitatively different neuroadaptations, relative to LgA, or even prolonged ShA, this should be an important consideration in the design of preclinical studies (Allain et al., 2015).

Individual differences in susceptibility to addiction

In addition to LgA, prolonged ShA cocaine self-administration experience is also reported to produce addiction-like behavior, but in only ~15% of individuals, based on an analysis of the number of 'addiction criteria' they meet (Deroche-Gamonet et al., 2004; Belin et

al., 2009; Belin and Deroche-Gamonet, 2012). We asked, therefore, whether the degree of incentive-sensitization seen in the present study, varied as a function of the number of 'addiction criteria' met. We found that on a number of measures 0 criteria and 2/3 criteria rats did not differ markedly in the degree to which their behavior *changed* with IntA experience. It is possible, therefore, that the prolonged ShA procedure may over-estimate the degree of individual variation in the development of addiction-like behavior, because the pharmacokinetics associated with ShA are not as effective in promoting neural sensitization, and thus pathological motivation for drug (Allain et al., 2015).

This issue clearly requires further investigation, but it is an interesting one, because it addresses the source of individual variation in addiction liability. Individual variation in susceptibility to addiction may not be due to differential susceptibility to drug-induced neuroadaptations that produce pathological motivation for drug. Perhaps any individual exposed to drugs intermittently, repeatedly and using routes of administration that result in rapid absorption would be susceptible to incentive-sensitization. If susceptibility to this form of drug experience-dependent plasticity, per se, is not the critical factor, then the important susceptibility factors for addiction may be those that determine, after initial use, whether a given individual continues to take drug, especially using routes of administration, doses and patterns of use that produce neuroadaptations that facilitate incentive-sensitization (Allain et al., 2015). This may depend more on social, personality and contextual factors.

Indeed, this may explain why we found that after prolonged IntA self-administration experience both STs and GTs underwent incentive-sensitization to the extent that they no longer differed on any measure of addiction-like behavior. We originally hypothesized that STs may be more susceptible to addiction than GTs (Flagel et al., 2009; Saunders and Robinson, 2013), at

least in part because they differed in susceptibility to sensitization (Flagel et al., 2008). However, all of our previous studies involved relatively limited exposure to drugs. The results here suggest an alternative hypothesis. Individuals with a ST phenotype may indeed be more susceptible to addiction, but not because they are especially vulnerable to incentive-sensitization, at least after IntA self-administration experience. Rather, they may be more susceptible to addiction because they are initially more motivated to take cocaine (Saunders and Robinson, 2011), they are especially sensitive to cocaine cues (Saunders and Robinson, 2010; Saunders et al., 2013), are more impulsive (Tomie et al., 2008; Lovic et al., 2011), are high novelty-seekers (Beckmann et al., 2011), are resistant to Pavlovian extinction (Beckmann and Chow, 2015; Ahrens et al., 2016), are initially more likely to choose drug (cocaine) over non-drug rewards (Tunstall and Kearns, 2015), and importantly, they have relatively poor top-down executive control over behavior (Paolone et al., 2013). All of these characteristics would increase the probability that individuals with a ST phenotype, after initial casual drug use, continue to use drugs, which would eventually expose them to incentive-sensitization, and addiction (Robinson and Berridge, 1993).

In summary, we report that IntA cocaine self-administration experience produces robust incentive-sensitization and other addiction-like behavior, and does so despite much less total drug intake than with the popular LgA model. We suggest, as have others (Zimmer et al., 2012; Allain et al., 2015), that 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 better match patterns of use in humans. We readily acknowledge some may view this suggestion as provocative, but it is important to question and modify our animal models in the face of new evidence. To paraphrase

the statistician, George Box (Box et al., 2005), “All models are wrong but some models are useful", and it behooves us to determine as best we can which animal models of addiction are more useful, and for what purpose.

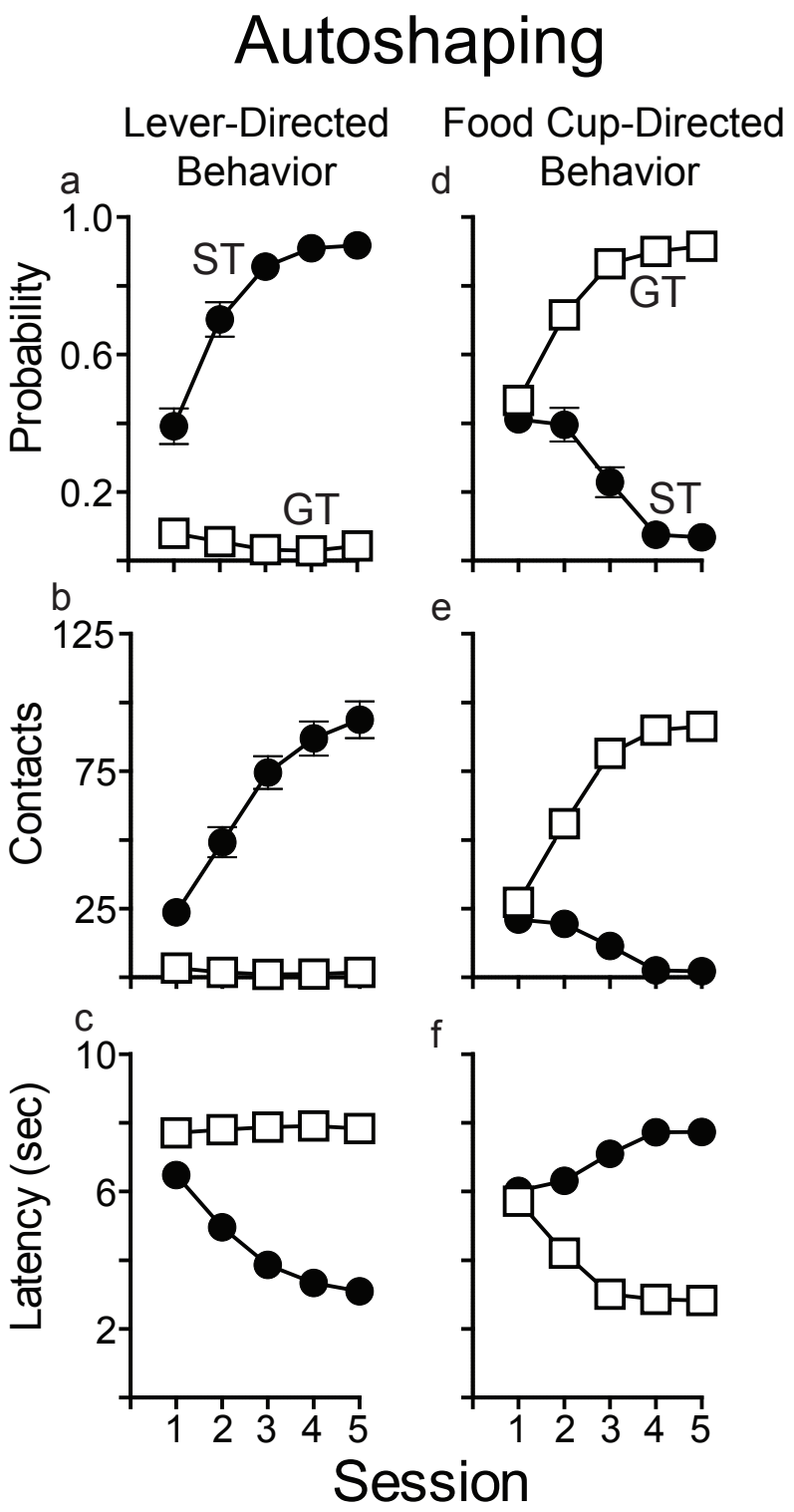

Autoshaping
Lever-Directed Behavior
Food Cup-Directed Behavior
a
d
b
e
c
f
Probability
1.0
0.6
0.2
ST
GT
GT
ST
Contacts
125
75
25
Latency (sec)
10
6
2
1 2 3 4 5
1 2 3 4 5
Session

Figure 2.1. Autoshaping. The development of Pavlovian conditioned approach behavior in rats classed as sign-trackers (STs) (n=37) or goal-trackers (GTs) (n=37) in the present experiment. As reported many times, pairing a lever-CS with a food reward results in some rats (STs) showing lever-directed behavior, as indicated by an progressive increase in the probability of deflecting the lever on any given trial (**a**), the number of lever contacts (deflections; **b**) and a decrease in the latency to contact the lever (**c**). During the CS period other rats (GTs) direct their behavior towards the food magazine, as indicated by a progressive increase in the probability of entering the food magazine on any given trial (**d**), the number of magazine entries (**e**) and a decrease in the latency to enter the magazine (**f**). Values represent means ± SEMs.

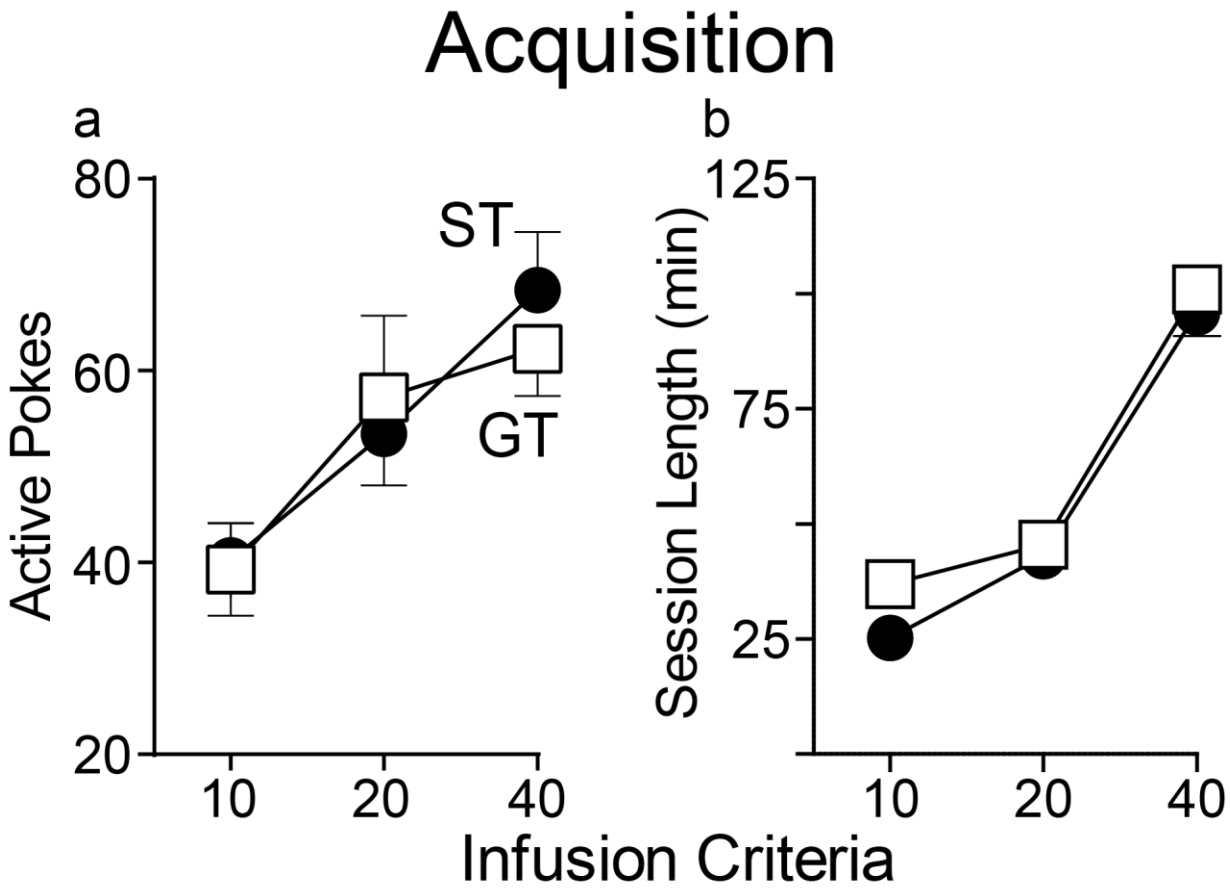


Figure 2.2. Acquisition. Acquisition of cocaine self-administration behavior using an Infusion Criterion procedure (see Methods). There were no differences between STs (n=36) and GTs (n=36) in the acquisition of cocaine self-administration as indicated by either the number of active responses (**a**) or the time to meet each criterion number of injections (session length; **b**). Values represent means ± SEMs.

Cocaine Demand After Limited Drug Experience

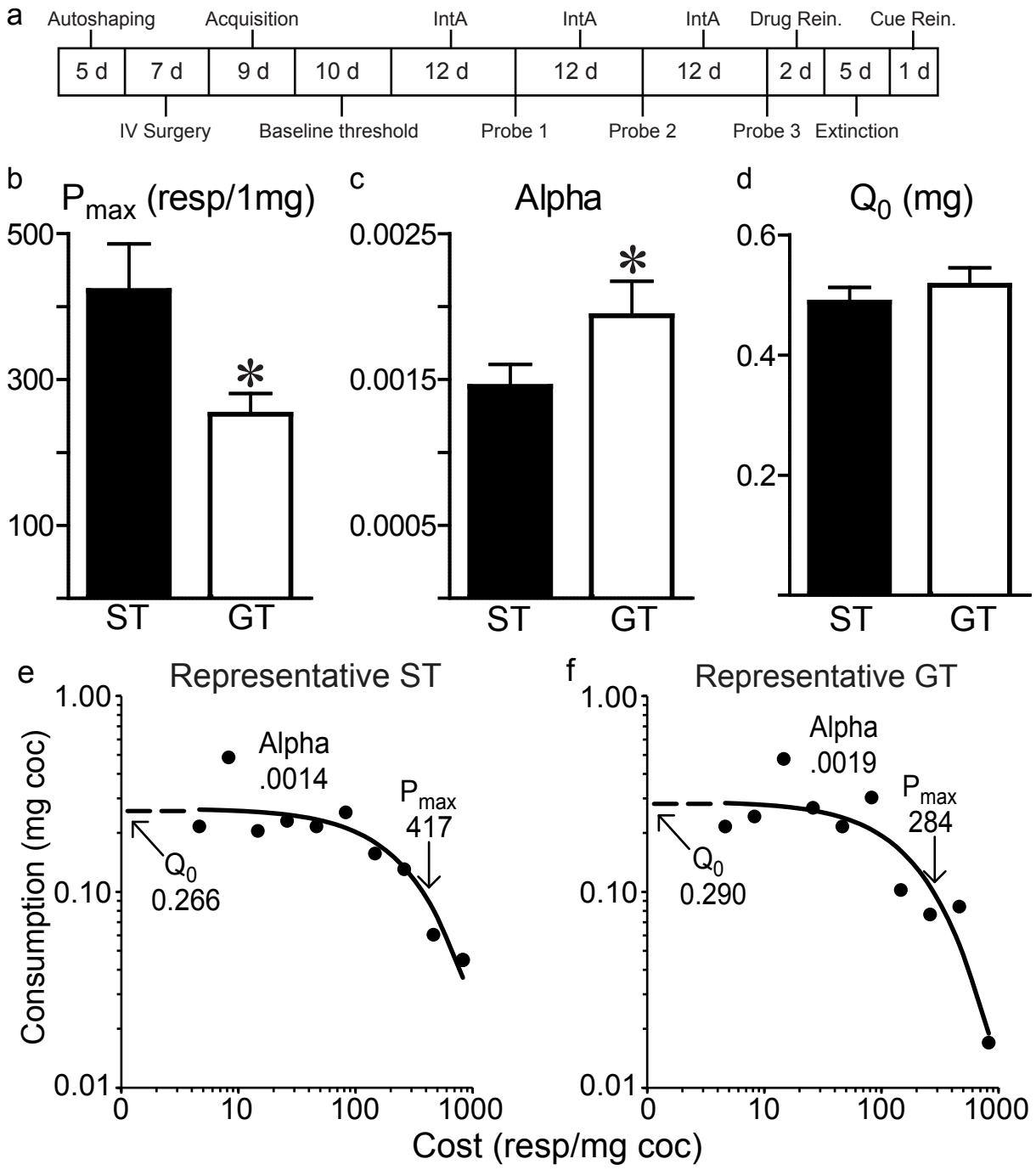


Figure 2.3. Baseline demand prior to IntA. Cocaine demand in STs (n=32) and GTs (n=33) after only limited drug experience. The flow diagram at the top (**a**) shows the overall experimental design and timeline for the entire experiment. The data shown here were obtained after acquisition, at the point indicated by 'Baseline threshold tests' in Panel **a,** and after 14-19 days of self-administration experience on a FR1 schedule. Relative to GTs, STs had a higher P_{max} (**b**), lower α (**c**) but there was no difference in Q_0 (**d**). Values represent the means ± SEMs. Panels **e** and **f** show demand curves for a representative ST and GT rat, respectively.

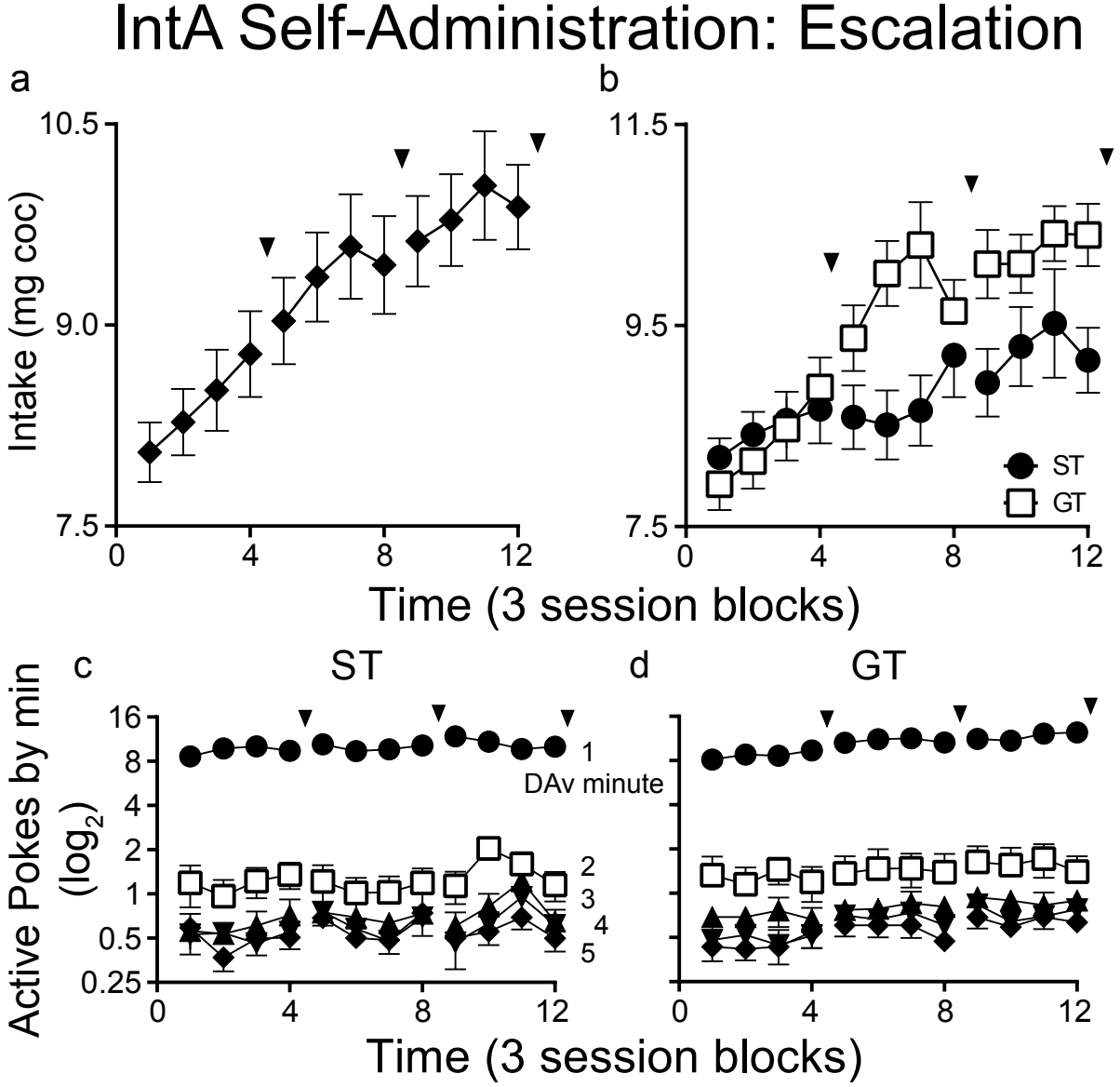

IntA Self-Administration: Escalation
a
b
10.5
9.0
7.5
11.5
9.5
7.5
Intake (mg coc)
0
4
8
12
ST
GT
Time (3 session blocks)
c
ST
d
GT
16
8
4
2
1
0.5
0.25
Active Pokes by min
(log2)
1
DAv minute
2
3
4
5
0
4
8
12
Time (3 session blocks)

Figure 2.4. IntA self-administration. Cocaine self-administration behavior during the 36 Intermittent Access (IntA) sessions (see Panel a in Fig. 2.3) averaged over 3 session blocks. After every 12 IntA sessions there was a probe test of cocaine demand using the threshold procedure. The timing of the threshold tests is indicated by the arrowheads. Panel **a** shows the progressive escalation of cocaine intake over time with STs and GTs pooled. Panel **b** shows that both STs and GTs escalated their cocaine intake over time, and although it appears that GTs may have escalated intake to a greater degree than STs, there were no statistically significant group differences. Panels **c** and **d** show the number of active responses during each minute of the 5-min Drug Available periods, in 3-session blocks, over the 36 days of IntA self-administration, in STs and GTs, respectively. Note the Log_2 scale, which was required to visualize data for each minute on the same graph. Both STs and GTs took nearly all their drug infusions during the first minute of the 5-min Drug Available periods. Values represent means ± SEMs. Respective n-values: ST: session 1= 32, session 36 = 20; GT: session 1= 33, session 36= 25.

IntA Self-Administration: Discrimination

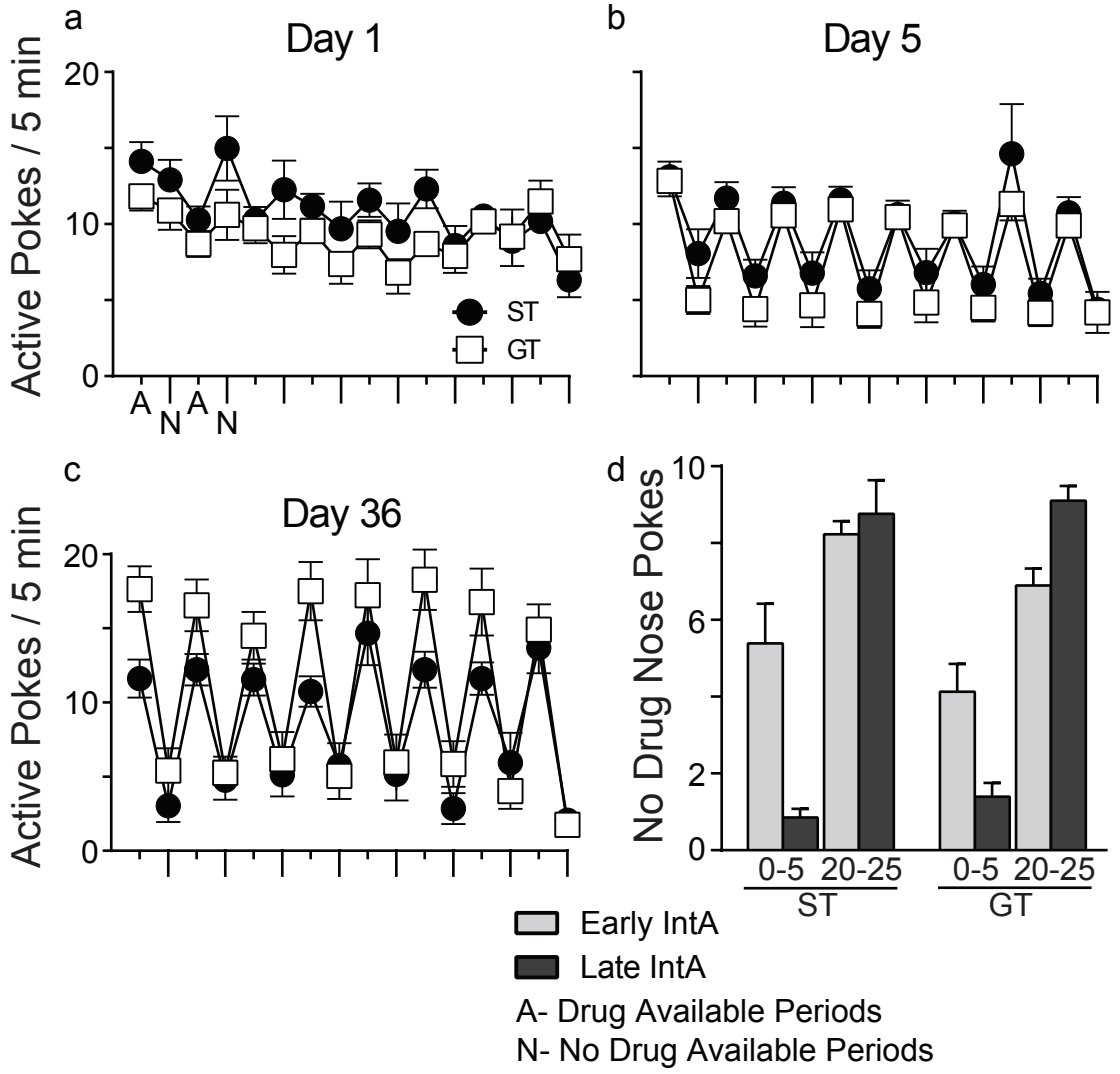


Figure 2.5. IntA Discrimination. Both STs and GTs quickly came to discriminate between the 5-min Drug Available periods (A) and the 25-min No Drug Available periods (N), which were signaled by changes in chamber illumination. Panels **a**, **b**, and **c** show the number of responses (nose pokes) per 5 min during the first day of IntA self-administration (n=32 STs, 33 GTs) (**a**) and then again after 5 days of IntA experience (n=32 STs, 33 GTs) (**b**) and yet again on the last (36^{th}) day of IntA self-administration (n=20 STs, 25 GTs) (**c**). Discrimination was evident by the 5^{th} day of IntA. Panel **d** compares the number of responses during the first 5-min of No Drug Available periods (0-5) early during IntA training (Early IntA; first 3 session block) and then again, at the end of IntA (Late IntA; last 3-session block), with the number of responses during the last 5-min of No Drug Available periods (20-25), again early and then late during IntA. It can be seen that as the discrimination was learned (compare Early and Late values) both STs and GTs greatly decreased responding during the first 5-min of the No Drug Available periods. However, responding during the last 5-min did not decrease, even though the discrimination was well learned. This may be indicative of anticipatory responding even when it is known that drug is not available (e.g., Deroche-Gamonet et al., 2004). Values represent means ± SEMs.

Cocaine Demand with Prolonged IntA Drug Experience

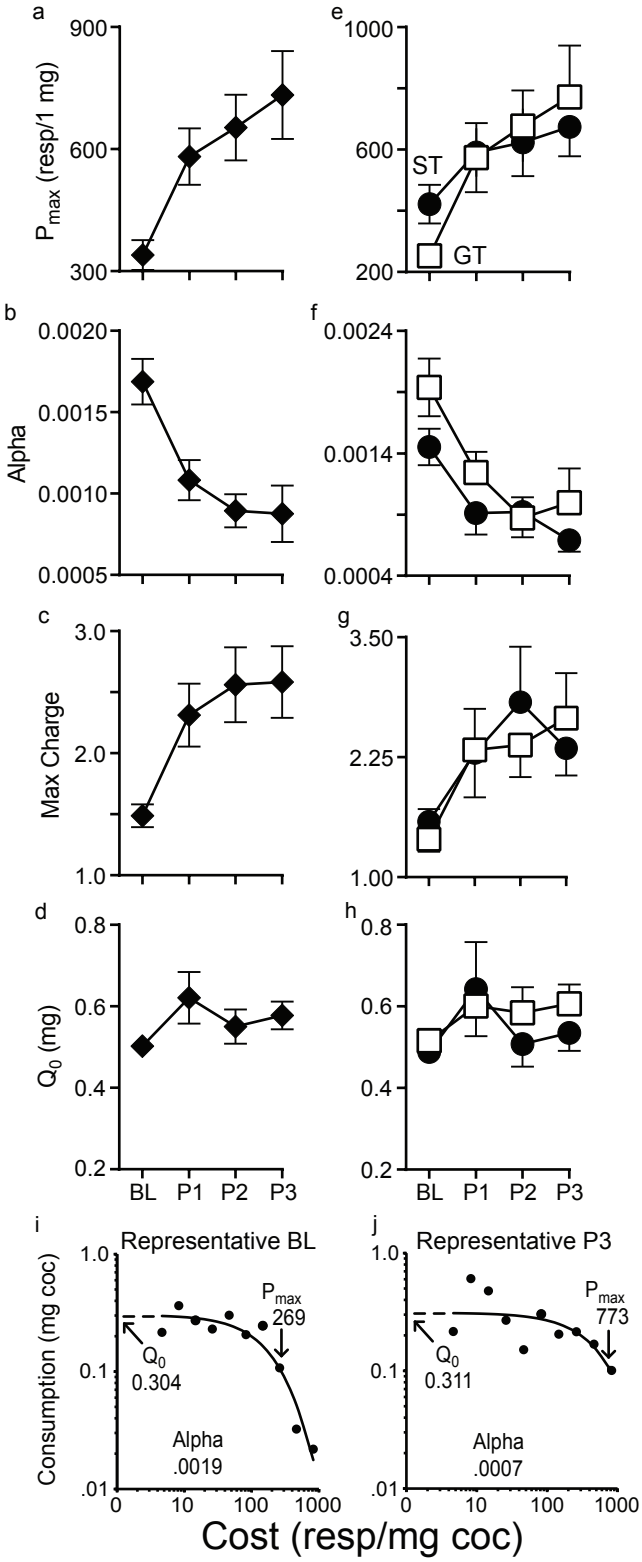


Figure 2.6. Cocaine demand following IntA. Changes in cocaine demand as a function of IntA self-administration experience. Panels **a-d** show the four metrics calculated from the demand curves during the Baseline (BL) threshold test, and then again after each of the three Probe threshold tests (P1-P3), which were conducted after every 12 IntA sessions (see Panel a in Fig. 2.3). There was a progressive increase in P_{max} (**a**), decrease in α (**b**), increase in Max Charge (**c**), but no change in Q_0 (**d**). Panels **e-h** show the performance of STs (n=20) and GTs (n=25) separately on the same four metrics. Notably the groups did not differ on any of the measures. Values represent means ± SEMs. Panels **e** and **f** show demand curves for a representative animal (a ST) on its Baseline (BL) test and then again from the same animal during last threshold test (P3), after 36 days of IntA experience, respectively.

Extinction and Reinstatement Tests

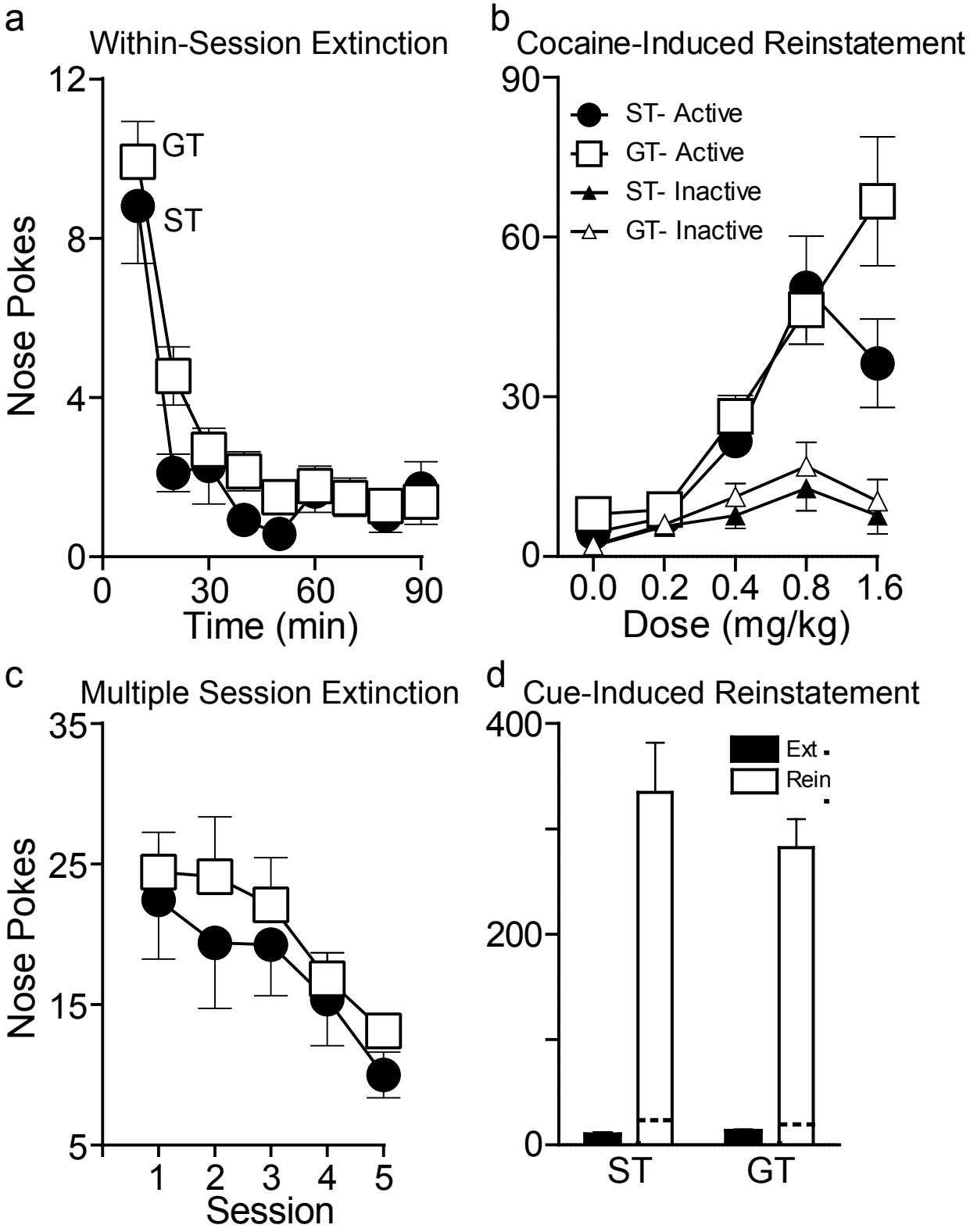


Figure 2.7. Reinstatement. Instrumental extinction, drug prime and cue-induced reinstatement tests. After 36 days of IntA self-administration all rats first underwent one day of extinction training, and then on the next day, further extinction before testing for drug-induced reinstatement of cocaine seeking behavior. Panel **a** shows that on the first day of extinction training both STs (n=20) and GTs (n=25) rapidly decreased instrumental responding (the number of active nose pokes), and there were no group differences in the rate of instrumental extinction (see Ahrens et al., 2015 for a comparison of Pavlovian vs. instrumental extinction in STs and GTs). Panel **b** shows the effect of three successive doses of cocaine, separated by 30 min, on responding, under extinction conditions. The values for the dose of 0 are the means ± SEMs for the 30-min immediately prior to the priming injections. The priming injections dose-dependently increased active responses, relative to inactive responses, and although there appears to be a group difference after the highest dose, this was not statistically significant. Panel **c** shows active responses on each of the 5 days of further extinction training following the drug-induced reinstatement test (n=19 STs, 23GTs). Again, there were no group differences in instrumental extinction. Panel **d** shows the results of the cue reinstatement test, when responding now resulted in presentation of the cue previously paired with cocaine delivery during self-administration sessions, but no cocaine was delivered. The black bars show responding at the end of extinction training and the white bars the number of active responses during the 2-hour reinstatement test. The dashed lines within the white bars indicate the mean number of inactive responses during the reinstatement test. It is clear that the cue was a highly effective conditioned reinforcer, reinstating high levels of responding, in both STs and GTs, which did not differ. Values represent means ± SEMs.

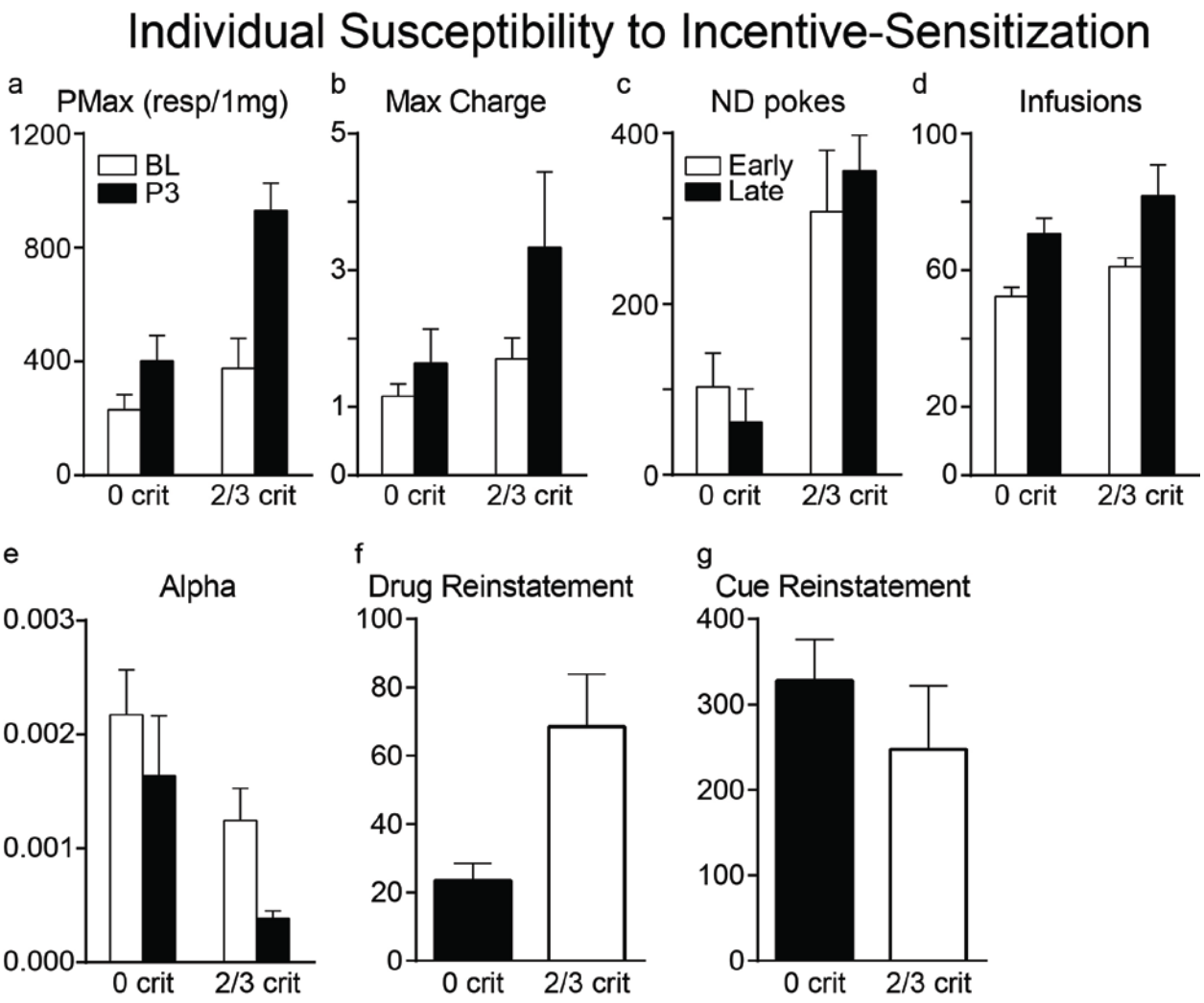


Figure 2.8. Addiction criteria. Analysis based on addiction criteria. Differences in the extent to which measures of motivation for cocaine *changed* on the baseline threshold and punishment tests (BL) and the last test after 36 days of IntA experience (P3) in 2/3 criteria (n=9) vs. 0 criteria rats (n=11) would be indicated by significant interaction effects. This was found for P_{max}, but not for any other measure. Also, although 2/3 criteria rats showed more robust drug-induced reinstatement the 2/3 and 0 criteria rats did not differ in the degree of cue-induced reinstatement.

Study	Procedure	Consumption
Present Study*	Prolonged IntA	10.0 mg
Zimmer et al., 2012	IntA	~7.0 mg
Calipari et al., 2013	IntA	~5.7 mg
Zimmer et al., 2012*	LgA	~25 mg
Calipari et al., 2013	LgA	~21.4 mg
Ahmed and Koob, 1998*	LgA	~27.5 mg
Ahmed and Koob, 1999*	Prolonged LgA	~35 mg
Zimmer et al., 2012	ShA	~5.0 mg
Calipari et al., 2013	ShA	~7.1 mg

Table 2.1. Cocaine consumption across various self-adminsitration studies. A comparison of average daily total drug consumption by the end of testing in selected studies using IntA, LgA, and ShA procedures**. *** Indicates study reported escalation and consumption data is taken from the late (post-escalation) session.

Study	Cue Rein (responses, 1 hour)
Prolonged IntA (present study)	200
Deroche et al., 2004 (Hi Rein- top 40%)	200
Grimm et al., 2003	1 day: 10, 30 day: 80, 60 day: 85
Saunders et al., 2010	60 (STs: 80)
Yager et al., 2013	80 (STs: 90)
Kippin et al., 2006 - LgA	~60 (*2 hours)

Table 2.2. Cue-induced reinstatement. Comparison of the magnitude of cue-induced reinstatement of cocaine seeking in the present study to that seen in other selected studies.

CHAPTER III

SEX DIFFERENCES IN ADDICTION-LIKE BEHAVIOR FOLLOWING INTERMITTENT COCAINE SELF-ADMINISTRATION

INTRODUCTION

There is considerable evidence from both human and pre-clinical studies suggesting that the addiction process occurs differently in males and females, and that females are more susceptible to develop certain symptoms of addiction than males (for reviews see: Fattore et al., 2008; Becker, 2016; Becker and Koob, 2016). For example, females tend to seek treatment sooner than males across a number of drug classes (Anglin et al., 1987a; Griffin et al., 1989; Brady and Randall, 1999), and when they seek treatment, females present with more severe problems (Anglin et al., 1987b; Kosten et al., 1993). Taken together these findings suggest what has been described as a "telescoping effect"- that is, females progress from casual drug use to addiction faster than males and have a smaller window for medical intervention and treatment (Piazza et al., 1989; Brady and Randall, 1999). In addition, and possibly related to the "telescoping effect", there is evidence that females report higher levels of craving induced by the presentation of cocaine-associated cues (Robbins et al., 1999; Elman et al., 2001; Kennedy et al., 2013). And interestingly, women, relative to men, report greater difficulty in controlling their

cocaine use, and more frequently report using more cocaine than they intended (Kennedy et al., 2013). Kennedy and colleagues (2013) also reported that women were more likely to have been tempted to use cocaine in the past hour relative to testing, suggesting more frequent cravings in females. Further, Kennedy et al., (2013) reported that males and females did not differ in the amount of cocaine they used per use event, but females provided fewer cocaine-negative samples than males, suggesting that females used cocaine more frequently.

Findings from rat self-administration studies have also indicated that females may have an increased susceptibility to develop certain addiction-like behaviors. Perhaps mirroring the "telescoping effect" that exists in humans, female rats are more likely to acquire cocaine self-administration (Lynch and Carroll, 1999; Hu et al., 2004) and when given extended access to cocaine, females self-administer more drug (Lynch and Taylor, 2004). Also, when measured on a progressive-ratio test, females show enhanced motivation to obtain cocaine after fewer self-administration sessions than males (Lynch and Taylor, 2004; Ramôa et al., 2013; Lynch, 2018) and when males and females are exposed to the same amount of self-administration access, females are more motivated than males (Roberts et al., 1989; Cummings et al., 2011).

Another behavioral measure with relevance to addiction is psychomotor sensitization. Psychomotor sensitization is a persistent increase in the ability of a drug to induce psychomotor activity as a result of past experience with that drug. Psychomotor sensitization is thought to develop through the same neurobiological changes that drive pathological drug wanting (incentive-sensitization) (Robinson and Berridge, 1993). Interestingly, female rats show greater psychomotor sensitization following exposure to a number of different drugs, including cocaine (Robinson, 1984; Robinson and Becker, 1986; van Haaren and Meyer, 1991). This is particularly interesting in light of a recent study conducted in male rats that showed that the degree of

psychomotor sensitization during Intermittent Access (IntA) cocaine self-administration experience was highly predictive of subsequent motivation for cocaine (Allain et al., 2017).

The IntA self-administration procedure has been used in a number of studies and is particularly effective at producing incentive-sensitization and addiction-like behavior, in spite of producing far less total drug-intake than other comparable self-administration models of addiction (Zimmer et al., 2012; Allain et al., 2015, 2017; chapter 2). However, to this point no study has examined females during and following IntA cocaine experience. Given that in humans, females appear to undergo incentive-sensitization more rapidly than males, and in rats, females show greater psychomotor sensitization, females may be particularly susceptible to develop addiction-like behaviors following IntA cocaine self-administration. Therefore, we hypothesized that females would undergo greater incentive-sensitization and show greater susceptibility to develop addiction-like behavior. We tested this hypothesis using behavioral economic indicators of cocaine demand before and after IntA self-administration experience.

MATERIALS AND METHODS

A total of 52 (28 males; 24 females) Sprague-Dawley rats (Envigo, Haslett, MI) weighing 250-275 g on arrival were housed individually on a reverse 12-h light/12-h dark cycle (lights on at 20:00) in a climate-controlled colony room. Males and females were housed in separate but identical housing rooms. All testing was conducted during the 12-hour lights off period. After arrival, rats were given 1 week to acclimate to the colony room before testing began. Water and food were available *ad libitum* throughout the experiment. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Apparatus

Behavioral testing was conducted in standard (22x18x13 cm) test chambers (Med Associates, St Albans, VT, USA) located inside sound-attenuating cabinets. A ventilating fan masked background noise. Within the test chambers, two nose poke ports were located 3 cm above the floor on the left and right side of the wall. A red house light was located at the top, center of the wall opposite the nose ports. During self-administration portions of the experiment, a nose poke into the active port was detected by an infrared photo beam inside the hole and resulted in an intravenous cocaine infusion, delivered by a pump mounted outside the sound attenuating chamber, through a tube connected to the rat's catheter back port. The infusion tube was suspended into the chamber via a swivel mechanism, allowing the rat free movement. All measures were recorded using Med Associates software.

Intravenous catheter surgery

Male and female rats underwent intravenous catheter surgery as described previously (Crombag et al., 2000). Briefly, rats were anesthetized using ketamine hydrochloride (90 mg/kg i.p.) and xylazine (10mg/kg i.p.) and a catheter was inserted into the right jugular vein and tubing was run subcutaneously to a port located on the rat's back. During recovery from surgery rats were administered the analgesic carprofen (5 mg/kg s.c.). Following surgery, catheters were flushed daily with 0.2 ml sterile saline containing 5 mg/ml gentamicin sulfate (Vedco, MO). Catheter patency was tested periodically with intravenous injection of 0.1 ml methohexital sodium (10 mg/ml in sterile water, JHP Pharmaceuticals). If a rat did not become ataxic within 10 seconds of the injection, the catheter was considered not patent and the rat was removed from the study.

Self-administration: acquisition

Rats were given ~7 days to recover from the catheter surgery, and then self-administration training commenced. The rats were placed in the chamber with the house light illuminated and the beginning of each session was signaled by the house light being extinguished. At that time a nose poke into the active port resulted in an intravenous infusion of cocaine hydrochloride (NIDA) dissolved in 0.9% sterile saline (0.4mg/kg/infusion in 50 µl delivered over 2.6 seconds) on a fixed ratio-1 (FR-1) schedule. Each infusion was paired with the illumination of a cue light in the active nose port for 20 seconds. Nose pokes during this time were recorded but had no consequences. An inactive nose port was also present at all times and pokes there were recorded but had no consequences. To ensure that during initial training all rats received the same amount of drug and cue exposure, an infusion criteria (IC) procedure was imposed on self-administration sessions, as described previously (Saunders and Robinson, 2010). During these sessions, session length was determined by how long it took each rat to reach the predetermined number of infusions, not by an explicit time limit. Each rat had 3 sessions at IC10 and 5 sessions at IC40. A total of 4 rats (3 males, 1 female) were excluded during acquisition training because they failed to reach the infusion criteria or failed to discriminate between the active nose port and the inactive nose port.

Self-administration: within-session threshold procedure

The day after the final acquisition session, rats were trained on a within-session threshold procedure, as described previously (Oleson and Roberts, 2009; Oleson et al., 2011; Bentzley et al., 2013; chapter 2). Briefly, each session (one per day) was 110 minutes in length, FR-1 throughout, and every 10 minutes the dose of drug was decreased on a quarter logarithmic scale (1.28, 0.72, 0.40, 0.23, 0.13, 0.072, 0.040, 0.023, 0.013, 0.007, 0.004 mg/kg/infusion). During

the threshold procedure, the nose port cue light was illuminated for the duration of each infusion. Importantly, there was *no timeout period* following each infusion. As during acquisition, the house light was illuminated when the rats were placed in the chambers and the beginning of the session was signaled by the house light being extinguished.

Demand curve fitting. As described previously (Bentzley et al., 2013; see chapter 2), demand curves were generated from the threshold procedure using a focused-fitting approach. Briefly, each animal's brain cocaine concentration was calculated to determine relative stability during a session. Demand data points that failed to meet stability criteria were truncated before demand curves were fit by standard techniques (Bentzley et al., 2013). This typically resulted in elimination of the data point from the first 10-min bin, during which the subject 'loaded' on cocaine (Oleson et al., 2011), and elimination of all data points that occurred more than 20-min (two data points) after P_{max}, when the brain cocaine concentration had dropped significantly (Bentzley et al., 2013). Using this focused-fit approach, the values α and Q_0 in the exponential demand equation (Hursh and Silberberg, 2008) were manipulated to minimize the residual sum of squares, i.e., the square of the difference between the logarithm of the experimentally measured demand and the logarithm of the demand predicted by the exponential demand equation was found for each price and then summed across all prices.

This procedure yields values for a number of metrics, here we report Q_0 and α. $\mathbf{Q_0}$ is a theoretical measure of consumption when no effort is required; that is, an inherent extrapolation of the animal's consumption at very low prices (Hursh and Silberberg, 2008; Oleson et al., 2011; Bentzley et al., 2013). In addition, $\boldsymbol{\alpha}$ is a measure of normalized demand elasticity and is equivalent to the slope of the demand curve – it is often taken to reflect the "essential value" of a commodity (Hursh and Silberberg, 2008; Bentzley et al., 2013). α is a uniquely unambiguous

measure of motivation because it is normalized with respect to Q_0. Thus, changes in motivation that are accompanied by changes in Q_0 (Bentzley et al., 2014), can be determined with greater confidence than by just P_{max} (another behavioral economic metric not reported here), or even breakpoint on a progressive ratio schedule (Hursh and Silberberg, 2008; Bentzley et al., 2013, 2014). Motivation is inversely proportional to α, meaning a lower α value corresponds to greater essential value (i.e. higher motivation).

For the initial (baseline) test, each rat was tested daily using the threshold procedure for a minimum of four sessions and until it produced three consecutive sessions with less than +/-25% variation in α. For baseline data analysis, α and Q_0 values were averaged over these last 3 sessions for each rat. Each probe test that followed the baseline test consisted of testing each rat for two days using the threshold procedure. Data (not shown) from other experiments have shown that after initial training the rats no longer require multiple days for their behavior to stabilize. For probe test data analysis, α and Q_0 values were averaged over the 2 sessions for each rat. A total of 7 rats (2 males, 5 females) were excluded during the baseline threshold procedure because their behavior failed to stabilize or their catheters failed.

Self-Administration: intermittent access procedure (IntA)

After completion of the baseline threshold test the rats were allowed to continue to self-administer cocaine using an intermittent access (IntA) procedure, similar to that described previously (Zimmer et al., 2012; chapter 2). Briefly, the rats were placed into the chamber with the house light illuminated. The beginning of the first 5-min Drug-Available period started 2 minutes after the rats were placed into the chamber and was signaled by extinguishing the house light. During the Drug-Available period a nose poke into the active port resulted in an intravenous infusion of cocaine hydrochloride (NIDA) dissolved in 0.9% sterile saline

(0.4mg/kg/infusion in 50 µl delivered over 2.6-sec) on a FR-1 schedule. Each infusion was paired with the illumination of a cue light in the nose port for the duration of the infusion. Pokes that were made during the 2.6-sec infusion period were recorded but not additionally reinforced. It is important to note that there was *no timeout period* following the infusion, so the rats could earn another infusion as soon as the preceding infusion ended. After the 5-min Drug-Available period, the house light turned on and signaled a 25-min No-Drug Available period. During the No-Drug Available period nose pokes were recorded but had no consequences. After 25-min, the house light was extinguished and another 5-min Drug-Available period began.

In this study we adjusted the traditional IntA procedure in an attempt to make it more intermittent and less predictable. To this end, two of the No-Drug Available periods each day were 50 minutes long instead of 25 minutes. Which two periods were extended was randomly determined each day. Each IntA session consisted of *8 Drug-Available and 8 No-Drug Available periods* (six 25-min and two 50-min), resulting in a 4-hr and 50-minute session. An inactive port was also present at all times and pokes there had no consequences.

Each rat underwent one IntA session/day for an average of 5 days/week. We varied the number and pattern of days off each week to accentuate the intermittency - for example, one week rats may have had only 1 day off and then the next week the rats may have had 3 days off. However, rats were never given the day directly before a probe test off. The rats were given a total of 30 IntA sessions and underwent probe tests, using the threshold procedure, after the 10^{th} and 30^{th} IntA sessions and then again after a 14-day abstinence period (see Fig. 3.1). A total of 41 rats began IntA testing, but 7 (5 males, 2 females) lost catheter patency before the reinstatement tests, and therefore the N is lower for those later tests. In all, the rats self-

administered cocaine for a total of approximately 50 days, combining acquisition (mean of 7 days), four threshold tests (mean of 11 days), and IntA (30 days).

Cocaine-induced reinstatement test

Following the final probe test (that followed the 14-day abstinence period), rats were tested for cocaine-induced reinstatement using procedures similar to those described previously (Deroche et al., 1999; chapter 2). On the first day of this two day test, rats were placed in the self-administration chambers with the house light illuminated. When the session started two minutes later, the house light was extinguished. All nose pokes during this test were recorded but had no consequences (that is, neither drug nor cue was presented). After a 90-min extinction period the rats received four IV saline infusions (25, 50, 100, 200 µl), each separated by 30-min. The following day the rats were tested using the same procedure, except the saline was replaced by a cocaine solution (0.2, 0.4, 0.8, 1.6 mg/kg).

Extinction and cue-induced reinstatement test

After the cocaine-induced reinstatement test the rats underwent two hour extinction sessions (1/day) for at least 5 days and until they made less than 20 active nose pokes for two consecutive sessions. The rats were placed into the chamber with the house light on and the session started two minutes later. Upon the session starting, the house light turned off and remained off for the duration of the session. Responses into the nose ports during these sessions were recorded but had no consequences. The day after a rat met the extinction criterion it underwent a day of testing identical to extinction except on this day pokes in the active port were reinforced by the illumination of the cue light for 2.6-sec.

Statistical analysis

Linear mixed-models (LMM) analyses were used for all repeated measures data. The best-fitting model of repeated measures covariance was determined by the lower Akaike information criterion score (West et al., 2007). Depending on the model selected, the degrees of freedom may have been adjusted to a non-integer value. Data for the α measure was not normally distributed and therefore all statistical tests involving α were run on log transformed data, consistent with previous reports (Bentzley et al., 2014). Planned post-hoc contrasts (and Bonferroni corrections) were done to compare data between the two sexes from the baseline threshold test. In addition, data from the first three probe tests were analyzed together to test the effects of increasing IntA experience. The fourth probe test was analyzed separately and compared only to the third probe test to test the effect of an abstinence period on motivation following IntA. Statistical significance was set at $p<0.05$.

RESULTS

Males and females did not differ in the acquisition of cocaine self-administration using an Infusion Criteria procedure

Rats were first trained to nose poke for cocaine. Due to the nature of the Infusion Criteria procedure differences in the acquisition of self-administration would appear in the number of responses made or the length of each session. The number of responses at the active nose port increased across sessions (as the infusion criteria increased) (effect of IC, $F(1,46)=18.9$, $p<0.001$; Fig. 3.2a), but there was no effect of sex on the number of active pokes ($F(1,46)=0.82$, $p=0.37$). Further, despite the number of infusions increasing, the rats finished their sessions faster with increasing experience (effect of IC, $F(1,46)=8.27$, $p=0.006$; Fig. 3.2b), but there was no effect of

sex on session length ($F(1,46)=0.23$, $p=0.636$). Responding at the inactive nose port was low in all rats and there was no effect of sex ($F(1,46)=0.03$, $p=0.87$) or IC ($F(1,45)=3.85$, $p=0.056$; data not shown) on the number of responses made.

Female's motivation for cocaine did not differ from that of males after limited cocaine experience

After limited cocaine self-administration, females have been shown to be more motivated to self-administer cocaine than males (Roberts et al., 1989). Therefore, we separately analyzed data from the baseline threshold test to identify sex differences that may exist after limited cocaine experience and before IntA experience. Males and females did not differ in the number of sessions required to stabilize on the threshold procedure. Females showed a trend toward a lower α (greater motivation) than males ($F(1,87)=3.32$, $p=0.072$; Fig. 3.2c). In contrast, females and males did not differ in their preferred level of consumption when cost was not a factor (Q_0) ($F(1,28)=0.02$, $p=0.885$; Fig. 3.2d).

Both males and females quickly learned to discriminate Drug Available and No-Drug Available periods during IntA self-administration

Following the baseline threshold test all rats underwent 30 IntA self-administration sessions. All rats quickly learned to discriminate between the alternating Drug Available and No-Drug periods, which were signaled by off/on cycles of the house light, respectively (Fig. 3.3a and b). In addition, both males and females made more active nose pokes during the Drug Available period with increasing IntA experience, indicating an escalation of responding (effect of session, $F(29,98.9)=5.03$, $p<0.001$; Fig. 3.3c). Males and females did not differ in the number of active nose pokes made during the Drug Available period ($F(1,10.6)=1.4$, $p=0.262$). Also, both males and females decreased the number of active nose pokes they made during the No-

Drug periods of IntA sessions (effect of session, $F(29,79)=2.84$, $p<0.001$; Fig. 3.3d). Females made more active nose pokes during the No-Drug period than males ($F(1,23.8)=10.0$, $p=0.004$). After the first 5 IntA sessions the majority of No-Drug responding came in the last 5 minutes of the 25-minute period (data not shown), as we have reported previously (see chapter 2), suggesting persistent, anticipatory responding. It is important to note that the number of inactive nose pokes during the Drug Available period and No-Drug period did not increase with increasing IntA experience nor was there a difference between males and females in the number of inactive responses made during either the Drug Available or No-Drug period (all p-values>0.1; Fig. 3.3c and d)

Females took more infusions during IntA and displayed a different pattern of intake

All rats consumed more cocaine as a function of increasing IntA experience (effect of session, $F(29,128.2)=5.23$, $p<0.001$; Fig. 3.4a). When analyzed separately, both males ($F(29,93.4)=2.28$, $p=0.002$) and females ($F(29,94.4)=4.03$, $p<0.001$) increased the number of infusions earned per session. In addition, females took more infusions than males (effect of sex, $F(2,97.3)=10.6$, $p=0.002$). However, males and females did not differ in the rate at which their intake escalated (sex X session interaction, $F(29, 128.2)=1.18$, $p=0.265$).

In light of the difference in the number of infusions earned, we analyzed the pattern of intake in males and females (Fig. 3.4b-f). The number of infusions earned in the 1st minute of the Drug Available period increased as a function of IntA experience (effect of session, $F(29,212.8)=10.5$, $p<0.001$) . However, males and females did not differ in the number of infusions earned in the 1st minute of the Drug-Available period (effect of sex, $F(1,17.3)=1.98$, $p=0.18$). Further all of the 'escalation of intake' that occurred took place in the first minute, as there was no effect of session on the number of infusions earned in the 2nd, 3rd, 4th, or 5th minute

of the Drug Available period (all p-values>0.05). But, females did take more infusions in minutes 2 ($p<0.001$), 3 ($p<0.001$), 4 ($p<0.001$), and 5 ($p=0.007$).

Motivation for cocaine increased with IntA experience in both males and females and females were more motivated than males following IntA experience and an abstinence period

Following acquisition, rats were tested for their motivation to self-administer cocaine using the within-session threshold procedure prior to IntA experience, after 10 IntA sessions, and then again after 30 IntA sessions. The motivation of all rats increased with IntA experience, indicated by a significant main effect of probe test on α ($F(2,62.8)=7.01$, $p=0.002$; Fig. 3.5a). In addition, females were more motivated than males on these probes tests indicated by a lower α (effect of sex, $F(1,32.2)=14.4$, $p=0.001$). When analyzed across all 3 of these probe tests, the motivation of females did not change to a greater extent than that of males (session X sex interaction, $F(2, 62.8)=2.21$, $p=0.119$). However, to assess how motivation changed with limited IntA experience we also compared only the baseline probe test and 1st probe test (after 10 IntA sessions). This yielded a significant interaction (session X sex interaction, $F(1,32.6)=4.16$, $p=0.049$), suggesting motivation for cocaine increased more rapidly in females than males. This same pattern of results was also evident when P_{Max} was analyzed (data not shown).

Rats were also tested on the within-session threshold procedure after a 14-day abstinence period that followed 30 IntA sessions. A test comparing motivation (α) on the 3rd probe test to the 4th probe test (post-abstinence) indicated a significant increase in motivation following the abstinence period ($F(1,57)=2.6$, $p=0.048$) and that females remained more motivated than males (effect of sex, $F(1,57)=25.8$, $p<0.001$), but no interaction ($F(1,57)=0.825$, $p=0.37$).

Another measure that can be extracted from the within-session threshold procedure is Q_0, a measure of preferred drug consumption when cost is nil (Fig. 3.5b). There was no change in Q_0 with increasing IntA experience ($F(2,41.8)=0.27$, $p=0.763$), nor was there a difference in Q_0 between males and females ($F(1,49)=0.99$, $p=0.325$). In addition there was no change in Q_0, and no difference between males and females, following the 14-day abstinence period (all p-values>0.1).

Figure 3.5c and d show demand curves for a representative male and representative female from the final probe test (following the 14-day abstinence period).

There was no correlation between IntA intake and α but there was a correlation between IntA intake and Q_0

One possible interpretation of our results was that the greater motivation (α) that was observed in females was the result of them consuming more cocaine relative to body weight than males during IntA. In order to test this, we correlated α values from the 2rd probe test (post-30 IntA sessions) with the average number of infusions self-administered across the last 3 IntA sessions in both males and females (Fig. 3.6a and b). There was no correlation in males ($R^2=0.06$, $p=0.3$) or females ($R^2=0.01$, $p=0.73$) suggesting that IntA intake and motivation on the within-session threshold procedure are dissociable.

In light of this result we tested whether Q_0 from the within-session threshold procedure was correlated with IntA intake (Fig. 3.6c and d). To do this we took the average intake during a Drug-Available block in both males and females from early-IntA (sessions 1-3) and correlated this with Q_0 values from the baseline probe test (prior to IntA). Our results showed a significant correlation between preferred cocaine consumption (Q_0) and IntA intake ($R^2=0.12$, $p=0.03$; Fig 3.6c). In addition to the correlation, it can be seen that rats 'load-up' to similar levels during each

Drug-Available block of IntA as they do during the within-session threshold procedure. Further, when Q_0 was measured after 30 IntA sessions and correlated with intake from the last 3 IntA sessions these measures were still significantly correlated (R^2=0.19, p=0.02; Fig 3.6d), perhaps suggesting that these two measures of consumption reflect similar processes before and after escalation of cocaine intake.

Males and females showed comparable levels of drug- and cue-induced reinstatement

Following the 3rd, and final probe test, the rats underwent a 2-day drug-induced reinstatement test. The first day consisted of a 90-minute extinction period followed by saline infusions and the second day consisted of a 90-minute extinction period followed by a series of cocaine infusions (see methods). Males and females did not differ in their extinction responding on the first day of extinction (effect of sex, F(2,27.3)=3.75 p=0.063; sex X time interaction, F(17,55.9)=1.67, p=0.08; Fig. 3.7a) or the second day of extinction (data not shown). The cocaine-priming injections dose dependently reinstated drug-seeking behavior in both males and females, as measured by responses in the previously active nose port (effect of dose, F(4,26.4)=8.34, p<0.001; Fig. 3.7b) but not to a different extent in males and females (F(1,25.0)=2.89, p=0.1). Notably, there was no effect of dose on responses at the inactive nose port (effect of dose, p>0.1).

Following the drug-induced reinstatement test, rats underwent additional extinction training for at least 5 days and then a test for cue-induced reinstatement of cocaine-seeking behavior (conditioned reinforcement). Again, males and females did not differ in either the number of responses made during extinction or the number of sessions required to meet a predetermined extinction criteria (all p-values>0.1; Fig. 3.7c). Both males and females showed robust cue-induced drug-seeking at the previously active nose port, compared to the last day of

extinction (effect of session, $F(1,23.0)=106$, $p<0.001$; Fig. 3.7d). In addition, there was no effect of sex on cue-induced reinstatement (effect of sex, $F(1,23.0)=0.58$, $p=0.45$; session X sex interaction, ($F(1,23.0)=0.62$, $p=0.44$). Notably, there was no effect of session on responses directed towards the inactive nose port (effect of session, $p>0.1$).

DISCUSSION

There are a number of factors that contribute to the transition from casual drug use to addiction including the temporal pattern by which drugs reach the brain (Robinson and Becker, 1986; Allain et al., 2015) and the sex of the user (Fattore et al., 2008; Becker, 2016; Becker and Koob, 2016). Given the importance of these two factors, we asked whether female and male rats differ in the development of addiction-like behavior following self-administration experience, using a procedure that models the intermittency seen in human cocaine use. Females and males demonstrated marked differences on several addiction-like behaviors. First, during IntA both males and females 'escalated their intake' to a similar degree, but females consumed more cocaine and responded in a different temporal pattern during the Drug Available periods. That is, in both males and females escalation of intake was confined to the first minute of the Drug Available periods and they consumed the same amount of cocaine during this first minute. However, while males largely stopped responding after the first minute, females continued to respond and took more cocaine throughout the rest of the Drug Available periods (minutes 2-5). Similar sex differences in cocaine intake and patterns of responding have been seen using different self-administration procedures (Lynch and Taylor, 2004). Also, females made more responses during the No-Drug periods (especially at the end of these periods)- which we interpret as responding in anticipation of the next Drug Available period (see chapter 2 discussion).

Further, females were more motivated to self-administer cocaine (lower α) throughout IntA self-administration experience and early IntA experience increased female's motivation for cocaine more rapidly than for male's. However females and males did not differ in the acquisition of self-administration, responding under extinction conditions, drug-induced reinstatement, or cue-induced reinstatement.

Interestingly, despite robust sex differences in α and changes in α with increasing IntA experience, males and females did not differ in Q_0, a measure of preferred consumption when cost is nil, nor did Q_0 change in either sex with increasing IntA experience or following a 14-day abstinence period. This suggests that IntA produced robust changes in motivation for cocaine ("wanting") without altering the desired effects produced by the preferred brain level of cocaine, consistent with previous reports (chapter 2). Given that Q_0 is often referred to as the 'hedonic set-point' (e.g., Bentzley et al., 2013), this suggests IntA experience increases drug "wanting" without a commensurate change in drug "liking", also consistent with previous reports (Singer et al., 2018; chapter 2). Relatedly, the increased motivation in females relative to males was not due to changes or differences in the preferred dose of cocaine, as this did not differ between the sexes at any point in the experiment. To this end, in humans the degree of enjoyment derived from cocaine use does not differ between the sexes, despite other reported differences in addiction vulnerability (Kennedy et al., 2013). Further, male and female rats do not differ in their metabolism of cocaine so this is unlikely to contribute to the differences reported in our study (Bowman et al., 1999).

Are females *especially* susceptible to the effects of intermittent drug experience?

The "telescoping effect" describes the observation that females transition from first drug use to addiction more rapidly and to a more severe extent than males (Anglin et al., 1987a,

1987b; Griffin et al., 1989; Kosten et al., 1993; Brady and Randall, 1999). Our present findings show that after only limited IntA experience, the motivation of females for cocaine (α) increased more than for males, and that starting from their first IntA session females consumed more cocaine. These are both consistent with the telescoping effect and with several other pre-clinical studies (Lynch and Carroll, 1999; Hu et al., 2004). It should be noted that female's motivation for cocaine also increases following other, less intermittent, self-administration procedures (Lynch and Taylor, 2004); however, it appears that IntA increased female's motivation, relative to male's, to a greater extent than other self-administration procedures.

Further evidence that females are more susceptible to intermittent drug exposure comes from studies using experimenter administered drug injections. While self-administration studies have better face-validity, daily experimenter administered drug injections provide a useful model of intermittent drug exposure. Females show greater psychomotor sensitization (Robinson, 1984; Robinson and Becker, 1986; van Haaren and Meyer, 1991) and more rapidly induced conditioned place preference (Russo et al., 2003) following repeated, experimenter administered psychomotor stimulant drug injections.

It is worth considering why the telescope effect exists in females and how this is impacted by the temporal pattern of drug delivery. Given that no individual starts taking a drug with an addiction-like use pattern, the progression from first use to addiction is necessarily intermittent. It is possible that the effects of intermittent drug use on the brain are more pronounced in females, and thus females are more likely to progress from casual use to addiction. One potential explanation for this is that it has been reliably shown that the reinforcing effects of cocaine differ with the phase of the estrous cycle (see below). When females self-administer cocaine across multiple days, the drug-experience varies and is thus necessarily

intermittent in nature, relative to males. In pharmacology intermittency often refers to discrete periods of a subject being on-drug and off-drug, but in the case of female self-administration, intermittency could include different magnitudes of effects or completely different effects of the drug altogether, based on phase of the estrous cycle. Intermittent bouts of drug use are particularly effective at producing a transition from casual drug use to addiction-like behavior (Zimmer et al., 2012; Allain et al., 2015; chapter 2) and the intermittent effects of cocaine in females (based on the estrous cycle) could share these properties. Thus in the present study when females were freely cycling, and we used a self-administration procedure that accentuated intermittency, females experienced a 'double-dose' of intermittency. If the intermittency of drug effects due to the estrous cycle in females does indeed share the ability of intermittent drug use to facilitate the transition from casual drug use to addiction, then this helps to explain the telescope effect. And further, the 'double-dose' of intermittency experienced in this study explains the especially robust addiction-like behavior we observed in females in this study.

Males and females did not differ in rate of acquisition or reinstatement

In this study males and females did not differ in the rate that they acquired the self-administration behavior nor in the number of responses made during acquisition (Fig. 3.2a and b). This runs contrary to several previous reports that females acquired self-administration faster than males (Lynch and Carroll, 1999; Hu et al., 2004). However, this discrepancy can most likely be attributed to procedural differences in how acquisition was carried out. In this study we used an "infusion criteria" procedure that was developed explicitly to minimize individual differences (Saunders and Robinson, 2010). Indeed, the advantage of using this procedure is that it ensures that all subjects have the same amount of drug exposure and CS-US pairings prior to any subsequent testing. We believe that our results from the first probe test (after acquisition, prior to

IntA) are a better reflection of motivation after very limited cocaine experience (Fig. 3.2c). Notably, on this test females were more motivated (lower α) than males, although it did not reach statistical significance.

Another interesting finding here is that males and females did not differ during extinction or on the drug- or cue-induced reinstatement tests (Fig. 3.7). The majority of extinction-reinstatement studies find that females respond more during the early stages of extinction but do not differ in the number of extinction sessions required to meet a predetermined criteria (Fuchs et al., 2005; Kippin et al., 2005; Lynch et al., 2005; Feltenstein et al., 2011). Similar to these studies, we did not see any sex difference in the number of sessions required to meet an extinction criteria. However, our results differed slightly in that we did not see increased responding in females during early extinction. We believe this is most likely due to differences in the self-administration procedure used in these different studies. Here we used the IntA procedure and these rats were well accustomed to periods of No-Drug availability (essentially extinction conditions) and both males and females stopped responding during extinction relatively quickly. This is best illustrated during the within-session extinction (Fig. 3.7a) when both males and females reached very low levels of responding within the first ten minutes of their first extinction session. We suggest that this expedited extinction of responding is due to extensive experience with the No-Drug periods during IntA.

There is a lack of a consensus on whether females are more motivated by cocaine-paired cues following cocaine self-administration. There are several reports in humans that cocaine-associated cues induce stronger craving in females (Robbins et al., 1999; Elman et al., 2001; Kennedy et al., 2013) although this is not always the case (Negrete and Emil, 1992; Avants et al., 1995). In pre-clinical models, we were unable to find evidence of females exhibiting greater

cocaine-paired, cue-induced reinstatement than males. There appears to be more evidence that, at least in rats, there is no sex difference or that males may even show greater cue-induced reinstatement (Fuchs et al., 2005; Lynch et al., 2005; Kerstetter et al., 2008; Feltenstein et al., 2011). Our results here (Fig. 3.7d) add to the literature suggesting that female rats are not more susceptible to cue-induced reinstatement than male rats.

Studies on cocaine-induced reinstatement more consistently demonstrate increased drug-seeking in females following a cocaine priming injection, particularly during estrus (Lynch and Carroll, 2000; Kippin et al., 2005; Kerstetter et al., 2008). Given these results, we were surprised that we did not find a sex difference in cocaine-induced reinstatement (Fig. 3.7b). It may be that females that were in estrus showed the highest levels of cocaine-induced reinstatement but we were unable to capture this because we did not track the phase of the estrous cycle (see below). Alternatively, IntA self-administration may leave males and females equally sensitive to the conditioned motivating effects of cocaine.

The discrepancy between ongoing self-administration behavior (IntA and threshold tests), when females were far more motivated than males, and extinction/reinstatement responding, during which we report no sex differences, is interesting. There could potentially be dissociable properties that contribute to the motivation to obtain cocaine versus the reinstatement of a drug-seeking response in the absence of ongoing reinforcement. In regard to addiction vulnerability, it is unlikely that females are more susceptible to develop every symptom of addiction-like behavior, and more likely that they have increased susceptibility to the neurobiological changes that drive several specific aspects of addiction. Future research will be required to determine how the neurobiological mechanisms underlying these behaviors interact and also how different behavioral aspects of addiction develop differently in males and females.

Female behavior was not more variable than male behavior despite not measuring estrous phase

As noted in the methods section, we did not monitor the estrous cycle. A great deal of work has been done to elucidate how circulating hormones interact with the brain and the role this plays in addiction (for reviews see: Becker et al., 2012; Lynch, 2018; Yoest et al., 2018). Based on this large body of literature it is likely that our behavioral measures would have been affected by phase of the cycle. However, the magnitudes of the effects of estrous cycle in females are small relative to the effects of sex and IntA training on motivation that we observed here. It is also important to note that at no point in our results was the variability of female behavior larger than the variability of male behavior, consistent with a recent meta-analysis of data collected from rats that concluded that female behavior was not more variable than male behavior, even when females are tested without regard for estrous phase (Becker et al., 2016).

In summary, we add to a growing body of literature showing that IntA self-administration produces robust addiction-like behavior despite much less total intake than other pre-clinical models of addiction (Zimmer et al., 2012; Allain et al., 2018; chapter 2). This finding also extends to female rats. Not only does it extend to female rats, but it appears that females may be particularly susceptible to the incentive-sensitization effects of intermittent drug exposure. This provides a useful model for research on the telescope effect and other sex differences in the development of addiction. Future research will be needed to determine how the temporal pattern of drug use and the sex of the user interact to produce the neurobiological changes that drive addiction.

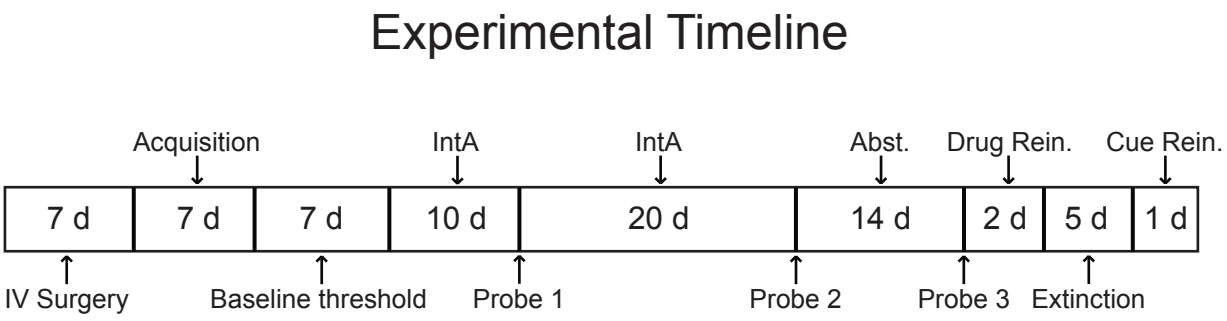


Figure 3.1. Timeline. The flow diagram shows the overall experimental design and timeline for the entire experiment. Each "Probe" was a 2-day probe test using the within-session threshold procedure. (IntA: Intermittent Access, Abst: Abstinence, Rein: Reinstatement)

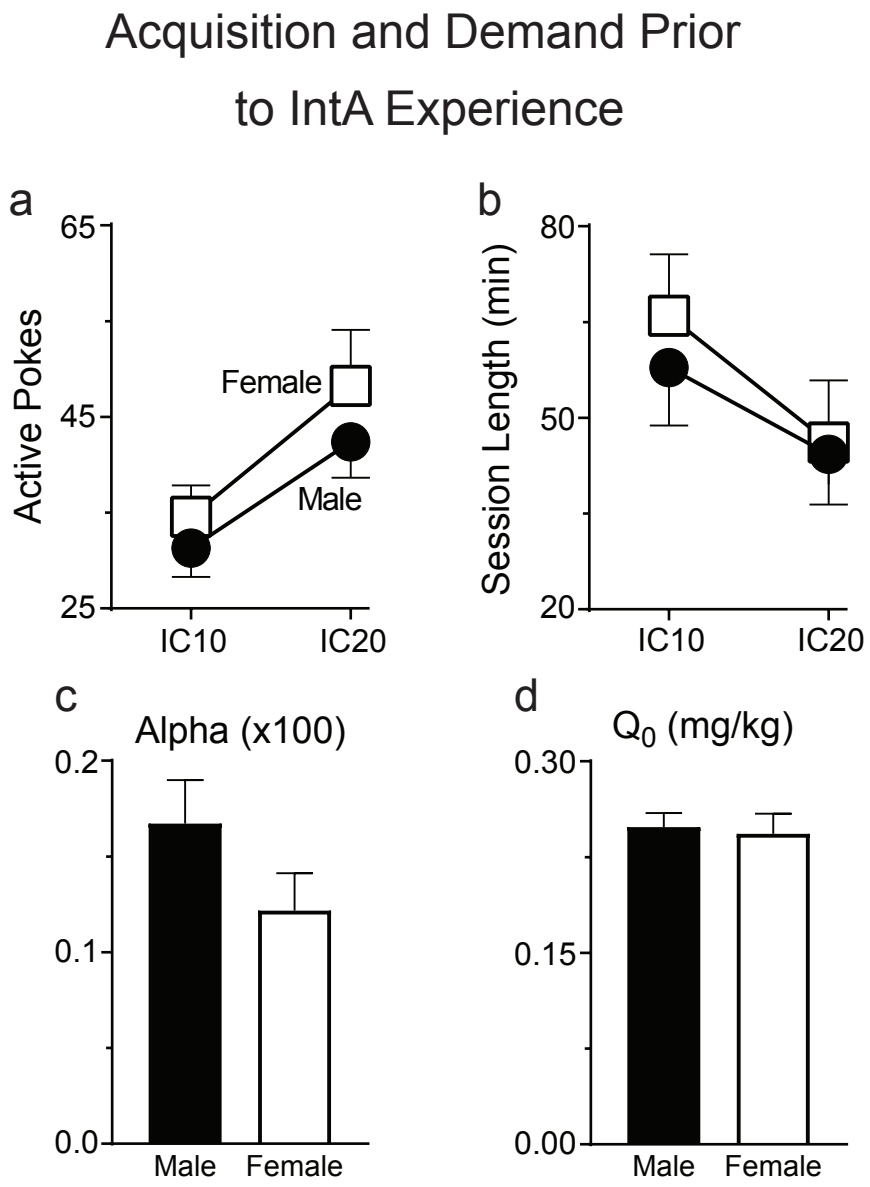


Figure 3.2. Acquisition and baseline demand. Acquisition of cocaine self-administration behavior using an Infusion Criterion procedure (see Methods) and baseline demand for cocaine prior to IntA experience. There were no differences between males (n=25) and females (n=23) in the acquisition of self-administration as indicated by active nose pokes (**a**) or the time to meet each criterion number of injections (**b**). Baseline demand (prior to IntA experience) did not differ between males (n=23) and females (n=18) indicated by α (**c**) or Q_0 (**d**). Values represent means ± SEMs.

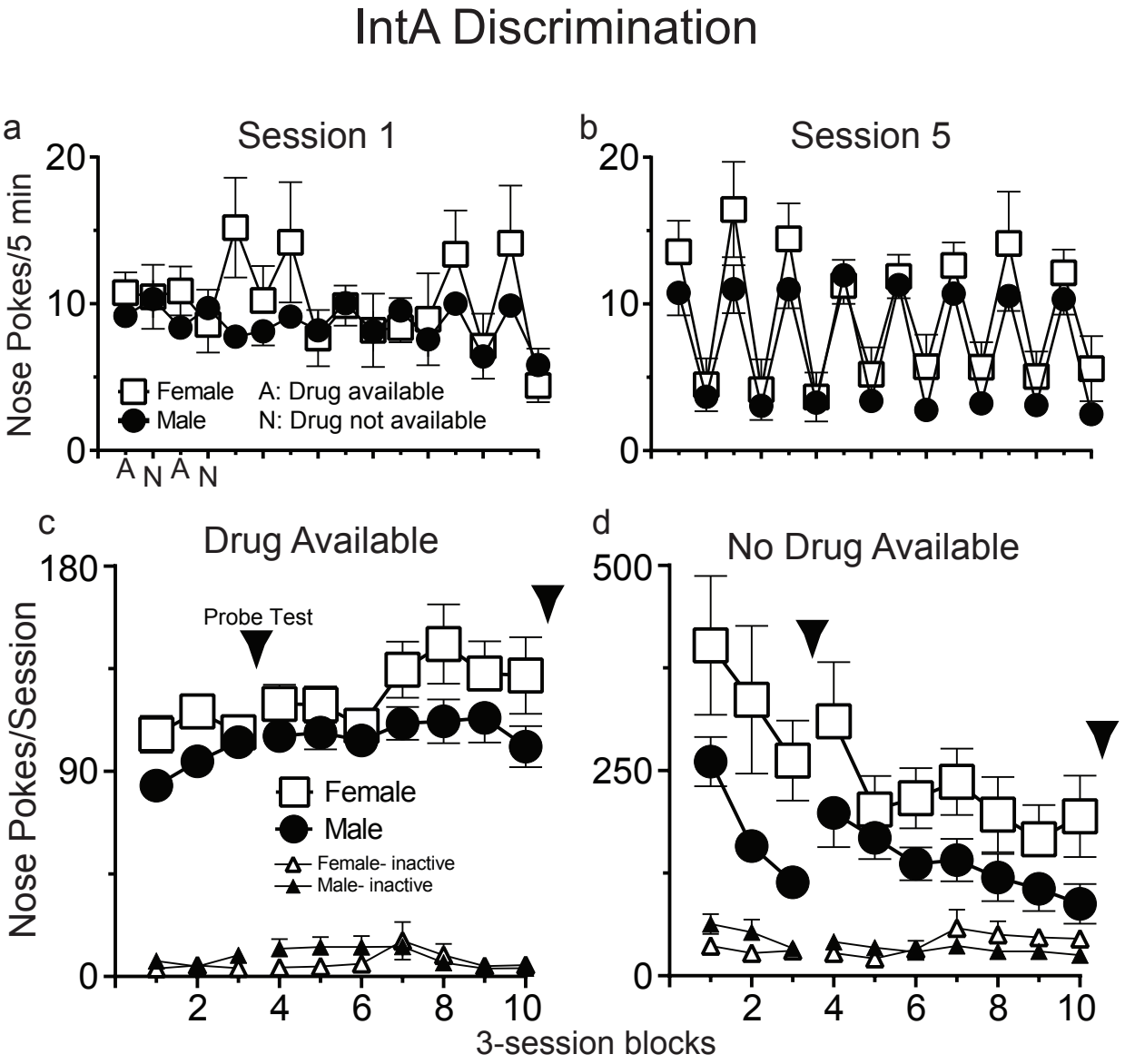


Figure 3.3. IntA discrimination. Both males and females quickly learn to discriminate between the Drug Available periods and No-Drug periods. Discrimination between the periods is not evident on the first day of IntA experience (n=23 males, 18 females) (**a**) but is evident after five sessions (**b**). Across all 30 IntA sessions both males and females make more responses during the Drug Available periods (**c**) and fewer responses during the No-Drug periods (**d**) as a function of increasing experience, but males and females do not differ on either measure. In panels c and d each point represents the average of three consecutive IntA sessions and arrowheads mark when Probe Tests were conducted (Session 30: n= 18 males, 16 females). Values represent means ± SEMs.

Minute X Minute Infusions During IntA

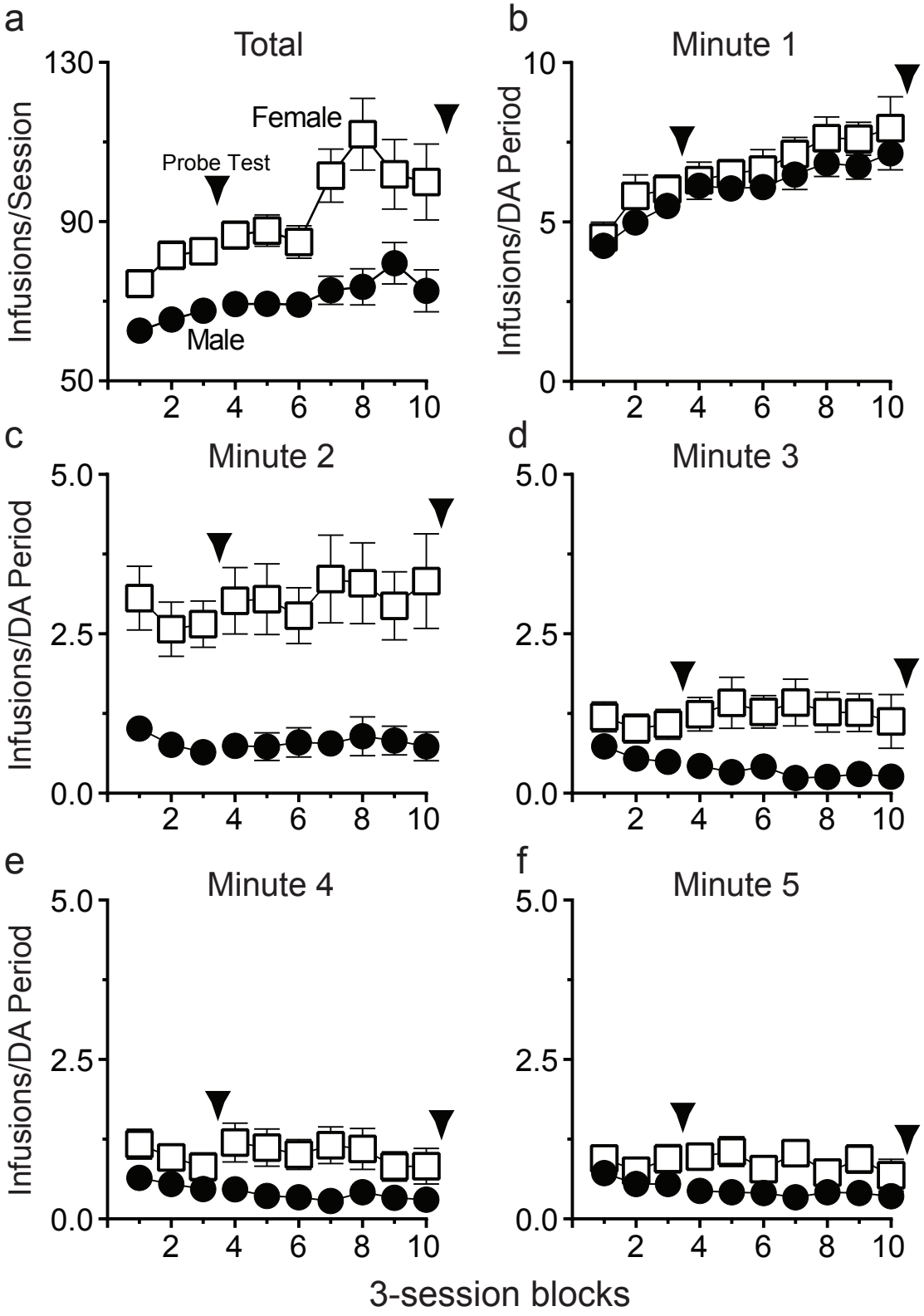


Figure 3.4. IntA infusions/minute. Each Drug Available (**DA**) period during IntA lasts for five minutes. Across all 5 minutes, males and females both consume more cocaine as a function of increasing IntA experience ('escalation of intake') and females consume more cocaine per session (**a**). All of the escalation of intake in both sexes occurs in the first minute of the DA periods and males and females consume similar amounts of cocaine in minute 1 of the DA periods (**b**). No escalation of intake occurs in minutes 2-5 of the DA periods but females consume more cocaine than males in minutes 2-5 (**c-f**). Each point represents the average of three consecutive IntA sessions and arrowheads mark when Probe Tests were conducted. Values represent means ± SEMs.

Cocaine Demand with Prolonged IntA Experience

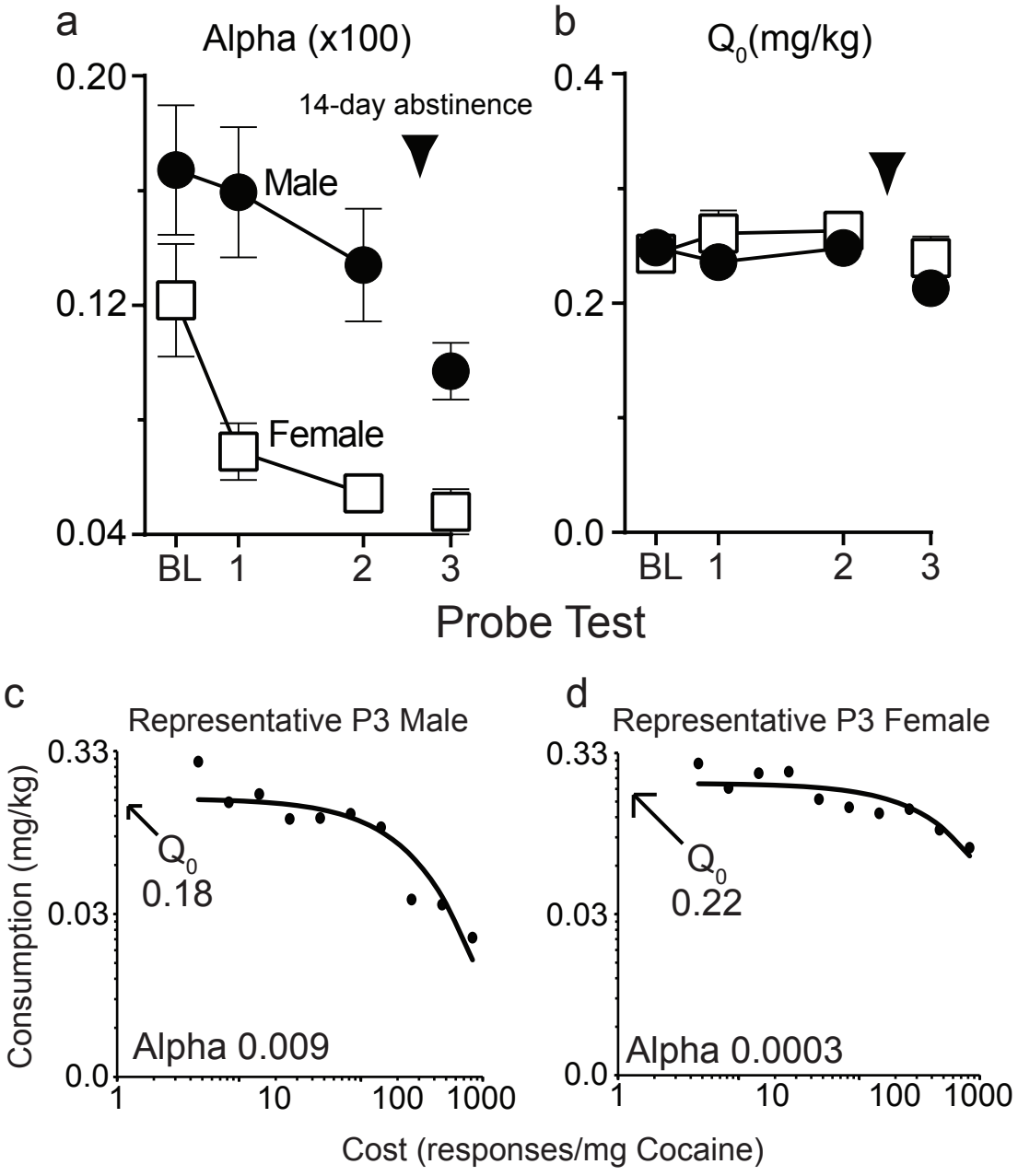


Figure 3.5. Cocaine demand in males and females. Cocaine demand was assessed prior to IntA experience (BL), after 10 (Probe Test 1) and 30 (Probe Test 2) IntA sessions, and after a 14-day abstinence period (Probe Test 3; n=18 males, 16 females). Alpha (α) decreased (motivation increased) in both males and females with increasing IntA experience and further decreased following the abstinence period. Females had lower α values throughout, and 10 IntA sessions decreased α to a greater extent in females than males (**a**). Q_0 (preferred cocaine intake when cost is not a factor) did not change with increasing IntA experience and did not differ between males and females (**b**). Panels c and d show representative demand curves generated by a male (**c**) and female (**d**) from Probe Test 3 (**P3**) after 30 IntA sessions and the abstinence period. In panels a and b the arrowhead marks a 14-day drug-free abstinence period. Values represent means ± SEMs.

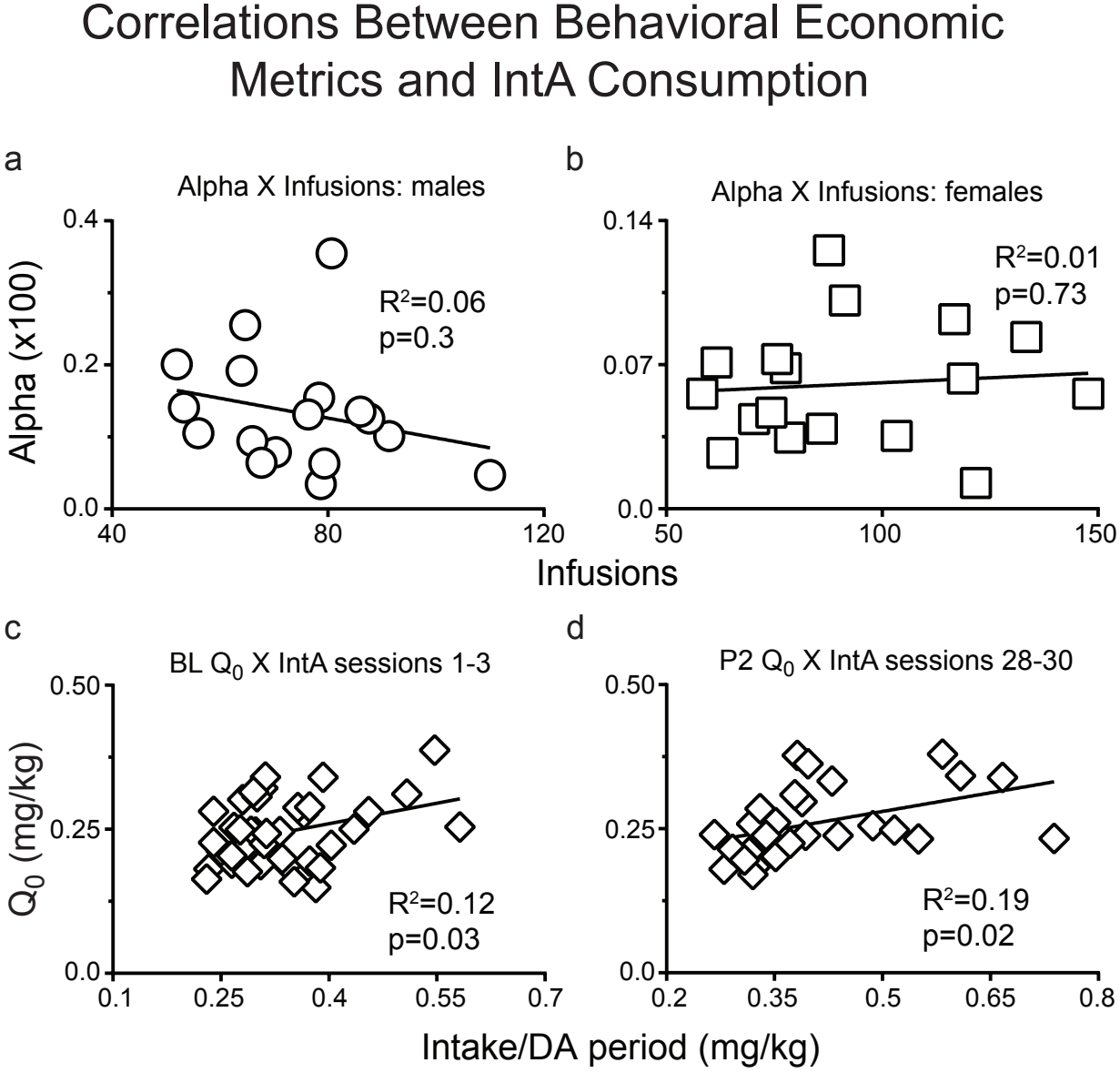


Figure 3.6. Behavioral economic metrics and IntA consumption. Females consumed more cocaine during IntA self-administration but that is not responsible for the increased motivation seen in females relative to males. Each rats' alpha (α) value from Probe Test 2 (after 30 IntA sessions) was correlated with the average number of infusions that rat self-administered over the last 3 IntA sessions in males (**a**) and females (**b**). Alpha was not correlated with IntA intake in either males or females. Q_0 (preferred cocaine intake when cost is not a factor) taken from the Baseline Probe Test (**BL**) correlated with cocaine intake in each 5-minute Drug Available (**DA**) period early in IntA experience (**c**) and Q_0 taken from Probe Test 2 (**P2**) was similarly correlated with cocaine intake from the last 3 IntA sessions (**d**). As Q_0 did not change between Probe Tests but intake increased across IntA sessions, Q_0 and intake per Drug Available period were more similar prior to escalation of intake (**c**).

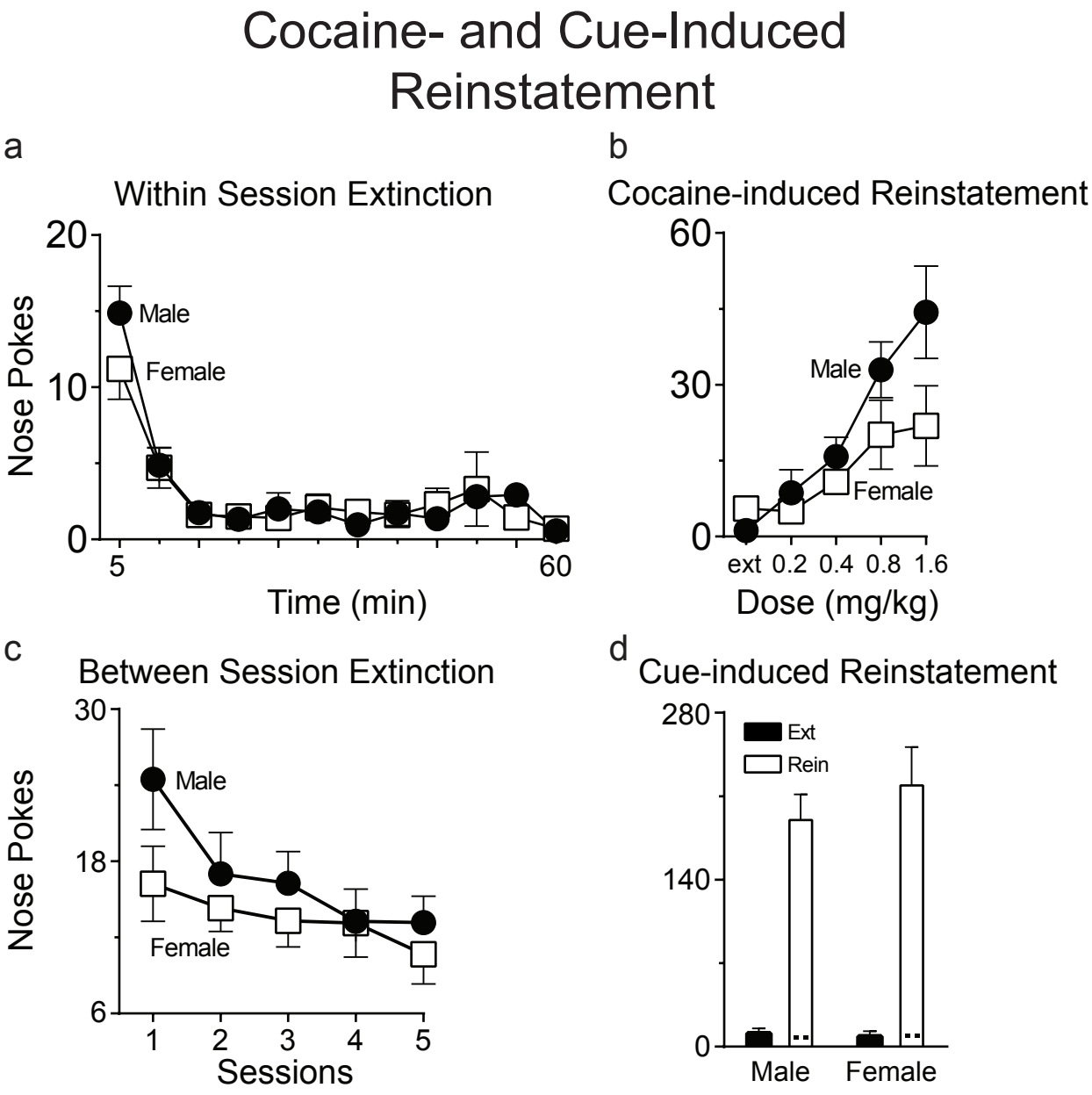


Figure 3.7. Cocaine- and cue-induced reinstatement of drug seeking. Nose pokes are shown during two separate extinction procedures and two separate reinstatement tests. Males and females did not differ in responding during a within session extinction procedure (see Methods) (**a**) or in the extent to which multiple doses of cocaine delivered IV reinstated drug-seeking under extinction conditions (**b**). Males and females did not differ in responding throughout a between session extinction procedure (**c**). In a test for conditioned reinforcement, when a nose poke in the previously active port was reinforced by presentation of the cue that had previously been associated with cocaine but not cocaine itself, males and females did not differ in the number of responses made (**d**). Nose pokes at the inactive nose port are represented by the dashed lines. N=18 males, 16 females. Values represent means ± SEMs.

CHAPTER IV

TEMPORAL PATTERN OF COCAINE SELF-ADMINISTRATION AFFECTS THE DEVELOPMENT OF ADDICTION-LIKE BEHAVIOR AND COCAINE-EVOKED DOPAMINE RELEASE

INTRODUCTION

Drug addiction is a chronic disease that affects millions of people worldwide and in 2015 alone the world's population lost an estimated 28 million years of "healthy life" as the result of drug use (UNODC, 2016). Pre-clinical models are necessary for the study of addiction and have proved invaluable in elucidating the biopsychology underlying addiction. It is encouraging then that in recent years there have been great advances in the development of pre-clinical models that reflect the clinical condition of addiction (Ahmed and Koob, 1998; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Ahmed, 2012; Zimmer et al., 2012). Procedures aimed at modeling the development of cocaine addiction in rats include, but are not limited to, **prolonged access-** that emphasizes the amount of drug consumed by stressing the number of self-administration sessions, **long access-** that also emphasizes the amount of drug consumed but through manipulating the length of each self-administration session, and **intermittent access** (IntA)**-** that stresses the temporal pattern of drug delivery.

Currently the most widely used procedure to model the transition to addiction is the 'long access' (LgA) self-administration procedure. The LgA procedure allows rats to freely self-administer cocaine for 6+ hours/day and during a typical LgA session, a rat will rapidly self-administer cocaine in the early stages of the session to elevate their brain cocaine concentration, and then maintain this level for the remainder of the (6-hour) session (Ahmed and Koob, 1998, 2005). Rats that are trained on the LgA procedure are typically compared to rats trained on a 'Short Access' (ShA) self-administration procedure (1-2 hours/day). Allowing rats to self-administer cocaine for a longer period each day, resulting in greater total intake, produces a number of addiction-like behaviors not seen with ShA procedures (for reviews see Ahmed, 2011, 2012). For example, LgA rats escalate their cocaine intake across sessions while ShA rats do not (Ahmed and Koob, 1998). Also, there are reports that rats that are trained on LgA are more motivated on a progressive ratio test than rats trained on ShA (Paterson and Markou, 2003; Wee et al., 2008; but see Liu et al., 2005; Oleson and Roberts, 2009; Quadros and Miczek, 2009; Willuhn et al., 2014(supplementary)) and exhibit greater cue-induced reinstatement of cocaine seeking following extinction (Kippin et al., 2006). Although the rationale for the use of the LgA procedure is that the neurobiological changes underlying addiction are contingent upon the user consuming large amounts of drug (Ahmed, 2012; Edwards and Koob, 2013), recent evidence suggests that the amount of drug consumed is just one of several factors that contribute to the development of addiction.

The IntA procedure allows rats brief periods (5 min) to self-administer cocaine separated by longer periods (25 min) during which cocaine is not available- thus producing successive spikes in brain cocaine concentrations (Zimmer et al., 2012). Compared to LgA, the IntA procedure better reflects the temporal pattern of drug use seen in human cocaine users (Ward et

al., 1997; Beveridge et al., 2012; Allain et al., 2015). Interestingly, in one of the few studies that directly compared ShA, LgA, and IntA, after 14 self-administration sessions rats with IntA experience were more motivated to seek cocaine than LgA rats (Zimmer et al., 2012). Further, the effects of LgA experience on motivation appear to be rather transient, dissipating after several days (Bentzley et al., 2014; James et al., 2018) while the effects of IntA on motivation last at least 50 days into abstinence (James et al., 2018).

A series of studies primarily conducted in Dr. Sara Jones' lab using voltammetry in brain slices containing the nucleus accumbens core has shed light on the potential neurobiological mechanisms underlying the behavioral differences that follow LgA and IntA experience. A history of LgA or LgA-like self-administration produces tolerance in dopamine neurotransmission (Ferris et al., 2011; Calipari et al., 2013, 2014), including a *decrease* in cocaine-induced inhibition of dopamine (DA) uptake when compared to drug-naïve controls (Calipari et al., 2014). In addition, voltammetry conducted *in vivo* during ongoing self-administration has shown a progressive decrease in DA release with increasing LgA experience (Willuhn et al., 2014). Conversely, a history of IntA produces sensitization in DA neurotransmission- resulting in an increase in cocaine-induced inhibition of DA uptake relative to drug-naïve rats and rats trained using ShA (Calipari et al., 2013). Further, the effects on DA transmission following LgA are not present after a 14-day abstinence period (Siciliano et al., 2016), but the effects on DA transmission produced by IntA are increased following an abstinence period (Calipari et al., 2015).

The development of multiple pre-clinical models of addiction, that all place emphasis on different aspects of the clinical condition, has led to questions regarding how they relate to one another and which best models addiction in humans. To date there are few studies that compare

the neurobiological consequences of LgA and IntA. The studies that have been done with a focus on DA have almost exclusively relied on *ex vivo* measures, and it is important to determine if similar effects are seen *in vivo* in awake, behaving rats. Here we directly compare how prolonged LgA and prolonged IntA self-administration affect the development of several addiction-like behaviors. Then we sought to compare how prolonged LgA and prolonged IntA, relative to a limited ShA procedure, affect DA release in the nucleus accumbens core *in vivo* in response to self-administered cocaine and cocaine-paired cues.

MATERIALS AND METHODS

A total of 50 Sprague-Dawley rats (Envigo, Haslett, MI) weighing 250-275 g on arrival were housed individually on a reverse 12-h light/12-h dark cycle (lights on at 20:00) in a climate-controlled colony room. All testing was conducted during the 12-hour lights off period. After arrival, rats were given 1 week to acclimate to the colony room before surgery. Water and food were available *ad libitum* until 2 days before the first day of self-administration, at which point the rats were mildly food restricted to maintain a stable body weight for the remainder of the experiment (20-24 grams/day). All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Apparatus

Behavioral testing was conducted in standard (22x18x13 cm) test chambers (Med Associates, St Albans, VT, USA) located inside sound-attenuating cabinets. A ventilating fan masked background noise. Within the test chambers, two nose poke ports were located 3 cm above the floor on the left and right side of the wall. A red house light was located at the top, center of the wall opposite the nose ports. During self-administration portions of the experiment,

a nose poke into the active port was detected by an infrared photo beam inside the hole and resulted in an intravenous cocaine infusion, delivered by a pump mounted outside the sound attenuating chamber, through a tube connected to the rat's catheter back port. The infusion tube was suspended into the chamber via a swivel mechanism, allowing the rat free movement. All measures were recorded using Med Associates software.

Microdialysis test sessions were conducted in separate but identical chambers as those described above. The only difference was that the swivel system in these boxes allowed for the microdialysis inlet tubing, outlet tubing, and the drug delivery tubing to be connected simultaneously.

Intravenous catheter surgery

Rats underwent intravenous catheter surgery as described previously (Crombag et al., 2000). Briefly, rats were anesthetized using ketamine hydrochloride (90 mg/kg i.p.) and xylazine (10mg/kg i.p.) and a catheter was inserted into the right jugular vein and tubing was run subcutaneously to a port located on the rat's back. During recovery from surgery rats were administered the analgesic carprofen (5 mg/kg s.c.). Following surgery, catheters were flushed daily with 0.2 ml sterile saline containing 5 mg/ml gentamicin sulfate (Vedco, MO). Catheter patency was tested periodically with intravenous injection of 0.1 ml methohexital sodium (10 mg/ml in sterile water, JHP Pharmaceuticals). If a rat did not become ataxic within 10 seconds of the injection, the catheter was considered not patent and the animal was removed from the study.

Self-administration: acquisition

Rats were given ~7 days to recover from the catheter surgery, and then self-administration training commenced. The rats were placed in the chamber with the house light illuminated and the beginning of each session was signaled by the house light being

extinguished. At that time a nose poke into the active port resulted in an intravenous infusion of cocaine hydrochloride (NIDA) dissolved in 0.9% sterile saline (0.4mg/kg/infusion in 50 µl delivered over 2.6 seconds) on a fixed ratio-1 (FR-1) schedule. Each infusion was paired with the illumination of a cue light in the active nose port for 20 seconds. Nose pokes during this time were recorded but had no consequences. An inactive nose port was also present at all times and pokes there were recorded but had no consequences. To ensure that during initial training all rats received the same amount of drug exposure and CS-US pairings an infusion criteria (IC) procedure was imposed on self-administration sessions, as described previously (Saunders and Robinson, 2010). During these sessions, session length was determined by how long it took each rat to reach the predetermined number of infusions, not by an explicit time limit. Each rat had 2 sessions at IC10, 3 sessions at IC20, and 4 sessions at IC40. A total of 5 rats were excluded during acquisition training because they failed to reach the infusion criteria or failed to discriminate between the active nose port and the inactive nose port.

Self-administration: within-session threshold procedure

The day after the final acquisition session, all rats were trained on a within-session threshold procedure and demand curves were generated, exactly as described in chapters 2 and 3. Briefly, each session (one per day) was 110 minutes in length, FR-1 throughout, and every 10 minutes the dose of drug was decreased on a quarter logarithmic scale (1.28, 0.72, 0.40, 0.23, 0.13, 0.072, 0.040, 0.023, 0.013, 0.007, 0.004 mg/kg/infusion). During the threshold procedure, the nose port cue light was illuminated for the duration of each infusion.

Each rat was tested daily for at least four sessions and until it produced three consecutive sessions with less than 25% variation in α. For baseline data analysis α, P_{Max}, and Q_0 values were averaged over these last 3 sessions for each rat. Groups (ShA, IntA, LgA) were determined

following this baseline test so that there were no baseline differences between groups in α, P_{Max}, or Q_0. A total of 3 rats were excluded for failure to stabilize or loss of catheter patency.

For the Prolonged Access groups (IntA and LgA groups), the probe test that followed prolonged self-administration experience consisted of testing each rat for two days using the threshold procedure and values were averaged across those two days.

Microdialysis

Intracranial placement of cannula for microdialysis

After their behavior stabilized on the threshold procedure, the rats in the ShA group (n=10) underwent surgery to implant a microdialysis guide cannula above the nucleus accumbens core. Rats were anesthetized with a ketamine xylazine cocktail as described above and placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA). A guide cannula (CMA, CMA12 Guide Cannula) was implanted that terminated just above the nucleus accumbens core, such that a 2mm probe would extend into the center of the region. The coordinates used were +1.6mm anterior, +/-1.6mm lateral, and -6.2mm ventral, relative to bregma. Hemisphere (right/left) was counter-balanced across all groups to avoid lateralization effects. To prevent clogging, a stainless steel stylet was inserted into the cannula. The guide cannula was then secured to the skull using three metallic screws and acrylic dental cement. The rats were administered the analgesic carprofen (5 mg/kg s.c.) during their recovery from surgery. Rats were allowed at least three days of recovery before any subsequent testing.

Probe construction and test session

After recovering from the microdialysis cannula surgery each rat in the ShA group was habituated to the microdialysis chamber in which they would be tested for ~1 hour. Then these rats were given three more IC40 self-administration sessions and at least one of these sessions

was conducted in the microdialysis chamber in which they would be tested. Thus each rat had at least one habituation session and one self-administration session in their microdialysis chamber. Due to complications on the test day one rat had to be excluded, so we successfully collected from 9 ShA rats.

The microdialysis probes were custom made similar to those described previously (Pitchers et al., 2017). Briefly, two silica capillaries (75µm inner diameter; 150 µm outer diameter; TSP075150; Polymicro Technologies) were glued together and inserted into a 24-gauge stainless steel tube that served as the shaft to be inserted into the guide cannula. The portion of each capillary tube that was not inserted into the shaft was sheathed in 22-gauge stainless steel tubing and one was used for the inlet capillary and one for the outlet capillary. The capillary tip extending from the shaft was sheathed in an 18 kDa molecular weight cutoff regenerated cellulose membrane (Spectrum Labs). The tip and base of the membrane was sealed with an epoxy resin. The membrane extended 2 mm in length beyond the shaft.

The microdialysis test session was conducted 1-3 days after the last IC40 self-administration session. Approximately 12-16 hours before the microdialysis test session, the stylet was removed from the guide cannula and a probe was inserted (see Fig. 4.3a). The animal was placed in the microdialysis test chamber with the house light on. The probe was perfused at a rate of 0.5 µL/min with artificial cerebrospinal fluid (aCSF) overnight and the rate was increased to 1 µL/min approximately four hours prior to collection. The aCSF was comprised of 145 mM NaCl, 2.7 mM KCl, 1.0 mM $MgSO_4$, 1.2 mM $CaCl_2$, 1.55 mM $NaHPO_4$, and 0.45 mM NaH_2PO_4. In addition, $^{13}C_6$-DA was added to the aCSF which allowed for in vivo calibration of the probes (Hershey and Kennedy, 2013) in all but a small subset of the rats (3 ShA, 4 IntA). In these rats recovery rates for each probe were calculated by dipping the membrane in known

concentrations of DA and correcting for the concentration collected in the dialysate sample. These rats did not differ from rats tested with $^{13}C_6$-DA added to the aCSF and thus were combined for analysis. Approximately four hours prior to collection the rats were also attached to the drug delivery tubing. Dialysate samples were collected every 3 minutes.

The test session and collection started with ten baseline samples (30 minutes). After 30 minutes, the house light turned off- signaling that cocaine was available, as it had during self-administration sessions. No samples were collected until the animal had made a response in the active nose port and self-administered a cocaine infusion. Each animal was allowed to self-administer a single 1.25 mg/kg infusion and no discrete cue was presented during the infusion. At the end of the infusion the house light turned back on. We collected 20 samples (1 hour) that corresponded to the onset of the cocaine infusion and the following hour. One hour after the cocaine infusion, the house light again turned off and the light in the active nose port that had previously been a conditioned stimulus paired with cocaine delivery during self-administration was flashed 14 times for 2.6 seconds per flash over a three minute period. We collected a sample that corresponded to this three minute cue-presentation and five more samples following the cue presentation. Nose pokes in both the active and inactive nose ports were recorded throughout the test session.

Analysis of neurochemical levels in dialysate samples using high performance liquid chromatography coupled with mass spectrometry (HPLC-MS)

All reagents, drugs, and chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. Samples were analyzed using benzoyl chloride derivatization and a modified LC-MS method previously described by the Kennedy lab (Song et al., 2012). Briefly, the 3 µL samples were derivatized by adding 1.5 µL of 100 mM sodium carbonate monohydrate

buffer, 1.5 µL of 2% benzoyl chloride in acetonitrile, and 1.5 µL of an internal standard mixture (to improve quantification), in order, briefly vortexing between each addition. A Thermo Fisher (Waltham, MA) Vanquish UHPLC system automatically injected 5 µL of the sample onto a Phenomenex Kinetex C18 HPLC column (2.1mm X 100mm, 1.7 µm). Mobile phase A consisted of 10 mM ammonium formate and 0.15% formic acid. Mobile phase B was pure acetonitrile. Analytes were detected with a Thermo Fisher TSQ Quantum Ultra triple-quadrupole mass spectrometer operating in positive multiple-reaction monitoring (MRM) mode.

Inclusion of $^{13}C_6$ dopamine in the aCSF perfusate allowed us to calculate an extraction fraction (E_d) for each sample (Hershey and Kennedy, 2013). The E_d ($E_d=1-(C_{in}/C_{out})$ is the ratio of the amount of the isotope that exits the probe to the amount retained in the dialysate sample. Absolute extracellular concentrations of dopamine were determined by dividing the dialysate concentration of dopamine by the E_d value. Finally, in a subset of rats we switched the perfusate to aCSF that lacked $^{13}C_6$ dopamine at the conclusion of the test session. After allowing this aCSF to run for ~one hour we collected several samples in the same manner as the samples collected during the test. We analyzed these samples and compared them to samples from the baseline portion of the test session. This analysis, along with comparisons between rats that did not have $^{13}C_6$-DA added to the aCSF at any point, confirmed that there were no effects of infusing $^{13}C_6$-DA on endogenous dopamine levels.

Histological Analysis

After the microdialysis test session, rats in the ShA group were anesthetized using sodium pentobarbital (270 mg/kg; i.p.) and perfused intracardially with 50 mL of 0.9% saline, followed by 500 mL of 4% paraformaldehyde in 0.1 M phosphate buffer (PB). After being perfused, brains were removed, post-fixed in the same paraformaldehyde solution for 2 hours,

then immersed in 20% sucrose and 0.01% sodium azide in 0.1 M PB for 48 hours at 4° C. Coronal sections (40 µm) were cut with a freezing microtome (SM 2000R; Leica), collected in PB, and mounted on to a slide immediately. Sections were imaged at 4x magnification using a Leica DM400B digital microscope to verify cannula placement.

Self-Administration: intermittent access or long access procedures

After their behavior stabilized on the threshold procedure, the rats in the Prolongeded Access groups started a stretch of 30 self-administration sessions (Fig. 4.1a). These rats were either trained using an intermittent access (IntA) procedure (n=20) similar to that described previously (Zimmer et al., 2012) and identical to the procedure described in chapter 2, or a long access (LgA) procedure (n=12) similar to that described previously (Ahmed and Koob, 1998). During the IntA procedure, the rats were placed into the chamber with the house light illuminated. The beginning of the first 5-min Drug-Available period started two minutes after the rats were placed into the chamber and was signaled by extinguishing the house light. During the Drug-Available period a nose poke into the active port resulted in an intravenous infusion of cocaine hydrochloride (NIDA) dissolved in 0.9% sterile saline (0.4mg/kg/infusion in 50 µl delivered over 2.6-sec) on a FR-1 schedule. Each infusion was paired with the illumination of a cue light in the nose port for the duration of the infusion. After the 5-min Drug-Available period, the house light turned on and signaled a 25-min No-Drug Available period. After 25-min, the house light was extinguished and another 5-min Drug-Available period began. Each IntA session lasted 4 hours (8 cycles of Drug-Availability).

During the LgA procedure, the rats were placed into the chamber with the house light illuminated. After two minutes the house light was extinguished and the rats were able to self-administer cocaine. Each LgA session lasted 6 hours and throughout the session a nose poke into

the active port resulted in an intravenous infusion of cocaine hydrochloride (NIDA) dissolved in 0.9% sterile saline (0.4mg/kg/infusion in 50 µl delivered over 2.6-sec) on a FR-1 schedule. Each infusion was paired with the illumination of a cue light in the nose port for the duration of the infusion and an additional 17.4 seconds, signaling a 20-second timeout period that followed each infusion. During this time nose pokes were recorded but had no consequences. With the exception of this timeout period, cocaine was available to the rats throughout the 6-hour session. An inactive port was also present at all times and pokes there had no consequences.

Rats in both groups underwent one self-administration session/day for an average of 5 days/week. The rats were given 25 self-administration sessions and then were tested using the within-session threshold procedure as described above. Two rats in the IntA group were removed from testing prior to this threshold test due to loss of catheter patency. After two threshold test sessions, the rats underwent surgery to implant a microdialysis guide cannula into the nucleus accumbens core, as described above. After three days of recovery the rats were given five additional IntA or LgA self-administration sessions. Also, each rat was habituated to the microdialysis chamber in which they would be tested for ~1 hour and at least one of the five additional self-administration sessions was conducted in the microdialysis chamber in which they would be tested. Thus each rat had at least one habituation session and one self-administration session in their microdialysis chamber. Then 1-3 days after the last self-administration session these rats underwent a microdialysis test and collection session identical to that described for the ShA rats. Due to complications during surgery or the microdialysis test session 2 IntA rats and 3 LgA rats were excluded, leaving us with 16 and 9 successful collections, respectively.

Extinction and cue-induced reinstatement test

Following the microdialysis test session, a subset of the Prolonged Access rats were tested for the ability of the cocaine-paired discrete cue (light in the nose port) to reinstate drug-seeking (n=10 IntA; 9 LgA rats). The rats underwent two hour extinction sessions (1/day) for at least 5 days and until they made less than 20 active nose pokes for two consecutive sessions. The rats were placed into the chamber with the house light on and the session started two minutes later. Upon the session starting, the house light turned off and remained off for the duration of the session. Responses into the nose ports during these sessions were recorded but had no consequences. The day after a rat met the extinction criterion it underwent a day of testing identical to extinction except on this day pokes in the active port were reinforced by the illumination of the cue light for 2.6-sec. After the cue-induced reinstatement test these rats were perfused to verify cannula placement as described above.

Statistical analysis

Linear mixed-models (LMM) analyses were used for all behavioral repeated measures data. The best-fitting model of repeated measures covariance was determined by the lowest Akaike information criterion score (West et al., 2007). Depending on the model selected, the degrees of freedom may have been adjusted to a non-integer value. Data for the α measure was not normally distributed and therefore all statistical tests involving α were run on log transformed data, consistent with previous reports (Bentzley et al., 2014). Planned post-hoc contrasts (and Bonferroni corrections) were done to compare between the different self-administration procedures and within a group across the two test periods. Active and inactive nose pokes from the cue-induced reinstatement test were compared to the last day of extinction and between the IntA and LgA groups. Similar LMM analysis and planned post-hoc analysis

were used to analyze neurochemical levels from the microdialysis test session. Statistical significance was set at $p<0.05$.

RESULTS

Rats acquired self-administration using the Infusion Criteria procedure

Rats were first trained to nose poke for cocaine. Acquisition of the self-administration behavior was defined as meeting the infusion criterion and making at least twice as many active nose pokes as inactive nose pokes. Rats that did not meet this criterion were excluded. Overall the number of response at the active nose port increased across sessions (effect of IC, $F(2,40.4)=38.7$, $p<0.001$; Fig. 4.1b), and the number of inactive nose pokes decreased across sessions (effect of IC, $F(2,75.1)=3.5$, $p=0.04$).

Rats trained on the LgA procedure consumed more cocaine than rats on the IntA procedure but rats in both groups escalated their intake to a similar extent

A subset of rats was given 30 self-administration sessions using either the IntA or LgA procedure. Rats in the IntA group quickly learned to discriminate between the Drug-Available and No-Drug periods as previously reported (data not shown; see chapters 2 and 3). When cocaine intake from the IntA and LgA rats was analyzed together there was a main effect of test session ($F(29,70.7)=4.8$, $p<0.001$; Fig. 4.1c) suggesting that rats in both groups increased their cocaine intake per session. Further analysis confirmed that when each group was analyzed separately both IntA ($p=0.01$) and LgA ($p=0.02$) rats took more infusions as a function of increasing self-administration experience. In addition, the rate at which consumption increased in both groups did not differ (group X session interaction, $F(29,70.7)=1.2$, $p=0.24$). There was

however a robust difference in the amount of cocaine consumed between the two groups (effect of group, $F(1,92.3)=823$, $p<0.001$; Fig. 4.1c and d).

Prolonged IntA and LgA experience differentially affect motivated behavior

All rats underwent a baseline threshold test to quantify their motivation to self-administer cocaine following only limited self-administration experience (Fig. 4.2). Groups (ShA, IntA, LgA) were determined following this test so that there were no differences between the groups in any of the measures derived from this test. The rats that were placed into the IntA and LgA groups underwent 25 self-administration sessions and then another threshold probe test to determine how motivation changed as a function of IntA or LgA experience. Thus, in our analysis there was one time point for rats in the ShA group and two time points for rats in the IntA and LgA groups.

The metric α measures the elasticity of the demand curve generated during the within-session threshold procedure, and is inversely proportional to motivation (Fig. 4.2a). Prolonged IntA and LgA differentially affected α indicated by a significant group X probe test interaction ($F(1,69)=7.2$, $p=0.009$). Post-hoc within subjects analysis revealed that IntA experience decreased α (increased motivation) (effect of probe test, $p=0.02$) but LgA experience did not change α (effect of probe test, $p=0.12$). Further, on the second probe test IntA rats had a lower α than LgA rats (effect of group, $p=0.04$), indicating that they were more motivated.

The metric P_{Max} is a measure of motivation that reflects the maximum price an individual is willing to pay (in effort) to obtain a reinforcer (Fig. 4.2b). Again, prolonged IntA and LgA differentially affected P_{Max} indicated by a significant group X probe test interaction ($F(1,33.1)=10.4$, $p=0.003$). Post-hoc within subjects analysis revealed that IntA experience increased P_{Max} (effect of probe test, $p=0.001$) but LgA experience did not (effect of probe test,

p=0.27). Motivation, as measured by P_{Max}, was greater in IntA rats than LgA rats following prolonged experience on the given procedure (effect of group, p=0.005).

Another metric derived from the threshold procedure is Q_0 which measures the preferred level of cocaine consumption when cost is nil (Fig. 4.2c). Again there was a significant group X probe test interaction (F(1,28.7)=7.7, p=0.009) indicating that IntA and LgA differentially affected Q_0. Q_0 differed from α and P_{Max} as post-hoc within subjects analysis revealed that LgA experience increased Q_0 (effect of probe test, p=0.01) but IntA experience did not change Q_0 (effect of probe test, p=0.25). Finally, LgA rats had higher Q_0 than IntA rats after prolonged self-administration (effect of group, p=0.03) These differential effects of LgA and IntA on Q_0 are consistent with several reports (Oleson and Roberts, 2009; Bentzley et al., 2014; Kawa et al., 2016; Singer et al., 2018).

ShA, IntA, and LgA rats did not differ in responding during the microdialysis test session but IntA rats showed greater cue-induced reinstatement than LgA rats.

Active nose pokes that were made in the hour following the single self-administered cocaine infusion during the microdialysis test session were recorded and analyzed as a measure of cocaine-induced drug seeking (see ordinate axis of Fig. 4.4a). A one-way anova comparing the three groups revealed no effect of group on nose pokes, although there was a trend towards IntA rats making more responses than ShA or LgA rats (effect of group, F(2)=2.7, p=0.08).

Following the microdialysis test session, the rats in the IntA group and the LgA group underwent extinction training followed by a test for cue-induced reinstatement of cocaine-seeking (conditioned reinforcement) (Fig. 4.2e). There were no group differences during extinction training in the number of responses made or the number of sessions required to reach extinction criteria (all p-values>0.1). On the test day, when responding was reinforced by the

presentation of the cue that had previously been paired with cocaine, both IntA rats and LgA rats reinstated their drug-seeking relative to extinction levels ($F(1,20.1)=100$, $p<0.001$), specifically at the active nose port (effect of inactive nose port X session, $F(1,18.1)=2.3$, $p=0.15$). Further, contingent presentation of the cocaine-paired cue spurred greater seeking in IntA rats than LgA rats (effect of group, $F(1,20.1)=22$, $p<0.001$).

Prolonged IntA sensitized cocaine-induced dopamine release.

Following limited ShA, prolonged IntA, or prolonged LgA rats were tested for their neurochemical response to a self-administered cocaine infusion. First, we analyzed the average raw DA concentrations for ten baseline samples and ten post-cocaine samples. There was a main effect of group ($F(2,326)=10.2$, $p<0.001$) and the cocaine infusion increased extracellular DA in all groups (effect of cocaine, $F(1,331)=8.93$, $p=0.003$; Fig. 4.3b). Post-hoc analysis revealed that following the cocaine infusion, DA levels were elevated in IntA rats relative to LgA rats ($p<0.001$) and ShA rats ($p=0.004$). However, DA levels in LgA rats and ShA rats did not differ following the cocaine infusion ($p=0.21$). Further, there were no differences between any of the groups in baseline DA levels (all p-values>0.1).

Given that there were no differences in baseline DA levels between the groups we averaged the baseline values together for each group and compared the peak percent change from baseline induced by cocaine in each group (Fig. 4.3d). Peak change was defined as the largest change from baseline that occurred within 3 samples of the cocaine infusion. There was a main effect of group ($F(2,672)=6.04$, $p=0.003$) and the cocaine infusion increased extracellular DA in all groups ($F(1, 673)=40$, $p<0.001$). In addition, when analyzed as a percent of baseline, the cocaine infusion increased DA levels to a greater extent in IntA rats than LgA or ShA rats (group

X cocaine interaction, $F(2,672)=6.04$, $p=0.003$; post-hoc analysis revealed this to be driven by IntA rats).

Cocaine-seeking on multiple tests of addiction-like behavior predicted cocaine-evoked dopamine release.

Given the role of DA in motivated behavior we next sought to determine if DA release in response to cocaine correlated with cocaine-seeking behavior (Fig. 4.4). We found that across all rats both P_{Max} ($R^2=0.32$, $p<0.001$) and α ($R^2=0.13$, $p=0.04$) predicted DA release as a percent of baseline. When this analysis was restricted to IntA rats only (data not shown), P_{Max} was correlated with DA release ($R^2=0.29$, $p=0.03$) and α showed a trend towards significance ($R^2=0.19$, $p=0.09$). In addition, across all three groups, nose pokes in the active nose port following the cocaine infusion on the microdialysis test day were correlated with DA release as a percent of baseline ($R^2=0.14$, $p=0.03$). In contrast, Q_0 did not correlate with DA release when all groups were combined ($R^2=0.07$, $p>0.1$), nor in any individual group (all p-values>0.1).

IntA reliably produces multiple addiction-like behaviors and this has been shown to be particularly robust in susceptible individuals (Kawa et al., 2016; Singer et al., 2018). Here we separated rats that were trained with the IntA procedure into those that met 2/3 ‘addiction criteria’ and those that met 0/1 ‘addiction criteria’, as described previously (Deroche-Gamonet et al., 2004; Kawa et al., 2016; Singer et al., 2018). Briefly, a rat met the criteria for addiction if it was within the top third of the population on a given measure. The measures used here were α, nose pokes during the No-Drug period of IntA, and cocaine-induced nose pokes during the microdialysis test session (Fig. 4.5). We found that 9 rats met 0/1 criteria and 7 rats met 2/3 criteria. Not surprisingly, 0/1 and 2/3 criteria rats differed in their performance on the behavioral measures for which they were classified. Notably, a t-test revealed that 2/3 criteria rats showed

greater cocaine-evoked DA release as a percent of baseline than 0/1 criteria rats ($p=0.05$; Fig. 4.5d). Finally, in 2/3 criteria IntA rats the cocaine infusion increased DA to a greater extent than it did in LgA rats or ShA rats (group X cocaine interaction, $F(2,484)=19.5$, $p<0.001$; Fig. 4.5e).

Self-administered cocaine increased extracellular glutamate and 3-MT levels.

Microdialysis allows for the analysis of a number of neurochemicals in addition to DA (Table 4.1). To analyze these neurochemicals of interest all values were normalized to baseline levels and cocaine-induced changes relative to baseline were determined. Self-administered cocaine increased extracellular glutamate levels relative to baseline in all groups (effect of cocaine, $F(1,671)=3.97$, $p=0.04$), but not to a different extent between groups ($p>0.1$). In addition, self-administered cocaine increased extracellular 3-MT, a DA metabolite, in all groups (effect of cocaine, $F(1,581)=10.5$, $p<0.001$). Further, cocaine increased 3-MT to a greater extent in IntA rats than LgA or ShA rats (group X cocaine interaction, $F(2,580)=4.93$, $p=0.008$; post-hoc analysis revealed this to be driven by IntA rats). We also analyzed extracellular GABA, ACh, DOPAC, and HVA levels but none of these differed between groups or changed significantly following cocaine.

Presentation of the cocaine-paired cue did not affect extracellular neurochemical levels.

One hour after the self-administered cocaine infusion, the conditioned stimulus that had been paired with cocaine infusions during self-administration training was presented non-contingently for three minutes. Surprisingly there were no changes in any of the neurochemicals that we measured in response to the presentation of the cue (data not shown).

DISCUSSION

Recent findings using the intermittent access (IntA) self-administration procedure have shed new light on a number of widely held theories regarding pre-clinical models of addiction. The IntA procedure stresses the temporal pattern of drug use while most other self-administration procedures modeling the development of addiction stress the amount of drug consumed. Here we sought to directly compare how prolonged IntA and prolonged 'long access' (LgA) affected multiple addiction-like behaviors and cocaine and cue-evoked neurochemical release in awake, behaving rats. Prolonged IntA and prolonged LgA both produced similar escalation of cocaine intake across self-administration sessions, but as expected, rats trained with the LgA procedure consumed far more total cocaine (Fig. 4.1d). Further, as has been reported previously, IntA experience produced robust increases in motivation as measured by P_{Max} and α (Singer et al., 2018; chapter 2), but LgA experience did not change motivation as measured by either of these metrics (Fig. 4.2). Accordingly, IntA rats were far more motivated to self-administer cocaine following prolonged self-administration experience and showed greater cue-induced drug seeking than LgA rats. Prolonged LgA did increase the preferred level of cocaine intake when no effort was required (Q_0), while IntA experience produced no change in this measure, again consistent with previous reports (Bentzley et al., 2014; chapter 2).

A single self-administered injection of cocaine (1.25 mg/kg I.V.) increased dopamine (DA) release in the core of the nucleus accumbens following prolonged IntA experience, relative to rats trained with a limited ShA procedure or rats trained with prolonged LgA, assessed using *in vivo* microdialysis (Fig. 4.3). Cocaine-evoked DA release did not differ between ShA rats and LgA rats. In addition, in all groups the percent change from baseline of cocaine-evoked DA release predicted a number of addiction-like behaviors, including P_{Max}, α, and cocaine-induced drug seeking. However, DA release did not predict intake during self-administration experience

or preferred level of intake when cost was not a factor (Q_0). Finally, within the rats trained with IntA, cocaine-evoked DA release was greatest amongst those rats that displayed the most robust addiction-like behaviors, assessed using an addiction-criterion scoring system (Deroche-Gamonet et al., 2004; chapter 2).

IntA rats are more motivated than LgA rats despite far less total cocaine intake

Rats trained using the LgA self-administration procedure often show a number of addiction-like behaviors when compared to rats trained using the ShA procedure (Edwards and Koob, 2013). Relative to ShA rats, LgA rats have been reported to escalate their intake across sessions (Ahmed and Koob, 1998), expend more effort for cocaine (Paterson and Markou, 2003; Wee et al., 2008), take more cocaine in the face of adverse consequences (Xue et al., 2012; Bentzley et al., 2014), and show greater reinstatement following extinction (Mantsch et al., 2004, 2008; Ahmed and Cador, 2006; Kippin et al., 2006). The development of these addiction-like behaviors following LgA, but not ShA, has been attributed to the increased amount of cocaine consumed during LgA (Ahmed, 2012; Edwards and Koob, 2013). However the amount of cocaine intake is only one factor that affects motivated behavior, and the pharmacokinetics of cocaine use may be even more important than the amount of drug consumed (Hatsukami and Fischman, 1996; Allain et al., 2015).

Here we showed that after prolonged self-administration experience, LgA rats had consumed approximately 3x as much cocaine as IntA rats (Fig. 4.1d). However, despite this discrepancy in cocaine intake, IntA rats escalated their intake to a similar extent (Fig. 4.1c), were more motivated to take cocaine assessed using a within-session threshold procedure (Fig. 4.2a and b), and showed greater cue-induced drug-seeking (Fig. 4.2e). These results add to an emerging body of literature suggesting that IntA experience results in the development of far

more severe addiction-like behavior than LgA experience (Zimmer et al., 2012; Allain et al., 2018; chapter 2). That LgA did not increase motivation as measured by P_{Max} or α relative to ShA was surprising given reports to the contrary using threshold procedures (Zimmer et al., 2012) and Progressive Ratio (PR) tests (Paterson and Markou, 2003; Wee et al., 2008). However these studies reported significant, but generally modest, increases in motivation in LgA rats. Further, a number of studies have reported no change in motivation following LgA experience relative to ShA experience using threshold procedures (Oleson and Roberts, 2009) or PR tests (Liu et al., 2005; Quadros and Miczek, 2009; Willuhn et al., 2014 (supplementary)). In addition, the modest increases in motivation following LgA persist for only a few days after the last self-administration session (Bentzley et al., 2014; James et al., 2018). Taken together these studies suggest that relatively modest increases in motivation following LgA are short-lasting and are heavily influenced by the specific conditions of the experiment. This does not appear to be the case following IntA experience. IntA self-administration has now been shown in a number of studies, across several labs, to reliably and robustly increase motivation for cocaine (Zimmer et al., 2012; Allain and Samaha, 2018; Allain et al., 2018; Singer et al., 2018; chapters 2, 3, 4). The unreliable changes in motivation following LgA put the changes following IntA into context and further stress just how robust the development of addiction-like behavior is following IntA.

One change in the effects of cocaine that appears to be reliably induced by LgA experience is a persistent increase in the "consummatory" aspects of behavior. Responding for cocaine under Fixed Ratio (FR) schedules of reinforcement has been suggested to reflect consummatory aspects of drug taking as rats will titrate their responding within a range of doses to achieve a preferred brain cocaine concentration (Gerber and Wise, 1989; Ahmed and Koob, 1999; Lynch and Carroll, 2001). Also, preferred consumption when cost is not a factor, derived

from the within-session threshold procedure, may reflect similar consummatory aspects of behavior (Oleson and Roberts, 2009; Bentzley et al., 2013). Both escalation of intake and an increase in preferred consumption when effort is not a factor are demonstrated following LgA, and both have been attributed to changes in rats' "hedonic setpoint" (Ahmed and Koob, 1998; Bentzley et al., 2014; James et al., 2018; Fig. 4.2c). That LgA experience produces changes in consummatory behaviors, often in the absence of changes to motivational measures, and IntA does exactly the opposite, indicates that consummatory and "appetitive" or motivational aspects of behavior are psychologically (and neurobiologically) dissociable, as previously suggested (Nicola and Deadwyler, 2000; Sharpe and Samson, 2001; Oleson et al., 2011; Guillem et al., 2014).

IntA, but not LgA, self-administration sensitizes cocaine-evoked dopamine release

A series of recent studies utilizing slice voltammetry in rats with a history of IntA self-administration has demonstrated sensitized cocaine-evoked DA release in the nucleus accumbens core (Calipari et al., 2013, 2015). Here we showed that DA release following a self-administered cocaine infusion is also sensitized following prolonged IntA in awake, behaving rats (Fig. 4.3). In addition, cocaine-evoked DA release as a percent of baseline correlated with a number of addiction-like behaviors. This is the first study to report *in vivo* neurobiological changes following IntA and the correlation of DA release with motivated behavior suggests that the sensitized DA release following IntA may be at least one cause for the development of addiction-like behavior (see below). The only other reported neurobiological consequence of IntA experience is dysregulated mGluR2/3 receptor function, and activation of these receptors following IntA decreased motivation for cocaine (Allain et al., 2017).

Baseline DA levels were not different between IntA, ShA, or LgA rats. *In vivo* DA levels have not been measured following IntA before, but this finding is consistent with microdialysis studies showing that LgA experience does not alter baseline DA concentrations (Ahmed et al., 2003; Calipari et al., 2013), although reductions in basal DA levels have been reported using other self-administration procedures (Weiss et al., 1992; Mateo et al., 2005; Ferris et al., 2011). In addition, baseline DA levels did not correlate with any of our behavioral measures, consistent with several studies (Hurd et al., 1989; Ahmed et al., 2003). Further, following the self-administered cocaine infusion DA increased in both the LgA rats and ShA rats but not do a different extent. This was somewhat surprising given recent literature that suggests LgA self-administration results in decreased cocaine-evoked DA release relative to drug-naïve and ShA rats (Calipari et al., 2013, 2014; Willuhn et al., 2014). However, the majority of these studies used voltammetry to measure DA release and the one subset of experiments that used microdialysis featured an experimenter administered, I.P. injection of cocaine and compared LgA rats to drug-naïve controls (Calipari et al., 2014). Differences certainly exist between experimenter administered drugs and self-administered drugs (e.g., Stuber et al., 2005), and it is also possible that if we had a drug-naïve control group, relative DA release would have been decreased in both the ShA and LgA groups. In addition, the tolerance-like effects of cocaine on DA release reported in these studies could be very short lasting and impossible to detect using microdialysis, reflecting differences in DA release as a function of the timeframe being measured. Finally, in the Willuhn et al. (2014) study it is unclear if the reported decreased DA release is in response to cocaine or the cocaine-paired cue as it occurred immediately after the rats made an active response. Our results are consistent with a microdialysis study that compared

the effects of self-administered cocaine in rats with LgA and ShA experience, and found no differences between the groups (Ahmed et al., 2003).

We were surprised to find that presentation of the cocaine-paired cue did not change DA levels in any of our groups. We believe this could be the result of limitations of the technique as it may be difficult to detect cue-induced changes in DA using microdialysis coupled with HPLC-MS analysis. *In vivo* voltammetry, which is more sensitive to small changes in DA, may be better suited for studying differences in cue-induced DA release between different self-administration procedures. In addition, presenting the cue after the rats had been in the chamber under extinction conditions for ~16 hours may have failed to attract the rats' attention or motivate them and thus it would not engage the neurobiological motivational circuitry. Indeed, we did not observe any behavioral effect of the cue presentation in any of our groups.

Implications for hyper vs. hypodopaminergic theories of addiction

A number of theories attempt to explain how casual drug use eventually progresses to addiction in a subset of individuals. Interestingly, two very distinct theories of addiction currently exist in the field that are relevant to the findings presented here. One group of theories we will refer to colloquially as "opponent-process" theories of addiction. These theories propose that initial drug use is motivated by the drug's "pleasurable" effects but as drug use continues and becomes compulsive, these effects gradually decrease while effects in the opposite direction (negative hedonic effects) get larger. Thus loss of control of drug use and addiction is driven by a desire to alleviate the unpleasant state of drug withdrawal (Himmelsbach et al., 1935; Dackis and Gold, 1985; Koob and Le Moal, 1997, 2001; Volkow et al., 2016). Further, these theories suggest that prolonged drug use leads to decreased DA levels (a *hypodopaminergic* state) in the mesolimbic "motivational circuitry" both during withdrawal and in response to drugs. This

hypodopaminergic state contributes to the aversive withdrawal state. Therefore individuals suffering from addiction use drugs to "self-medicate" and return DA from a hypodopaminergic state to "normal" levels.

The incentive-sensitization theory of addiction presents a very different explanation for how drug use progresses from recreational to addictive (Robinson and Berridge, 1993). This theory posits that drugs of abuse share the ability to induce long-lasting changes in the brain and that these changes (among other effects) render the brain's reward system hypersensitive to drugs and drug-paired cues. Further, the systems that are rendered hypersensitive are explicitly not responsible for the hedonic or "pleasurable" effects of drugs. Thus with each subsequent drug use, it becomes increasingly difficult to resist the craving induced by presentation of the drug itself or cues associated with the drug. Robinson and Berridge have argued that mesolimbic DA plays an important role in addiction and is responsible for the attribution of incentive-salience to drugs and drug cues, and mediates "wanting" or craving (Berridge et al., 1989; Berridge and Valenstein, 1991; Robinson and Berridge, 1993, 2001). Collectively, the incentive-sensitization theory argues that mesolimbic DA release is sensitized with repeated drug use (a *hyperdopaminergic* state) and this is responsible for "wanting" and contributes to the development of addiction.

Studies that have demonstrated tolerance-like effects on the mesolimbic DA system to cocaine following LgA self-administration support opponent-process theories of addiction (Caprioli et al., 2014; Willuhn et al., 2014; Koob and Volkow, 2016). However, other studies demonstrate sensitization-like effects following drug self-administration and are largely supportive of incentive-sensitization theory (e.g., Grimm et al., 2003; Vezina, 2004; Ferrario et al., 2005; Evans et al., 2006; Cox et al., 2009). The results presented here are largely consistent

with the latter. First, we found that prolonged LgA self-administration did not produce addiction-like behavior, making it difficult to interpret the importance of DA alterations following LgA cocaine self-administration. Second, we found that baseline DA levels tested only 1-3 days after the last self-administration session did not differ between ShA, LgA, or IntA rats and did not predict motivated behavior. This suggests that the increased severity of withdrawal associated with long-term drug use and implicated in the development of addiction by opponent-process theories of addiction may not involve DA and basal DA levels, even during periods associated with withdrawal, have little impact on motivated behavior. Third, we found that the magnitude of cocaine-evoked DA release, relative to baseline, predicted a number of addiction-like behaviors and was sensitized to the greatest extent in animals that displayed the most robust addiction-like behaviors, which is exactly the opposite of what would be predicted by opponent-process theories if blunted DA was causal in the transition to addiction.

Taken together we believe the findings presented here demonstrate the critical importance of the temporal pattern of drug delivery in modeling the development of addiction and its underlying neurobiological basis. The IntA procedure, which better models the temporal pattern of drug use seen in humans, produced robust addiction-like behavior and produced a sensitized DA response to cocaine.

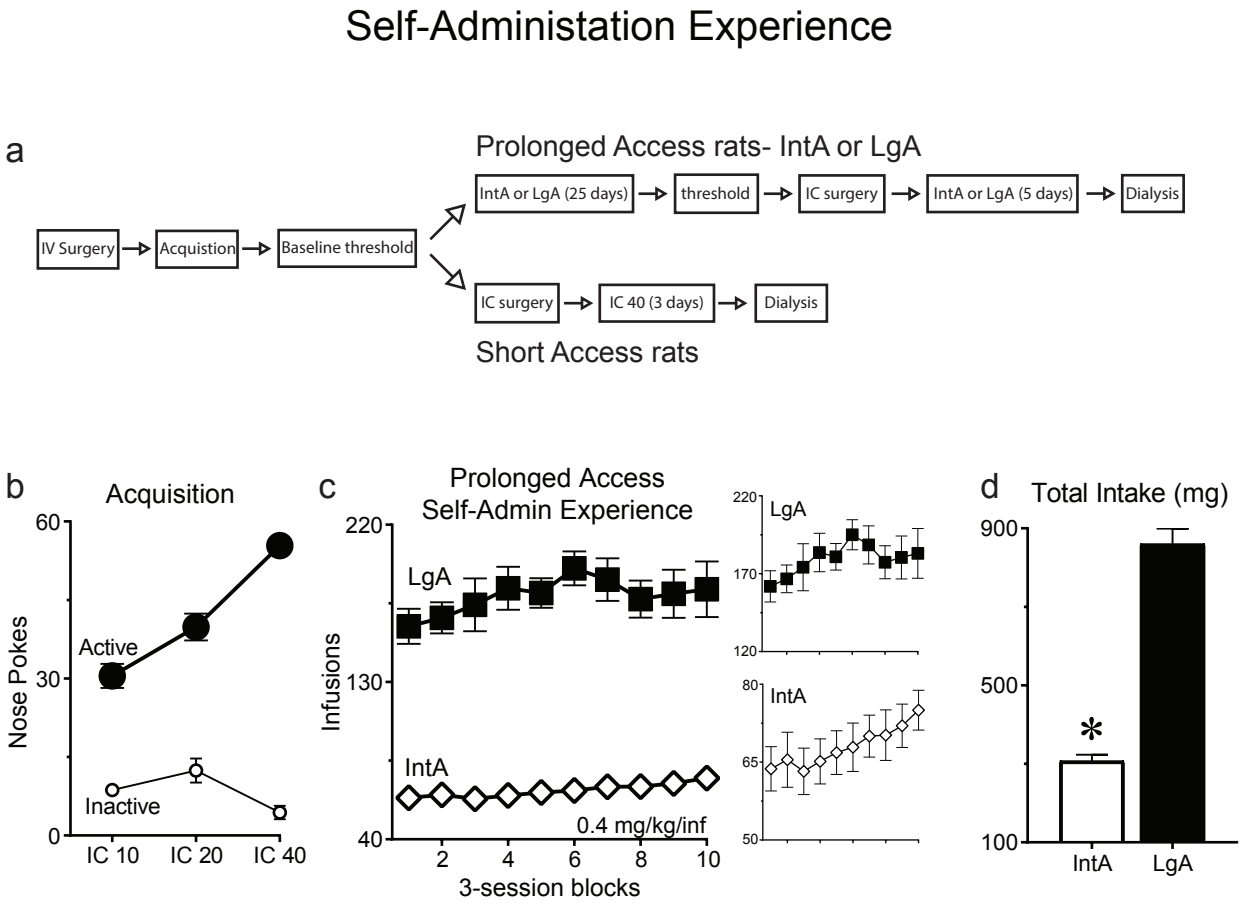


Figure 4.1. Timeline and self-administration experience. The flow diagram shows the overall experimental design and timeline for the experiment (**a**) (see methods for details; the cue-induced reinstatement test that followed dialysis in the Prolonged rats is not shown as it was only conducted in a subset of rats). An Infusion Criterion (**IC**) procedure was used for the acquisition of cocaine self-administration (**b**). IntA rats and LgA rats both escalated their intake to a similar extent across all 30 self-administration sessions (**c**). LgA rats consumed far more cocaine on average than IntA rats over the course of 30 self-administration sessions under their respective procedures (**d**). (IntA: Intermittent Access, LgA: Long Access, IC surgery: Intracranial (microdialysis guide cannula) surgery). In panel c each point represents the average of three consecutive self-administration sessions. See methods for the number of rats included in each group and panel. Values represent means ± SEMs.

Motivation Following IntA and LgA

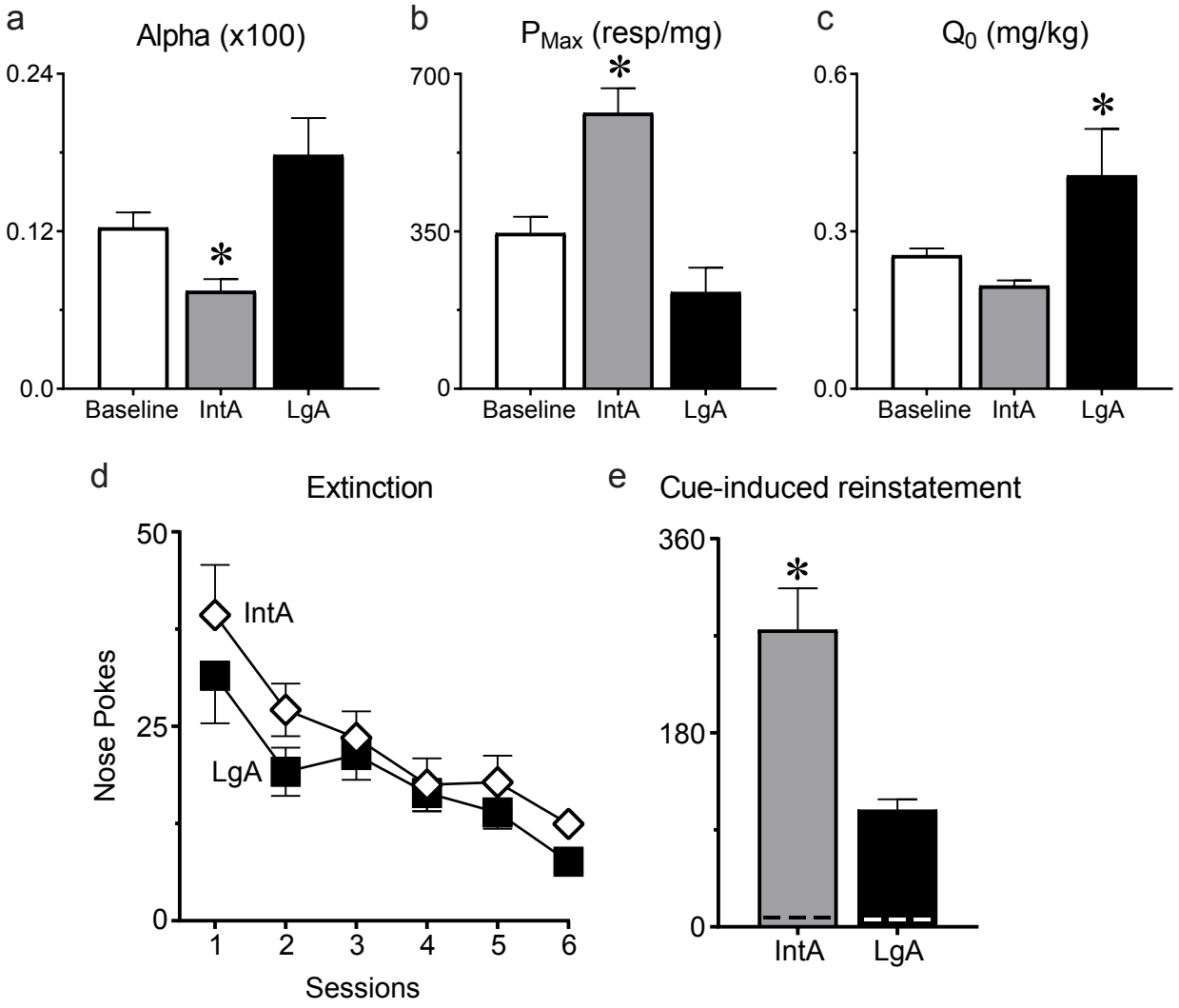


Figure 4.2. Cocaine demand in rats with different self-administration experience. Cocaine demand was assessed using a threshold procedure in rats with different self-administration experience. In panels a-c all rats are included in the 'Baseline' group (n=42) and a subset of rats went on to experience IntA self-administration (n=18) while a separate group of rats went on to LgA self-administration (n=12). IntA experience decreased alpha (α; increased motivation) relative to LgA experience (**a**) and increased P_{Max} (increased motivation) relative to LgA experience (**b**). LgA experience increased Q_0 (preferred cocaine intake when effort is not a factor) relative to IntA experience (**c**). IntA rats (n=10) and LgA rats (n=9) did not differ in responding during extinction training that occurred in a subset of rats following the microdialysis test session (**d**). In a test for conditioned reinforcement, when a nose poke in the previously active port was reinforced by presentation of the cue that had previously been associated with cocaine but not cocaine itself, IntA rats made more responses than LgA rats (**e**). Nose pokes at the inactive port are represented by the dashed lines. Values represent means ± SEMs, * represents a significant difference ($p<0.05$) between IntA rats and LgA rats.

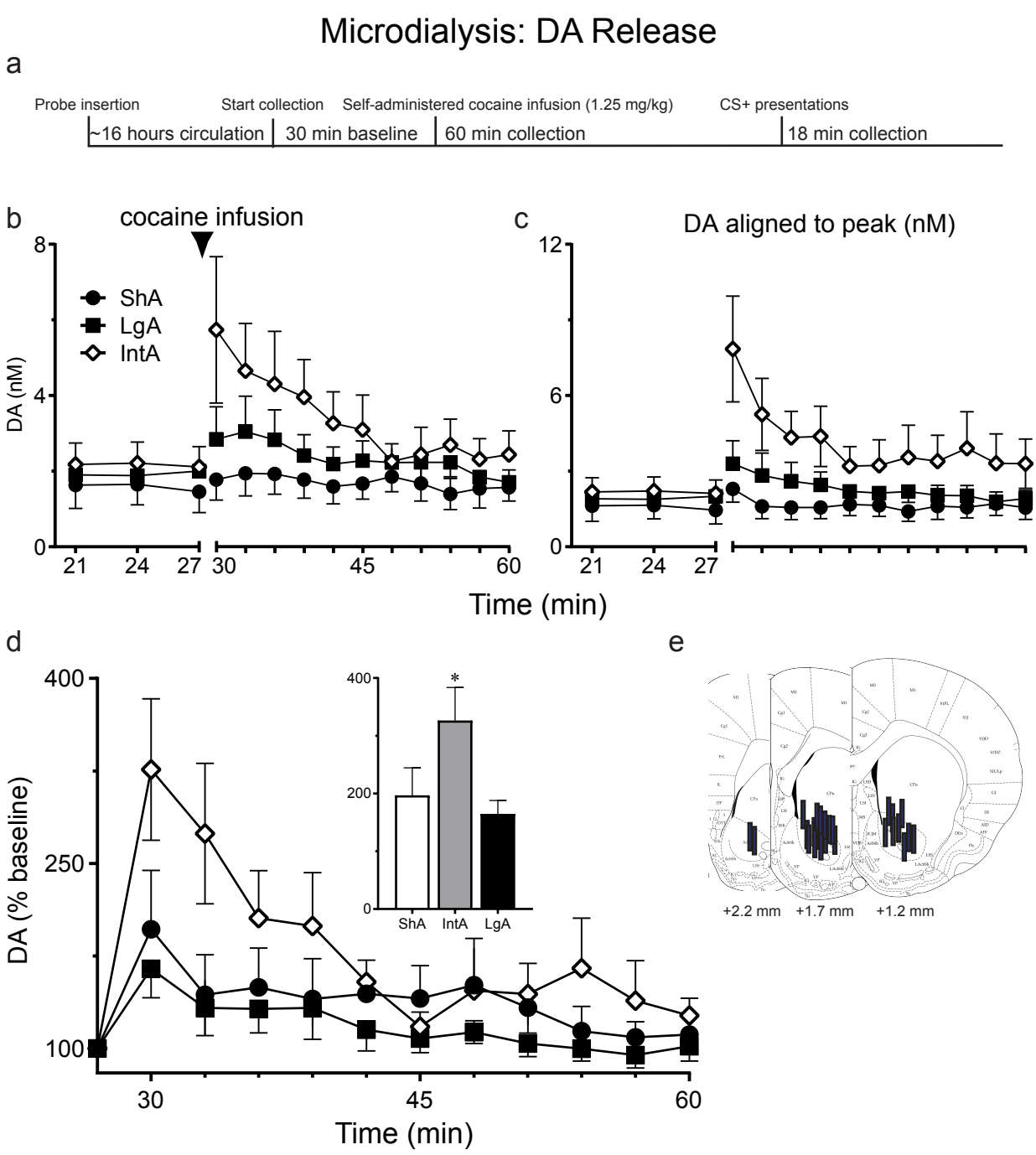

Microdialysis: DA Release
a
Probe insertion
Start collection
Self-administered cocaine infusion (1.25 mg/kg)
CS+ presentations
~16 hours circulation
30 min baseline
60 min collection
18 min collection
b
cocaine infusion
ShA
LgA
IntA
DA (nM)
Time (min)
c
DA aligned to peak (nM)
d
DA (% baseline)
Time (min)
ShA
IntA
LgA
e
+2.2 mm
+1.7 mm
+1.2 mm

Figure 4.3. Dopamine release measured with *in vivo* microdialysis. Dopamine (DA) release in response to a self-administered cocaine infusion was tested in rats with different self-administration experience. A timeline of the microdialysis test day is shown in panel **a**. Data from the CS presentation is not shown as there was no change in DA levels. Cocaine-evoked DA release was larger in rats trained with IntA (n=16) than rats trained with LgA (n=9) or ShA (n=9) (**b**). Panel **c** shows the same data with each rat's peak response to cocaine aligned. When peak DA release was analyzed as a % of baseline, cocaine evoked a larger DA response in IntA rats than LgA or ShA rats (**d**). Microdialysis probes were targeted to the nucleus accumbens core (**e**). Values represent means ± SEMs.

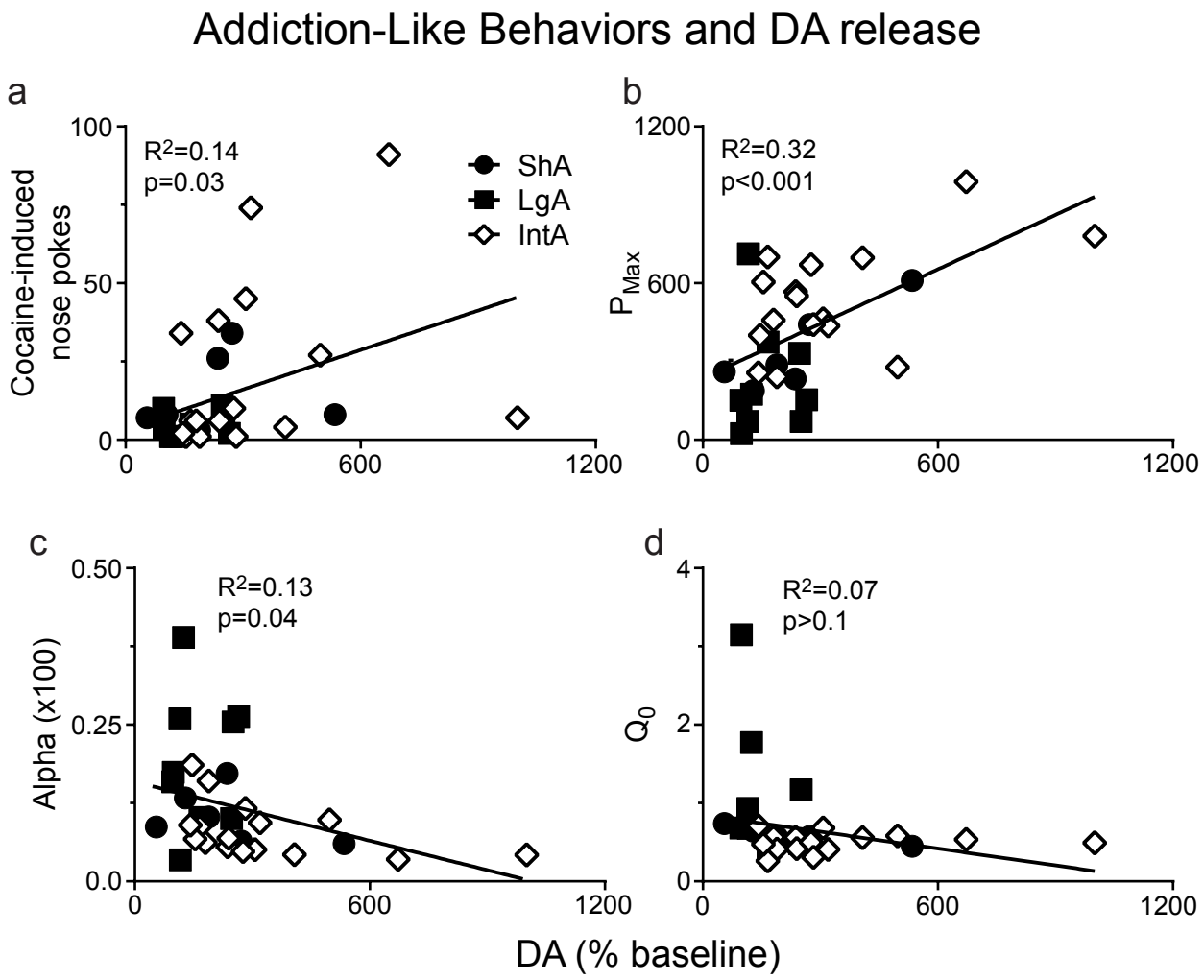


Figure 4.4. Addiction-like behaviors and dopamine release. Each rat's dopamine (DA) release in response to a self-administered cocaine infusion was correlated with that rat's performance on several tests of addiction-like behavior. When all animals were analyzed collectively DA release, as a percent of baseline, predicted cocaine-induced nose pokes (measured during the microdialysis test session) (**a**), P_{Max} (**b**), and alpha (α) (**c**). DA release, as a percent of baseline, did not predict Q_0 (**d**).

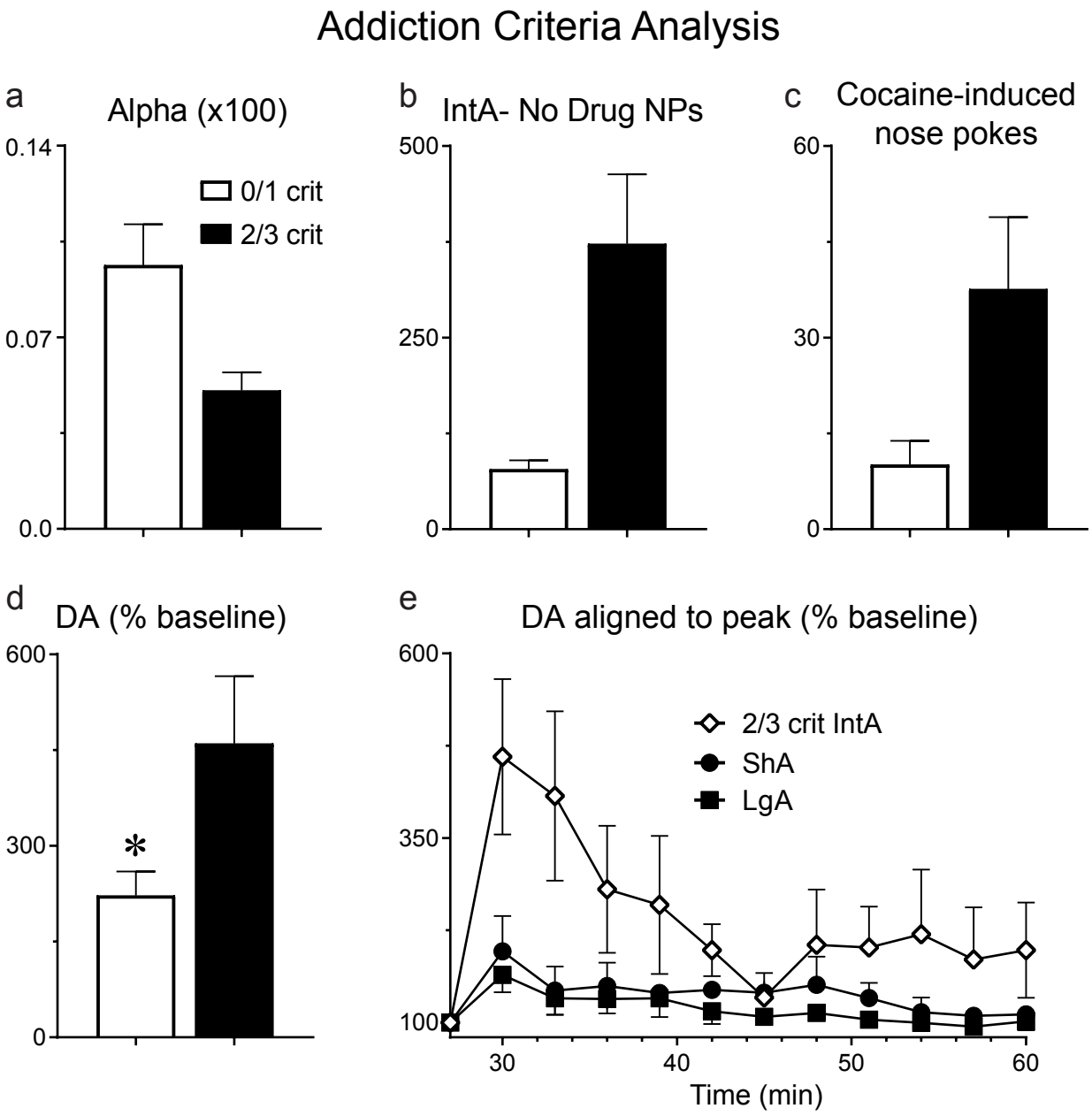

Addiction Criteria Analysis
a
Alpha (x100)
0.14
0.07
0.0
0/1 crit
2/3 crit
b
IntA- No Drug NPs
500
250
0
c
Cocaine-induced
nose pokes
60
30
0
d
DA (% baseline)
600
300
0
*
e
DA aligned to peak (% baseline)
600
350
100
2/3 crit IntA
ShA
LgA
30
40
50
60
Time (min)

Figure 4.5. Addiction criteria and dopamine release. Analysis based on addiction criteria. IntA rats were separated based on the number of 'addiction-criteria' they met (see methods). Rats that met 2/3 criteria (n=7) showed increased motivation relative to rats that met 0/1 criteria (n=9) as indicated by alpha (α) (**a**), nose pokes (**NPs**) made during the No Drug periods of IntA (**b**), and cocaine-induced nose pokes (measured during the microdialysis test day) (**c**). A self-administered infusion of cocaine evoked greater peak dopamine (DA) release, shown as a % of baseline, in 2/3 criteria rats than 0/1 criteria rats (**d**). Panel **e** shows the timecourse of DA release (% baseline) following cocaine in 2/3 criteria IntA rats, ShA rats, and LgA rats. Values represent means ± SEMs.

Neurotransmitter	Group Differences	Peak cocaine-induced change from baseline	Std. Error
Glutamate	No	+32%*	+/- 10.3%
GABA	No	+1.4%	+/-4.6%
ACh	No	-10%	+/-33%
3-MT	Yes	IntA: +98%* ShA&LgA: +25%*	+/-26% +/-9.1%
DOPAC	No	-9.9%	+/- 4.1%
HVA	No	+0.5%	+/-5.2%

Table 4.1. Neurochemicals of interest following a cocaine infusion. Microdialysis allows for the analysis of a number of neurochemicals in addition to dopamine. Select neurochemicals of interest are shown here. A self-administered cocaine infusion increased extracellular levels of Glutamate and 3-MT (a dopamine metabolite) in all animals. Cocaine-evoked 3-MT was greater in IntA rats than ShA rats or LgA rats. * indicates a significant ($p<0.05$) change in neurochemical level following the cocaine infusion relative to baseline.

CHAPTER V

GENERAL DISCUSSION

Summary of Results

The experiments included here characterize the effects of prolonged intermittent cocaine self-administration on motivated behavior in sign-trackers (STs) and goal-trackers (GTs) and in male and female rats. We then attempted to identify potential neurochemical mechanisms underlying the robust development of addiction-like behaviors that was seen following prolonged intermittent self-administration.

In 2011-12 Benjamin Zimmer, working in the lab of Dave Roberts, introduced the 'Intermittent Access' (IntA) self-administration procedure (Zimmer et al., 2011, 2012). The IntA procedure was developed to better model in rats the temporal pattern by which humans that suffer from addiction use cocaine. That is, even within a cocaine binge humans do not elevate their brain cocaine concentration and maintain these levels for prolonged periods of time. Instead, cocaine users will take a 'hit' of cocaine and then wait a period of time, allowing their blood levels to fall, and then take another hit (Cohen and Sas, 1994; Ward et al., 1997; Beveridge et al., 2012; Allain et al., 2015). In the first study that compared IntA to other self-administration procedures, Zimmer et al., (2012) showed that rats with a history of IntA were more motivated to

work for cocaine than rats that were trained with the widely used 'Long Access' (LgA) self-administration procedure, despite much less total drug consumption.

Based on this exciting finding we designed a study that utilized the IntA procedure in the investigation of individual differences in addiction susceptibility. At that time, a great deal of evidence suggested that individuals that attributed incentive-salience to reward-paired cues (sign-trackers; STs) were more susceptible to develop addiction than animals for which the cue held only predictive value (goal-trackers; GTs) (for reviews see Flagel et al., 2009; Saunders and Robinson, 2013). However all of the studies contributing to this hypothesis had been done with relatively limited drug exposure and we sought to test this hypothesis using a procedure that better modeled the development of addiction. We found that after limited drug experience, STs were more motivated to work for cocaine than GTs, consistent with a previous report (Saunders and Robinson, 2011). Surprisingly, with prolonged IntA self-administration experience both STs and GTs underwent robust incentive-sensitization for cocaine to the point that motivation was equally high in both STs and GTs on a number of addiction-like behaviors.

These findings added to the data surrounding individual differences in addiction liability and forced us to reshape our hypothesis. As is often the case, with more data the story became more complex. Our findings suggested that differences in addiction liability exist at certain points in the development of addiction, but these differences do not necessarily generalize to all stages of addiction. Indeed, we still believe that STs are more susceptible to develop addiction for a number of reasons (see chapter 2 discussion) including that after relatively limited experience they may be more likely to continue drug use and develop problematic patterns of use. However, STs and GTs may not differ in their propensity to undergo incentive-sensitization

following intermittent drug exposure. Under these conditions there is nothing *protective* in the GT phenotype against the development of addiction.

Given the robust development of multiple addiction-like behaviors that we saw in the experiments described in chapter 2, we turned our attention to how intermittent cocaine self-administration may interact with sex differences in addiction susceptibility. There is a great deal of evidence in humans and pre-clinical populations that females are more susceptible to develop certain symptoms of addiction than males (Fattore et al., 2008; Becker, 2016; Becker and Koob, 2016). In our studies we found that males and females showed considerable differences on a number of measures. Primarily, females consumed more cocaine during IntA and were more motivated to work for cocaine following IntA self-administration. Based on these findings we theorized that females were more susceptible to rapid and potent incentive-sensitization, and may be *particularly* susceptible to intermittent drug exposure.

Interestingly, even after prolonged IntA (and an abstinence period) females were still more motivated than males to self-administer cocaine, and if anything this difference was accentuated with increasing IntA experience. This pattern was very different than what we observed in STs and GTs- that increasing IntA attenuated individual differences. This inconsistency provides further evidence that it is unlikely that any one population has an increased liability at every stage of the progression to addiction, but that individual populations have an increased risk, relative to the general population, at specific stages or to specific aspects of the addiction process.

Finally, we were interested in identifying the neurochemical changes that resulted from prolonged IntA self-administration in individuals that developed addiction-like behaviors. We sought to compare cocaine and cue-induced neurochemical release in the nucleus accumbens

core of rats that were given prolonged IntA to rats given prolonged LgA and rats given limited exposure to cocaine self-administration ('Short Access'; ShA). We found that prolonged LgA did not change motivation for cocaine, but did increase the preferred level of intake when no effort was required. In the rats trained on the IntA procedure, we replicated our previous finding that prolonged IntA resulted in a number of addiction-like behaviors. Further, we found that LgA rats did not differ from ShA rats in cocaine-evoked dopamine (DA) release. However, IntA rats showed a sensitized DA release in response to a self-administered cocaine infusion, relative to both LgA and ShA rats. In all animals, the magnitude of DA release, relative to baseline DA levels, predicted several measures of motivation, but did not correlate with the rat's preferred intake when no effort was required. Based on these results we concluded that the addiction-like behaviors observed following prolonged IntA are due at least in part to a *sensitized* dopaminergic system in the brain.

An Emerging Literature Using IntA Self-Administration

A number of studies have been published using the IntA procedure since the first papers were published by Dave Roberts' group. The results presented in chapter 2 represent the first examination of the progression of addiction-like behaviors with increasing IntA experience. The results therein have been replicated and extended in several studies now from multiple labs. A recent study from our lab similarly demonstrated the development of multiple addiction-like behaviors following prolonged IntA (Singer et al., 2018). In this study, as in chapter 2, IntA produced (i) escalation of intake; (ii) a progressive increase in the amount of effort rats are willing to expend to maintain preferred blood levels of cocaine; (iii) a decrease in the elasticity of the cocaine demand curve; (iv) continued drug-taking in the face of an adverse consequence; (v) continued responding when drug is not available; and (vi) especially robust cue-induced

reinstatement of drug-seeking. Also, IntA did not change the preferred level of cocaine intake when no effort was required. In addition, we showed that the development of these addiction-like behaviors is not contingent on the formation of stimulus-response habits, nor a shift in control of motivated behavior from ventral to dorsal striatum (Singer et al., 2018), although this has been suggested to be necessary for the development of addiction (Everitt and Robbins, 2005, 2016).

Increased motivation following IntA has also been shown using a progressive-ratio (PR) test, indicating that this effect is not unique to the within-session threshold procedure (Allain et al., 2017). Allain et al. (2017) also demonstrated that IntA self-administration produces psychomotor sensitization and the degree of psychomotor sensitization predicts subsequent motivation for cocaine on the PR test. There are also now reports showing persistent drug-seeking even when the drug is not available (Allain et al., 2018) and 'burst-like' patterns of drug use (Allain and Samaha, 2018; Allain et al., 2018) following IntA experience.

In addition to these findings, a provocative series of studies using IntA from Anne-Noel Samaha's lab has demonstrated the importance, or lack thereof, of the amount of cocaine consumed during self-administration and escalation of intake. A number of studies have shown that consuming the large quantities of cocaine associated with LgA is not necessary to produce escalation of intake as rats will escalate their intake across IntA sessions to a similar extent as rats trained with LgA self-administration (Kawa et al., 2016; Allain et al., 2017, 2018, Pitchers et al., 2017a, 2017b; Allain and Samaha, 2018; Singer et al., 2018). Interestingly, Allain et al., (2018) developed an 'IntA-Limited' procedure that limited the amount of cocaine the rats could self-administer to a maximum of two infusions per Drug-Available period which precluded escalation of intake. Rats trained on this IntA-Limited procedure were just as motivated as rats trained using the traditional IntA procedure, and still more motivated than rats trained on the

LgA procedure (Allain et al., 2018). Similar results were shown by decreasing the length of the IntA procedure to 2 hours (4 cycles of drug availability), which did not produce escalation of intake, but produced psychomotor sensitization and levels of motivation for cocaine and cocaine-induced reinstatement that were similar to those seen with a 6-hour IntA session (Allain and Samaha, 2018). Taken together these studies suggest, "*Taking large and escalating quantities of cocaine does not appear necessary to increase incentive motivation for the drug. Taking cocaine in an intermittent pattern—even in small amounts—is more effective in producing this addiction-relevant change,"* and thus *"Escalation might be a consequence, rather than a cause in the transition to addiction*" (Allain et al., 2018).

One potential explanation for the robust addiction-like behaviors that are observed following IntA self-administration is that they are the result of the rats experiencing successive withdrawal states during the No-Drug periods of IntA. It is very difficult to unequivocally rule this possibility out, but we believe it is unlikely to be the main driving force behind the development of addiction-like behaviors following IntA. First, there is a long history of studies showing that intermittent drug exposure tends to produce sensitization, while tolerance and withdrawal are maximally produced following long, sustained elevations in drug levels- exactly the opposite of the pattern produced by IntA (Post, 1980; see Chapter 1). Second, the No-Drug period used in IntA is 25-minutes long, which is just long enough for brain cocaine levels to approach baseline levels, but withdrawal typically manifests hours to days after the last cocaine use in humans (Gawin and Kleber, 1986) and rats (Markou and Koob, 1991; Parsons et al., 1995). Further, acute withdrawal states are associated with decreased locomotor activity (Baldo et al., 1999; Koeltzow and White, 2003) and reduced craving (Gawin and Kleber, 1986; Koob et al., 2004), neither of which are observed during the No-Drug period of IntA (Allain et al., 2017;

chapters 2 &3). Finally, the studies using IntA-Limited and 2-hour IntA, which dramatically reduce the amount of cocaine rats can consume, should also reduce any corresponding withdrawal symptoms and thus if these were driving the progression to addiction one would expect motivation to be decreased following these procedures relative to traditional IntA, which is not what is observed (Allain and Samaha, 2018; Allain et al., 2018). We believe it is much more likely that the addiction-like behaviors produced by IntA are the result of sensitized motivational systems in the brain, known to be induced by intermittent drug exposure (Robinson and Berridge, 1993).

One criticism of IntA self-administration is that it imposes experimenter-determined constraints on how rats take cocaine that do not exist in human cocaine use. The argument follows that if humans take cocaine intermittently within a binge to maximize the pleasurable effects, rats should eventually do the same during LgA self-administration. While rats do not appear to take cocaine intermittently even after prolonged LgA, when rats were allowed truly unrestricted access to cocaine, 24-hours/day over the course of 30 days, one of the defining characteristics that emerged was erratic, intermittent patterns of intake within a day and across days (Deneau et al., 1969; Johanson et al., 1976; Bozarth and Wise, 1985). No procedure perfectly models the human condition of drug-taking. For example, humans are never presented with repeated, free-access to cocaine as rats are in self-administration studies, and it is reasonable to assume the pattern of intake in humans may be very different if they were. We argue that it is important to study the effects of intermittent drug exposure *given* that this is how humans consume drug, even if it requires experimenter-imposed constraints.

Using the Within-Session Threshold Procedure to Quantify Cocaine Consumption and Motivation

Throughout the studies presented here we relied heavily on behavioral economic analysis of data derived from the within-session threshold procedure. Thus it is worth discussing the merits and potential weaknesses of this procedure. Self-administration behavior using fixed-ratio (FR) schedules of reinforcement are difficult to interpret as changes in either direction of responding (increases or decreases) can be interpreted as increases in the reinforcing effects of the drug, or vice versa. This is illustrated by changes in response rate that occur in opposite directions following manipulations on the same neurobiological system (e.g., Yokel and Wise, 1975; Roberts et al., 1980). One better way of testing the reinforcing effects of a commodity is through the use of a Progressive-Ratio (PR) schedule of reinforcement that progressively increases the number of responses required for each subsequent reward delivery (Hodos, 1961; for review see Richardson and Roberts, 1996). Alternatively, the response requirement can be held constant and the dose of drug delivered can be manipulated (Threshold Procedure) (Yokel and Pickens, 1974; Zittel-Lazarini et al., 2007; Oleson and Roberts, 2009). Both procedures yield data that are more informative than response rate during a traditional FR schedule of reinforcement (Bickel et al., 1990; Richardson and Roberts, 1996).

The use of behavioral economics to analyze drug reinforcement dates back a number of years (Bickel et al., 1990; Hursh, 1991). One of the early findings using behavioral economic analysis of food- and drug-maintained responding was that response requirement manipulations (PR) and dose manipulations (threshold) have functionally equivalent effects on food and drug responding (Collier et al., 1986; Hursh et al., 1988; Bickel et al., 1990; but see Gan et al., 2010). That is, both manipulations equivalently affect ‘unit price’ of a commodity and individuals will

generally titrate their responding similarly in both cases (see below). One advantage of using threshold procedures over PR procedures is that they differentiate between “appetitive” and “consummatory” aspects of drug self-administration (Oleson and Roberts, 2009; Oleson et al., 2011). That is, threshold procedures yield metrics for the preferred level of drug consumption when little, or no effort is required, and separately, the amount of effort a subject is willing to expend for drug when the amount of effort required is increased. Distinct psychological and neurobiological systems have been implicated in the appetitive and consummatory aspects of behavior (Brebner et al., 2000; Nicola and Deadwyler, 2000; Sharpe and Samson, 2001; España et al., 2010; Gan et al., 2010; Oleson et al., 2011; Guillem et al., 2014).

Recently a ‘within-session threshold procedure’ was developed that allowed for the assessment of both appetitive and consummatory aspects within a single session (Oleson et al., 2011). The within-session threshold procedure is comprised of ten-minute bins and every ten minutes the dose of drug delivered per response is decreased. In addition to the advantages already mentioned, this shortened the length of experiments and allowed experimenters to test the effects of different manipulations on appetitive and consummatory aspects within a single session. In addition, this procedure asked a slightly different question than PR or between-session threshold procedures. It allowed the rats to ‘load-up’ to a preferred brain level of drug early in the session, when the unit price of drug was very low, and then asked how hard the rat was willing to work to defend that preferred drug level. This is different from a PR test that asks how hard a subject is willing to work for an experimenter-determined dose of drug. A great deal of work has been put into validating the within-session threshold procedure including: studies that show manipulations similarly and predictably affect the ‘appetitive’ measures on the within-session threshold procedure and ‘breakpoint’ from a PR test (Oleson et al., 2011), economic

demand (but not preferred consumption) derived from the within-session threshold procedure predicts other addiction-like behaviors (Bentzley et al., 2014), and finally decreased responding during the within-session threshold procedure is the result of increased 'unit price' and not session length or satiation- demonstrated by presenting doses in an ascending order or with a 20-minute time-out (intermittent threshold procedure) between each unit price (Porter-Stransky et al., 2017).

One drawback of both PR and the within-session threshold procedure is that they both measure motivation for drug while the user is on the drug itself, and motivation is often highest after a small, priming injection of drug (e.g., Deneau et al., 1969; Fitch and Roberts, 1993). However, as reviewed above, similar findings using between-session, intermittent, and within-session threshold procedures suggest that while motivation may be higher when a subject is on drug, this is correlated with the subject's motivation in a drug-free state.

Also, while both PR and threshold procedures similarly affect 'unit price', it remains possible that a smaller dose of drug has different subjective/hedonic effects than a larger dose and this is responsible for the decrease in responding seen during the threshold procedure. This potential confound is largely avoided in PR studies when a single dose is used throughout a test session. However, we do not believe that decreases in the subjective effects of cocaine across the within-session threshold procedure largely affected our measures of motivation. First, as has been mentioned previously, changes to unit-price through manipulations of response requirements or dose similarly affect responding (Collier et al., 1986; Hursh et al., 1988; Bickel et al., 1990). Along those lines, PR breakpoint and the appetitive measures from the threshold procedure are tightly correlated (Rodefer and Carroll, 1997; Oleson and Roberts, 2009; Bentzley et al., 2013). To the extent that PR tests control for the subjective effects of the drug, these data

suggest that the appetitive aspects measured during threshold procedures are not driven by the hedonic properties of the drug.

In addition, the within-session threshold procedure may largely avoid this concern. Early in the session the rats 'load-up' to a preferred level of drug consumption when the cost is very low. Presumably, this preferred level of consumption maximizes the pleasurable effects of the drug. For some duration of the session rats will titrate their responding such that this level of consumption remains 'inelastic', in that it does not change as the dose decreases. Thus while the subjective effects of each single infusion are changing, the subjective effects the rats are experiencing are not. Rats maintain this preferred level of consumption until the effort required to do so increases to such a point that they stop responding. In so much as every response a rat makes is the manifestation of a cost-benefit analysis of the appetitive effects of the drug versus the effort required, the effects of each infusion on the within-session threshold procedure are influenced heavily by the existing brain cocaine concentration. This means that no one 'unit price' is isolated from the preceding 'unit price' and the decision the rat must make is based upon their existing brain cocaine concentration. In this way the within-session threshold procedure minimizes the impact of the subjective value of each individual infusion. Therefore the question being asked at each unit-price is if maintaining the existing (preferred) level of consumption is worth the effort required to do so, and not if the subjective "pleasurable" effects of a single infusion (in isolation) are worth it. To further address this concern we adopted a behavioral economic "focused fitting" method for analyzing our data that only included data points that occurred at relatively stable consumption levels and to date is the most accurate method for generating demand curves based on within-session threshold data (Bentzley et al., 2013).

Temporal Pattern of Stimuli and Neural Plasticity

While the fact that IntA and LgA self-administration, two proposed models of addiction, have very different effects on the brain (e.g., Calipari et al., 2013; chapter 4) may at first appear surprising, when these results are viewed in the broader context of neural plasticity they are less surprising. The importance of the temporal pattern of stimuli impinging on the brain in shaping neural plasticity and behavior has long been acknowledged across the breadth of experimental psychology. Early studies on human memory showed that the *amount of training* was only one factor that determined retention, and the *temporal distribution* of training trials also affected later performance, as "spaced" trials proved more effective than "massed" trials (Ebbinghaus, 1885; Woodworth, 1938; Davis, 1970). On a neuro-cellular level, the importance of temporal pattern has been acknowledged dating back to the original studies on synaptic plasticity. These studies demonstrated that increases in the excitability of neurons were observed following only specific patterns of electrical stimulation (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973; Larson et al., 1986). Critically, many patterns of electrical stimulation that did not yield increased excitability were later found to have the *opposite* effect, and decrease excitability in neurons (Dudek and Bear, 1992). It is then not surprising that different temporal patterns of drug use can have equally diverse impacts on neural plasticity and behavior.

Drug Self-Administration and Theories of Addiction

The role of dopamine (DA) in addiction has been the focus of countless studies and implicated in numerous theories of addiction (Koob, 1982; Wise and Bozarth, 1987; Robinson and Berridge, 1993; Everitt et al., 2001). Interestingly two very different, nearly opposite, theories regarding how drug use changes the DA system and how this contributes to the progression from casual drug use to addiction still have support in the field. The opponent-

process theory, or anhedonia theory, is very similar to the early theories of addiction, referred to as “escape theories”, discussed in Chapter 1. Generally, these theories posit that initial drug use is driven by a drug’s primary, rewarding effects, but escalation of drug use, and chronic, addictive use is driven by a desire to alleviate the unpleasant state of drug withdrawal. Across time the frequency of drug use increases and the amount of drug consumed escalates which leads to withdrawal symptoms that are more severe and thus the desire to alleviate these symptoms grows stronger. In addition, such theories propose that relapse can occur even after physical withdrawal has dissipated in response to conditioned withdrawal prompted by the presentation of cues previously associated with drug use.

The opponent-process theory and related theories often propose that chronic drug use results in decreased functioning of the mesolimbic DA system. This *hypodopaminergic* state is proposed to contribute to the dysphoria associated with withdrawal and drives cocaine seeking in an effort to restore normal DA levels (Dackis and Gold, 1985; Koob and Le Moal, 2001; Volkow et al., 2016). The tolerance-like effects of LgA cocaine self-administration on the mesolimbic DA system reviewed in chapter 1 have been taken as evidence in support of opponent-process theories of addiction (Caprioli et al., 2014; Willuhn et al., 2014; Koob and Volkow, 2016). Our studies presented in chapter 4, and others, have shown that LgA reliably changes aspects of consummatory behavior- resulting in increases in a subjects’ preferred level of cocaine intake (Q_0) and pronounced escalation of intake across sessions (e.g., Ahmed and Koob, 1998; Bentzley et al., 2014; James et al., 2018). Thus it is possible that the hypodopaminergic state produced by LgA may affect consummatory aspects of behavior (Guillem et al., 2014). This is supported by studies that show L-Dopa treatment reverses escalation of intake (Willuhn et al., 2014) and pretreatment with a DA antagonist increased Q_0 (but decreased P_{Max}) (Oleson et al., 2011).

However, this conclusion is not supported by data that suggests that following the cessation of drug use the hypodopaminergic state produced by LgA is relatively short lasting (Guillem et al., 2014; Siciliano et al., 2016) but changes to Q_0 and escalation of intake appear to endure for a longer duration of time (Ahmed and Koob, 1998; Bentzley et al., 2014; James et al., 2018). Also, in considering how these findings relate to broader theories of addiction it is important to note that while there is a strong relationship between Q_0 and escalation of intake, neither reliably predicts other addiction-like behaviors (chapters 3 and 4; Oleson et al., 2011; Bentzley et al., 2014; Allain et al., 2018). Further, LgA did not change the motivational aspects measured in chapter 4 and this is consistent with other reports using threshold procedures (Oleson and Roberts, 2009) and it does not reliably increase breakpoint on PR procedures (Liu et al., 2005; Quadros and Miczek, 2009; Willuhn et al., 2014 (supplementary)). In addition, opponent-process theories fail to adequately explain how drug-induced *hypodopaminergic* states and drug-associated, cue-induced *hyperdopaminergic* states both trigger drug craving. As stated by Volkow et al. (2016), "environmental stimuli that are repeatedly paired with drug use… may all come to elicit conditioned, fast surges of dopamine release that trigger craving for the drug, motivate drug-seeking behaviors, and lead to heavy "binge" use of the drug. These conditioned responses become deeply ingrained and can trigger strong cravings for a drug long after use has stopped," (p. 366).

Therefore we believe that a *hypodopaminergic* state may produce increases in consummatory aspects of behavior, although questions surrounding the time course of these actions and correlation versus causation remain, but these consummatory aspects (and the hypodopaminergic *state*) may have only minor or secondary contributions to the development of addiction.

The incentive-sensitization theory of addiction (described in chapter 1) presents a very different view of addiction and the underlying neural processes involved. Briefly, the incentive-sensitization theory proposes that the incentive motivational effects of drugs and drug cues increase with increasing drug experience. These incentive motivational effects are long lasting and can contribute to drug craving and relapse even years after the cessation of drug use. The incentive-sensitization theory proposes that these incentive-motivational effects are caused by long-lasting sensitization of the neural systems involved in reward and motivation. One key component of this motivational circuit is the mesolimbic DA system (Berridge et al., 1989; Berridge and Valenstein, 1991; Robinson and Berridge, 1993). Thus in the presence of drugs and drug cues, the incentive-sensitization theory proposes a sensitized DA system, akin to a *hyperdopaminergic* state, is responsible for "wanting" and addiction.

The data presented in chapters 2, 3, and 4 suggest that prolonged IntA results in robust incentive-sensitization indicated by increases in P_{Max} and decreases in α, along with a number of other addiction-like behaviors. In addition, IntA self-administration produces a progressive increase in the psychomotor activating effects of cocaine, and the degree of this psychomotor sensitization predicts subsequent motivation for cocaine assessed by breakpoint on a PR schedule (Allain et al., 2017). Interestingly, IntA does not alter the preferred level of drug consumption when cost is nil (Q_0), which has been suggested to reflect a 'hedonic setpoint' (Bentzley et al., 2013; James et al., 2018; Singer et al., 2018). The dissociation between measures of cocaine demand (motivation) and Q_0 suggests IntA experience may increase drug "wanting", without any associated change in drug "liking", a central tenet of the incentive-sensitization theory of addiction (Robinson and Berridge, 1993; Berridge and Robinson, 2016). Thus we suggest that

the development of addiction-like behavior with IntA experience reflects the process of incentive-sensitization.

If IntA is producing incentive-sensitization then studies of the neurobiological effects of IntA should indicate a sensitized DA system. In chapter 4 we observed in awake, behaving rats that prolonged IntA produced a sensitized DA response to cocaine, relative to rats trained with LgA or ShA. This data supports findings collected *ex vivo* following IntA experience (Calipari et al., 2013, 2014; Calipari and Jones, 2014). In addition we found that DA release in response to cocaine reliably predicted a number of addiction-like behaviors, including α, P_{Max}, and cocaine-induced drug seeking. Further, cocaine-induced DA release was also significantly increased in animals that showed the most robust addiction-like behavior, using an addiction-criterion scoring system (Deroche-Gamonet et al., 2004). Notably, cocaine-induced DA release did not predict Q_0 or intake during IntA. In addition, baseline DA levels did not correlate with any addiction-like behaviors, suggesting that a tonic *hypodopaminergic* state is not responsible for these addiction-like behaviors.

Implications for Preclinical Models of Addiction

The studies summarized above clearly establish that the temporal pattern by which self-administered cocaine impinges on the brain has an enormous influence on its ability to *change* brain and psychological function in ways that are relevant for the transition to addiction. Indeed, studies using IntA self-administration procedures challenge fundamental assumptions underlying the most widely accepted animal model for studying the transition to addiction- the LgA procedure. Contrary to what had previously been believed, the studies herein reveal that the development of addiction-like behavior- including high motivation for drug, continued drug use in the face of an adverse consequence, robust cue-induced reinstatement of drug-seeking, and

even escalation of intake- does not require the ingestion of the large amounts of cocaine associated with LgA self-administration. These findings provide new ways of thinking about what constitutes an appropriate animal model to study the development of drug addiction.

As put by the statistician George Box (Box et al., 2005), "*All models are wrong but some models are useful*". It is clearly problematic for the field if two purported animal models of addiction produce such different, and even opposite effects. However based on the findings presented here and their context in a broader literature, we believe that the IntA procedure more effectively produces addiction-like behavior through the sensitization of the mesolimbic DA system, while also more accurately modeling the temporal pattern by which humans use cocaine.

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